

# HealthMED

Journal of Society for development of teaching and business processes in new net environment in B&H

## EDITORIAL BOARD

|                     |                                                |
|---------------------|------------------------------------------------|
| Editor-in-chief     | <i>Mensura Kudumovic</i>                       |
| Execute Editors     | <i>Mostafa Nejati</i><br><i>Azra Kudumovic</i> |
| Editorial assistant | <i>Jasmin Musanovic</i>                        |
| Secretaries         | <i>Dzenana Jusupovic</i>                       |
| Technical editor    | <i>Eldin Huremovic</i>                         |
| Lectors             | <i>Mirnes Avdic</i>                            |

## Members

*Farah Mustafa (Pakistan)*  
*Yann Meunier (USA)*  
*Forouzan Bayat Nejad (Iran)*  
*Suresh Vatsyayann (New Zealand)*  
*Maizirwan Mel (Malaysia)*  
*Budimka Novakovic (Serbia)*  
*Bakir Mehic (Bosnia & Herzegovina)*  
*Diaa Eldin Abdel Hameed Mohamad (Egypt)*  
*Damir Marjanovic (Bosnia & Herzegovina)*  
*Farid Ljuca (Bosnia & Herzegovina)*  
*Emina Nakas-Icindic (Bosnia & Herzegovina)*  
*Ago Omerbasic (Bosnia & Herzegovina)*  
*Slavica Ibrulj (Bosnia & Herzegovina)*  
*Fatima Jusupovic (Bosnia & Herzegovina)*  
*Aida Hasanovic (Bosnia & Herzegovina)*  
*Dijana Avdic (Bosnia & Herzegovina)*  
*Selma Alicelebic (Bosnia & Herzegovina)*  
*Bozo Banjanin (Bosnia & Herzegovina)*

Address of the Sarajevo, Bolnicka BB  
Editorial Board phone/fax 00387 33 640 407  
healthMed\_bih@yahoo.com  
<http://www.healthmedjournal.com>

Published by DRUNPP, Sarajevo  
Volume 4 Number 1, 2010  
ISSN 1840-2291

## Sadržaj / Table of Contents

|                                                                                                                                                                                                               |       |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| <b>A comparative evaluation of communication apprehension among pharmacy and non-pharmacy undergraduates</b> .....                                                                                            | 3-11  |
| <i>Ejaz. MA, Azmi.S, Khan.TM</i>                                                                                                                                                                              |       |
| * * *                                                                                                                                                                                                         |       |
| <b>Vaginal douching practices in women and influential factors</b> .....                                                                                                                                      | 12-20 |
| <i>Güngör Güler, Mine Bekar, Hatice Ulusoy, Semra Kocatas</i>                                                                                                                                                 |       |
| * * *                                                                                                                                                                                                         |       |
| <b>Relationship between sociodemographic characteristics and migraine in working women</b> .....                                                                                                              | 21-28 |
| <i>Svetlana Simic, Dragan Simic</i>                                                                                                                                                                           |       |
| * * *                                                                                                                                                                                                         |       |
| <b>The impact of various factors on self-care of elderly</b> .....                                                                                                                                            | 29-37 |
| <i>Danica Zeleznik, Uros Zeleznik, Jadranka Stricevic</i>                                                                                                                                                     |       |
| * * *                                                                                                                                                                                                         |       |
| <b>Generic medicines: Perceptions of Pharmacists in Basrah, Iraq</b> .....                                                                                                                                    | 38-44 |
| <i>Adheed Khalid Sharrad, Mohamed Azmi Hassali, Asrul Akmal Shafie</i>                                                                                                                                        |       |
| * * *                                                                                                                                                                                                         |       |
| <b>Salt content in meals of boarding schools and students' restaurants in Novi Sad</b> .....                                                                                                                  | 45-51 |
| <i>Ljiljana Trajkovic-Pavlovic, Budimka Novakovic, Natasa Dragnic, Ljilja Torovic</i>                                                                                                                         |       |
| * * *                                                                                                                                                                                                         |       |
| <b>Performance of regional tuberculosis dispensaries in a province of Turkey in pre-DOTS era (1989-2002)</b> .....                                                                                            | 52-57 |
| <i>Sefa Levent Ozsahin, Sulhattin Arslan, Omer Tamer Dogan, Serdar Berk, İbrahim Akkurt</i>                                                                                                                   |       |
| * * *                                                                                                                                                                                                         |       |
| <b>Effects of Plateau Environment on Social Interaction</b> .....                                                                                                                                             | 58-62 |
| <i>Qun Yang, Rui'an Wang, Baoxin Shi, Wenbin Zhang, Tao Ke, Yebing Yang, Xiang jun Chen, Baohua Cao, Xiwen Liu, Yinling Zhang, Chungping Ni, Xina Yuan, Kaiyuan Li, Yulong Ma, Jingyuan Chen, Wenjing Luo</i> |       |

HealthMED is indexed in:

- Thomson Reuters ISI web of Science
- Science Citation Index-Expanded
- EBSCO Academic Research Premier
- Index Copernicus
- getCITED, and etc.



# Sadržaj / Table of Contents

|                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                              |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ***                                                                                                                                                                                                                                                | ***                                                                                                                                                                                                                                                                                                                          |
| <b>Risk factors of allergic rhinitis: a case-control study</b> ..... 63-70<br><i>Natasa Maksimovic, Vesna Tomic-Spiric, Slavenka Jankovic, Aleksandra Jovic-Vranes, Zorica Terzic-Supic, Janko Jankovic</i>                                        | <b>Determination of mean glandular dose from routine mammography for two age groups of patients</b> ..... 125-131<br><i>Suad Kunosic, Denis Ceke, Mustafa Kopric, Lidija Lincender</i>                                                                                                                                       |
| ***                                                                                                                                                                                                                                                | ***                                                                                                                                                                                                                                                                                                                          |
| <b>A Qualitative Evaluation of Nurses' Perception towards the Role of Pharmacist in Healthcare Setup in Pakistan</b> ..... 71-76<br><i>Azhar. S, Hassali. MA, Izham MI &amp; Khan .TM</i>                                                          | <b>The serum fructosamine concentration at patients with diabetes mellitus</b> ..... 132-137<br><i>Serdarevic N., Pepic E., Musanovic J., Pleho-Kapic A.</i>                                                                                                                                                                 |
| ***                                                                                                                                                                                                                                                | ***                                                                                                                                                                                                                                                                                                                          |
| <b>An epidemiological study of physical and mental health of rescuing soldiers in 5.12 Wenchuan earthquake</b> ..... 77-83<br><i>Wei qiang Zhang, Tian Sheng Sun, Jing Zhao, Ju qiang Han, Yong Hong Yang, Shu Jun Li, Ya Qun Ma</i>               | <b>Dosage of Erythropoietin and iron-replacement therapy in patients on chronic hemodialysis</b> ..... 138-142<br><i>Edin Ostrvica, Dzenana Ostrvica, Vahidin Katica</i>                                                                                                                                                     |
| ***                                                                                                                                                                                                                                                | ***                                                                                                                                                                                                                                                                                                                          |
| <b>Lessons from an IV Drug abuser: Reform the blood safety surveillance measures</b> ..... 84-86<br><i>Mehrdad Jalalian Hosseini, Reyhaneh Bazargani, Sadegh Shokri, Latiffah Latiff, Syed Tajuddin Syed Hassan, Mohammad Hosseinpour</i>          | <b>Application of the PowerPlex®S5 MINISTR System in Forensic DNA Analysis of Human Telogenic Hair</b> ..... 143-151<br><i>Lejla Kovacevic, Dzenisa Buljugic, Mirela Dzehverovic, Adaleta Durmic-Pasic, Damir Marjanovic</i>                                                                                                 |
| ***                                                                                                                                                                                                                                                | ***                                                                                                                                                                                                                                                                                                                          |
| <b>Dexamethasone / human chorionic gonadotrophin (hCG) and dexamethasone / nafarelin as a valid diagnostic tests in reproductive aged women with polycystic ovarian syndrome (PCOS)</b> ..... 87-94<br><i>Hajder E., Hajder M.</i>                 | <b>Evaluation of Serologic tests for diagnosis of Brucellosis</b> ..... 152-157<br><i>Sukrija Zvizdic, Ramiz Velic, Tarik Bajrovic, Lejla Velic, Sadeta Hamzic, Salih Tandir, Jasmin Musanovic, Azra Kudumovic</i>                                                                                                           |
| ***                                                                                                                                                                                                                                                | ***                                                                                                                                                                                                                                                                                                                          |
| <b>Results of treatment of simple unstable uncomplicated lower leg shaft fractures treated operatively and nonoperatively</b> ..... 95-101<br><i>Fuad Dzankovic, Dijana Avdic, Amra Macic-Dzankovic</i>                                            | <b>Modern Information Communication Technologies and educational technologies applied to education of medicine</b> ..... 158-162<br><i>Mensura Kudumovic, Dzafer Kudumovic, Nevres Mesanovic, Eldin Huremovic</i>                                                                                                            |
| ***                                                                                                                                                                                                                                                | ***                                                                                                                                                                                                                                                                                                                          |
| <b>Comparison in Mycobacterium Tuberculosis Positivity between Broncholaveolar Lavage and Sputum Examination - the Importance of Broncholaveolar Lavage in Diagnostic of Pulmonary Tuberculosis</b> ..... 102-105<br><i>Vesna Cukic</i>            | <b>Alopecia areata: New treatment modalities</b> ..... 163-167<br><i>Emina Kasumagic-Halilovic, Asja Prohic, Semra Cavaljuga</i>                                                                                                                                                                                             |
| ***                                                                                                                                                                                                                                                | ***                                                                                                                                                                                                                                                                                                                          |
| <b>The influences of traumatic brain injuries in largeness of fussion convergency</b> ..... 106-110<br><i>Raif Serdarevic, Emina Alimanovic-Halilovic</i>                                                                                          | <b>Sustained attention in Bosnian war veterans diagnosed with PTSD</b> ..... 168-174<br><i>Aida Sarac – Hadzihalilovic, Amela Kulenovic, Abdulah Kucukalic</i>                                                                                                                                                               |
| ***                                                                                                                                                                                                                                                | ***                                                                                                                                                                                                                                                                                                                          |
| <b>Bioavailability of antioxidants from tea infusions with honey addition</b> ..... 111-115<br><i>Aldina Kesic, Zorica Hodzic, Aida Crnkic, Mirzeta Saletovic, Benjamin Catovic</i>                                                                | <b>Re-affirmation of traditional values of space for the sake of healthier manner of life – analysis of traditional and modern materials, their influence to the human health and giving directions for future actions in the materialization of contemporary facilities</b> ..... 175-186<br><i>Chabbouh-Aksamija Lemja</i> |
| ***                                                                                                                                                                                                                                                | ***                                                                                                                                                                                                                                                                                                                          |
| <b>Metabolic syndrome: comparative analysis of dermatological, biochemical and electroneurographic parameters of n.medianus and n.ulnaris in patients of both sexes</b> ..... 116-124<br><i>Edin Suljagic, Nermina Hadzigraphic, Emir Tupkovic</i> | <b>New information about Von Willebrand's factor?</b> ..... 187-194<br><i>Nermina Babic, Jasminko Huskic, Emina Nakas-Icindic, Suzana Tihic-Kapidzic, Miralem Music</i>                                                                                                                                                      |
|                                                                                                                                                                                                                                                    | ***                                                                                                                                                                                                                                                                                                                          |
|                                                                                                                                                                                                                                                    | <b>Instructions for the autors</b> ..... 195-196                                                                                                                                                                                                                                                                             |

# A comparative evaluation of communication apprehension among pharmacy and non-pharmacy undergraduates

Ejaz. MA<sup>\*1</sup>, Azmi.S<sup>1</sup>, Khan.TM<sup>1,2</sup>

<sup>1</sup> School of Pharmaceutical sciences, Universiti Sains Malaysia, Malaysia

<sup>2</sup> Department of Pharmacy, Island college of technology, Malaysia

## Abstract

The goals of the present study were to assess and compare the level of communication apprehension (CA) among pharmacy undergraduate and non-pharmacy undergraduate students. To accomplish this aim a modified version of Zimbardo's scale was used. A total of 501 students showed participation in this study. A higher participation was observed from the respondents from the age group 21-25 years. About 235 (46.9%) of the respondents were Chinese followed by Malay, Indian and other ethnic minorities. Majority 53.5% of the students were from school of pharmacy. Findings demonstrate a high level of CA among pharmacy students. However, in general Chinese female were found at a high risk of CA. Race, gender and study discipline were found to be the factor associated with CA.

**Key words:** Communication apprehension, pharmacy undergraduate, non-pharmacy undergraduate

## Introduction

Communication is a dynamic way to share information and feelings to others (Cathcart, 1988). Communication can be either intentional or unintentional by adopting either verbal or non-verbal way (Berko et al., 1992). According to Berko et al., 1992 as an adult, one spends 42% of the communication time as a listener and 40% as a speaker. However, about 15% of the time is spent in reading and 1% in writing (Berko, et al., 1992).

Nowadays good communication skills are the assets of a professional. Especially in pharmacy practice effective communication skill are vital for a pharmacist (Baldwin et al. 1979). Whilst offering the pharmacy practice services pharmacist interact with different individuals like patients, their family member, medical staff and other pharmacists. For affective patient care it is essential for a pharmacist to have good communication skill. However, sometimes an affective communication is hindered due to the shyness or lack of confidence to communicate. In other words CA on the part of pharmacist act as a barrier to affective communication (Baldwin et al. 1979). According to Baldwin et al. 1979 this CA can be a possible factor compelling a pharmacist to avoid; patient communication, counseling or discussion forums with professional peers.

McCroskey, 2001 has defined CA as "The level of fear or anxiety associated with either real or anticipated communication with another person or a group of person" (McCroskey, 2001). There are two types of CA; writing apprehension and oral CA (Elias, 1999). According to Stanga and Ladd (1990) oral CA is due to an intense personal fear or anxiety about communicating. In other words, experts view oral CA as a deficiency to communicate verbally with other people which afterward distort effectual communication. However, Zimbardo, 1977 has associated CA with the shyness. Shyness is seen as, the lack of tendency to talk and engage in communication with others, Which 'may result from high CA, lack of verbal skills, or other causal factors (McCroskey & Ri-

chmond, 1980). Oral CA is considered to be an issue of more importance. Oral CA can be reduced by means of cognitive intercession to ensure better quality future professionals. The cognitive intercession strategies consist of the systematic desensitization; cognitive restructuring and assertiveness training (Stanga & Ladd, 1990).

Communication apprehension exists in every one; the level of shyness or hesitation to participate in the discussion is a marker to define the CA in to different level (Mc,Croskey & Richmond, 1980). This study aims to evaluate and compare the level of CA among the pharmacy undergraduates and non pharmacy undergraduates' students.

### Methodology

In August 2007 a cross sectional study was conducted among the pharmacy and non- pharmacy students at university Sains Malaysia (USM). USM is a public university with an estimated student's number five thousand over. Mainly four groups are at USM Malays, Chinese, Indians and foreigners. However, at undergraduate level there is a high percentage of Malays, Chinese and Indians.

### Participants

This study encompasses a non-experimental method. A cluster random sampling method was used to approach the potential participants. Two clusters were defined on the basis of types of participants; pharmacy undergraduates and non-pharmacy graduate. The non-pharmacy graduates were mainly from the school of arts, social sciences, maths, computer sciences, management and linguistics. Those willing to participate were the part of study. Ethical approvals from the research ethics committee of University Sains Malaysia were taken for this study. Moreover, a verbal consent was also taken from the respondents in order to assure the confidentiality of the information.

### Study tool

Face to face interviews were conducted using a prevalidated Zimbardo's scale. Zimbardo's, 1977 comes up with a scale to evaluate the CA among the students. This was scale previously validated by McCroskey 1970; 1977 & F1cCroskey, 1982. In order to make the Zimbardo's scale compliant with the Malaysian students' content validation was conducted.

The content validation process was comprised of two phases; phase one was the preliminary content validation conducted by the professionals at the school of pharmacy, USM. After phase one twenty two items out of twenty four were selected to be the best to attain the objectives of the study. A three item likert scale (Agree, Undisclosed and Disagree) was used in order to calculate the level of communication apprehension among the students. After the phase one content validation the questionnaire was translated in to Malay language in order to make the questionnaire easier to understand by the respondents. The translation of the questionnaire to Malay language was done by the experts at the school of linguistics, USM. The translation was rechecked by the professionals at school of pharmacy in order to check the appropriateness of the word according the study objectives. After the phase one content validation the questionnaire was subjected to the phase two validation. The phase two content validations involves a pilot survey conducted among the USM students. For the pilot study two clusters were defined; one pharmacy undergraduates and two non-pharmacy undergraduates. A total of fifty students were approached (twenty five from every cluster). Keeping in view the responses the reliability scale was applied and internal consistency of the study tool was estimated on the basis of Cronbach's Alpha ( $\alpha = 0.63$ ). Furthermore to assure the validity of the contents factor analysis was carried out. The content validity was estimated by using Bartlett's test of sphericity and Kaiser-Mayer-Olkin measure of sampling adequacy. The results appear that Bartlett's test of sphericity was significant 0.0000 and Kaiser-Mayer-Olkin measure of sampling adequacy was 0.790. According to Sheridan and Lyndall (2001), a measure of more than 0.6 reflects the adequacy of the contents of the questionnaire. Thus these results showed a considerable evidence of reliability and validity of the sampling tool.



### Contents of the questionnaire

The questionnaire comprised of two parts. One demographic part and second the part evaluating the shyness level. The demographic part comprises of four questions i.e age, sex, race and school. However, the second part comprise of twenty two items. Of these twenty, only sixteen items were used to evaluate the level of CA among the students. Furthermore on the basis of the responses form this section scoring was done. The list of the items considered for scoring are illustrated in Table 2. However, self evaluation and personal perception about shyness was estimated by using two items i.e Are you facing some problem in communication with your colleagues and teachers and what do you think

communication apprehension is a problem. The personal perception of the respondents about the importance of communication was evaluated by using a four item mentioned in Table 1.

### Response for the survey and Scoring of the responses

A total of seven hundred participants were approached using the cluster random sampling method (n=350 from every cluster). Of seven hundred respondents, n=501 respondents participated in this survey. The responses obtained from the items mentioned in Table 2 were used to evaluate the level of communication apprehension.

Table 1. Personal perception about the importance of communication

| Statement                                                            | Not important | Moderately important | Very important |
|----------------------------------------------------------------------|---------------|----------------------|----------------|
| 1. How you rate the importance of good communication in conversation |               |                      |                |
| 2. How you rate the important one to one interpersonal communication |               |                      |                |
| 3. How you rate importance of good group communication               |               |                      |                |
| 4. How you rate importance of good public speaking                   |               |                      |                |

Table 2 Evaluation of the shyness level among students

| Statements                                                                               | Scoring of responses |             |          |
|------------------------------------------------------------------------------------------|----------------------|-------------|----------|
|                                                                                          | Agree                | Undisclosed | Disagree |
| 1. I dislike to participate in the group discussion                                      | 2                    | 1           | 0        |
| 2. I feel nervous and tense while participating in a group discussion                    | 2                    | 1           | 0        |
| 3. Group discussion with new people makes me more tense and nervous                      | 2                    | 1           | 0        |
| 4. Usually I am nervous when I have to participate in a meeting                          | 0                    | 1           | 2        |
| 5. I am very calmed and relaxed when I am called upon to express my opinion at a meeting | 0                    | 1           | 2        |
| 6. I feel afraid to express myself at meetings                                           | 2                    | 1           | 0        |
| 7. Communication at meeting with my colleagues make me uncomfortable                     | 2                    | 1           | 0        |
| 8. I stay relaxed and confident while answering the questions in a meeting               | 0                    | 1           | 2        |
| 9. Participation in a conversation with a new colleague make me nervous                  | 2                    | 1           | 0        |
| 10. I feel confidents in a conversation and express my views without any fear            | 0                    | 1           | 2        |
| 11. I have no fear in giving speech                                                      | 0                    | 1           | 2        |
| 12. I feel tense while giving speech                                                     | 2                    | 1           | 0        |
| 13. My body shake while giving speech                                                    | 2                    | 1           | 0        |
| 14. I have confused and jumbled thoughts when I am giving a speech                       | 2                    | 1           | 0        |
| 15. I face the prospect of giving a speech with confidence                               | 0                    | 1           | 2        |
| 16. while giving speech I get so nervous , I forget eh facts I really know               | 2                    | 1           | 0        |

These responses were scored in order to categorise the communication apprehension in to further levels. The maximum possible score for the questionnaire was 32. However, the maximum score attained by the respondents was 21. Quartiles were applied to classify the communication apprehension in to sub levels i.e no communication apprehension, slight communication apprehension, moderate communication apprehension and high communication apprehension. Those score zero were in the criteria of no communication apprehension. However, those scored 1-5 ranked with slight communication apprehension, 6-10 ranked with moderate communication apprehension, 11-15 ranked with high communication apprehension and those with 16 and over with the highest communication apprehension.

**Data analysis**

For the purpose of data analysis, the Statistical package for social sciences (SPSS13.0®) was used. A parametric statistics has been used. In order to compare the difference in CA in gender and among the non pharmacy and pharmacy students' student *t- test* was applied. However, in order to compare the CA level among the ethnic groups One way *ANOVA* was used. Moreover, to identify the communication among the different racial groups *Post-Hoc* analysis was conducted.

**Results**

A total of N=501 respondents participated in the survey with a response rate of 71.6%. Majority 302 (60.3%) of the respondents were females. A higher participation was observed from the respondents from the age group 21-25 years. About 235 (46.9%) of the respondents were Chinese followed by Malay, Indian and other ethnic minorities. Details about the demographics of the respondents are mentioned in table 3.

*Table 3. Demographics of respondents*

| Demographics                              |         | N (%)       |
|-------------------------------------------|---------|-------------|
| <b>Gender</b>                             | Male    | 199 (39.7%) |
|                                           | Female  | 302 (60.3%) |
| <b>Race</b>                               | Malay   | 218 (43.5%) |
|                                           | Chinese | 235 (46.9%) |
|                                           | Indian  | 22 (4.4%)   |
|                                           | Other   | 26 (5.2%)   |
| <b>Pharmacy Students</b>                  |         | 268 (53.5%) |
| <b>Non-Pharmacy Students</b>              |         | 233 (46.5%) |
| <b>Age [Range=18-40]<br/>Mean=22 ±3.4</b> |         |             |
|                                           | 18-20   | 207 (41.3%) |
|                                           | 21-25   | 252 (50.3%) |
|                                           | 26-30   | 26 (5.2%)   |
|                                           | 31-35   | 10 (2.0%)   |
|                                           | 36-40   | 6 (1.2%)    |

*Table 4. Students perceptions towards the importance of communication*

| Statement                                                            | Students           | Not important | Moderately important | Very important |
|----------------------------------------------------------------------|--------------------|---------------|----------------------|----------------|
| 1. How you rate the importance of good communication in conversation | Pharmacy (268)     | 9             | 30                   | 229            |
|                                                                      | Non-Pharmacy (233) | 16            | 56                   | 157            |
| 2. How you rate the importance of interpersonal communication        | Pharmacy (268)     | 9             | 50                   | 209            |
|                                                                      | Non-Pharmacy (233) | 10            | 66                   | 157            |
| 3. How you rate importance of good group communication               | Pharmacy (268)     | 9             | 90                   | 169            |
|                                                                      | Non-Pharmacy (233) | 6             | 92                   | 135            |
| 4. How you rate importance of good public speaking                   | Pharmacy (268)     | 29            | 111                  | 128            |
|                                                                      | Non-Pharmacy (233) | 33            | 190                  | 110            |

### Perception about the importance of communication

Overall, most of the students consider good communication as a very vital factor for the oral, group and interpersonal communication. However, the turn out for the importance of communication in general public communication was low. Details about the student's perceptions toward the importance of communication are described in Table 4.

### Evaluation of communication apprehension

Communication apprehension among both groups was evaluated on the basis of the sixteen items mentioned in Table 2. The minimum score of the respondents was two and maximum was 21. Those score zero were in the criteria of no communication apprehension. However, those scored 1-5 ranked with slight communication apprehension, 6-10 ranked with moderate communication apprehension, 11-15 ranked with high communication

Table 5. Students responses about communication apprehension

| Statements                                                                               | Students           | Scoring of responses |             |          |
|------------------------------------------------------------------------------------------|--------------------|----------------------|-------------|----------|
|                                                                                          |                    | Agree                | Undisclosed | Disagree |
| 1. I dislike to participate in the group discussion                                      | Pharmacy (268)     | 35                   | 64          | 169      |
|                                                                                          | Non-Pharmacy (233) | 38                   | 41          | 154      |
| 2. I feel nervous and tense while participating in a group discussion                    | Pharmacy (268)     | 72                   | 57          | 139      |
|                                                                                          | Non-Pharmacy (233) | 48                   | 59          | 126      |
| 3. Group discussion with new people makes me more tense and nervous                      | Pharmacy (268)     | 126                  | 60          | 82       |
|                                                                                          | Non-Pharmacy (233) | 71                   | 61          | 101      |
| 4. Usually I am nervous when I have to participate in a meeting                          | Pharmacy (268)     | 135                  | 41          | 92       |
|                                                                                          | Non-Pharmacy (233) | 80                   | 49          | 104      |
| 5. I am very calmed and relaxed when I am called upon to express my opinion at a meeting | Pharmacy (268)     | 66                   | 64          | 138      |
|                                                                                          | Non-Pharmacy (233) | 84                   | 78          | 71       |
| 6. I feel afraid to express myself at meetings                                           | Pharmacy (268)     | 125                  | 67          | 76       |
|                                                                                          | Non-Pharmacy (233) | 62                   | 58          | 113      |
| 7. Communication at meeting with my colleagues make me uncomfortable                     | Pharmacy (268)     | 90                   | 67          | 111      |
|                                                                                          | Non-Pharmacy (233) | 54                   | 63          | 116      |
| 8. I stay relaxed and confident while answering the questions in a meeting               | Pharmacy (268)     | 57                   | 99          | 112      |
|                                                                                          | Non-Pharmacy (233) | 86                   | 86          | 61       |
| 9. Participation in a conversation with a new colleague make me nervous                  | Pharmacy (268)     | 142                  | 63          | 63       |
|                                                                                          | Non-Pharmacy (233) | 73                   | 74          | 86       |
| 10. I feel confidents in a conversation and express my views without any fear            | Pharmacy (268)     | 68                   | 72          | 128      |
|                                                                                          | Non-Pharmacy (233) | 93                   | 69          | 71       |
| 11. I have no fear in giving speech                                                      | Pharmacy (268)     | 49                   | 55          | 164      |
|                                                                                          | Non-Pharmacy (233) | 82                   | 62          | 89       |
| 12. I feel tense while giving speech                                                     | Pharmacy (268)     | 96                   | 71          | 101      |
|                                                                                          | Non-Pharmacy (233) | 58                   | 60          | 115      |
| 13. My body shake while giving speech                                                    | Pharmacy (268)     | 167                  | 49          | 52       |
|                                                                                          | Non-Pharmacy (233) | 104                  | 67          | 61       |
| 14. I have confused and jumbled thoughts when I am giving a speech                       | Pharmacy (268)     | 133                  | 70          | 65       |
|                                                                                          | Non-Pharmacy (233) | 81                   | 70          | 82       |
| 15. I face the prospect of giving a speech with confidence                               | Pharmacy (268)     | 70                   | 116         | 82       |
|                                                                                          | Non-Pharmacy (233) | 94                   | 85          | 54       |
| 16. while giving speech I get so nervous , I forget eh facts I really know               | Pharmacy (268)     | 144                  | 55          | 68       |
|                                                                                          | Non-Pharmacy (233) | 83                   | 62          | 88       |

apprehension and those with 16 and over with the highest communication apprehension. Detailed evaluation of the respondents is mentioned in Table 5 & Table 6. Overall, a high communication apprehension was found among the pharmacy students in comparison to non-pharmacy students and the difference were found statistically significant ( $p = <0.001$ ,  $t = -51.538$ ) (Table 6). In term of gender female ( $p = <0.001$ ,  $t = -53.430$ ) were found to face CA more in comparison to males (Table 6). However, on racial grounds Chinese student were found to have higher communication apprehension in comparison to others (Table 7).

**Discussion**

*Philosophy of Communication apprehension*

CA is found in almost every individual at a certain level. Not only the students they face problems but any one involve in any type of social or professional communication is at risk (McCroskey, 1977). Those facing CA are not only at the risk of low academic profile but also at the risk of poor self esteem and lack of social skills. Friedman, 1980 has associated CA with anxiety; few relate it to personality problem while McCroskey,

Table 6. Communication apprehension among different groups

| Demographics               | Slight CA<br>1-5 | Moderate CA<br>6-10 | High CA<br>11-15 | Highest CA<br>16 and over | Total | p-value                     |
|----------------------------|------------------|---------------------|------------------|---------------------------|-------|-----------------------------|
| <b>Race</b>                |                  |                     |                  |                           |       |                             |
| Malay                      | 24               | 49                  | 97               | 48                        | 218   | <0.001*<br>F=8.243<br>df= 3 |
| Chinese                    | 13               | 43                  | 97               | 82                        | 235   |                             |
| Indian                     | 4                | 7                   | 8                | 3                         | 22    |                             |
| Other                      | 5                | 8                   | 11               | 2                         | 26    |                             |
| Total                      | 46               | 107                 | 213              | 135                       | 501   |                             |
| <b>Gender</b>              |                  |                     |                  |                           |       |                             |
| Male                       | 23               | 59                  | 87               | 30                        | 199   | <0.001*<br>t=-53.430        |
| Female                     | 23               | 48                  | 126              | 105                       | 302   |                             |
| Total                      | 46               | 107                 | 213              | 135                       | 501   |                             |
| <b>School</b>              |                  |                     |                  |                           |       |                             |
| Pharmacy<br>Graduates      | 19               | 40                  | 113              | 96                        | 268   | <0.001*<br>t=-51.538        |
| Non- Pharmacy<br>Graduates | 27               | 67                  | 100              | 39                        | 233   |                             |
| Total                      | 46               | 107                 | 213              | 135                       | 501   |                             |

Mean score= 12.5 ± 4.6, (range; Minimum 2, Maximum 21),  $\square$  student t-test,  $\bar{I}$  one way ANOVA, p-value significant at 0.05

Table 7 Comparison of Communication apprehension among racial groups

| Race (A) | Compared Race group (B) | Mean Difference (A-B) | p value |
|----------|-------------------------|-----------------------|---------|
| Malay    | Chinese                 | -1.15075              | 0.036*  |
|          | Indian                  | 2.196831              | 0.134   |
|          | Other                   | 2.343684              | 0.062   |
| Chinese  | Malay                   | 1.150752              | 0.036*  |
|          | Indian                  | 3.347582              | 0.005*  |
|          | Other                   | 3.494435              | 0.001*  |
| Indians  | Malay                   | 2.19683               | 0.134   |
|          | Chinese                 | -3.34758              | 0.005*  |
|          | Other                   | 0.146853              | 0.999   |
| Others   | Malay                   | -2.34368              | 0.062   |
|          | Chinese                 | -3.49444              | 0.001*  |
|          | Indian                  | -0.14685              | 0.999   |

Post Hoc tukey, p-value significant at 0.05



1977 has associated CA with the situation. However, McCroskey, 1980; Bond, 1984 believe that CA can be a result of the following seven items.

- Lack of intellectual skills or low intellectual skills
- Poor speech skills
- Social shyness
- Social isolation
- Nervousness while conducting a communication
- Low social self-esteem
- Ethnic/cultural issues

In spite of all these facts, one can not ignore the Negative Cognitive Appraisal Model (NCAM) (Glaser, 1981). In NCAM, Glaser, 1981 stated that CA is a result of poor early language performance of the individual. In response to the poor language performance that individual fear to speak and this lead to CA. Bond, 1984 has further refined this aspect of CA and associated it with the negative remarks on the poor language performance by the classmates, teacher or family member. Such response put an individual at a risk of high CA (Bond, 1984)

#### *Communication apprehension among Pharmacy and Non pharmacy students*

Findings of this study report a higher CA among pharmacy students. Majority 60.3% of the respondents were female and in term of ethnic majority 46.9% were Chinese. About a half of the respondents 53.5%, were from school of pharmacy (Table 2). In response to evaluation of the student's

perception regarding communication majority has positive perception toward the importance of communication (Table 4). Rating of responses in terms of educational background i.e (Pharmacy and Non-Pharmacy), the importance of ; good communication in conversation, interpersonal communication, group communication & good public speaking was observed higher among pharmacy students in comparison to non-pharmacy students. However, CA among professional peers, meetings, speech and group discussion pharmacy students were on the top (Table 5). Especially if the evaluation is done on the basis of problems faced while delivering a speech, majority facing these problems were pharmacy students. Scoring on the basis of the responses mentioned in table 5 revealed the significant CA among pharmacy and non-pharmacy students ( $<0.001$ ,  $t=-51.538$ ) (Table 6). Further exploration on the basis of gender showed that the CA was significant among female (0.001,  $t=-53.430$ ) (Table 6) (Rosan, 1994). Findings demonstrated that regardless of gender and school, Chinese students were found to have a higher level of CA than Malays and Indians (Table 7). Majority (64.2%) of the pharmacy students were facing the problem of communication apprehension.

#### *Possible cause of communication apprehension among pharmacy students*

This is one of the difficult issues to debate. What are the factors resulting CA among pharmacy students? Either student is the only one responsible for it or there is some cultural or social factors contributing in this situation. There is a

*Table 8. Self evaluation and personal perception of students regarding communication apprehension*

| Statement                                                                      | Response | Pharmacy (268) | Non-Pharmacy (233) | t     | df  | P Value |
|--------------------------------------------------------------------------------|----------|----------------|--------------------|-------|-----|---------|
| Are you facing some problem in communication with your colleagues and teachers | Yes      | 172            | 119                | 1.420 | 500 | 0.156   |
| What do you think communication apprehension is a problem                      | Yes      | 147            | 90                 | 5.991 | 289 | <0.001* |

*Student t-test, p-value significant at 0.05*

possibility that the communication apprehension among the pharmacy students is a blend of the social and cultural factors. As the findings of the study revealed that majority reporting a higher level of CA were Chinese. May be the some underlying cultural issues are the possible factors behind this. However, from my teaching experience and the experience of my other professional peer at different Malaysian universities, Chinese students were found at a higher risk of CA than others. Overall a lack of participation is observed among the Chinese students in the question answer session in the lecture hall, however Chinese students are observed to be more comfortable in asking the questions and discussing problems individually with the lecturer in his office or discussion room instead of asking the questions in the class. While discussing the issue of CA among the pharmacy and non-pharmacy graduates a possible reason can the lack of participation of pharmacy students in the extracurricular activities in the campus. The students from the other disciplines were found more involved in the games, cultural shows, and recreation in comparison with the pharmacy students. Lack of participation of pharmacy students can be a possible factor contributing to the high level of CA.

### **Conclusion**

Findings demonstrate a high level of CA among pharmacy students. However, in general Chinese female were found at a high risk of CA. Race, gender and study discipline were found to the factor associated with CA.

### **Recommendation**

There is an immediate need to focus on the communication aspect of pharmacy. Negligence in this regards may result lack of confidence in the future pharmacist to conduct an affective counselling session for the patients (ZmagoTurk & Eva-Turk ,2009). Moreover, the communication apprehension also hinders the learning process of the pharmacist which will result inability to share and retrieve patient and pharmacy oriented knowledge

from the professionals and colleagues. Furthermore future studies should focus on the exploration of the factors responsible for the communication apprehension among the pharmacy students. In addition to this evaluation of the academic staff attitude with the students is also essential which may be one of the main factors

## Reference

1. Baldwin; H. J., McCroskey, J. C. & TnuVson, T. J. (1979) *Communication apprehension in the pharmacy student. American Journal of Pharmaceutical Education.* 43; 91-93.
2. Berko, R.M., Wolvin, A.D., & Wolvin, D.R. (1992) . *Communicating: A social and career focus (5th ed.)*. Boston: Houghton Mifflin Company.
3. Bond, B. D. (1984). *Silent incarceration. Contemporary Education*, 55,95-101.
4. Cathcart., Robert. S., & Samovar, L. A. (1998). *Small group communication (5<sup>th</sup> ed.)* Dubuque, IA: Brown.
5. Elias, R. Z. (1999). *An examination of nontraditional accounting students' communication apprehension and ambiguity tolerance. Journal of education for business.* Vol. 75(1), pp. 38-41
6. Glaser, S. R. "Oral Communication Apprehension and Avoidance: (1981)*The Current Status of Treatment Research.*" *COMMUNICATION EDUCATION* 30: 321-41.
7. McCroskey, J. C. "Quiet Children in the Classroom: (1980) *On Helping Not Hurting.*" *COMMUNICATION EDUCATION* 29: 239-44.
8. McCroskey, J. C. ( 1978). *Validity of the PRCA as an index of oral communication apprehension. Communication Monographs*, 45, 192-203.
9. McCroskey, J. C. (1970). *Measures of communicative-bound anxiety. Speech Monographs.* Vol. 37, pp. 269-277.
10. McCroskey, J. C. (1977). *Oral communication apprehension: A summary of recent theory and research. Human communication research.* Vol. 4, pp. 78-96.
11. McCroskey, J.C., & Richmond, V. (1980) *The quiet ones: Communication Apprehension and shyness.* Dubeque, Iowa: Commcomp, Gorsuch Scaresbuch, 1 pp. 2 1).
12. McCroskey, J.C. (2001). *An introduction to rhetorical communication. 8th Ed., Massachusetts: Prentice Hall.* Monograph, 37, (pp. 269-277).
13. Rosna, A.H. (1994). *Factors affecting communication apprehension among second language learners. Paper presented at the Thai TESOL 15<sup>h</sup> annual convention: Diversity in the classroom, Bangkok, Thailand.*
14. Sheridan, J. C. & Lyndall, G. S. (2001). *SPSS analysis without anguish version 10.0 for Windows, Singapore: John Wiley & Sons Australia, Ltd.*
15. Stanga, K. G. & Ladd, R. T. (1990). *Oral communication apprehension in beginning accounting majors: An exploratory study. Issues in Accounting education.* Vol. 5(2), pp. 180-194.
16. Zimbardo, P. (1977). *Shyness: What it is; what to do about it.* (Reading, Mass: Addison-Wesley, (pp. 92).
17. ZmagoTurk & EvaTurk (2009) *Our experience with evaluation of communication among older patients and health workers HealthMED* 3(3); 195-203.

### Corresponding author:

Tahir Mehmood Khan

Lecturer, Department of Pharmacy

Island College of technology

Malaysia

e-mail: tahir.pks@gmail.com

# Vaginal douching practices in women and influential factors

Güngör Güler<sup>1</sup>, Mine Bekar<sup>2</sup>, Hatice Ulusoy<sup>3</sup>, Semra Kocatas<sup>1</sup>

<sup>1</sup> Cumhuriyet University, Faculty of Health Sciences, Department of Nursing, Turkey

<sup>2</sup> Cumhuriyet University, Faculty of Health Sciences, Department of Midwifery, Turkey

<sup>3</sup> Cumhuriyet University, Faculty of Health Sciences, Department of Health Management, Turkey

## Abstract

**Objective:** Vaginal douching can be very harmful to women's health and can lead to health problems because of the introduction of many pathogenic microorganisms into the ascending pathways. This research was conducted to investigate the vaginal douching practices of women and influential factors of this practices.

**Materials and methods:** The research population of this cross-sectional research, consisted of 665 women, attending a primary healthcare centre, between March - May in 2006. A questionnaire, developed by researchers, was used as a data collection tool.

**Results:** The prevalence of vaginal douching of the women was found to be 54.3%. The major reason for douching was found to be for general body hygiene. In total, 83.1% of the women applied vaginal douching after each elimination (defecation and urination). Of the participants 12.2% reported that they have learned this practice from health care personnel.

**Conclusion:** the prevalence of vaginal douching was found to be high. Practicing vaginal douching after each elimination may expose high risk to various genital infections.

**Key words:** Perineal hygiene; Traditional intravaginal practices; Vaginal douching

## Introduction

**Objectives of the Study:** The purpose of this study was to investigate the vaginal douching practices of women and influential factors of this practices.

**Background:** Vaginal douching is a commonly applied, traditional practice among women as a means of 'cleansing' the vagina. It is defined as washing the inside of the vagina (inserting of fingers into vagina) with water or another liquid. Women in many developed and developing countries use vaginal douching for personal cleanliness and general hygiene, for decreasing complaints related to genital infections, preventing sexually transmitted diseases, preventing pregnancy, and for esthetic and religious reasons (Çalışkan, 2005; Demirbağ, 2000; Foch, McDaniel & Chacko, 2001; Karatay & Özvarış, 2006; Ness et al., 2002; Rupp, Short, Head-Caroll & Rosenthal, 2006).

International studies on this subject have reported a prevalence of vaginal douching between 27% and 80% (Cottrell, 2006; Foch, McDaniel & Chacko, 2001; Kim Oh, Merchant & Brown, 2002; Simpson, Merchant, Grimley & Kim Oh, 2004; Vermund et al., 2001). Nationally, although it is a well known and common practice among Turkish women, only five studies have been found and none of them was carried out in a health centre. These studies reported prevalence of vaginal douching as 50.2% (Çalışkan, 2005), 58.0% (Karaer, Avşar, Özkan, Bayır & Sayan, 2005), 61.5% (Ege, Timur, Zincir & Egri, 2007), 64.0% (Hodoglugil, Aslan & Bertan, 2000; Karaer, Boylu & Avsar, 2005). In these studies it was reported that Turkish women practice vaginal douching as part of personal hygiene, as part of religious practices, or after menstruation. Previous works have shown that vaginal douching can be very harmful to women's health and can lead to health problems from the introduction of many pathogenic microorganisms into the ascending pathways. Vaginal

douching disturbs the normal vaginal flora that exists in the vagina to protect it against infection and leads the foundation for infection. Vaginal douching causes many health problems including complaints of vulvovaginal irritation, such as itching, burning and dyspareunia, infection, vulvovaginal candidiasis, trichomonas vaginalis, abnormal and malodorous vaginal drainage, cervical erosion, pelvic inflammatory disease (PID) and related health problems, ectopic pregnancy, decreased fertility and infertility, risk of sexually transmitted diseases, bacterial vaginosis, chlamydia, Herpes Simplex Type II, Chlamydia, Human Papilloma virus (HPV), preterm birth, low birth weight, and cervical cancer (Chiaffarino, Parazzini, De Besi & Lavezzari, 2004; Cottrell, 2006; Fiscella, Franks, Kendrick, Meldrum & Kieke, 2002; Fiscella, Franks, Kendrick & Bruce, 1998; Foch, McDaniel & Chacko, 2001; Joesoef et al., 2001; Karatay & Özvarış, 2006; Misra & Trabert, 2007; Ness et al., 2002; Newton, Piper, Shain, Perdue & Peairs, 2001; Scholes et al., 1998; Sun et al., 2005; Vermund et al., 2001).

There are various investigations about the origin of knowledge about vaginal douching. Simpson, Merchant, Grimley and Kim Oh (2004) proposed that 43% of women learned about this practice from close relatives. However Cottrell (2006) found that 70.5% of women learned from close relatives and 6.2% from physicians and nurses. In a study by Foch, McDaniel and Chacko (2001) 70% of the women learned about this practice from their mothers.

**Research Questions:** What are the frequency (timing of the vaginal douching), prevalence and the reasons behind vaginal douching practices among women in Sivas province.

## Methods

**Design:** This study was a cross-sectional type. The research population was comprised of all married, divorced and widowed women who attended a Primary Health Center (PHC) in Sivas province between March-May 2006. Information obtained from employees of the health center and from the health center records revealed that March and May is the busiest season for the health center.

Therefore, data was collected during this period. Sampling method was not used, all 665 women who agreed to participate in this study were included. Single women were not included due to strong cultural values placed on having an intact hymen. That is, single women, who are not virgin, are not approved of in society and would not openly admit that they were not virgins. Therefore, they were not included in the research sample.

The PHC, where the research carried out, serves some 20.000 people around the centre. In Turkey PHCs are the important parts of providing primary health care services. Health protection, health promotion and outpatient treatment are given to all people covered by centre. Patients, who do not have health insurance or have low income, prefer these centers since they provide cheaper health care services as compared with hospitals. Especially women and children have priority in using these services provided by PHCs. Nurses and midwives employed in PHCs, work closely with women in the community, and play an increasingly prominent role in the provision of health care. They have a significant role in health education, advice and counselling, and in preventing harmful traditional practices such as vaginal douching.

The region covered by the PHC, where the research is performed, is a rural area and have primarily lower socio-economic level residents. In such regions, it is well known that vaginal douching practice is widespread.

**Measures:** A questionnaire was developed by the researchers used as a data collection tool. In the questionnaire, there were 20 descriptive questions about the women's age, occupation, educational level, number of children, as well as questions about the women's vaginal douching practices and factors that may have an influence on vaginal douching. The questionnaire was piloted, in another PHC in Sivas province, in order to ensure the validity and reliability of the questionnaire. In the main study, the questionnaire was applied using a face-to-face interview technique with the women. The questionnaire was administered to the research population by the researchers. The interviews took place in an appropriate place in the health center so that the women could be alone with the interviewer in a quiet environment. Before collecting data from the women they were informed



by the interviewer about the purpose and length of the interview, and that the results would not be used for any other purpose. It was also explained that the participation to the research was voluntarily. All research data were obtained by asking the women themselves. Health center records and other sources of data were not used.

Before beginning data collection written permission was obtained from the relevant authorities. The women gave their verbal consent to participate in the research.

**Statistical Analysis** Data was evaluated in the SPSS 13.0 software program using percentage and Chi square. Statistical tests were conducted at the 5% significance level.

## Results

The descriptive characteristics of the women are shown in Table 1. One fifth of the women were under 25 years of age. The percentage of women in the 26-30 (40.4%) and over 40 year old (40.8%) age groups were nearly the same. The majority of the women (97.2%) were housewives, 33.5% had less than a primary school education, more than half (58%) had a primary or middle school education, 13.5% had no social security health insurance, 66.3% were in nuclear families, 32.5% were in extended families, one third had four or more children, and 27.8% described their income level as low. In the examination of their husbands' demographic data, it was seen that 62.9% had primary to middle school level education, and 5.1% were unemployed.

In this study, 6.9% of the women did not use any method of family planning method, and 54.6% used some method. However 22.9% of the second group used ineffective methods, such as withdrawal. Furthermore, the prevalence of vaginal douching of the women was found to be 54.3%. Of the women who use vaginal douching (inserting of fingers into vagina), 89.5% used just water and 10.5 % use soapy water. The women used vaginal douching while cleaning the perineum, after sexual intercourse, while bathing, and at the end of their menstrual cycles (Table 2).

*Table 1. Descriptive Characteristics of Women Included in the Research*

| Descriptive Characteristics             | n   | %     |
|-----------------------------------------|-----|-------|
| <b>Age</b>                              |     |       |
| <25                                     | 125 | 18.8  |
| 26-39                                   | 269 | 40.4  |
| ≥40                                     | 271 | 40.8  |
| <b>Educational level</b>                |     |       |
| <Primary school                         | 223 | 33.5  |
| Primary-middle school                   | 386 | 58.0  |
| ≥High school                            | 56  | 8.5   |
| <b>Occupation</b>                       |     |       |
| Housewife                               | 647 | 97.2  |
| Civil servant                           | 10  | 1.5   |
| Laborer                                 | 4   | 0.6   |
| Self-employed                           | 3   | 0.5   |
| Farmer                                  | 1   | 0.2   |
| <b>Husband's educational level</b>      |     |       |
| <Primary school                         | 69  | 10.4  |
| Primary-middle school                   | 418 | 62.9  |
| ≥High school                            | 178 | 26.7  |
| <b>Husband's occupation</b>             |     |       |
| Unemployed                              | 34  | 5.1   |
| Civil servant                           | 87  | 13.1  |
| Laborer                                 | 139 | 20.9  |
| Self-employed                           | 266 | 40.0  |
| Farmer                                  | 25  | 3.8   |
| Retired                                 | 101 | 15.2  |
| Not answered                            | 13  | 1.9   |
| <b>Social security health insurance</b> |     |       |
| Yes                                     | 575 | 86.5  |
| No                                      | 90  | 13.5  |
| <b>Family type</b>                      |     |       |
| Nuclear                                 | 441 | 66.3  |
| Extended                                | 216 | 32.5  |
| Broken                                  | 8   | 1.2   |
| <b>Number of children</b>               |     |       |
| None                                    | 47  | 7.1   |
| One child                               | 80  | 12.0  |
| Two children                            | 169 | 25.4  |
| Three children                          | 163 | 24.5  |
| ≥Four children                          | 206 | 31.0  |
| <b>Income level</b>                     |     |       |
| Low                                     | 185 | 27.8  |
| Middle                                  | 453 | 68.1  |
| High                                    | 27  | 4.1   |
| Total                                   | 665 | 100.0 |

*Table 2. Time When Women Did Vaginal Douche (n=361)*

| Vaginal douche time          | n   | %    |
|------------------------------|-----|------|
| While washing perineum       | 300 | 83.1 |
| After having sex             | 278 | 77.0 |
| While bathing                | 260 | 72.0 |
| During menses and at the end | 209 | 57.9 |
| All the time                 | 18  | 12.2 |
| When doing ritual cleansing  | 4   | 1.1  |
| When having extra drainage   | 1   | 0.3  |

In the examination of the reasons why women use vaginal douching, it was determined that the overwhelming majority (82.5%) used vaginal douching for cleanliness, but 6.4% used vaginal douching for religious reasons (Table 3).

*Table 3. Reasons Why Women Used Vaginal Douche*

| Reasons for vaginal douche                  | n          | %            |
|---------------------------------------------|------------|--------------|
| Ensure cleanliness                          | 298        | 82.5         |
| Ensure cleanliness and protect from disease | 36         | 10.0         |
| Religious belief                            | 23         | 6.4          |
| Doesn't know                                | 3          | 0.8          |
| To prevent pregnancy                        | 1          | 0.3          |
| <b>Total</b>                                | <b>361</b> | <b>100.0</b> |

When the women were asked where they learned about vaginal douching it was determined that 40.9% learned on their own, 31.3% from relatives, and 12.2% from health care personnel (nurses, midwives and physicians) (Table 4).

*Table 4. People From Whom the Women Learned about Vaginal Douching*

| People from whom learned vaginal douching       | n          | %            |
|-------------------------------------------------|------------|--------------|
| Learned on her own                              | 148        | 40.9         |
| Relatives                                       | 113        | 31.3         |
| Health personnel (midwife, nurse and physician) | 44         | 12.2         |
| Neighbors                                       | 32         | 8.9          |
| Religious publication                           | 18         | 5.0          |
| Husband                                         | 4          | 1.1          |
| TV, magazine, book                              | 2          | 0.6          |
| <b>Total</b>                                    | <b>361</b> | <b>100.0</b> |

There was a significantly higher percentage of vaginal douching application in the following groups (Table 5): younger women under 25 years old,

those with lower educational level (primary school or lower), those whose husbands were farmers or self-employed, those without health insurance, those with a low income level, those living in an extended family, those with four or more children, and those who used Intra Uterin Devices (IUD) or injection as their method of family planning.

Of the women, 71.4% had symptoms of genital infection. This prevalence of genital infection was 55.2% in women who used vaginal douching. Comparative overview of Table 5 exhibited that 61.3% of the women, who used vaginal douching, had itching, 58.1% had pain, burning and leakage when urinating, 55.3% had pain in the abdomen or groin, the same percentage had odor, and 54.3% had abnormal drainage.

## Discussion

Douching or douche products may disrupt the normal vaginal microbial flora, increasing susceptibility to pathogenic microorganisms capable of rapid proliferation. The repetitive and frequent use of douche products over time may change the pH of vaginal secretions and reduce the prevalence and concentration of hydrogen peroxide-producing lactobacilli, creating a less protective vaginal environment (Bruce, Fiscella & Kendrick, 2000). The prevalence of vaginal douching was found to be 54.3%. Karaer, Avsar, Özkan, Bayır and Sayan (2005) also proposed the similar results. Demba et al. (2005) determined that 57.6% used just water and 22.2% used soapy water for vaginal douching. Misra, Trabert and Atherly-Trim (2006) proposed that 90.8% of African American women used vaginal douching with vinegar and water. Rupp, Short, Head-Carroll and Rosenthal (2006) exhibited that 92% of the population, most of which was composed of African American women, used the commercial douche products. On the other hand, in a similar work, these commercial products were used by 58% of the research group, 55% of whom were African American (Cottrell, 2006). Sun et al. (2005) suggested that women, who used salty water or detergent, had a higher HPV positivity than women who used tap water.

In the examination of time when women use vaginal douching (Table 2), it was seen that the

Table 5. Characteristics of Women According to Douching Practice (n=665)

| Characteristics                         | Women who douche |       | Women who don't douche |      | p     |
|-----------------------------------------|------------------|-------|------------------------|------|-------|
|                                         | n                | %     | n                      | %    |       |
| <b>Age</b>                              |                  |       |                        |      |       |
| ≤25                                     | 73               | 58.4  | 52                     | 41.6 | 0.491 |
| 26-39                                   | 147              | 54.6  | 122                    | 45.4 |       |
| ≥40                                     | 141              | 52.0  | 130                    | 48.0 |       |
| <b>Educational level</b>                |                  |       |                        |      |       |
| <Primary school                         | 133              | 59.6  | 90                     | 40.4 | 0.020 |
| Primary-middle school                   | 206              | 53.4  | 180                    | 46.6 |       |
| ≥High school                            | 22               | 39.3  | 34                     | 60.7 |       |
| <b>Husband's education</b>              |                  |       |                        |      |       |
| <Primary school                         | 38               | 55.1  | 31                     | 44.9 | 0.021 |
| Primary-middle school                   | 242              | 57.9  | 176                    | 42.1 |       |
| ≥High school                            | 81               | 45.5  | 97                     | 54.5 |       |
| <b>Husband's occupation*</b>            |                  |       |                        |      |       |
| Unemployed                              | 18               | 52.9  | 16                     | 47.1 | 0.013 |
| Civil servant                           | 38               | 43.7  | 49                     | 56.3 |       |
| Laborer                                 | 65               | 46.8  | 74                     | 53.2 |       |
| Self-employed                           | 163              | 61.3  | 103                    | 38.7 |       |
| Farmer                                  | 17               | 68.0  | 8                      | 32.0 |       |
| Retired                                 | 53               | 52.5  | 48                     | 47.5 |       |
| <b>Social security health insurance</b> |                  |       |                        |      |       |
| Yes                                     | 300              | 52.2  | 275                    | 47.8 | 0.004 |
| No                                      | 61               | 67.8  | 29                     | 32.2 |       |
| <b>Family type</b>                      |                  |       |                        |      |       |
| Nuclear                                 | 237              | 53.7  | 204                    | 46.3 | 0.882 |
| Extended                                | 120              | 55.5  | 96                     | 44.5 |       |
| Broken                                  | 4                | 50.0  | 4                      | 50.0 |       |
| <b>Number of children</b>               |                  |       |                        |      |       |
| None                                    | 20               | 42.6  | 27                     | 57.4 | 0.001 |
| One child                               | 45               | 56.2  | 35                     | 43.8 |       |
| Two children                            | 91               | 53.8  | 78                     | 46.2 |       |
| Three children                          | 71               | 43.6  | 92                     | 56.4 |       |
| ≥Four children                          | 134              | 65.0  | 72                     | 35.0 |       |
| <b>Income level**</b>                   |                  |       |                        |      |       |
| Low                                     | 105              | 56.8  | 80                     | 43.2 | 0.463 |
| Middle                                  | 244              | 53.9  | 209                    | 46.1 |       |
| High                                    | 12               | 44.4  | 15                     | 55.6 |       |
| <b>Family planning method</b>           |                  |       |                        |      |       |
| IUD                                     | 69               | 61.1  | 44                     | 38.9 | 0.398 |
| Condom                                  | 64               | 58.2  | 46                     | 41.8 |       |
| Withdrawal                              | 38               | 45.8  | 45                     | 54.2 |       |
| Tubal ligation                          | 17               | 58.6  | 12                     | 41.4 |       |
| Pill                                    | 12               | 52.2  | 11                     | 47.8 |       |
| Injection                               | 3                | 60.0  | 2                      | 40.0 |       |
| <b>Symptoms of genital infection</b>    |                  |       |                        |      |       |
| Pain, burning with urination            | 140              | 58.1  | 101                    | 41.9 | 0.192 |
| Abdominal, groin pain                   | 182              | 55.3  | 147                    | 44.7 | 0.916 |
| Abnormal drainage                       | 146              | 54.3  | 123                    | 45.7 | 0.658 |
| Odor                                    | 109              | 55.3  | 88                     | 44.7 | 0.949 |
| Itching                                 | 100              | 61.3  | 63                     | 38.7 | 0.050 |
| Pain during sexual intercourse          | 2                | 100.0 | -                      | -    | 0.201 |

\* 13 women did not provide any answer

\*\* Defined according to women's own statements

top four were while cleaning the perineum, after sexual intercourse, while bathing, and at the end of their menstrual cycles. The overwhelming majority (82.5%) use vaginal douching for cleanliness (Table 3). It was also determined that 6.4% of the women use vaginal douching for religious reasons. In many studies, similar reasons were reported by women for douching: to feel clean, for personal hygiene, to remove blood and odor at the end of menstruation, after sexual intercourse, and to eliminate symptoms of odor and drainage (Blythe, Fortenberry & Orr, 2003; Cottrell, 2006; Foch, McDaniel & Chacko, 2001; Karaer, Avsar, Özkan, Bayır & Sayan, 2005; Kim Oh, Merchant & Brown, 2002; Simpson, Merchant, Grimley & Kim Oh, 2004). Women who use vaginal douching after sexual intercourse are at risk for sexually transmitted infections and recurrent infections (Bruce, Fiscella & Kendrick, 2000; Çalışkan, 2005; Demirbağ, 2000; Karatay & Özvarış, 2006; Kim Oh, Merchant & Brown, 2002; Ness et al., 2002). Sun et al. (2005) found that women who use vaginal douching after sexual intercourse have a 50% greater risk for HPV.

Although only 6.4% of the women in this study used vaginal douching for religious reasons alone, it is likely that a significant percentage of the women who used vaginal douching for cleanliness also used it for religious reasons, since, the practice of cleanliness is very important according to Islamic religious beliefs: individuals are required to bathe the entire body (referred to as "gusül" in Turkish), particularly after menstruation and sexual intercourse. It was supported by 77% of the women reporting that they practice vaginal douching after sexual intercourse and 57.9% at the end of their menstrual cycle (Table 2).

The most striking finding of the present research was that 83.1% of the women use vaginal douching when they are cleaning their perineum after elimination, that is, nearly every day. Taking into consideration that it is a common practice in Turkish society to clean the perineal region by hand after having a bowel movement, those who are using a vaginal douche during perineal cleaning can be said to be at significantly increased risk for genital infections. In addition, this is assisted by the results in this study that 71.4% of the women had symptoms of genital infection.

It was stated from this work that there was a lower percentage who learned about vaginal douching from relatives but a higher percentage who learned from health care personnel as compared with the findings of previous studies (Cottrell, 2006; Foch, McDaniel & Chacko, 2001; Simpson, Merchant, Grimley & Kim Oh, 2004). A lower percentage of women stated that they learned about vaginal douching on their own compared to the Karaer and his co-workers' findings (Karaer, Avsar, Özkan, Bayır & Sayan, 2005). Even though approximately 41% of the women in this study stated that they learned about vaginal douching on their own, it can be said that this practice is learned during the socialization process from those around them, including their relatives and friends. In spite of the fact that many health problems, primarily infections, are related to vaginal douching, it is an interesting finding that in this research 12.2% of the women were encouraged to douche by health care personnel. The majority of these were nurses and midwives (7.5%), followed by physicians (2.5%), and "health workers" (2.2%). This finding makes it clear that all health care personnel, particularly nurses and midwives, need to be taught about this subject in continuing educational programs.

In some studies, the percentage of women at an early age who used vaginal douching was found to be lower than those in older age groups (Blythe, Fortenberry & Orr, 2003; Karaer, Avsar, Özkan, Bayır & Sayan, 2005). However in this study, although not reaching statistical significance, a higher percentage of the younger women were found to use vaginal douching. This result can be explained for reasons such as the younger women being more active sexually and menstruating, because a significant number of women stated that they practiced vaginal douching for cleanliness during and after menstruation and after sexual intercourse (Tables 2 and 3). For this reason, young women, who are menstruating and sexually active, would practice vaginal douching more than older women.

The prevalence of women using vaginal douching was found to be higher in women with low educational level in this study. This result is similar to that reported from other studies (Cottrell, 2006; Karaer, Avsar, Özkan, Bayır & Sayan, 2005). A

similar result was found in the evaluation of their husbands' educational level. Both results were found to be statistically significant. It is known that as educational level increases, the likelihood of individuals practicing negative behaviors in the health area, as in other areas, decreases.

When the prevalence of douching was compared to the occupations of the women's husbands, it was observed that there was a higher prevalence of vaginal douching in the wives of men who were farmers and self-employed. This was followed by the wives of men who were unemployed and retired. The lowest prevalence was in the wives of men who were civil servants and laborers. This result may be related to the higher educational level of civil servants and laborers compared to farmers and self-employed people. A statistically significant relationship was found according to husband's occupation.

In the comparison of those who use vaginal douching with the presence of social security health insurance and income level, it was determined that women, who did not have social security health insurance and women with a low income level, had higher prevalence of vaginal douching. The difference between those who did and did not have social security health insurance was found to be statistically significant. This result is likely to be related to the fact that women, who do not have social security health insurance, generally have low income levels and are unable to benefit from health care services (Cottrell, 2006; Karaer, Avsar, Özkan, Bayır & Sayan, 2005; Simpson, Merchant, Grimley & Kim Oh, 2004).

Comparative investigation of vaginal douching prevalence exhibited that women living in extended families had a higher prevalence of vaginal douching, but this difference was not found to be statistically significant. The increased prevalence of this traditional health practice in extended types of families may have caused this result. For this reason, priority needs to be given to educate women living in extended families.

Prevalence of vaginal douching with number of children was striking that the women with four or more children had a higher prevalence of vaginal douching. This was followed by women with one and two children. The difference between these groups was found to be statistically significant. It

is suggested that as the number of children increase the number of women who use this practice to prevent pregnancy also increases. This theory is supported by the finding that 77% of the women who used vaginal douching did so after sexual intercourse. However in the examination of reasons for vaginal douching it was seen that only 0.3% of the women use vaginal douching to prevent pregnancy (Table 3). Although more women stated that they use vaginal douching for cleanliness, they may include the removal of sperm from the vagina to prevent pregnancy as a basis for this cleanliness when they douche after sexual intercourse.

No statistically significant difference was found for practicing vaginal douching according to whether or not the women used a method of family planning ( $p = 0.35$ ). Although the difference was not statistically significant, the percentage of women who used vaginal douching was higher in women who use IUD and injections as a method of family planning. The reason why women with IUDs may practice vaginal douching may be more related to the IUD causing an increase in vaginal drainage and for this reason the women would use vaginal douching for the purpose of genital hygiene. In the study by Karaer, Avsar, Özkan, Bayır and Sayan (2005) it was determined that more women use vaginal douching who have IUDs, but in the study by Hodoglugil, Aslan and Bertan (2000) no difference was found in prevalence of vaginal douching between women who did and did not use IUD.

Karaer, Avsar, Özkan, Bayır and Sayan (2005) found a significantly higher prevalence of symptoms of vaginal infection in women who use vaginal douching and of complaints of vulvovaginal irritation, such as itching, burning and dyspareunia. Although the women, in this study, who used vaginal douching, had a higher prevalence of symptoms of genital infection, only the difference for itching between those, who did and did not use vaginal douching, was found to be statistically significant ( $p = 0.05$ ). However, many other studies have found a correlation between vaginal douching and genital infections (Blythe, Fortenberry & Orr, 2003; Chiaffarino, Parazzini, De Besi & Lavezzari, 2004; Corsello et al., 2003; Cottrell, 2006; Joesoef et al., 2001; Karaer, Boylu & Avsar, 2005; Newton, Piper, Shain, Perdue & Peairs, 2001; Sun et al., 2005; Vermund et al., 2001).



## Conclusion

There are several clinical implications of this study. First, the practice of vaginal douching was more prevalent in women under 25 years of age, who had low educational and income levels, who did not have health insurance, whose husband was a farmer or self-employed, and who had four or more children. Second, the most common reason for vaginal douching application was for general hygiene. A significant percentage of women who practice vaginal douching did so after they had a bowel movement. Therefore, this practice brings with it many potential health dangers. For this reason, nurses and midwives can help women, who attend to PHC, by informing them about problems that can occur from vaginal douching and can encourage them to give up this practice. Third, the primary basis for the practice of vaginal douching was religious beliefs. Therefore it is suggested that the health care personnel should cooperate with religious leaders when they plan public educational programs for women as they work to change incorrect beliefs about this practice. Finally, some of the women had been encouraged to practice vaginal douching by health care personnel. Thus doctors, nurses and midwives need to be taught about this subject in continuing education programs. In addition assessment of women on vaginal douching in details, by nurses and midwives, during their visit to the HCSs, should be as part of routine health history taking activity.

## References

1. Blythe, M. J., Fortenberry, J. D., & Orr, D. P. (2003). Douching behaviors reported by adolescent and young adult women at high risk for sexually transmitted infections. *J Pediatr Adolesc Gynecol*, 16(2), 95-100.
2. Bruce, F. C., Fiscella, K., & Kendrick, J. S. (2000). Vaginal douching and preterm birth: an intriguing hypothesis. *Med Hypotheses*, 54(3), 448-452.
3. Chiaffarino, F., Parazzini, F., De Besi, P., & Lavezzari, M. (2004). Risk factors for bacterial vaginosis. *Eur J Obstet Gynecol Reprod Biol*, 117(2), 222-226.
4. Corsello, S., Spinillo, A., Osnengo, G., Penna, C., Guaschino, S., Beltrame, A., et al. (2003). An epidemiological survey of vulvovaginal candidiasis in Italy. *Eur J Obstet Gynecol Reprod Biol*, 110(1), 66-72.
5. Cottrell, B. H. (2006). Vaginal douching practices of women in eight Florida panhandle counties. *JO-GNN*, 35(1), 24-33.
6. Çalışkan, D. (2005). Geleneksel intravaginal uygulama "vajinal duş, lavaj" yapılmalı mı? yapılmamalı mı? (Should or shouldn't traditional intravaginal douching be done?). *Sürekli Tıp Eğitimi Dergisi*, 14(1), 15-18.
7. Demba, E., Morison, L., Van Der Loeff, M. S., Awasana, A. A., Gooding, E., Bailey, R., et al. (2005). Bacterial vaginosis, vaginal flora patterns and vaginal hygiene practices in patients presenting with vaginal discharge syndrome in The Gambia, West Africa. *BMC Infect Dis*, 5, 1-12.
8. Demirbağ, C. C. (2000). Kadınlarda idrar yolu enfeksiyonlarında hijyenik alışkanlıklarla ilgili risk faktörleri (Risk factors associated with hygienic practices for women's urinary tract infections). *Cumhuriyet Üniversitesi Hemşirelik Yüksekokulu Dergisi*, 4(2), 52-58.
9. Ege, E., Timur, S., Zincir, H., Egri, M., & Reeder, B. S. (2007). Women's douching practices and related attitudes in eastern Turkey. *J Obstet Gynecol Res*, 33, 353-359.
10. Fiscella, K., Franks, P., Kendrick, J. S., & Bruce, F. C. (1998). The risk of low birth weight associated with vaginal douching. *Obstet Gynecol*, 92(6), 913-917.

11. Fiscella, K., Franks, P., Kendrick, J. S., Meldrum, S., & Kieke, B. A. (2002). Risk of preterm birth that is associated with vaginal douching. *Am J Obstet Gynecol*, 186(6), 1345-1350.
12. Foch, B. J., McDaniel, N. D., & Chacko, M. R. (2001). Racial differences in vaginal douching knowledge, attitude, and practices among sexually active adolescents. *J Pediatr Adolesc Gynecol*, 14(1), 29-33.
13. Hodoglugil, N. N. S., Aslan, D., & Bertan, M. (2000). Intrauterine device use and some issues related to sexually transmitted disease screening and occurrence. *Contraception*, 61(6), 359-364.
14. Joesoef, M. R., Karundeng, A., Runtupalit, C., Moran, J. S., Lewis, J. S., & Ryan, C. A. (2001). High rate of bacterial vaginosis among women with intrauterine devices in Manado, Indonesia. *Contraception*, 64(3), 169-172.
15. Karaer, A., Avsar, A. F., Özkan, Ö., Bayır, B., & Sayan, K. (2005). Vaginal douching practice in Turkish women: who is douching, and why?. *Australian and New Zealand J Obstet Gynaecol*, 45(6), 522-525.
16. Karaer, A., Boylu, M., & Avsar, A. F. (2005). Vaginitis in Turkish women: symptoms, epidemiologic-microbiologic association. *Eur J Obstet Gynecol Reprod Biol*, 121(2), 211-215.
17. Karatay, G., & Özvarış, Ş. B. (2006). Bir sağlık merkezi bölgesindeki gecekondularda yaşayan kadınların genital hijyene ilişkin uygulamalarının değerlendirilmesi (Evaluation of genital hygiene practices of women who live in a shanty town in a public health clinic's coverage area). *Cumhuriyet Üniversitesi Hemşirelik Yüksekokulu Dergisi*, 10(1), 7-14.
18. Kim Oh, M., Merchant, J. S., & Brown, P. (2002). Douching behavior in high-risk adolescents: what do they use, when and why do they douche?. *J Pediatr Adolesc Gynecol*, 15(2), 83-88.
19. Misra, D. P., Trabert, B., & Atherly-Trim, S. (2006). Variation and predictors of vaginal douching behavior. *Women Health Iss*, 16, 275-282.
20. Misra, D. P., & Trabert, B. (2007). Vaginal douching and risk of preterm birth among African American women. *Am J Obstet Gynecol*, 140.e1-140.e8.
- 21.
22. Ness, R. B., Hillier, S. L., Richter, H. E., Soper, D. E., Stamm, C., & McGregor, J. (2002). Douching in relation to bacterial vaginosis, lactobacilli, and facultative bacteria in the vagina. *Obstet Gynecol*, 100(4), 765-772.
23. Newton, E. R., Piper, J. M., Shain, R. N., Perdue, S. T., & Peairs, W. (2001). Predictors of the vaginal microflora. *Am J Obstet Gynecol*, 184(5), 845-855.
24. Rupp, R., Short, M. B., Head-Carroll, Y., & Rosenthal, S. L. (2006). Intergenerational transfer of douching information. *J Pediatr Adolesc Gynecol*, 19, 69-73.
25. Scholes, D., Stergachis, A., Ichikawa, L. E., Heidrich, F. E., Holmes, K. K., & Stamm, W. E. (1998). Vaginal douching as a risk factor for cervical chlamydia trachomatis infection. *Obstet Gynecol*, 91(6), 993-997.
26. Simpson, T., Merchant, J., Grimley, D. M., & Kim Oh, M. (2004). Vaginal douching among adolescent and young women: more challenges than progress. *J Pediatr Adolesc Gynecol*, 17(4), 249-255.
27. Sun, C-A., Hsiung, C. A., Lai, C-H., Chen, C-A., Chou, C-Y., Ho, C-M., et al. (2005). Epidemiologic correlates of cervical human papillomavirus prevalence in women with abnormal pap smear tests: a Taiwan Cooperative Oncology Group (TCOG) study. *J Med Virol*, 77, 273-281.
28. Vermund, S. H., Sarr, M., Murphy, D. A., Levin, L., Abdalian, S. E., Ma Y., et al. (2001). Douching practices among HIV infected and uninfected adolescents in the United States. *J Adolesc Health*, 29 (Suppl.), S80-S86.

Corresponding author:

Güngör Güler  
 Cumhuriyet University,  
 Faculty of Health Sciences,  
 Department of Nursing,  
 Turkey  
 e-mail: guler89@yahoo.com

# Relationship between sociodemographic characteristics and migraine in working women

Svetlana Simic<sup>1,2</sup>, Dragan Simic<sup>3</sup>

<sup>1</sup> University of Novi Sad, School of Medicine, Serbia

<sup>2</sup> Clinical Centre of Vojvodina, Clinic of Neurology, Serbia

<sup>3</sup> University of Novi Sad, Faculty of Technical Sciences, Serbia

## Abstract

Migraine is the most common type of headache in working women of reproductive age. It is classified according to the internationally recognized criteria. Menstrual migraine is not included into the main body of the classification, but in its appendix, indicating the need for further research in this field.

The aim of the study was to determine the prevalence of migraine and its subtypes in working women and to evaluate the significance of sociodemographic characteristics.

The study was done on a sample of 715 working women aged 20-65 years who filled in a questionnaire. The general questionnaire was used to collect relevant data. The collected data were analyzed using appropriate mathematical and statistical methods.

The results obtained show the prevalence of migraine in working women to be 20.69%. Among the women suffering from migraine, 4.73% had pure menstrual migraine and 56.76% had menstrually-related migraine, while non-menstrual migraine was present in 38.51%. We found a significant difference among some of the three studied subtypes of migraine in relation to age, level of education, family history of headache, and presence of another chronic disease. On the other hand, the three studied subtypes of migraine did not show significant differences in relation to marital status, children, type of employment, overtime work, or smoking. Migraine in women deserves special attention. Migraine subtypes differ in certain sociodemographic characteristics.

**Key words:** migraine, women, working population, sociodemographic characteristics

## Introduction

Migraine is a type of primary headache, manifested as intermittent, usually unilateral headache accompanied by vegetative disturbances, and frequently also by transient neurological symptoms (1). Migraine is diagnosed using the International Classification of Headache Disorders (ICHD-II) criteria, published in *Cephalalgia*, Vol. 24, Suppl.1, 2004 (2, 3). The classification encompasses three parts. Part one presents primary headaches, coded 1-4; part two presents secondary headaches, coded 5-12; and part three, i.e., chapter 13, presents cranial neuralgias and central causes of facial pain. Headache types and subtypes that do not fulfill the diagnostic criteria stated in the chapters 1-13 are listed in chapter 14 (14.1 Headache not else classified), including headaches that need more information in order to be classified (14.2 Headache unspecified). At the end of the classification there is an appendix, which has been introduced in the second edition with the main purpose of providing research criteria for the new entities that still need further validation in order to be formally accepted (4).

In women, migraine without aura is frequently closely associated with menstruation. Based on the current knowledge menstrual migraine is classified into two subtypes: pure menstrual migraine and menstrually-related migraine (5). Pure menstrual

migraine occurs only during menses. MacGregor has proposed the following definition of menstrual migraine: an attack of migraine without aura that occurs regularly on the first day of menstruation or on two days before or after that day, and at no other times (6). There is another opinion, that this is migraine headache occurring regularly every month and only in the period between two days before menstruation and the end of menstruation (7). Menstrually-related migraine may occur as pure menstrual migraine during the perimenstrual period, as well as at other times during a menstrual cycle (8). Since it is not clear whether these two types of migraine are different entities, menstrual migraine is included in the appendix, suggesting the need for further elucidation.

All epidemiological research of migraine clearly indicates that the prevalence of migraine is associated with sex and age (9). The most frequently reported data is that migraine is three times more common in women than in men, although some studies present different ratios. In China, Zhao et al found this ratio to be 5:1 to the disadvantage of female sex (10). The prevalence of migraine does not change significantly throughout childhood, however, in adolescence it increases in women (11). Given the ratio of migraine in women and men, the question arises as to whether and how ovarian hormones affect migraine. Abnormal response of the central nervous system exposed to normal hormonal fluctuations during a menstrual cycle is probably the mechanism underlying menstrual migraine (12, 13). It has been established that oscillations in the oestrogen levels are the key factor in the increased prevalence of migraine in women. The absolute oestrogen levels are not so important as are the sudden changes in the levels occurring, among others, in physiological states such as a menstrual cycle and after childbirth.

The prevalence of menstrual migraine has been reported to range from 4% to 73%, depending on the criteria used for defining the onset of migraine attack (14, 15).

Our paper, therefore, focuses on migraine in working women in our environment, and aims at contributing to the research of this important medical problem.

## Material and methods

The study was done on a sample of 715 working women aged 20-65 years, using a general questionnaire. The questionnaire contained general questions and questions about sex, age, type of employment, marital status, children, level of education, overtime work, smoking, family history of headache, and presence of another chronic disease. The last two questions of the questionnaire were about the occurrence of headache during the previous year and month. Subjects who answered positively to one or both of these questions, i.e., who had had a headache in the previous year and/or month, were included for further study. The questionnaire was made on the basis of our clinical experience and scientific research findings, respecting the accepted knowledge about headache, with an emphasis on primary headache.

The data was analyzed using multivariate analysis of variance (MANOVA), discriminative analysis, and other parametric procedures and methods. Univariate analyses included Roy's test, Pearson's contingency coefficient ( $\chi$ ), and the multiple correlation coefficient (R). Calculation of discrimination coefficients discriminates characteristics that determine specificity of subsamples. The purpose of the mathematical and statistical analysis was to determine characteristics of each subsample and the homogeneity and distance between subsamples in relation to the discriminative characteristics.

The collected data were checked for possible formal or logical errors. The data obtained through the statistical analysis are presented in tables and figures, accompanied by comments.

## Results

The results of the study show that out of 715 subjects 148 (20.69%) had migraine (Figure 1). Of the 148 women suffering from migraine, 7 (4.73%) had pure menstrual migraine (PMM), 84 (56.76%) had menstrually-related migraine (MRM), and 57 (38.51%) had non-menstrual migraine (NMRM).



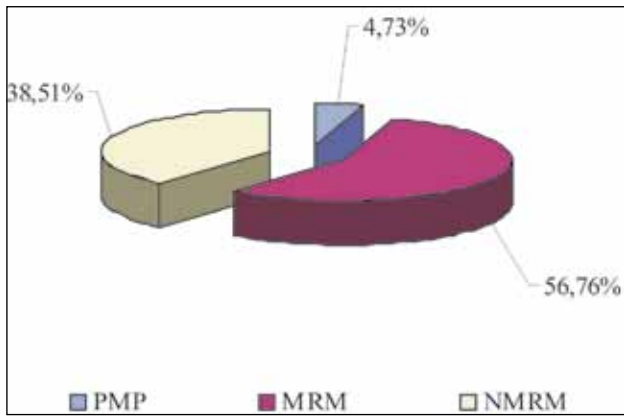


Figure 1. Prevalence of migraine and migraine subtypes in working women

Table 1 and table 2 show cumulative responses to general questions. Multivariate analysis of variance (MANOVA) showed a significant difference between the three studied migraine subtypes for some of the general questions ( $p = 0.008$ ).

Table 3 shows that in subjects suffering from pure menstrual migraine the most prevalent was age 41-50, present in 57%.

Regarding menstrually-related migraine, the prevalence of ages 41-50 years was 38%, which is significantly higher than the prevalence of ages 20-30, in 14.3% of subjects suffering from migraine ( $p=0.001$ ), and the prevalence of ages 51-60, in 13.1% of subjects suffering from migraine ( $p=0.000$ ).

Regarding non-menstrual migraine, the prevalence of ages 41-50 was 35.1%, which is significantly higher than the prevalence of ages 20-30, in 10.5% of subjects suffering from migraine ( $p=0.002$ ).

Since  $p = 0.078$  it can be said that there is an association, with an increased risk for making conclusions, between the migraine subtypes and age structure.

Table 1. Cumulative responses to general questions (age, marital status, children, education)

| Age   |    | Marital status |     | Children |     | Education            |    |
|-------|----|----------------|-----|----------|-----|----------------------|----|
| 20-30 | 18 | Single         | 25  | yes      | 114 | Elementary school    | 8  |
| 31-40 | 45 | Married        | 106 | no       | 34  | Secondary school     | 88 |
| 41-50 | 56 | Divorce        | 13  |          |     | 2-year college       | 17 |
| 51-60 | 29 | Widower        | 4   |          |     | University education | 35 |

Table 2. Cumulative responses to general questions (type of company, overtime work, smoking, family history, and chronic disease)

| Type of company | Overtime work | Smoking | Family history | Chr. disease |
|-----------------|---------------|---------|----------------|--------------|
| Public          | 121           | Yes 56  | Yes 83         | Yes 49       |
| Private         | 21            | No 92   | No 65          | No 99        |
| Mixed           | 6             |         |                |              |

Table 3. Prevalence of migraine subtypes across age, expressed as numbers (n) and percentage (%)

|              | 51-60 |       | 41-50 |      | 31-40 |      | 20-30 |      |
|--------------|-------|-------|-------|------|-------|------|-------|------|
|              | n     | %     | n     | %    | n     | %    | n     | %    |
| <b>PMM</b>   | 0     | 0.0   | 4     | 57.1 | 3     | 42.9 | 0     | 0.0  |
| <b>MRM</b>   | 11    | 13.1  | 32    | 38.1 | 29    | 34.5 | 12    | 14.3 |
| <b>NMRM</b>  | 18    | 31.6* | 20    | 35.1 | 13    | 22.8 | 6     | 10.5 |
| <b>Total</b> | 29    | 19.6  | 56    | 37.8 | 45    | 30.4 | 18    | 12.2 |

Table 4. Prevalence of migraine subtypes across levels of education

|              | Elementary school |       | Secondary school |      | 2-year college |      | University education |      |
|--------------|-------------------|-------|------------------|------|----------------|------|----------------------|------|
|              | n                 | %     | n                | %    | n              | %    | n                    | %    |
| <b>PMM</b>   | 1                 | 14.3* | 5                | 71.4 | 1              | 14.3 | 0                    | 0.0  |
| <b>MRM</b>   | 2                 | 2.4   | 49               | 58.3 | 10             | 11.9 | 23                   | 27.4 |
| <b>NMRM</b>  | 5                 | 8.8   | 34               | 59.6 | 6              | 10.5 | 12                   | 21.1 |
| <b>Total</b> | 8                 | 5.4   | 88               | 59.5 | 17             | 11.5 | 35                   | 23.6 |



Since  $p = 0.078$  of  $\chi^2$  - test, and  $\chi = 0.267$  with confidence interval (0.141- 0.393) it can be said that there is weak association between migraine subtypes and age structure.

By observing presented data (Table 4), it can be noticed that in the whole sample of working women suffering from migraine in relation to educational level, the most prevalent was secondary school 59.46%, which is significantly higher than the prevalence of university education 23.65%  $p=0.000$ , 2-year college 11.49%  $p=0.000$ , and elementary school education 5.41%  $p=0.000$ . Regarding individual migraine types, the results obtained for pure menstrual migraine were the most different.

Since  $p = 0.396$  it can be said that the method of  $\chi^2$  test did not determine association between migraine subtypes and educational level.

Since  $p = 0.396$  of  $\chi^2$  - test, and  $\chi = 0.201$  with confidence interval (0.077 - 0.325) it can be said that there is weak association between migraine subtypes and educational level.

Since (Table 5)  $p > 0.1$ , this means that no significant difference was observed between the three migraine subtypes for responses related to type of employment ( $p=0.258$ ), marital status ( $p=0.328$ ), family status ( $p=0.158$ ), overtime work ( $p=0.286$ ), or smoking ( $p=0.843$ ).

Since  $p < 0.1$ , this means there was a significant difference between some of the three migraine subtypes in responses related to age structure ( $p=0.007$ ), level of education ( $p=0.056$ ), family history of headache ( $p=0.069$ ), and presence of another chronic disease ( $p=0.030$ ).

Discriminative analysis was applied, where  $p=0.009$ , which means there is a significant difference and a clear delineation between some of the migraine subtypes.

Discrimination coefficient shows that the greatest contribution to discriminating migraine subtypes (i.e. that the difference was greatest) with regard to the responses obtained to the questions about family history of headache (0.053), followed by age structure (0.051), level of education (0.045), family status (0.045), marital status (0.033), another chronic disease (0.022), type of employment (0.016), overtime work (0.014), and smoking (0.011).

### Discussion

Primary headaches are the most prevalent among professionally and reproductively active population. Over the past two decades significant advances have been made in the data collection and understanding of the pathophysiology, pharmacology, genetics and epidemiology of migraine, although there are still numerous issues that need further clarification. New developments in each of these fields will contribute to a more comprehensive understanding of this significant medical problem. However, in our region epidemiological data that could shed light on the magnitude of the problem are still scarce, and our study therefore aimed at contributing to clarification of the problem. The results we obtained are in accord with other similar studies.

The most frequently reported data is that over 10% of the world's populations suffer from migraine, with migraine being three times more prevalent in women than in men (16, 17). American Migraine Study II (AMS II) published in 2001 and based on the research done in 1999, ten years after the first study (AMS I), shows the prevalence of migraine in women to be 18.2%. An epidemi-

Table 5. Significance of differences between migraine subtypes in relation to responses to general questions

|                    | $\chi$ | R     | F     | p     |
|--------------------|--------|-------|-------|-------|
| Age                | 0.267  | 0.256 | 5.083 | 0.007 |
| Type of employment | 0.148  | 0.136 | 1.367 | 0.258 |
| Marital status     | 0.131  | 0.124 | 1.125 | 0.328 |
| Children           | 0.157  | 0.158 | 1.868 | 0.158 |
| Education          | 0.201  | 0.197 | 2.941 | 0.056 |
| Overtime work      | 0.130  | 0.131 | 1.261 | 0.286 |
| Smoking            | 0.048  | 0.049 | 0.171 | 0.843 |
| Family history     | 0.187  | 0.190 | 2.729 | 0.069 |
| Chronic disease    | 0.212  | 0.217 | 3.578 | 0.300 |

ological study of migraine carried out in Sivas, Turkey, in the first half of 1999, by interviewing 1320 persons suffering from headache older than 7 years, showed migraine prevalence in women of 17.1% (18).

In our study of working women aged 20-65 years, 148 (20.69%) out of 715 subjects had migraine. We can therefore conclude that the prevalence of migraine in our region is similar to that found in other studies, and a somewhat higher prevalence was expected considering the age of the studied population in our study.

The significantly higher prevalence of migraine in women and its decline with advancing age indicates that migraine attacks in women are associated with the hormonal status. Numerous physiological mechanisms during a menstrual cycle have been proposed as a possible basis of menstrual migraine. Decrease in oestrogen levels, magnesium deficiency and changes in the neurotransmission system during menses are possible causes of attacks of menstrual migraine (19).

Migraine without aura in women is frequently associated with menstruation. The latest classification of headache disorders includes menstrual migraine without aura in its appendix, indicating that further research in this field is needed before this entity can be moved into the main body of the classification (4). Inconsistent classification criteria are one of the reasons why the prevalence of menstrual migraine ranges from 4% to 73% in literature data, depending on the criteria used for defining the onset of a migraine attack (14, 15). As mentioned before, menstrual migraine can be classified into two types: pure menstrual migraine and menstrual-related migraine (5). In our study we divided migraineurs into those suffering from pure migraine, those suffering from menstrually-related migraine, and those suffering from non-menstrual migraine. This division was governed by the assumption that pure menstrual migraine occurs only during menstruation. For the diagnosis of pure menstrual migraine, we used MacGregor's definition that distinguishes menstrual migraine as an attack of migraine without aura, occurring regularly on the first day of menstruation or on two days before or after menstruation, and at no other times (6). By these criteria, 4.73% of all women migraineurs in our study had pure menstrual migraine.

The prevalence of menstrually-related migraine has been reported to range between 35% and 68% (20, 21). In our study, the prevalence of this type of migraine was 56.76%, whereas non-menstrual migraine was present in 38.51% of subjects suffering from migraine. Moreover, we found a significant difference between the three studied subtypes of migraine in relation to age, level of education, family history of headache, and presence of another chronic disease.

The prevalence of migraine varies with age, it starts increasing with menarche and reaches its peak at the age of 42, after which it declines (9, 12). The prevalence is the highest during the fourth decade of life in both sexes, and according to Lipton et al it is 7.4% in men and 24.4% in women (22). In the AMS I the highest prevalence was found between the ages of 35 and 45 years (12). The age groups in our study were formed using a different methodology and subjects were only women, so our comparison can only be indirect. Our results show that 68.25 % of women suffering from migraine were 31-50 years old; 30.41% in the age group 31-40 years, and 37.84% in the age group 41-50. Considerably fewer migraineurs were found in ages 20-30 – 12.16%, and 51-60 years – 19.59%. There were no subjects suffering from migraine older than 60 years. Furthermore, we found significant differences in the prevalence of the three migraine subtypes in relation to age. Namely, no subject in the age categories 20-30 and over 50 years suffered from pure menstrual migraine. The groups of subjects suffering from menstrually-related migraine and non-menstrual migraine had a typical distribution of prevalence that first increases with age and then declines. In addition, in the group with non-menstrual migraine the prevalence in the oldest age category was significantly higher compared to the other two types of migraine, which was expected considering menopause.

Previous studies have not found association between the level of education and the prevalence of migraine (18). Our study corroborates that the level of achieved education has no significant impact on the occurrence of migraine; however, it can contribute to differentiation of migraine subtypes. Namely, regarding education, there was no difference in the distribution of prevalence

between the whole study sample and the sample of migraineurs, with the prevalence being the highest among subjects with secondary education, followed by those with college and university education, whereas the lowest prevalence was found among those with elementary school education. Regarding the distribution across the levels of education in relation to migraine subtypes, only pure menstrual migraine showed deviation from this pattern of distribution. Namely, in pure menstrual migraine, the highest number of subjects had completed secondary school education, there were no subjects with a university diploma, and there were equal numbers of subjects with college and elementary school education, with the difference between pure menstrual migraine and other migraine subtypes being significant only in relation to the prevalence of subjects with elementary school education.

A strong genetic predisposition to migraine has been well established. If only parents and children with migraine are taken into account, 46% have a positive family history of migraine (23), and the percentage further increases with inclusion of other relatives. The information about the presence of headache in relatives is of great importance for prediction of migraine, and it can be of use in determining the subtype of migraine. In contrast to menstrually-related migraine and non-menstrual migraine, in which almost two thirds of sufferers have relatives who also suffer from headache, in pure menstrual migraine only 14.3% of sufferers have relatives with headache, which is similar to the percentage present in healthy population. This finding requires a larger study sample suffering from pure menstrual migraine.

A thorough history of previous and current diseases is essential for a complete insight into possible contraindications and for the choice of a treatment option. Moreover, a complete medical history is necessary for determining possible presence of more than one type of headache and indications for supplementary diagnostic procedures in order to exclude or confirm a secondary headache (24, 25). In our study 33% of migraine sufferers had a chronic disease. Furthermore, we found that other chronic diseases were less frequent in menstrually-related migraine (26.2%) than in non-menstrual migraine (45.6%). Chronic dise-

ases were the least frequent in subjects suffering from pure menstrual migraine (14.3%), with a statistically significant difference compared to the other studied subtypes.

The analysis of study results did not show a significant difference between the three migraine subtypes in relation to marital status, family status, type of employment, overtime work, and smoking.

A study of 1320 residents of Sivas, Turkey, suggested that marital status had no significance for the prevalence of migraine (18). We found that both in the overall study sample and individual migraine subtypes the greatest number of sufferers was among those who were married, followed by single women, divorcees, and widows. However, there was no significant difference among the groups, or between each group and the whole sample.

In addition to marital status, we investigated the influence of family status, i.e., the subjects were asked whether they had any children, and if so, how many. The analysis of results did not show association between the presence and subtypes of migraine on one hand and family status on the other.

Considering that our country is in a state of social and political turmoil, our study paid attention to the type of enterprise the subjects were employed at: public, private or mixed. The greatest number of subjects, both in the overall sample and migraineurs, were employed in public companies; however, there was no significant association between the type of employment and the presence of migraine or its subtype.

An everyday occurrence nowadays is that a considerable number of employees work overtime, either voluntarily or compulsory and we took this fact into account in our study. Among our subjects suffering from migraine, 37.84% reported overtime work, while significantly higher percentage of sufferers, 62.16%, did not work overtime. However, we found no association between overtime work and migraine subtype.

It is well known that healthy lifestyles can contribute to reducing frequency and severity of headache. It is recommended that a person suffering from migraine should avoid smoking and staying in smoky rooms, caffeine and alcohol, and stressful situations, and should practice regular physical exercise and respect normal rhythm of sleep

and rest (23). While some of the factors can be influenced and corrected, others cannot.

Smoking is a bad habit, to say the least, and it is also a significant risk factor for numerous diseases, and in some cases it is also an addiction disorder. Given the limited number of studies on the relationship between smoking and headache, we analyzed the structure of our subjects in relation to smoking. In the overall sample of women migrainers, there were an equal number of smokers and non-smokers. Regarding the migraine subtype, the highest number of smokers was found among women with non-menstrual migraine (52.6%) and the lowest among those with pure menstrual migraine (42.9%). However, there was no significant association between the studied migraine subtypes and smoking.

### Conclusion

Migraine in women deserves special attention. The prevalence of migraine in professionally active women in our region is 20.69%. Among the women suffering from migraine, 4.73% have pure menstrual migraine, 56.76% have menstrually-related migraine, and 38.51% have non-menstrual migraine.

Our study corroborates the relationship between sociodemographic characteristics and migraine. The established specificities and differences between the migraine subtypes in relation to sociodemographic characteristics among working women suggest the need for further more extensive research in this field.

### References

1. Olesan J, Lipton RB. *Headache classification updates 2004. Current Opinion in Neurology* 2004; 17:257-82.
2. *Headache Classification Subcommittee of the International Headache Society. The International Classification of Headache Disorders, Second Edition. Cephalalgia* 2004; 24 Suppl. 1:1-160.
3. Šarenac S, Šarenac D. Šta je novo u klasifikaciji glavobolja, Zbornik sažetaka 12. kongresa neurologa SCG sa međunarodnim učešćem, Novi Sad 6-10 oktobar 2004.g.
4. Zidverc-Trajković J. Klasifikacija glavobolja. Klasifikacije i kriterijumi u neurologiji, Beograd 2006, 134-142.
5. *About menstrual Migraine* <http://www.menstrual-migraine.org/about.html>.
6. MacGregor EA. *Menstruation, sex hormones, and migraine. Neurol Clin* 1997; 15:125-141.
7. Jelovsek FR *Menstrual Migraine Headaches* <http://www.wdxcyber.com/ngen03.htm>
8. Vincent TM. *Menstrual Migraine: New Approaches to Diagnosis and Treatment.* Available at [www.AmericanHeadacheSociety.org](http://www.AmericanHeadacheSociety.org)
9. Prencipe M, Casini AR, Ferretti C, Santini M, Pezzella F, Scaldaferrri N, Culasso F. *Prevalence of headache in an elderly population: attack frequency, disability, and use of medication. J Neurol Neurosurg Psychiatry*, 2001;70(3):377-81.
10. Lichten EM, Lichten JB, Whitty AJ. *The Use of Leuprolide Acetate in the Diagnosis and Treatment of Menstrual Migraine: The Role of Artificially Induced Menopause. Headache Quarterly, Current Treatment and Research.* 1995; 6:4:313-17.
11. *What are the Different Types of Headache? The Complete Guide to Headache* <http://www.headaches.org/consumer/aduc...dules/completeguide/differenttypes.html>
12. Randolph W. Evans, Ninan T. Mathew. *Handbook of Headache Second Edition, Lippincott Williams and Wilkins*, 2005.
13. Loder E. *Menstrual migraine 1092-8480. 2001. ; 189-200, PubMed, abstract*

14. National Headache Foundation «Headache Types / Hormone» <http://www.headaches.org/consumer/educationalmodules/completeguide/hormone3.html>
15. Fetters I. Menstrual migraine Methods of prevention and control. *Postgraduate medicine, Vol 101/ No5/May 1997.*
16. Nabih R. *Epidemiology and impact of migraine. Continuum Lifelong Learning in Neurology, Headache update, American Academy of Neurology, 78-92.*
17. Lipton RB, Sewart WF, Scher AL. *Epidemiology and economic impact of migraine. Curr Med Res Opin 2001;17(suppl 1):S4-12.*
18. Kececi H, Dener S. *Epidemiological and Clinical Characteristics of Migraine in Sivas, Turkey. Headache 2002; 42:275-280.*
19. Martin VT, Behbehani M. *Ovarian hormones and migraine headache: understanding mechanisms and pathogenesis. Headache 2006;46:3-23.*
20. Džoljić E, Šipetić S, Vlajinać H, et al. *Prevalence of menstrually related migraine and non-migraine primary headache in female students of Belgrade University. Headache. 2002; 42:185-193.*
21. Brandes JL. *The influence of estrogen on migraine: a systemic review. JAMA. 2006:1824-1830.*
22. Lipton RB, Bigal ME, Diamond M, et al; AMPP Advisory Group. *Migraine prevalence, disease burden, and the need for preventive therapy. Neurology. 2007;68:343-349.*
23. Martinović Ž. *Glavobolje klasifikacija i lečenje. Beograd, 2006.*
24. Silberstein SD. *Migraine. Lancet 2004; 363(9406):381-91.*
25. Evans RW. *„Diagnostic testing for headaches“ Med Clin North Am 2001;85(4):865-885.*

Corresponding author:

Svetlana Simić  
Clinical Centre of Vojvodina,  
Clinic of Neurology Novi Sad,  
Serbia  
e-mail: dsimic@eunet.rs

**Abbreviations:**

ICHD - International Classification of Headache Disorders

MANOVA - multivariate analysis of variance

PMM - pure menstrual migraine

MRM - menstrually-related migraine

NMRM - non-menstrual migraine

AMS - American Migraine Study



# The impact of various factors on self-care of elderly

Danica Zeleznik<sup>1</sup>, Uros Zeleznik<sup>2</sup>, Jadranka Stricevic<sup>3</sup>

<sup>1</sup> Faculty of Health Science, University of Maribor, Slovenia

<sup>2</sup> Health Centre dr. Adolfa Drolca Maribor, Slovenia

<sup>3</sup> Faculty of Health Science, University of Maribor, Slovenia

## Abstract

**Introduction:** This study is focused to describe the self-care of home-dwelling elderly people living in Slovenia and factors affecting to self-care. The purpose is to describe the experiences of the elderly people's ability to manage at home.

The aim of this study was to produce new knowledge about the self-care of home dwelling elderly people living in Slovenia and the factors connected with it. The knowledge could be used to develop elderly care and support elderly people who live at home and the knowledge could be also used to educate nurses to care for the elderly people.

**Methods:** The quantitative structured data was collected by interview of home-dwelling elderly people all over Slovenia. The sample consisted of 302 home-dwelling elderly people who were clients in domiciliary care. The selection criteria were: aged 75 years or over and the ability to communicate, no hearing problems, no severe mental problem/cognitive disability and gave full consent for their participation. Community nurses selected elderly people in their homes. Descriptive statistics was used for presentation of sample background information and cross tabulations for presentation of results. Factors were extracted by factor analysis.

**Results:** Extracted self-care factors (handling of everyday tasks, relationship with health care staff, medication handling, and medical treatment) were often in significant relations to elderly people's perceptions concerning either their past or their future (physical condition, family relations, meaningful and stimulating ageing, working habits etc).

**Discussion and conclusions:** Self-care is combined with functional capacity, stimulative ageing and acceptance of the future. Quality old age depends a great deal on the habits and approaches to life in the previous active years. A general preventive and other measures, and strivings for healthy habits and lifestyle, are the way to healthy ageing. It is upon each of us how the process will be directed.

The results may present the basis for care planning by caregivers, especially community nurses. On the basis of this results the model of nursing and social care for home - dwelling elderly people living in Slovenia could be planned.

**Key words:** self-care, factors, home-dwelling, elderly people

## Introduction

At the start of the twenty-first century, one of the most profound social changes to occur in developing societies is that the population is older (Backman, Hentinen 1999; Čačinovič Vogrinčič, 2000; Železnik, 2005; Dragoš, 2004; Hendry in McVittie, 2004, Kempen et al., 2006). This change is a result of social and scientific developments over the course of the previous century, resulting in the addition of 25 years to life expectancy (Ramonš, 2003; Dean, 2003). This demographic trend has meant that, in Europe, elderly people represent twenty per cent of the total population and demographic projections anticipate significant increases in this section of the population; these projections predict that the proportion of the elderly people in Europe as a whole will increase to twenty five per cent by the year 2025 (European

Commission, 2000). According to (Cheng, 2006; Baltes in Baltes, 1990), ageing is more than a series of biological changes. It is defined by gender, class, social standing, and culture rather than year alone. Ageing is for the individuals regarded as something unpleasant, useful, and unnecessary and above all, unwanted. Owing to economical crisis the whole relationship to people in older years has been changed. The old aged people feel themselves to be useless. There are still some stereotypes that old aged people are unnecessary and inferior (Mesec, 2000; Zupančič, 2004).

One important factor allowing an elderly people to live at home is self-care. Self-care means to take care of his/her own self it is a part of an individual lifestyle, which is shaped by values and beliefs learned in specific cultures (Slovar slovenskega knjižnjega jezika, 2005). Self care practices are seen to reflect elderly people's individual styles and adaptations specific to their personal histories, current circumstances and views of the future. According to Backman in Hentinen, (2001), self-care seems to be connected with the personal experiences of each old woman or man. Self-care is the personal care that individuals require each day to regulate their own functioning and development (Orem et al., 2001; Allender in Spradley, 2001). Self-care is supposed to be the key to health and illness care (Aggleton in Chalmers, 1985; Orem, 2001; Toljamo in Hentinen 2001; William, 2004, Parissopoulos in Kotzabassaki, 2004). The theory of self-care proposes that individuals learn and deliberately perform for themselves or have performed for them (dependent care) on a continuous basis those actions that are necessary to protect human integrity, physical and mental functioning, and development within norms essential for promoting life, health and well-being (McAuley et al. 2000; Denyes et al. 2001; Tomey in Alligood 2002; Allison, 2007). Physical activity seems to be an important factor when older people assess their health (Leinonen in Jylhä 2001). According to Teel in Leenerts, (2005), self-care responses appear to be learned within the social context early in life, be reinforced through the life cycle, and evolve through cooperation with both professional and lay persons.

Self-care has traditionally been defined as activities associated with health promotion (Back-

man, Hentinen 1999; Backman, 2003). It represents the range of behaviours undertaken by individuals to promote or restore their health. The activities of daily living, such as exercise, nutrition and relaxation, are often used to measure self-care. Orem, (1991) has started: "self-care means care that is performed by oneself for oneself when one has reached a state of maturity that enables consistent, controlled, effective, and purposeful action". The aim of such rational self-care is to maintain health. In this way, self-care is seen as a rational, conscious way to operate. In this presentation, self-care activities are not seen merely as rational ways to maintain health. Self-care is not only a conscious way to act, but partly also a subconscious routine that has been shaped in the course of life. Self-care is not a separate part of old men's or women's lives. It is associated closely with both their past life and the future. Such knowledge of the self-care of elderly people helps us to understand many aspects of self-care and its associations with vulnerability in later life.

## Methods

Quantitative research method was used. Quantitative study is needed to get a general picture and to describe what the self-care of home - dwelling elderly people is like in Slovenia, because we don't have any knowledge of that. Quantitative research methods assume that the world is stable and predictable, and phenomena can be measured empirically (Christensen, Kackrow, 2003). The positivist tradition of quantitative research derives from the biomedical sciences (Topping, 2006). Quantitative researchers focus on a very specific area and plan every detail (Polit, Beck, Hungler 2006). The purposive sample involves the conscious selection by a researcher of a certain criterion.

## Data collection

The data was collected in Slovenia by interviewing elderly people in their homes by structured instrument. The community nurses selected and interviewed the elderly people in their homes who fulfilled the criteria (over 75 years old, does not

have a profound hearing problem, does not have a severe mental problem/cognitive disability, can speak Slovene, can give fully informed consent of their participation). All the home-dwelling elderly people in this study were capable of describing their experiences of self-care and they were very interested to discuss and share their opinions.

### **Instrument**

The whole instrument consists of 91 items and covers background data, and the following subscales measuring the following factors: types of self-care, self care orientation, functional ability, life satisfaction and self-esteem. The original instrument had been used previously in the Finnish language, it was based on Backman's theory of the self-care of elderly people and it was used and tested in Finland. The reliability and validity was found to be good (Räsänen, Backman, Kyngäs, 2007). In order to use the instrument in this study, it had to be translated from Finnish into English and then to Slovene language following instructions by Harkness, (2003). Before using the instrument, it was pre-tested by five elderly people. The researcher asked the participants to read the instrument very carefully and give their comments. In their opinion, units, sentences and statements were logical, understandable and unambiguous.

Cronbach's alpha coefficient for the reliability of the instrument in this study was highly accepted for self-care orientation. The alpha values were as follow: self-care 0.75, self-care orientation 0.60, self-esteem 0.75, life-satisfaction 0.84 and functional capacity 0.95.

### **Data analyses**

The data were analysed by exploratory factor analyses - principal component analyses with varimax rotation and an unlimited number of factors (Burns in Grove 2005; Gillis in Jackson 2001). Before the final exploratory factor analyses, all items with low correlation coefficients (under 0.400) were omitted (Polit, Beck, Hungler 2006; Gillis in Jackson 2001). Items with factor loadings under 0.400 were also omitted, and missing values were

excluded likewise. Factors were extracted using the following guidelines: eigenvalues were greater than one in all factors and the factors showed a reasonable structure in terms of the theory underlying the instruments (Gillis in Jackson 2001; Holloway in Wheller 2002; Gerrish in Lacey 2006).

Sum variables have been made for each factor of self-care of home-dwelling elderly people and separated them into three 3 categories (good, moderate, poor), using percentiles. The procedures of sum variables calculations was done by adding together all the items of each factor divided by the total number of those items (Teeri et al. 2008). The relations between the factor variables were analyzed using cross-tabulations and  $\chi^2$  tests.

The statistical calculation was made by SPSS (Statistical Package for the Social Sciences) 12.0.1. SPSS supported all statistical methods used in this study. P-value<0.05 was considered significant.

### **Ethical considerations**

The Board of the Ethical Committee in Slovenia approved this research study into home-dwelling elderly people. The permission to use the instruments was given by the Ethical Committee in Slovenia. In this study it was emphasized that participation was completely voluntary. Written consent was obtained from each elderly person. Anonymity and confidentiality were assured and permission. Participants were advised that their participation was entirely voluntary and that they had the right to withdraw from the study at any time. Participants were assured that their confidentiality would be protected and findings reported anonymously. Quantitative information was obtained using anonymous instruments.

### **Results**

The factor analysis started with 42 items and 6 items were omitted based on the criterion. A twelve-factor solution was specified, 4 factors described self-care and 8 factors describe elderly people's perceptions concerning either the past or the future. For all extracted factors sum variables

were calculated and, according to percentiles, separated into 3 equal categories (poor, moderate and good). The relations between the factor variables were analysed using  $\chi^2$  tests. There were statistically significant connections between the self-care behaviour and experiences of health and ageing and attitudes towards other people (table 1, cells include p-values). These connections were present in nearly all calculations. Some relations were also found to ageing and the future and to elderly people's background.

The results of  $\chi^2$ -tests for the first extracted self-care behaviour factor (handling of everyday tasks) showed statistically significant connections to physical condition, family relations, meaningful and stimulating ageing and to working habits (table 2). More than 80 % of the elderly people who managed everyday tasks well had good (52 %) or moderate (30 %) physical condition. 60 % of the elderly people and more who managed everyday tasks well had good family relations. More than 80 % of the elderly people who managed everyday tasks well

Table 1. The self-care behaviour and factors connected to it (N=302)

| Elderly people background           | physical condition | family relations | meaningful and stimulating ageing | future perceptions | working habits | past events | confidence | future perspectives |
|-------------------------------------|--------------------|------------------|-----------------------------------|--------------------|----------------|-------------|------------|---------------------|
| handling of everyday tasks          | <.001              | <.001            | <.001                             | .453               | <.001          | .497        | .837       | .099                |
| Relationship with health care staff | .001               | <.001            | <.001                             | .542               | .003           | .079        | .708       | .046                |
| medication handling                 | .049               | .901             | .002                              | .033               | .241           | .124        | .229       | .298                |
| medical treatment                   | .365               | .019             | .024                              | <.001              | .262           | .051        | .211       | .076                |

Table 2. Handling of everyday tasks and the factors connected to it (N=302)

| Factors connected to the handling of every day tasks | Handling of everyday tasks |              |            |            |            |            | $\chi^2$ |
|------------------------------------------------------|----------------------------|--------------|------------|------------|------------|------------|----------|
|                                                      | Poor                       |              | Moderate   |            | Well       |            |          |
|                                                      | n                          | %            | n          | %          | n          | %          | P-value  |
| Physical condition                                   |                            |              |            |            |            |            | <0.001   |
| Poor                                                 | 50                         | 67           | 40         | 34         | 20         | 18         |          |
| Moderate                                             | 21                         | 28           | 48         | 40         | 32         | 30         |          |
| Good                                                 | 4                          | 5            | 31         | 26         | 56         | 52         |          |
| <b>Total</b>                                         | <b>75</b>                  | <b>100</b>   | <b>119</b> | <b>100</b> | <b>108</b> | <b>100</b> |          |
| Family relations                                     |                            |              |            |            |            |            | <0.001   |
| Poor                                                 | 28                         | 37           | 30         | 25         | 22         | 20         |          |
| Moderate                                             | 27                         | 36           | 43         | 36         | 21         | 20         |          |
| Good                                                 | 20                         | 27           | 46         | 39         | 65         | 60         |          |
| <b>Total</b>                                         | <b>75</b>                  | <b>100</b>   | <b>119</b> | <b>100</b> | <b>108</b> | <b>100</b> |          |
| Meaningful and stimulating ageing                    |                            |              |            |            |            |            | <0.001   |
| Poor                                                 | 39                         | 52.0         | 37         | 31         | 18         | 17         |          |
| Moderate                                             | 26                         | 34.7         | 54         | 45         | 47         | 43         |          |
| Good                                                 | 10                         | 13.3         | 28         | 24         | 43         | 40         |          |
| <b>Total</b>                                         | <b>75</b>                  | <b>100.0</b> | <b>119</b> | <b>100</b> | <b>108</b> | <b>100</b> |          |
| Working habits                                       |                            |              |            |            |            |            | <0.001   |
| Poor                                                 | 32                         | 43           | 23         | 20         | 22         | 20         |          |
| Moderate                                             | 35                         | 47           | 48         | 40         | 32         | 30         |          |
| Good                                                 | 8                          | 10           | 48         | 40         | 54         | 50         |          |
| <b>Total</b>                                         | <b>75</b>                  | <b>100.0</b> | <b>119</b> | <b>100</b> | <b>108</b> | <b>100</b> |          |

Table 3. Relations with health care staff and factors connected to them (N=302)

| Factors connected to relations with health care staff | Relation to health care staff |            |            |            |           |            | $\chi^2$ |
|-------------------------------------------------------|-------------------------------|------------|------------|------------|-----------|------------|----------|
|                                                       | Poor                          |            | Moderate   |            | Good      |            |          |
|                                                       | n                             | %          | n          | %          | n         | %          | P-value  |
| Physical condition                                    |                               |            |            |            |           |            | 0.001    |
| Poor                                                  | 59                            | 47         | 36         | 33         | 16        | 23         |          |
| Moderate                                              | 36                            | 29         | 44         | 41         | 20        | 29         |          |
| Good                                                  | 30                            | 24         | 28         | 26         | 33        | 48         |          |
| <b>Total</b>                                          | <b>124</b>                    | <b>100</b> | <b>108</b> | <b>100</b> | <b>69</b> | <b>100</b> |          |
| Family relations                                      |                               |            |            |            |           |            | <0.001   |
| Poor                                                  | 54                            | 43         | 21         | 20         | 5         | 7          |          |
| Moderate                                              | 37                            | 30         | 35         | 32         | 19        | 28         |          |
| Good                                                  | 33                            | 27         | 52         | 48         | 45        | 65         |          |
| <b>Total</b>                                          | <b>124</b>                    | <b>100</b> | <b>108</b> | <b>100</b> | <b>69</b> | <b>100</b> |          |
| Meaningful and stimulating ageing                     |                               |            |            |            |           |            | <0.001   |
| Poor                                                  | 63                            | 51         | 21         | 19         | 11        | 16         |          |
| Moderate                                              | 44                            | 35         | 53         | 49         | 28        | 41         |          |
| Good                                                  | 17                            | 14         | 34         | 32         | 30        | 43         |          |
| <b>Total</b>                                          | <b>124</b>                    | <b>100</b> | <b>108</b> | <b>100</b> | <b>69</b> | <b>100</b> |          |
| Working habits                                        |                               |            |            |            |           |            | 0.003    |
| Poor                                                  | 39                            | 31         | 27         | 25         | 11        | 16         |          |
| Moderate                                              | 54                            | 44         | 40         | 37         | 21        | 30         |          |
| Good                                                  | 31                            | 25         | 41         | 38         | 37        | 54         |          |
| <b>Total</b>                                          | <b>124</b>                    | <b>100</b> | <b>108</b> | <b>100</b> | <b>69</b> | <b>100</b> |          |
| Future perspectives                                   |                               |            |            |            |           |            | 0.046    |
| Poor                                                  | 52                            | 43         | 46         | 44         | 43        | 62         |          |
| Moderate                                              | 38                            | 31         | 38         | 37         | 18        | 26         |          |
| Good                                                  | 32                            | 26         | 20         | 19         | 8         | 12         |          |
| <b>Total</b>                                          | <b>124</b>                    | <b>100</b> | <b>104</b> | <b>100</b> | <b>69</b> | <b>100</b> |          |

had meaningful and stimulating ageing (good 40 % and moderate 43 %). Also nearly 80 % of the elderly people who managed everyday tasks well had good (50 %) or moderate (30 %) working habits.

The results of  $\chi^2$ -tests for the second extracted self-care behaviour factor (relationship with health care staff) showed statistically significant connections to physical condition, family relations, meaningful and stimulating ageing, working habits and future perspectives (table 3). Nearly 80 % of the elderly people who enjoyed good relations with health care staff had good (48 %) or moderate (29 %) physical condition. More than 60 % of the elderly people with good relationships with health care staff had good family relations. Over 80 % of the elderly people with good relationships with health care staff had meaningful and stimulating ageing (good 43 % and moderate 41 %). More than 80 % of the elderly people with good relationships with health care staff had good (54 %) or moderate (30 %) working habits. All results confirmed the results of the first self-care behaviour factor (handling of everyday tasks).

The only contradiction represented factor future perspectives, describing ageing and the future with only one item: "I am going to continue living at home, no matter how sick I may become". Nearly 90 % of the elderly people with good relationships with health care staff had poor (62 %) or moderate (26 %) future perspectives. The elderly people who had good relationships with health care staff (and also in most cases practice healthy self-care behaviour) had difficulty to accept that they will probably have to leave their homes at some stage.

The results of  $\chi^2$ -tests for the third extracted self-care behaviour factor (medication handling) showed statistically significant connections to physical condition, meaningful and stimulating ageing and future perceptions (table 4). Nearly 80 % of the elderly people with poor medication handling had good (37 %) or moderate (36 %) physical condition. These results show that the elderly people in good physical condition show some signs of irresponsible self-care, but correlation was not strong. More than 80 % of the elderly people with good medication handling had good (26 %)



or moderate (55 %) family relations. Nearly 80 % of the elderly people with good medication handling had good (38 %) or moderate (30 %) future perceptions, but also correlation was not strong.

The results of  $\chi^2$ -tests for the fourth extracted self-care behaviour factor (medical treatment) showed statistically significant connections to family relations, meaningful and stimulating ageing and to future perceptions (table 5). The elderly people with good medical treatment had good (38 %) or moderate (38 %) family relations. More than 80 % of the elderly people with good medical treatment had meaningless and unstimulating ageing (poor 35 % and moderate 46 %). More than 70 % of the elderly people with good medical treatment had good (42 %) or moderate (34 %) future perceptions.

### Discussion

Noted by Fung in Carstensen (2002), any synthesis of the self-care of the elderly people and related factors based on the existing research knowledge is hampered by the fact that self-care and related factors have been defined from different theoretical viewpoints and operationalized in a number of different ways. Roughly speaking, it can be said that advancing age and declining functional capacity are likely to affect self-care at some point of the life span (Norburn et al.,1995). The knowledge of elderly people home-dwelling self-care is not clear. Most studies are quantitative which measure and compare two or more factors which have been defined and measured in diffe-

Table 4. Medication handling and factors connected to it (N=302)

| Medication handling and factors connected to it | Medication handling |            |           |            |            |              | $\chi^2$ |
|-------------------------------------------------|---------------------|------------|-----------|------------|------------|--------------|----------|
|                                                 | Poor                |            | Moderate  |            | Good       |              |          |
|                                                 | n                   | %          | n         | %          | n          | %            | P-value  |
| Physical condition                              |                     |            |           |            |            |              | 0.049    |
| Poor                                            | 32                  | 27         | 36        | 46         | 42         | 41           |          |
| Moderate                                        | 43                  | 36         | 21        | 27         | 36         | 35           |          |
| Good                                            | 44                  | 37         | 21        | 27         | 25         | 24           |          |
| <b>Total</b>                                    | <b>119</b>          | <b>100</b> | <b>78</b> | <b>100</b> | <b>105</b> | <b>100.0</b> |          |
| Meaningful and stimulating ageing               |                     |            |           |            |            |              | 0.002    |
| Poor                                            | 42                  | 35         | 33        | 42         | 20         | 19           |          |
| Moderate                                        | 40                  | 34         | 30        | 39         | 58         | 55           |          |
| Good                                            | 37                  | 31         | 15        | 19         | 27         | 26           |          |
| <b>Total</b>                                    | <b>119</b>          | <b>100</b> | <b>78</b> | <b>100</b> | <b>105</b> | <b>100.0</b> |          |
| Future perceptions                              |                     |            |           |            |            |              | 0.033    |
| Poor                                            | 49                  | 41         | 23        | 29         | 34         | 32           |          |
| Moderate                                        | 46                  | 39         | 25        | 32         | 31         | 30           |          |
| Good                                            | 24                  | 20         | 30        | 39         | 40         | 38           |          |
| <b>Total</b>                                    | <b>119</b>          | <b>100</b> | <b>78</b> | <b>100</b> | <b>105</b> | <b>100</b>   |          |

Table 5. Medical treatment and factors connected to it (N=302)

| Medical treatment and factors connected to it | Medical treatment |              |           |            |           |            | X <sup>2</sup> |
|-----------------------------------------------|-------------------|--------------|-----------|------------|-----------|------------|----------------|
|                                               | Poor              |              | Moderate  |            | Good      |            |                |
|                                               | n                 | %            | n         | %          | n         | %          | P-value        |
| Family relations                              |                   |              |           |            |           |            | 0.019          |
| Poor                                          | 29                | 22           | 32        | 35         | 19        | 24         |                |
| Moderate                                      | 32                | 24           | 29        | 31         | 30        | 38         |                |
| Good                                          | 69                | 53           | 31        | 34         | 30        | 38         |                |
| <b>Total</b>                                  | <b>131</b>        | <b>100</b>   | <b>92</b> | <b>100</b> | <b>79</b> | <b>100</b> |                |
| Meaningful and stimulating ageing             |                   |              |           |            |           |            | 0.024          |
| Poor                                          | 32                | 24           | 35        | 38         | 28        | 35         |                |
| Moderate                                      | 52                | 40           | 38        | 41         | 36        | 46         |                |
| Good                                          | 47                | 36           | 19        | 21         | 15        | 19         |                |
| <b>Total</b>                                  | <b>131</b>        | <b>100.0</b> | <b>92</b> | <b>100</b> | <b>79</b> | <b>100</b> |                |
| Future perceptions                            |                   |              |           |            |           |            | 0.000          |
| Poor                                          | 67                | 51           | 21        | 23         | 19        | 24         |                |
| Moderate                                      | 35                | 27           | 39        | 42         | 27        | 34         |                |
| Good                                          | 29                | 22           | 32        | 35         | 33        | 42         |                |
| <b>Total</b>                                  | <b>131</b>        | <b>100</b>   | <b>92</b> | <b>100</b> | <b>79</b> | <b>100</b> |                |

rent ways. Because of the lack of knowledge of the levels of self care of home-dwelling elderly people this study is based on Backman's theory 1999 of the self care of home-dwelling elderly people. According to Backman's study 2003 and some other studies (Zasuszniewski, 1996; Rabiner et al., 1997), the self-care of elderly people is found to be linked to functional capacity, satisfaction with life and self-esteem (Toljamo, Hentinen, 2001; Isola et al., 2003; Fagerström et al., 2007). These factors were also studied here.

Based on factor analyses, four factors were found which described the self-care. All other factors described elderly people's perceptions concerning either the past or the future. The elderly people who were able to manage their daily activities/routines had good functional capacity, good family relations, live qualitatively, accept the future positively and clearly and are satisfied with their life because they can take care of themselves. Elderly people, whose life was full of heavy labour, are more responsible for self-care with high levels of life satisfaction. They are responsible for their health, therapies and maintaining functional capacity and they are satisfied with their own life. Careful treatment with medicaments is connected with stimulative ageing and clear acceptance of the future.

The elderly people who had good relations with health care staff were in good physical condition, had good family relations and had meaningful and stimulating ageing. They were also proud of their past and present working performance. The future perspectives factor describes the elderly people's desire to stay at home at any cost; this is the only negative experience of their self-care.

Estimation of elderly people should be founded on the level of their functionality and not according to chronological age. Functional level is the accurate indicator of the difficulties experienced by elderly people and the required interventions. Functional capabilities range from complete independence to complete dependence, accompanied by different/ various physical, cognitive, psychological and emotional deprivation (Hagberg et al., 2004).

According to Gerson, Berg, (2004) the increase in size of older groups of the population has set new demands for the development of existing established means of providing support to the elderly people which, at the same time, calls for an

organised approach to the development of new forms of care - educational forms in the field of gerontological nursing.

In order to understand self-care of the elderly people living in Slovenia it is necessary to understand that elderly people would like to live as long as possible at home and care for themselves in daily living. According to (Hobbs Leenerts et al., 2002; Teel in Leenerts, 2005; Allison, 2007), self-care consists of the action systems performed by individuals in time and in conformity with health care requirements that are associated with their growth and development, their state of health and health-related conditions, the environment, and other influencing factors.

## Conclusion

The self-care of home-dwelling elderly people who are able to manage their daily activities, are in good functional capacity and who have good family relations, live qualitatively and have meaningful stimulating ageing. They are proud of their past and present working performance. Their self-care is at a high level. Elderly people whose life was full of heavy labour are more responsible for self-care with high levels of life satisfaction. They are responsible for their health, therapies and maintaining functional capacity and they are satisfied with their own life.

In the process of planning public care for the elderly population it is of vital importance that the middle generation of today becomes prepared for their old age. Otherwise, social problems of the elderly population will be impossible to manage. Healthy way of life, better human and generational relationships, respectfulness, tolerance, solidarity, and before all, treating old age as equally valuable period of life are important for one's self-care in old age.

## References

1. Allender, J.A., Spradley, B.W. *Community Health Nursing: Concepts and Practice*. Philadelphia, New York, Baltimore. Lippincott. 2001.
2. Allison, S.E. *Self-Care Requirements for Activity and Rest: An Orem Nursing Focus. V: Nursing Science Quarterly*, letnik, 20. Thousand Oaks :Sage Publications . 2007; pp68 -76.
3. Backman, K., Hentinen, M. *Factors associated with the self-care of home-dwelling elderly*. Nordic College of Caring Sciences. V: *Scandinavian Journal of Caring Sciences*, letnik 15, Oxford: Blackwell Publishing Limited. 2001; pp 195-202.
4. Backman, K, Hentinen, M. *Model for the self-care of home-dwelling elderly. V: Journal of Advanced Nursing*, letnik 30, Oxford, London, Edinburgh, Melbourne: Blackwell Scietific Publications. 1999; pp 564-572.
5. Backman K. *Self-care of home-dwelling elderly persons from the view of their vulnerability. V: Mičetić Turk, D. in Kokol, P., (ed). V: International Symposium "New trends in modern nursing". Maribor: University College of Nursing Studies. 2003; pp29-34.*
6. Baltes, P.M. Baltes, M.M. *Psychological perspectives on successful ageing: The model of selective optimisation with compensation. V: Successful Aging Perspectives from the Behavioural Sciences, (Baltes, P.M. in Baltes, M.M., eds). New York. Cambridge University Press. 1990;1-34.*
7. Burns, N., Grove, S.K. *The practice of nursing research. V: Conduct, critique, & utilisation. The 4th edition. Philadelphia, the US: W.B. Saunders Company, 2005.*
8. Christensen, B.L. & Kackrow, E.O. *Foundations of nursing. St. Louis: Mosby, 2003.*
9. Cheng, C.Y. *Living alone. The choice and health of older women. V: Journal of Gerontological Nursing*, letnik 32 šte. 9, Thorofare: Slack ; 2006. pp16-23.
10. Čačinovič Vogrinčič, G. *Družina in star človek. V: Socialno delo*, letnik 39, št. 4-5. Ljubljana: Fakulteta za socialno delo. 2000; pp287-291.
11. Dean, M. *Growing older in the 21st Century Growing Older Programme*. Swindon. The ESRC Research programme on Extending the Quality of Life, 2003.
12. Denyes, M.J., Orem, D.E. & Bekel, G. *Self-care: a foundational science. Nursing Science Quarterly*, letnik 14, Thousand Oaks : Sage Publications. 2001; pp 28 - 54.
13. Dragoš, S. *Bo skrb za starost ženskega spola? Zdravniški Vestnik*, letnik 73, št. 6, Ljubljana: Slovensko zdravniško društvo.2004; pp531-534.
14. *European Commission Survey of the Current Status of Research into »Ageing« in Europe by the ad hoc Advisory Committee on Coordination of RTD Policies. European Commision, Brussels, 2000.*
15. Fagerström, C., Holst, G. & Hallberg, I.R. *Feeling hindered by health problems and functional capacity at 60 years and above. V: Archives of Gerontology & Geriatrics*, letnik 44, št. 2, Amsterdam: Elsevier Science Publishers B.V.2007; pp181-201.
16. Fung, H.H. & Carstensen, L.L. *In focus: The knowledge of our years. V: Ageing Today*, letnik 23, št 2 , San Francisco: American Society on Aging. 2002; pp9 -12.
17. Gerrish, K. & Lacey, A. *The Research Process in Nursing. Blackwell Oxford Publishing, 2006.*
18. Gerson, L. & Berg, J. *Enhancing Self-Care in Community Dwelling Older Adults. V: Geriatric Nursing*, letnik 25, št.5, Philadelphia : Philadelphia Elsevier Science .2004; pp272-276.
19. Gillis, A. & Jackson, W. *Research for nurses: Methods and Interpretation. Philadelphia. F.A. Davis Company, 2001.*
20. Hagberg, M., Hagberg, B. & Saveman, B.I. *The significance of personality factors for various dimensions of life quality among older people. V: Ageing & Ment Health*, letnik, 6, št. 2, Abingdon: Routledge. 2004; pp178-185.
21. Harkness, J.A. *Instrument translation. V: Harkness, J.A., van der Vijver, F.J.R., & Mohler, P.P., (ed). Cross -Cultural Survey Methods. New Jersey: John Wiley & Sons, 2003.*
22. Hendry, F. & McVittie, C. *Is quality of life a healthy concept? Measuring and understanding life experiences of older people. V: Qualitative Health Research*, letnik 14, Thousand Oaks: Sage Publications Inc. 2004; pp961-975.
23. Hobbs Leenerts, M., Teel, C. S., & Pendleton, M. K. *Building a Model of Self-Care for Health Promotion In Ageing. V: Journal of Nursing Scholarship*, letnik 34, št 4, Oxford : Blackwell Publishing Limited .2002; pp355-361.
24. Holloway, I., & Wheller, S. *Qualitative Research in Nursing. Oxford, Malden, Carlton: Blackwell Science, 2002.*

25. Kempen, G.I.J., Ranchor, A.V., van Sonderen, E., van Jaarsveld, C.H.M. & Sanderman, R. Risk and protective factors of different functional trajectories in older persons: are these the same? V: *Journal of Gerontology*, letnik 61, št.2, Oxford : Oxford University Press / UK . 2006; pp95-101.
26. Leinonen, R., Hekkinen, E., & Jylhä, M. Predictors of decline in self-assessments of health among older people - a 5-year longitudinal study. V: *Social Science & Medicine*, letnik 52, št. 9, Oxford : Pergamon Press. 2001; pp1329-1341.
27. McAuley, E., Blissmer, B., Marquez, D.X., Jerome, G.J. & Kramer, A.F. Social relations, physical activity, and well-being in older adults. V: *Preventive Medicine*, letnik 31, št. 5, Burlington : Academic Press Inc. 2000; pp608-617.
28. Mesec, B., Teorija Roberta Atchleya o kontinuiteti v starosti. V: *Socalo delo*, letnik 39, št. 4 -5, Ljubljana: Fakulteta za socialno delo. 2000; pp355-364.
29. Norburn, J.E.K., Bernard, S.L., Konrad, T.R. Self – care and assistance from others in coping with functional status limitations among a national sample of elder adults. V: *Journal of Gerontology*, letnik 50, Oxford : Oxford University Press / UK. 1995; pp101–109.
30. Orem, D.E., Taylor, S. & McLaughlin, K. *Nursing - concepts of practice*. St. Louis: Mosby, 2001.
31. Orem D.E. *Nursing Concepts of Practice*. StLouis. Mosby Year Book. 1991.
32. Parissopoulos, S. & Kotzabassaki, S. Orem's self-care Theory, transactional analysis and the management of elderly rehabilitation. *ICUS and Nursing Web Journal* 17: <http://www.nursing.gr/OREM.pdf> (11p), 2004.
33. Polit, D. E., Beck, C.T., Hungler, B.P. *Essentials of nursing research: methods, appraisals, and utilization*. 6th ed. Philadelphia, New York, Baltimore. J. B. Lippincott, 2006.
34. Rabiner, D.J., Konrad, T.R., DeFriece, G.H., Kincade, J. & Bernard, S.L. Metropolitan versus nonmetropolitan differences in functional status and self-care practice. Findings from a national sample of community-dwelling older adults. V: *Journal of Rural Health*, letnik 13, Oxford : Blackwell Publishing Limited. 1997;pp14-28.
35. Räsänen, P., Backman, K., Kyngäs, H . Development of an instrument to test the middle-range theory for the self-care of home-dwelling elderly. *Scandinavian Journal of Caring Sciences*, letnik 21, št. 3, Oxford: Blackwell Publishing Limited. 2007; pp397- 405.
36. Ramovš, J. *Kakovostna starost: socialna gerontologija in gerontagogika*. Ljubljana: Inštitut Antona Trstenjaka, 2003.
37. *Slovar slovenskega knjižnjega jezika*. Ljubljana: DZS, 2005.
38. Teel, C.S. & Leenerts, M.H. Developing and testing self-care interventions for older adults in caregiving roles. V: *Nursing Research*, letnik 54, št. 3, Baltimore: Lippincott Williams & Wilkins. 2005; pp193-201.
39. Teeri, S., Välimäki, M., Katajisto, J. & Leino-Kilpi, H. Maintenance of patients' integrity in long-term institutional care. V: *Nursing Ethics*, letnik 15, št. 4, : Sage Publications, Ltd.2008; pp523-535.
40. Toljamo, M. & Hentinen, M. Adherence to self-care and social support. *Journal of Clinical Nursing*, leto 10, št. 5, Oxford: Blackwell Publishing Limited. 2001; pp618-627.
41. Tomey, A.M. & Alligood, M.R. *Nursing theorists and their work (5th ed.)*. St. Louis: Mosby, 2002.
42. Topping, A. The quantitative – qualitative Continuum. V: Gerrisg K & Lacey A (eds). *The research process in nursing*. Oxford, Malden, MA, Blackwell Publishing. 2006; pp157-158.
43. William, L. The utility of self-care theory as a theoretical basis for self-neglect. V: *Journal of Advanced Nursing*, leto 34, št. 4, Oxford: Blackwell Publishing Limited. 2004; pp545-551.
44. Zasuszniewski, J.A. Self -help and help-seeking behavior patterns in healthy elders. V: *Journal of Holistic Nursing*, letnik 14, št. 3 Thousand Oaks : Sage Publications Inc. 1996; pp223-236.
45. Zupančič, M. Pozna odraslost. V: Marjanovič Umek, L. & Zupančič, M. (ur) *Razvojna psihologija*. Ljubljana. Filozofska fakulteta Univerza v Ljubljani, 2004.
46. Železnik, D. Kakovost življenja starostnikov. V: Špelič, M. & Špur, M. (ur.) *Paliativna oskrba bolnikov – Zbornik*, Ljubljana: Zbornica - Zveza zdravstvene in babiške nege Slovenije.2005; pp16 -17.

Corresponding author:

Danica Zeleznik,  
Faculty of Health Science,  
University of Maribor,  
Slovenia,  
e-mail: danica.zeleznik@gmail.com



# Generic medicines: Perceptions of Pharmacists in Basrah, Iraq

*Adheed Khalid Sharrad, Mohamed Azmi Hassali, Asrul Akmal Shafie*

School of Pharmaceutical Sciences, Universiti Sains Malaysia, Malaysia

## Abstract

**Background:** The use of cheaper generic medicines is a strategy promoted in many countries to reduce rising health care costs. The objective of this study was to investigate generic medicine dispensing trends and substitution practice from the perspective of pharmacists in Basra, Iraq.

**Methodology:** A qualitative approach was used to gather information from the pharmacists in the city of Basrah, Iraq. A purposive sample of ten pharmacist practicing in Basrah was interviewed face to face using a semi-structured interview guide.

**Results:** A total of ten pharmacists were interviewed. Thematic content analysis of the interviews identified five major themes: Generic Medicines substitution practices, Knowledge about generic medication, Quality and safety of generic medicines, Patient education by pharmacists regarding generic medicines, and Strategies to improve generic medicine utility.

**Conclusion:** The present study suggested that, in general pharmacists interviewed in Basrah city were positive towards the use of generic medicines. Patient should be educated to increase the use of generic medicines in the future.

**Key words:** Generic medicine, bioequivalence, perceptions, substitution

## Introduction

Generic medicines provide the opportunity for major savings in healthcare expenditure to both government and consumers, given that they are generally lower price than their counterpart branded<sup>1,2</sup>

In Iraq, the Ministry of Health (MOH) is responsible for importing medicines from companies

that are previously registered with KIMADIA (the state company for importation and distribution of drugs and medical appliances). The MOH distributes imported pharmaceuticals to the public and private sectors (private pharmacies) after obtaining permission from the Drug National Quality Control Laboratories (DNQCL). Iraqi law requires all drugs to be marketed via the KIMADIA system. For over 20 years, KIMADIA has been the sole body authorised to carry out management, planning, selection, quantification, procurement, storage and distribution of medicines and medical equipment. In 1989, it was estimated that 70% of drugs were imported. The other 30% came from Samara Drugs Industries (SDI), a government agency.<sup>3</sup> Until 1994; the supply of medicines was dominated by the public sector. Approximately 90% of the drugs purchased using public funding was allocated to the public sector. Currently, there is no social health insurance system exist in Iraq. There are very small, isolated health insurance programs for employees of specific companies. At present, however, there is no system for reimbursement for money spent by public on private prescriptions in the country.<sup>4</sup> Thus, ensuring the availability of high-quality drugs at affordable prices in Iraq is a public health priority. Prescribing drugs by generic name and encouraging pharmacists to dispense prescriptions with generic medicines is one frequently suggested means for lowering the costs of healthcare.<sup>5-7</sup> Various articles have discussed the implications of generic substitution and other strategies to reduce pharmaceutical expenditure.<sup>8-11</sup> The concept of dispensing and using generic medicines has been controversial, however.<sup>5</sup> Concern has been expressed by pharmacists elsewhere about the efficacy of generic medication.<sup>12,13</sup> This debate has centered on issues



related to bioequivalence and potential confusion that might arise when changes of medicine brands occur in some patient populations.<sup>14,9</sup> The theoretical framework for this study calls on bounded rationality theory which predicts that the quality of the decisions we make are limited by the available information and our ability to synthesize that information.<sup>15</sup> A review of the available literature revealed that a few studies have been carried out in the USA and France, but no studies have been performed in Iraq to explore pharmacists' perceptions regarding the use of generic medications by the consumer. As a result of differences in pharmacy practices and the health care system between Iraq and western countries, the results of such a study would be a significant contribution to the field. Therefore, the aim of this study was to explore perceptions held by Iraqi pharmacists towards the use of generic medicines.

## Method

Since little research has been carried out in Iraq to identify pharmacists' perceptions of generic medicines, qualitative methods were used to gain understanding of this issue.<sup>16,17</sup> A qualitative approach was adopted because it allows a flexible exploration of informants' attitudes and experiences and produces a richness of data that allows the researcher to gain a deeper understanding of social phenomena.,<sup>18,19</sup> One of the challenges faced in the process of recruitment was to obtain a varied sample of informants to participate in the interviews. To address this, purposive sampling procedure was used. The advantages of purposive sampling are that it is cheaper and time-saving to implement in comparison to other sampling procedure. Semi-structured interviews were conducted with pharmacists until we reached saturation of themes.<sup>20,21</sup> The interview guide was developed following an extensive review of the literature on similar studies conducted elsewhere. Pharmacists were approached personally by the researcher at their private pharmacy hospital. Ten pharmacists consented to be interviewed. The interviews focused on the following issues: generic medicine dispensing preferences, knowledge about and confidence in the generic medication, and patients' ac-

ceptance of generic prescriptions. Follow-up questions were used when necessary to get a more in-depth explanation and to draw out more complete ideas from the participants. They were given freedom to express additional views on the subject at the end of the interview time.<sup>22</sup>

All interviews were conducted at a place suitable for the participants: eight at their private pharmacy and two in hospital pharmacy in Basrah province. Interviews took an average of 20-30 minutes. They were conducted by the investigator in his native language (Arabic). Interviews were documented and transcribed verbatim by qualified transcribers. Transcripts were confirmed by the researcher, corrections (if needed) were made before copies of the transcripts were sent to the respective physicians for their endorsement. The researcher manually analysed all of the transcripts line-by-line for relevant content and themes.<sup>23,24</sup> Ten interviews were required to achieve saturation of the themes, and no new themes emerged in the last three interviews.

## Results

### Characteristics of participants

Ten pharmacists were interviewed. Eight of them worked in urban areas, and the other two were in rural settings. Demographic characteristics are summarised in Table 1.

*Table 1. Pharmacists' demographic characteristics (n=10)*

| Descriptions                   | n |
|--------------------------------|---|
| Gender:                        |   |
| Male                           | 7 |
| Female                         | 3 |
| Degree obtained from:          |   |
| Baghdad University             | 8 |
| Mosul University               | 2 |
| Work place                     |   |
| College of pharmacy (lecturer) | 2 |
| Government hospital            | 5 |
| Private pharmacy               | 3 |
| Experience in Basrah:          |   |
| More than 10 years             | 7 |
| Less than 10 years             | 3 |

All the interviews were conducted at the pharmacists' workplaces (two in hospital pharmacies and the others at the pharmacists' private pharmacies).

## Themes

Five major themes emerged when the interview content was reviewed: generic medication substitution practices, knowledge of and confidence with generic prescriptions, quality and safety of generic drugs, pharmacist's roles in educating patients regarding generic substitutions and strategies to improve their use of generic medications. The themes are presented below.

### Theme 1 Generic Medicines substitution practices

Nearly, all the pharmacists interviewed have recommended generic medications to consumers.

*"Normally, we dispense generic medicines, unless the patient requests to have of innovative medicine" (p01)*

*"I recommend the generic and innovative medicines and leave the choice to the patient" (p03)*

Interestingly, the relatively inexpensive cost of generic medications leads pharmacists and patients to choose generic over innovative drugs.

*"Medicine price is one of the important factors that makes the patient favour the generic medicine. For example, the price of Zantac® (Ranitidine 150 mg) is ID15000 (US\$=12.5) for sixty tablets, while the price of Histac is ID 2000 (US\$ 1.6)" (p10)*

*"I recommend generic medicine because it is cheap for the patient and more profitable to the pharmacy" (p07)*

A patient's income status affects the pharmacists' recommendations.

*"Patients with low income request a cheaper medicine; therefore offering generic medicine will help patients to buy such medicines" (p06).*

Another factor which encourages pharmacists to dispense generic medications is the availability of generic medicines in drug stores. Innovative drugs are more difficult to obtain.

*"The availability of generic medicines in drug stores constantly encourages the pharmacist to recommend them to the patients, in contrast to the innovator medicines, which are difficult to obtain continuously" (p02)*

Though most pharmacists recommend generic medications, certain circumstances make them reluctant to use the generic over the innovative drug. When the physician does not want generic medicines, pharmacists respect the physicians' recommendations. Furthermore, the nature of the disease affects pharmacists' willingness to use the generic form of a drug.

*"The doctor does not agree to switch medicines is one of the factors that prevent me from offering generic medicines to the patients" (p01)*

*"If the patient was satisfied and stabilized with the innovator medicine, I don't offer generic medicines, especially in the cases of chronic disease" (p05)*

In Iraq, patients' trust in their physicians' choice of innovative medicine was seen as a barrier to offering generic medicines.

*"Some patients are convinced with what the physician prescribes to them, so I don't offer generic medicine to these patients" (p 03).*

Confusion is one barrier that prevents pharmacists from recommending generic medications. This is especially pertinent with respect to elderly patients and patients treated with multiple medications.

*"I don't offer generic medicine to the elderly people who use innovative medicine and they are comfortable with it, or to those who take more than one type of medicine to avoid getting them confused" (p04).*

*"I do not offer generic medicines to any refill prescription because I am afraid of confusing the patient" (p09).*

Some pharmacists have realised that many patients, especially from those from rural areas, do not believe that generic medications are effective.

*“Actually, in my pharmacy I don’t recommend generic medicine to patients (especially those patients from rural areas) who do not believe in the effect of generic medicines” (p03).*

## **Theme 2**

### **Knowledge about generic medication**

Pharmacists were questioned about the bioequivalence criteria required by the WHO and/or FDA, KIMADIA and DNQCL in Iraq, for approval of a generic medication. We found that the pharmacists have little or no knowledge about the bioequivalence requirements.

*“I don’t have any idea” (p02).*

*“I do not care about these things, I depend on the DNQC” (p01).*

## **Theme 3**

### **Quality and safety of generic medicines**

Pharmacists were asked about their belief in therapeutic equivalence when comparing a brand-name (innovative) medication with generic medicine. Generally, the pharmacists expressed their belief that quality of a particular generic medication depends on the reputation of the company that produced it. In addition, all of the pharmacists included in this study reported a lot of confidence in the laboratory control program in Iraq (DNQCL).

*“I have great confidence in the generic medicines which come from European origin as well as the medicines that are being tested by the DNQCL” (p09.)*

## **Theme 4**

### **Patient education by pharmacists regarding generic medicines**

One of the most important responsibilities of the pharmacist is to educate patients about proper use of their medications. All of the pharmacists interviewed in this study agreed that educating patients about generic medicines may reduce misunderstandings about brand substitution when using generic drugs.

*“Answering the patients’ questions, if any is the most important duties of the pharmacist, also giving the patients all the information on the medicine leads to reduce the risk of the misuse of medications and confusion due to different brands of medications” (p07).*

## **Theme 5**

### **Strategies to improve generic medicine utility**

All of the pharmacists commented on the need for a law to regulate the substitution of generic medicines.

*“Legislating a law to regulate generic medicines substitution made by the pharmacist leads to increase the use of generic medicine by the patients and helps them to save money. This is because the price different between innovative and generic medicine” (p08).*

Furthermore, some pharmacists noted the usefulness of a drug guide that would contain a list of the generic equivalent of commonly-used innovative drugs. This guide could be used to guide brand substitutions in Iraq.

*“A drug guide containing all the innovative medicine and the medicines which are equivalent leads to regulate the generic medicine substitution. This will convince the physician to follow the proposed guide” (p10).*

Some pharmacists suggested that the use of the scientific name or “INN” in prescription writing by Iraqi physicians would increase the use of ge-

neric medicine. In addition, this would decrease patient confusion caused by name variations between brand-name and generic forms of a medication.

*“Many names of branded medicines confuse the patients, physicians and pharmacist and thus, the use of scientific names is the best” (p06).*

Currently, the Iraqi market is inundated with counterfeit medicines, which patients often confuse with generic medications. Decreasing the availability of counterfeit medication, by increasing oversight of pharmaceutical production, would help to prevent this Confusion.

*“The large numbers of the counterfeit medicines adversely affect the use of generic medicine and these counterfeit medicines are considered by most patients as generic medicines, and this is not true” (p03).*

## Discussion

Generic substitutions positively impact medication usage by making necessary prescriptions more affordable. Pharmacists play an important role in educating patients about generic medicines. This exploratory study of Iraqi pharmacists' perceptions about the use of generic medication highlights factors that interfere with utilising generic medicines in Iraq. Some of these issues include bio- and therapeutic equivalence, substitution practices, confusion due to the usage of different generic brands, and product labelling.

There is not currently any law in Iraq which regulates generic substitution. This decision is currently dictated by two main factors: the relationship between the pharmacist and physician and the relationship between the pharmacist and patient. In general, all of the pharmacists interviewed were willing to recommend generic medicines because generics are lower in price than their brand equivalents and supplier consistency enables pharmacists to feel secure that they are recommending a quality replacement. This supports previous studies published by Segal, Sanborn, and Hassali all of which highlighted the influence of price and sup-

plier consistency in pharmacists' decisions whether to recommend a generic substitution.<sup>25-27</sup> (Segal et al, 1989; Sanborn and White,1993;Hassali et al,2005).

Patients' reluctance to deviate from what their physicians have prescribed for them, and the reluctance of some physicians to recommend generic substitutions are two of the main barriers to the widespread usage of generic medicine.<sup>28,29</sup>(Mott and Cline,2000;Ludin,2000). In addition, previous studies have reported that many physicians are unwilling to recommend generic medications to avoid confusing their patients, especially elderly patients, those with chronic diseases, or those on multiple medications.<sup>27</sup> (Hassali et al.,2005) . These were the main pharmacist-reported patient concerns regarding medication substitution.

All of the pharmacists interviewed agreed that generic medications obtained from European or reputable Arab companies are bio and therapeutic equivalents of the innovative medicines. However, there are a lot of counterfeit medicines in the Iraqi market, which makes it very difficult to distinguish legitimate medications from counterfeit drugs. Ultimately, this may negatively affect the usage of generic medicines in Iraq.

When counselled by a pharmacist, patients are generally amenable to using generic medicine. Most Iraqi patients hold the baseless belief that generic medications are less effective than innovative medicines because generic versions are less expensive. Patients should be educated by their pharmacist about the equivalence of generic medicines to innovative medicines to prevent such misunderstandings. For example, an American study reports that only 35% of patients over 65 years of age believe that the quality of generic medicines does not equal that of innovative medicines.<sup>30</sup> (Rosendahl,1994).

Some of the pharmacists involved in this study suggested that using the scientific name (INN) for medications may increase the usage of generic medicine. This aligns with previous studies by Lagarce and Segal, which reported that pharmacists are more likely to dispense generic medicines for patients with a low income or when the physician writes the prescription using INN.<sup>25,31</sup> (Segal et al.,1989; Lagarace et al., 2005) to this end, the WHO International Non-proprietary Names



(INN) Committee works to develop generic names that will be accepted worldwide.<sup>32</sup>(WHO,2003) Similar results have been reported in the United Kingdom.<sup>33</sup>(Pharmacopia,2003).

Discussions between physicians and pharmacists regarding the availability of generic medications in pharmacies can increase prescriptions for generic medicines. A study by Knowlton and Knapp found out that pharmacists' interference led to a 6% increase in prescriptions for generic medicines.<sup>34</sup>(Knolton and Knapp,1994). Institution of a law to regulate generic substitutions in Iraq would likely increase the use of generic medications. In addition, a medicine guide containing innovative drugs and their therapeutically equivalent generics would facilitate generic substitution by both physicians and pharmacists.

### Study limitation

One of the limitation of qualitative methods is that the results cannot be extrapolated to the wider population.<sup>35</sup> (Creswell et al.,2004).This study was conducted in Basrah city in Iraq and the findings may not apply to Pharmacists living in other province in Iraq. We were unable to include pharmacists from other province due to limited funding.

### Conclusion

In general, the pharmacists interviewed in this study were positively inclined towards generic substitution because it gives the pharmacist an expanded role in the education and healthcare of the patient. The pharmacists felt that this process could change the negative predisposition that patients have toward pharmacists as businessmen or dispensers of drugs. This study shows the perceptions of ten pharmacists about the use of generic medicines. An understanding of these perceptions will be important to policy makers when they strategies to improve the utilisation of generic medicines in Iraq. In addition, an extra effort should be taken by regulatory and professional bodies to educate pharmacists on bioequivalence requirements, which currently cause confusion among Iraqi pharmacists.

### Reference

1. Fisher M.A., Avorn J. *Economic consequences of underuse of generic drugs: Evidence from Medicaid and Implications for prescription drug benefit plans.* *Health Ser Res* 2003;38(4):pp.1051-64.
2. Ford N., Hoen E. *Generic medicines are not substandard medicines.* *Lancet*. 2002; 359 (9314) p.1351.
3. USAID, *Pharmaceutical and Medical Products in Iraq, 2007: 2-8.*
4. USAID, *Pharmaceutical and medical products in Iraq, 2007:51.*
5. Karim, S., Pillai, G., Ziqubu-Page. (1996) 'Potential savings from generic prescribing and generic substitution in South Africa', *Health Policy and Planning* 1996, 11:198–205.
6. Tilyard MW and Dovey SM: 'General practitioners' views on generic medication and substitution', *New Zealand Medical Journal* 1990, 103:318–320.
7. King DR and Kanavos P: *Encouraging the use of generic medicines: Implications for transition economies.* *Croatian Medical Journal* 2002, 43:462–469.
8. Lofgren H: *Generics drugs: International trends and policy developments in Australia.* *Australian Health Review* 2004, 27:39–48.
9. Moulds R: *Generic prescribing — the pros.* *Australian Prescriber* 1992, 15:50.
10. Messenger A: *Substitution bitter pill to swallow for some: Australian Doctor* 1995, June 4:16.
11. Donovan J: *Is there a role for generic medicines in the pharmaceutical benefits scheme?* *Health Issues (Melbourne, Australia)* 2003, 75:9–11.
12. Brand P, Clear P, Keen, S. 'Brand substitution', *Australian Pharmacist* 1995, 14: 646–649.
13. Kirking DM, Gaither CA, Ascione FJ and Welage LS: *Physicians' individual and organizational views on generic medications.* *Journal of the American Pharmaceutical Association* 2001, 41:718–722.
14. McGavock H: *Generic substitution Issues relating to the Australian experience.* *Pharmacoepidemiology and Drug Safety* 2001, 10:555–556.



15. Simon, H. A. *Models of bounded rationality*. Cambridge, Massachusetts: MIT Press 1982.3
16. Berg B 'Designing qualitative research', in *Qualitative research methods for the social science*. 5th edn, Allyn and Bacon, Boston, MA, 1995: 37.
17. Smith F: *Health services research methods in pharmacy-Qualitative Interviews*. *International Journal of Pharmacy* 1998, 6: 97-108.
18. Smith F. *Research methods in pharmacy practice*. London: Pharmaceutical press; 2002. :119-122
19. Morse, J. and A. Field, *Qualitative Research Methods for Health Professionals*. Sage Publications, 2nd ed. London:1995:1 *Australasian Medical Journal* 2009, 1, 8, 58-64 63
20. Morse J: *The significance of saturation*. *Qualitative Health Research* 1995.5(2):147-9.
21. Glazer BG and Strauss AL: *The discovery of grounded theory: strategies for qualitative research*. New York: Aldine; 1997.
22. DeSilets L: *Using icebreakers to open communication*. *J Contin Educ Nurs* 2008, 39:292-293.
23. Burnard P: *A method of analysing interview transcripts in qualitative research*. *Nurse Educ Today* 1991, 11(6): 461-6.
24. Morse J and Field A: *Qualitative research methods for health professionals*. London: Sage Publications, 2nd ed.; 1995: 94
25. Segal R, Wantz D, Brusadin R. (1989) *Pharmacists' decision making in the selection of generic pharmaceuticals*. *J Pharm Mark Manage*, 4(1): 75-91.
26. Sanborn MD, White SJ. *Pharmacists' perceptions and practices regarding the purchase of multisource pharmaceuticals*. *Hosp Pharm* 1993; 28(2): 104-6, 109-13.
27. Hassali MA, David C. M. Kong., Kay Stewart. (2005) *Generic medicines: perceptions of community pharmacists in Melbourne, Australia, Vol. 1* 4(3): p27-45.
28. Mott DA and Cline RR, (2000) *Exploring generic drug use behavior: the role of prescribers and pharmacists in the opportunity for generic drug use and generic substitution*. *Med Care*. **40(8)**: p. 662-74.
29. Ludin D. *Moral hazard in physician prescription behavior*. *J Health Econ* 2000; 19(5): 639-662.
30. Rosendahl I. (1994) *Consumer on generic: some clues on their views from AARP*. *drug topics*. AARP, 54s-56s.
31. Lagarce L., Lusson-Brisset C. and Bruhat C. (2005) *General practitioner perception for generic medicine*. *therapie*, 60, 67-74.
32. WHO, (2003) *International non proprietary names: revised procedure* [online]. Available from URL: [http://www.who.int/gb/EB\\_WHA/PDF/EB110/EEB1103.pdf](http://www.who.int/gb/EB_WHA/PDF/EB110/EEB1103.pdf) [Accessed 2003 Mar 19].
33. Pharmacopoeia, B. (2003) *British approved names: guiding principles* [online]. Available from URL: <http://www.pharmacopoeia.org.uk/guiding.cfm> [Accessed 2003 Mar 19].
34. Knowlton C. & Knapp D. (1994) *community pharmacists help HMO cut drug costs*. *american pharmacy*, 34, 36-42.
35. Creswell JW, Fetter MD, and Lvankova NV: *Designing a mixed methods study in primary care*. *Annals of Family Medicine* 2004, 2: 7-12.

Corresponding author:

Adheed Khalid Sharrad,  
 Discipline of Social and Administrative Pharmacy,  
 School of Pharmaceutical Sciences,  
 Universiti Sains Malaysia,  
 Malaysia  
 E-mail: adheed\_k@yahoo.co.uk

# Salt content in meals of boarding schools and students' restaurants in Novi Sad

Ljiljana Trajkovic-Pavlovic<sup>1,2</sup>, Budimka Novakovic<sup>2</sup>, Natasa Dragnic<sup>1,2</sup>, Ljilja Torovic<sup>1,2</sup>

<sup>1</sup> Institute of Public Health of Vojvodina, Republic of Serbia

<sup>2</sup> School of Medicine University of Novi Sad, Republic of Serbia

## Abstract

Investigation brought evidence that salt intake was in a positive relationship with systolic blood pressure and that children with higher blood pressure were more susceptible to develop hypertension in adulthood. The purpose of this paper was to determine total sodium chloride content in average daily meal (breakfast, dinner and supper) prepared for adolescents in boarding schools and young adults in student's restaurants. In the meal time, samples of breakfast, dinner and supper, of 46 daily meals dedicated to adolescents and 63 daily meals dedicated to students were taken from boarding schools/students' restaurants. Standardized laboratory methods were applied to determine proteins, fats, ash and water in order to calculate energy value of meal. Titrimetric method with  $\text{AgNO}_3$ , and  $\text{K}_2\text{CrO}_4$  as indicator, was applied in order to determine chloride ion and after that a content of NaCl was calculated as % NaCl. Descriptive statistical methods were applied in order to present average salt content in the whole and in 100g and in 100 kcal of average daily meal. Student's t-test was applied to determine statistical differences of sodium chloride amount among meals. Energy value of the average adolescent's and student's daily meal was  $2938.8 \pm 457.3\text{kcal}$  (CV 15.6%) and  $3338.4 \pm 446.3\text{kcal}$  (CV 13.4%), respectively. Energy value of the average student's daily meal was significantly higher ( $p < 0.001$ ). Energy density of the average adolescent's and student's meal was  $130.0 \pm 22.5 \text{ kcal}/100\text{g}$  (CV 17.3%) and  $136.8 \pm 15.7 \text{ kcal}/100\text{g}$  (CV 11.5%), respectively. Sodium chloride content in average daily meal for adolescents

and students was  $18.0 \pm 3.3\text{g}$  (CV 18.4%) and  $16.8 \pm 3.7\text{g}$  (CV 22.0%). The difference was not statistically significant. Sodium chloride content in 100g of adolescent's and student's meals was  $0.8 \pm 0.1\text{g}$  (CV 18.4%) and  $0.7 \pm 0.1\text{g}$  (CV 21.3%), respectively. The difference was statistically significant. Sodium chloride content in 100kcal of the average adolescent's and student's meal was  $0.6 \pm 0.1\text{g}$  (CV 17.8%) and  $0.5 \pm 0.1\text{g}$  (CV 24.2%), respectively. The difference was statistically significant. Adolescents and young adults in Novi Sad that consume meals with 16-18g of salt are overloaded with sodium and it is reasonable to expect that some of them could not cope with sodium overload and might develop undesirable response to it.

**Key words:** salt, meals, adolescents, young adults

## Introduction

The estimation of the World Health Organization showed that total burden due to cardiovascular diseases (CVD), expressed as DALY (disability adjusted life years lost) in developing, low-income and middle-income countries accounted for more than 80% of total DALYs lost worldwide. High blood pressure is recognized the most important risk factor and it accounts for 62% of stroke cases and 49% of coronary heart events (1). Epidemiological investigations brought overwhelming evidence that dietary salt intake within population was in a positive correlation with average blood pressure and prevalence of hypertension (2,3,4). Significant positive association was obser-

ved between sodium excretion and stroke, cardiovascular and all causes of death (5,6,7). Sodium/calorie intake ratio was directly associated with all causes and CVD mortality (8). High salt intake was recognized as risk factor for end stage of renal failure (9,10). It is also in a positive association with stomach cancer and osteoporosis in adults (11,12,13). Population based intervention studies showed that reduction of salt intake was followed with systolic and diastolic blood pressure depletion (14,15,16), stroke and chronic heart disease mortality rate reduction and with increased life expectancy within controlled population (16). Non-personal health interventions of salt reduction were recognized as cost effective methods for reduction of health care costs of chronic cardiovascular diseases (17,18,19). On the global level non-personal governmental actions on reducing salt content in processed food are cost effective ways to reduce chronic cardiovascular diseases burden measured as disability-adjusted life years / DALYs (20). Although a high blood pressure is not common in adolescents and young adults, it was found that salt intake of children and adolescents was in a positive relationship with systolic, or pulse or diastolic blood pressure (21,22,23). Youths with higher blood pressure were more susceptible to develop hypertension in adulthood (24). Data from investigations in developed countries indicated that the main source of salt for the general population (25, 26, 27) was processed food.

In the Republic of Serbia cardiovascular diseases are leading cause of death. They participate with 55.2% in the total death cases. Ischemic heart diseases are responsible for 150 889 DALYs. Cerebral and other vascular diseases are responsible for further 136 090 DALYs of the population (28). The latest national health survey indicated that a prevalence of hypertension (systolic blood pressure  $\geq 140$  mmHg and diastolic blood pressure  $\geq 90$  mmHg) in the Republic of Serbia was 46.5% among adults aged 18 year and more (29). In the Province of Vojvodina prevalence of hypertension (applied criteria for measured blood pressure were the same as in Serbia) among adults aged 45 years and more was 48.7% and in the city of Novi Sad it was 69.8% (30).

Countries that have been conducting national programs on salt reduction had started with pro-

viding data on salt intake of the population (31). Population based investigation on salt intake, concerning adults, or children and adolescents in the Republic of Serbia have not been performed. Our previous data indicated that average daily meal, breakfast, snack and dinner, in kindergarten in Novi Sad salt content was higher than children's salt intake in developed countries and more than it is recommended for that age (21,32,33). In the city of Novi Sad there are two boarding schools and one student's centre. The boarding schools prepare approximately 200-220 and the student's centre near 3000 daily meals (breakfast, dinner and supper). We were interested to determine salt content in average daily meals prepared for adolescents and young adults/students.

### AIM

The purpose of this paper was to determine total sodium chloride content in average daily meals dedicated to adolescents aged 15-18y in boarding schools and young adults/students aged 20-27y and to determine salt density of the controlled meals.

### Method

**Sampling:** Trained samplers, during 2007 and 2008y took 46 daily meals dedicated to adolescents and 63 daily meals dedicated to students. They visited boarding schools /students' restaurants at meal time and took samples of breakfast, dinner and supper. Visits of samplers were not announced.

**Preparation of sample:** each sample of daily meal (breakfast, dinner and supper) was transferred into container of high-speed blender and mixed thoroughly. Test sample was stirred immediately before removing test portion for analysis. **Determination:** 10.0 g of prepared test sample was weighted into 100 ml volumetric flask and diluted to volume with distilled H<sub>2</sub>O. After mixing and filtering, 20 ml aliquot was transferred to 250 ml Erlenmeyer and 0.5 ml of chromate indicator (cold saturated K<sub>2</sub>CrO<sub>4</sub> solution) was added. Solution was titrated with standardized AgNO<sub>3</sub> soluti-

on until turned permanent brown-red. Calculation:  
 $\% \text{NaCl} = \text{ml AgNO}_3 \times 0.05844 \times 5 \times 100/\text{g tested portion}$ .

Standardized methods were applied in order to determine quantity of proteins, fats, ash and water. Obtained results were applied to calculate energy value (calories) of each daily meal. Energy density of the average daily meal was expressed as calories/100g ratio.

Sodium chloride content was presented by using descriptive statistical method including average  $\pm$  SD and CV of sodium chloride per daily meal. Sodium chloride density was calculated as sodium chloride (g) per 100g of daily meal and sodium chloride (g) per 100kcal of each daily meal, as well. The Student's t-test was applied to find out whether there were statistically significant differences of sodium chloride content, expressed in absolute number and as g /100g of daily meal and g/100kcal ratio.

## Results

Obtained results showed that the adolescent's and students' average daily meal weighted  $2285.3 \pm 299.5\text{g}$  (CV 13.1%) and  $2446.5 \pm 271.3$  (CV 11.1%), respectively. The average weight of the

student's average daily meal was statistically higher ( $p=0.003$ ). Energy value of the average adolescent's and student's daily meal was  $2938.8 \pm 457.3\text{kcal}$  (CV 15.6%) and  $3338.4 \pm 446.3\text{kcal}$  (CV 13.4%), respectively. Energy value of the average student's daily meal was statistically higher ( $p < 0.001$ ). Energy density of the average adolescent's and student's meal was  $130.0 \pm 22.5 \text{ kcal}/100\text{g}$  (CV 17.3%) and  $136.8 \pm 15.7 \text{ kcal}/100\text{g}$  (CV 11.5%), respectively. The difference was not statistically significant (Table 1).

The average sodium chloride content in average daily meal for adolescents and students was  $18.0 \pm 3.3\text{g}$  (CV 18.4%) and  $16.8 \pm 3.7\text{g}$  (CV 22.0%), respectively. The difference was not statistically significant. The average sodium chloride content in 100g of adolescent's and student's meals was  $0.8 \pm 0.1\text{g}$  (CV 16.4%) and  $0.7 \pm 0.1\text{g}$  (CV 21.3%), respectively. Sodium chloride content in 100kcal of the average adolescent's and student's meal was  $0.6 \pm 0.1\text{g}$  (CV 17.8%) and  $0.5 \pm 0.1\text{g}$  (CV 24.2%), respectively. When sodium chloride density was computed, it appeared that it was significantly higher in adolescent's daily meal compared to the student's one, regardless the density of sodium chloride was expressed per 100g or per 100kcal ( $p<0.001$ ) (Table 2.).

Table 1. Energy value and energy density of average daily meals in boarding schools and students' restaurants

|                  | Weight               |             |        |        | Energy value         |                |           |        | Energy density       |                     |                |        |
|------------------|----------------------|-------------|--------|--------|----------------------|----------------|-----------|--------|----------------------|---------------------|----------------|--------|
|                  | range (g)            | average (g) | SD (g) | CV (%) | range (kcal)         | average (kcal) | SD (kcal) | CV (%) | range (kcal/100g)    | average (kcal/100g) | SD (kcal/100g) | CV (%) |
| Students' centre | 1230-3066            | 2446.5      | 271.3  | 11.1   | 1304.4-4103.7        | 3338.4         | 446.3     | 13.4   | 104.6-175.7          | 136.8               | 15.7           | 11.5   |
| Boarding schools | 1508-3178            | 2285.3      | 299.5  | 13.1   | 2038.2-4014.8        | 2938.8         | 457.3     | 15.6   | 85.5-182.9           | 130.0               | 22.5           | 17.3   |
|                  | t = 3.026, p = 0.003 |             |        |        | t = 4.726, p = 0.000 |                |           |        | t = 1.890, p = 0.061 |                     |                |        |

Table 2. Salt content and salt density of average daily meals in boarding schools and students' restaurants

|                  | Salt content         |             |        |        | Salt content in 100g |             |        |        | Salt content in 100kcal |             |        |        |
|------------------|----------------------|-------------|--------|--------|----------------------|-------------|--------|--------|-------------------------|-------------|--------|--------|
|                  | range (g)            | average (g) | SD (g) | CV (%) | range (g)            | average (g) | SD (g) | CV (%) | range (g)               | average (g) | SD (g) | CV (%) |
| Students' centre | 7.9-23.1             | 16.8        | 3.7    | 22.0   | 0.4-1.0              | 0.7         | 0.1    | 21.3   | 0.2-0.8                 | 0.5         | 0.1    | 24.2   |
| Boarding schools | 9.7-25.6             | 18.0        | 3.3    | 18.4   | 0.4-1.1              | 0.8         | 0.1    | 16.4   | 0.3-0.9                 | 0.6         | 0.1    | 17.8   |
|                  | t = 1.757, p = 0.082 |             |        |        | t = 3.841, p = 0.000 |             |        |        | t = 4.862, p = 0.000    |             |        |        |



## Discussion

High blood pressure has been indicated as a major risk factor for stroke, ischemic heart disease, hypertensive disease and other cardiovascular diseases and all causes of deaths (1,5,6,7,34). More than 80% of the high blood pressure attributed burden of diseases was recorded in low-income and middle-income countries in the Eastern Europe and Central Asia with a greater proportion of the burden in young age groups (35). From the public health point of view, it is important to elucidate modifiable risk factors within population and to implement intervention programmes (36,37). The results came from community programmes, that were conducted with participation of food industry and followed by the educational programmes of the population, confirmed beneficial outcomes in reducing cardiovascular and stroke mortality rate (14,15,16). Although many studies suffer from methodological problems related to data collection on salt intake, the results suggest that higher sodium intake is related to higher blood pressure in children and adolescents (21,22). There is increasing evidence that blood pressure of children, adolescents and young adults is in a positive relationship with sodium intake, especially when it is associated with the increased body mass index (21,22,23,38).

Data obtained by our investigation showed that average daily meal which includes three meals (breakfast, dinner and supper), dedicated to adolescents aged 15-19y and students aged 20-27y in boarding schools and student's restaurants amounted  $18.0 \pm 3.3\text{g}$  and  $16.8 \pm 3.7\text{g}$  of salt, respectively and exceeded average salt intake of adolescents and young adults in developed countries (21,38). Detected amount of sodium in sodium chloride was not the whole one because the laboratory method, we applied in our investigation, was not able to identify all sodium presented in tested food samples. It detected only sodium that was bound to chlorine. Detected sodium chloride content in the controlled samples of the daily meal exceeds for more than three folds the amount of the established nutrient goal of the World Health Organization (36,37) and by more than two folds of the established achievable goals of the countries that have been conducting countrywide national pro-

grams (31). There is relatively little evidence of the salt sensitive proportion of the adolescents and young adults (39,40), but there is evidence that the salt sensitive proportion of the population is increasing with the age, body mass index and other associated diseases such are hypertension, diabetes mellitus and renal dysfunction (41,42,43). For us, it was important to note that in majority of the studies (40,41,42,43,44), that investigated salt sensitivity among humans, sodium load (high salt intake) was lower or similar to the sodium content in the meals dedicated to adolescents and young adults in boarding schools and students' restaurants in Novi Sad. These findings indicate that adolescents and young adults in Novi Sad that consume meals with 16-18g of salt are overloaded with sodium. It is reasonable to expect that some of them could not cope with sodium overload and might develop undesirable response to it. Our analyses showed that the average energy value of the controlled meals, although they were not energy dense (45) exceeded the latest WHO/FAO/UNU recommendation for energy intake of the adolescents girls and young adults of both gender (46). These observations suggest that the adolescents and young adults in Novi Sad who take high energy and salt dense meals are exposed to the unfavorable dietary pattern, i.e. diet that could lead to obesity, hypertension and other over nutrition related diseases (1,36,37). Our findings are in line with data suggested that in low-income and mild-income countries national and regional programs should be developed in order to support healthy start. Studies from developed counties clearly demonstrated beneficial effects from life style modifying trials and programs (1,31,34,47,48,49).

## Conclusion

Obtained results indicated that adolescents and young adults/students, throughout three meals received in boarding schools and students' centre, had received salt in a quantity that exceeds internationally established population goal for average daily intake and population achievable goal. This amount also exceeds daily salt intake of adolescent and young adults in developed countries. Detected salt quantity showed that the target gro-



ups of adolescent and young adults are salt overloaded and thus it was reasonable to expect that some of them would not be able to cope with it especially those who were overweight, who have positive family history of hypertension and other cardiovascular risk factors. Further, more specific, investigations are needed in order to provide data relevant to highlight a magnitude of risk factors within adolescent and young adult population that could be responsible for high blood pressure and related chronic diseases in adulthood in our community. Beneficial effects of that kind of investigations and implemented programmes already have been extensively evaluated.

## References

1. *World Health Organization: World health report 2002: reducing risks, promoting healthy life. Geneva (Switzerland): World Health Organization; 2002.*
2. *Intersalt Cooperative Research Group. Intersalt: an international study of electrolyte secretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. BMJ 1988;297:319-28.*
3. *Elliot P, Stamler J, Nichols AR, Stamler R, Kesteloot H, Marmot M. Intersalt revisited: further analyses of 24 hour sodium excretion and blood pressure within and across populations. BMJ 1996;312:1249-53.*
4. *Khaw KT, Bingham S, Welch A, Luben R, O'Brien E, Wareham N, Day N. Blood pressure and urinary sodium in men and women. The Norfolk cohort of the European prospective investigation into cancer (EPIC-Norfolk). Am J Clin Nutr 2004;80(5):1397-1403.*
5. *Nagata C, Takatsuka N, Shimizu N, Shimizu H. Sodium intake and risk of death from stroke in Japanese men and women. Stroke 2004;35:1543-7*
6. *Umesawa M, Iso H, Date C, Yamamoto A, Toyoshima H, Watanabe Y, Kikuchi S, Koizumi A, Kondo T, Inaba Y, Tanabe T, Tamakoshi A, and JACC study group. Relationship between dietary sodium and potassium intakes and mortality from cardiovascular diseases: the Japan collaborative cohort study for evaluation cancer risks. Am J Clin Nutr 2008;88(1)195-202.*
7. *Tuomilehto J, Jousilahti P, Rastenyte D, Moltchanov V, Tanskanen A, Pietinen P et al. Urinary sodium excretion and cardiovascular mortality in Finland: A prospective study. Lancet 2000 Mar 17; 357(9259):848-51.*
8. *Alderman MH, Cohen H, Madhavan S. Dietary sodium intake and mortality: the national health and nutrition examination survey (NHANES II). Lancet 1998;351(9105):781-5.*
9. *Johnson RJ, Herrera-Acosta, Schreiner FG, Rodriguez-Iturbe B. Subtle Acquired Renal Injury as a mechanism of salt sensitive hypertension. NEJM 2002;346:913-23.*
10. *Verhave JC, Hillege HL, Burgerhof JGM, Janssen WMT, Gansevoort RT, Navis G et al. Sodium intake affects urinary albumin excretion especially in overweight subjects. J Int Med 2004; 256:324-30.*

11. Tsugane S. Salt, salted food intake and risk of gastric cancer: epidemiological evidence. *Cancer Sci* 2005;96:1-6.
12. Nordin CEB, Need AG, Steurer T, Morris HA, Chatterton BE, Horowitz M. Nutrition, Osteoporosis and Aging. *Ann NY Acad Sci* 1998;854:336-51.
13. Heaney RP. Role of dietary sodium in osteoporosis. *J Am College Nutr*. 2006;25 (90003):271S-6S.
14. Forte JG, Miguel MJ, de Padua F, Rose G. Salt and blood pressure: a community trial. *J Hum Hypertens* 1989;3:179-84.
15. Takahashi Y, Sasaki S, Okubo S, Hayashi M, Tsugane S. Blood pressure change in a free living population-based dietary modification study in Japan. *J Hypertens* 2006;24:451-8.
16. Karppanen H, Mervaala E. Sodium intake and hypertension. *Prog Cardiovasc Dis* 2006;49:59-75.
17. Joffres MR, Campbell NR, Manns B, Tu K. Estimate of the benefits of a population-based reduction in dietary sodium additives on hypertension and its related health care costs in Canada. *Can J Cardiol* 2007;23(6):437-43.
18. Selmer R, Kristiansen IS, Haglerod A, Graf-Iversien S, Larsen H, Meyer HE et al. Cost and health consequences of reducing population intake of salt. *J Epidemiol Commun Health* 2000;54:657-702.
19. Asaria P, Chisholm D, Mathers C, Ezzeti M, Beaglehole R. Chronic disease prevention: health effects and financial cost strategies to reduce salt intake and control tobacco use. *Lancet* 2007 Dec 15; 70(9604):2044-53.
20. Murray CJ, Lauer JA, Hutubessy RC, Nissen L, Tomijima N, Rodgers A, Lawes CM, Evans DB. Effectiveness and costs of interventions to lower systolic blood pressure and cholesterol: a global and regional analysis on reduction of cardiovascular disease risk. *Lancet* 2003 Mar 1; 361(9359):717-25.
21. He FJ, Marrero NM, Mc Gregor GA. Salt and blood pressure in children and adolescents. *J Hum Hypertens* 2008 Jan; 22(1):1-8.
22. Simons-Morton DG, Obrazanek E. Diet and blood pressure in children and adolescents. *Pediatr Nephrol* 1997 Apr; 11:244-9.
23. Soudarssanane MB, Karthigean S, Stephen S, Sahai A. Key predictors of high blood pressure and hypertension among adolescents: a simple prescription for prevention. *Ind J Community Med* 2006;31(3):164-9.
24. Nelson MJ, Regland DR, Syme SL. Longitudinal prediction of adult blood pressure from juvenile blood pressure levels. *Am J Epidemiol* 1992;136:633-45.
25. Vandevijvere S, van Oyen H. Sodium intake in the Belgian population. Research limitations and policy implications. *Arch Pub H* 2008;66:187-95.
26. Beer-Bost S, Constanza MC, Pechere-Bertschi A, Morabia A. Twelve years trends and correlates of dietary salt intakes for general population of Geneva (Switzerland). *Eur J Clin Nutr* 2009;63(2):155-64.
27. Thomson B. Nutrition modelling: distribution of salt intake from processed food in New Zealand. *Brit J Nutr* 2009 Mar doi:10.1017/S000711450928901X Published online by Cambridge University Press, January 2009.
28. Ministry of Health of Republic Serbia. Burden of diseases and injuries in Serbia, Belgrade, 2003. Ministry of Health of Republic of Serbia: National Health Survey Serbia 2006. Key findings. Belgrade (Serbia); 2007.
29. Ministry of Health of Republic Serbia. National Health Survey: Serbia, 2006, Ministry of Health of Republic of Serbia, Belgrade (Serbia); 2007.
30. Novaković B, Božić D. Diabetes, Obesity and Hypertension in Vojvodina. Novi Sad (Serbia): School of Medicine University of Novi Sad; 2004. (Monograph; Vol 62)
31. World Health Organization. Reducing salt intake in populations. Report of a WHO Forum and Technical Meeting. Geneva (Switzerland): World Health Organisation; 2007.
32. Trajković-Pavlović Lj, Martinov-Cvejin M, Novaković B, Bijelović S, Torović Lj. Analysis of salt content in meals in kindergarten facilities in Novi Sad. *Srpski arhiv, in pressum*.
33. Scientific Advisory Committee on Nutrition, Standards Agency and Department of Health, Her Majesty's Sanitary Office: Salt and Health, Copyright Unit, Norwich (United Kingdom); 2003.

34. He FJ, MacGregor GA. A comprehensive review on salt and health and current experience of worldwide salt reduction programmes. *J Hum Hypertens* 2008;1-22.
35. Lawes CMM, Vander Hoorn S, Rodgers A. Global burden of blood pressure-related diseases, 2001. *Lancet* 2008;371(9623):1513-8.
36. World Health Organization. Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation. WHO, Technical Report Series No 916, Geneva (Switzerland); 2003.
37. World Health Organization. WHO European action plan for food and nutrition policy, WHO Regional Office for Europe, Copenhagen (Denmark); 2008.
38. Zhu H, Yan W, Ge D, Treiber FA, Harshfield GA, Kapuku G, Sneider H, Dong Y. Relationship of cardiovascular phenotypes with healthy weight, at risk overweight and overweight in US Youths. *Pediatrics* 2008;121(1):115-22.
39. Wilson DK, Bayer L, Sica DA. Variability in salt sensitivity classification in black male versus female adolescents. *Hypertension* 1996;28:250-5
40. Miyajima E, Yamada Y. Reduced sympathetic inhibition in salt sensitive Japanese young adults. *Am J Hypertens* 1999;12(12):1195-200.
41. Weinberger MH, Miller JZ, Luft FC, Grim CE, Finberg NS. Definitions and characteristics of sodium sensitivity and blood pressure resistance. *Hypertension* 1986;8(Suppl II):II-127-34.
42. Weinberger MH, Finberger N, Finberger E, Weinberger M. Salt sensitivity, pulse pressure and death in normal and hypertensive humans. *Hypertension* 2001;37:429-32.
43. Franco V, Oparil S. Salt sensitivity and treatment of blood pressure, cardiovascular disease and survival. *J Am Coll Nutr* 2006;25(3):247S-55S.
44. De la Sierra A, Giner V, Brauglat E, Coca A. Lack of correlation between two methods for the assessment of salt sensitivity in essential hypertension. *J Hypertens* 2002;16(4):355-60.
45. World Cancer Research Fund and American Institute for Cancer Research. Second Expert Report. Food, Nutrition, Physical Activity and Prevention of Cancer. A Global Perspective. American Institute for Cancer Research Washington DC; 2007.
46. Food and Agriculture Organization. Food and Nutrition Technical Report. Human energy requirements. Report of Joint FAO/WHO/UNU Expert Consultation. Rome; 2004.
47. Kaplan NM. Lifestyle modification for and prevention of hypertension. *J Clin Hypertens* 2004;6(7):716-9.
48. Sack F, Svetkey L, Vollmer W, Appel LJ, Bray GA, Harskha D, Obrazek E, Conlin PR, Miller ER, Simons-Morton DG, Karanja N, Lin PH. Effects on blood pressure of reduced sodium and dietary approach to stop hypertension (DASH) diet. *NEJM* 2001;344(1):3-10.
49. Reinivuo H, Valsta LM, Laatikainen T, Thumilehto J, Pietinen P. Sodium in the Finnish diet: II Trends in dietary sodium intake and comparison between intake and 24-h excretion of sodium. *EJCN* 2006;6:1160-7.

Corresponding author:

Ljiljana Trajkovic-Pavlovic,  
Institute of Public Health of Vojvodina  
Republic of Serbia  
E-mail: ljtp@eunet.rs

# Performance of regional tuberculosis dispensaries in a province of Turkey in pre-DOTS era (1989-2002)

Sefa Levent Ozsahin, Sulhattin Arslan, Omer Tamer Dogan, Serdar Berk, İbrahim Akkurt

Department of Chest Disease, Faculty of Medical, Cumhuriyet University SIVAS, Turkey

## Abstract

**Background:** In recent years, very few dispensary staff has been striving to apply directly observed therapy (DOT) elsewhere in Turkey, but the overwhelming majority of TB dispensaries still continue their usual practice today. Therefore, practice of any regional TB dispensary may mirror Turkey's Tuberculosis Programme practice profile.

**Method:** Statistical Year Books of Sivas Province Health Department (from 1989 to 2002) are the main sources of data. The mid-year population numbers obtained from Statistical Year Books of Sivas Province Health Department were preferred for calculating annual incidences. Pearson's correlation coefficients and, if data ordinal, Spearman's correlation coefficients were used to determine correlations.

**Results:** There are three TB dispensaries in the Sivas Province. Total staff number at Sivas TB dispensaries decreased within years ( $r = -0.92$ ,  $p < 0.001$ ). TB incidence declined within years, also ( $r = -0.87$ ,  $p < 0.0001$ ). Chest X-ray examinations gradually decreased within years from 20,416 examinations in 1989 to 5856 examinations in 2002 ( $r = -0.75$ ,  $p = 0.002$ ). Annual microscopic examinations for acid-fast bacilli (AFB) declined within years with 1400 microscopy done in 1989 decreased to 445 examinations in 2002 ( $r = -0.62$ ,  $p = 0.018$ ). Per each TB patient, the annual mean microscopy for AFB examination was  $4.49 \pm 1.47$  and the mean chest radiography was  $8.25 \pm 3.27$  at local dispensaries in this fourteen-year period. The annual tuberculin skin test (TST) and Bacillus Calmette-Guerin (BCG) vaccinations per 100,000 population performed by local dispensaries did

not statistically different within years ( $r = -0.45$ ,  $p = 0.106$  and,  $r = -0.15$ ,  $p = 0.605$ , respectively).

**Conclusion:** TB incidence declined within years, but this situation seems to be not related with improving of dispensaries performance.

**Key words:** tuberculosis, dispensary, pre-DOTS era, application, performance

## Introduction

The global burden of tuberculosis is still enormous. World Health Organization (WHO) Report 2006 on Global Tuberculosis Control pointed out that there were 8.9 million new cases of tuberculosis (TB) in 2004 (140/100 000 population), of which 3.9 million (62/100 000 population) were smear-positive. A total of 183 countries and territories implemented the Directly Observed Therapy Strategy (DOTS) during 2004. By the end of 2004, 83% of the world's population lived in countries, or parts of countries covered by DOTS.<sup>1</sup>

Mainly funded by international organisations, National Tuberculosis Programmes (NTPS) of high-burden countries were relatively successful in implementing DOTS, in case detection and treatment success rates.<sup>1</sup>

Turkey, a European region country with approximately 70 million populations, with a widely distributed tuberculosis dispensary network,<sup>2</sup> is lacked implementing DOTS in nation-wide yet. National Tuberculosis Programme (NTP) of Turkey reported to WHO that DOTS coverage was 2% in 2003, and 3% in 2004. There were 19 799 TB notified cases in Turkey in 2004, and 616 of them were under the supervision of DOTS.<sup>1</sup>



Tuberculosis dispensaries, first introduced in the 1920s, are peripheral units of NTP of Turkey. Although TB dispensaries are politically directed by TB Office of the Ministry of Health, their staff have been assigned to and supervised by Province Health Departments at regional levels. This dual management situation may cause lack of supervision of TB dispensaries applications and policies. In recent years, very few dispensary staff has been striving to apply directly observed therapy (DOT) elsewhere in Turkey, but the overwhelming majority of TB dispensaries still continue their usual practice today. Therefore, practice of any regional TB dispensary may mirror Turkey's NTP practice profile.

On the other hand, a DOTS programme was implemented by our Province Health Department Communicable Diseases Section in the late 2003, we will be able to compare DOTS performance with the pre-dots era applications in a few years later.

### Materials and methods

Statistical Year Books of Sivas Province Health Department (from 1989 to 2002) are the main sources of data. All the available data related to TB dispensary applications have to be recorded into these year books. However, there were only the number of annual TB cases recorded; neither affected organs, nor patient gender or age were reported. In these written sources, the term "follow-up without medication" was used instead of the terms "cure" or "treatment completed" to mean that patient treatment was completed under the supervision of the dispensary.

As censuses in Turkey are applied every 5 years, and there has been a slight tendency of immigration in the region, the mid-year population numbers obtained from Statistical Year Books of Sivas Province Health Department were preferred for calculating annual incidences.

### Statistics

Data were entered into an Excel Spreadsheet. SPSS (version 12.0) was used for statistical analyses. Data are expressed as mean  $\pm$  SD. Pearson's

correlation coefficients and, if data ordinal, Spearman's correlation coefficients were used to determine correlations. All p values are two sided, with  $p < 0.05$  considered statistically significant.

### Results

There are three TB dispensaries in Sivas province (~ 650 000 populations). Total staff number at Sivas TB dispensaries decreased within years. Province-wide health sector staff was 1645 persons in 1989, and gradually increased to 2530 persons in 2002. In contrast, dispensaries had 50 staff in 1989, and gradually declined to 37 persons in Sivas TB dispensaries in 2002 ( $r = -0.923$ ,  $p < 0.001$ ). The number of the doctors working at TB dispensaries varied within years, with a minimum of three doctors in 1994 and, a maximum of ten doctors in 1996. During that time, dispensaries doctor/province-wide doctor ratios gradually declined within years ( $r = -0.73$ ,  $p = 0.003$ ). The annual mean population per each TB dispensary doctor was  $99139 \pm 36123$  in these 14 study years.

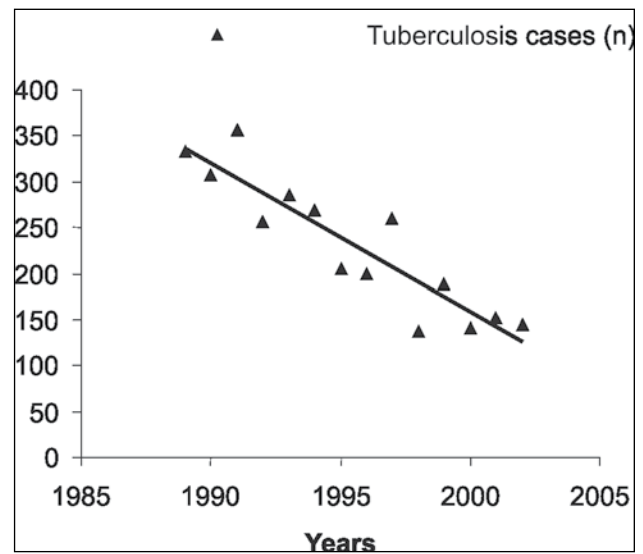


Figure 1. Tuberculosis incidence per 100 000 population by dispensaries registries in Sivas Province between 1989 and 2002 ( $r = -0.868$ ,  $p < 0.001$ ).

Totally 3235 TB cases were registered at local dispensaries between 1989 and 2002, with a maximum of 357 cases in 1991 and a minimum of 137 cases in 1998 (Table 1). Both total TB cases and



TB incidences (per 100 000 population) as shown in Figure 1 declined within years ( $r = -0.881$ ,  $p < 0.0001$ , and  $r = -0.868$ ,  $p < 0.0001$ , respectively). There was a mean annual TB incidence of 35.5/100 000, with an annual reduction of 1.9%. Figure 1 indicates the changes in patient number registered in dispensaries within years. According to TB dispensaries registries, the TB incidence per 100 000 population decreased from 47.7 to 23.3 in the years 1989 and 2002 respectively (Table 1). *Table 1. Annual numbers, and, annual incidences (per 100 000 population) of tuberculosis cases and annual treatment completed cases registered at local dispensaries of Sivas Province in Turkey between 1989 and 2002.*

| Year | Tuberculosis cases (n) | Incidence | Treatment completed (n) |
|------|------------------------|-----------|-------------------------|
| 1989 | 332                    | 44.7      | 476                     |
| 1990 | 307                    | 44.4      | 475                     |
| 1991 | 357                    | 52.6      | 379                     |
| 1992 | 255                    | 38.4      | 324                     |
| 1993 | 285                    | 43.3      | 218                     |
| 1994 | 269                    | 41.9      | 218                     |
| 1995 | 205                    | 32.2      | 135                     |
| 1996 | 200                    | 31.3      | 198                     |
| 1997 | 260                    | 42.1      | 260                     |
| 1998 | 137                    | 22.2      | 177                     |
| 1999 | 189                    | 30.8      | 196                     |
| 2000 | 141                    | 22.8      | 155                     |
| 2001 | 152                    | 24.5      | 137                     |
| 2002 | 145                    | 23.3      | 197                     |

The annual TB incidence in our region correlates with Turkey's national incidence (data not shown) between 1989 and 2002 ( $r = 0.816$ ,  $p < 0.001$ ).

The highest number of patients examined at Sivas TB dispensaries ( $n = 26\ 537$ ) was in 1991 while 2002 was the year with the least number of patients ( $n = 7\ 304$ ) with a mean of  $9,766 \pm 8\ 451$  for the 14 years period. The 14-year average of the annual patient number examined per 100 000 population is  $2\ 237 \pm 688$  with a minimum of 1 174 and a maximum of 3 913. Naturally, there is a highly positive correlation between the number of the patients admitted to dispensaries per 100 000 population and those with TB notification per 100 000 population ( $r = 0.737$ ,  $p = 0.003$ ).

Annual microscopic examinations for acid-fast bacilli (AFB) declined within years with 1 400 microscopy done in 1989 decreased to 445 examinations in 2002. Annual microscopic AFB examination numbers per 100 000 population at local tuberculosis dispensaries between 1989 and 2002 are shown in Figure 2.

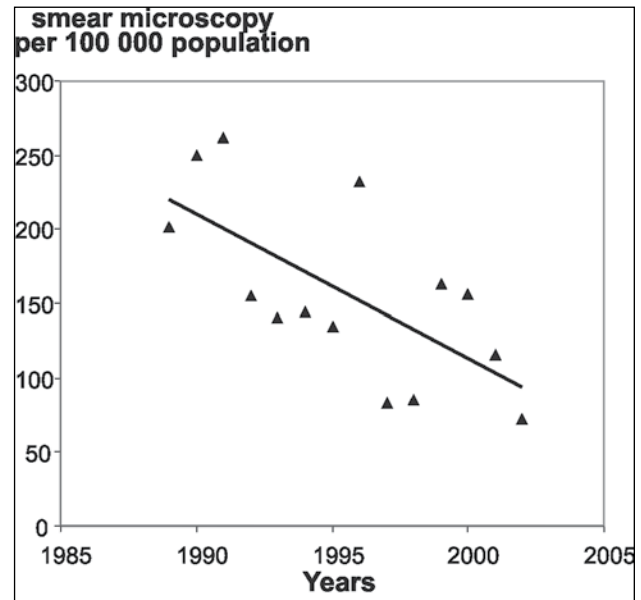


Figure 2. Annual microscopic examination per 100 000 population at Sivas dispensaries between 1989 and 2002 ( $r = -0.622$ ,  $p = 0.018$ ).

Annual chest X-ray examinations decreased within years from 20 416 examinations in 1989 to 5 856 examinations in 2002. Chest X-ray examinations per 100 000 population at TB dispensaries as shown in figure 3, gradually decreased within time ( $r = -0.754$ ,  $p = 0.002$ ).

Per each TB patient, the annual mean microscopy for AFB examination was  $4.49 \pm 1.47$  and the mean chest radiography was  $8.25 \pm 3.27$  at local dispensaries of Sivas in this fourteen-year period. In 1997, for example, only two microscopic examinations for AFB and, only three chest radiographies had been performed per each TB patient.

The mean chest radiography per AFB microscopy at Sivas TB dispensaries in these fourteen years was  $12.6 \pm 4.6$  and it did not change within years ( $r = -0.147$ ,  $p = 0.615$ ). People who admitted to TB dispensaries were examined by chest radiography rather than AFB microscopy.

The annual microscopy for AFB per person examined at dispensaries during these 14 years is

$0.07 \pm 0.022$  while the mean chest radiography per person examined is  $0.82 \pm 0.24$ . The number of both microscopy ( $r = -0.132$ ,  $p = 0.654$ ) and chest radiography ( $r = 0.216$ ,  $p = 0.459$ ) per person examined at dispensaries has not changed within this 14 year-period as well.

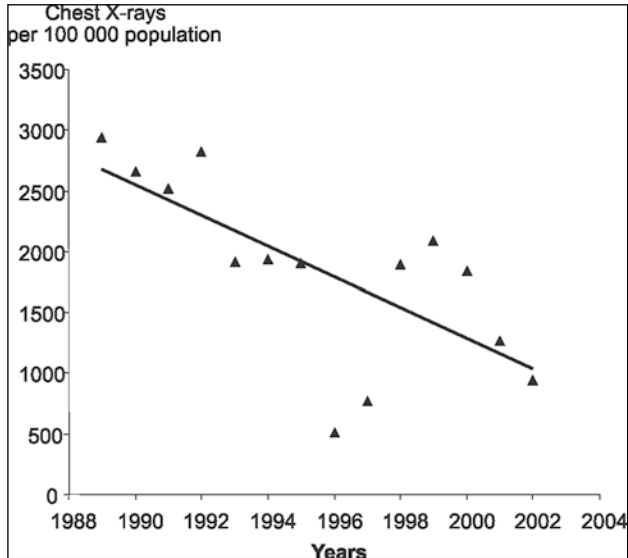


Figure 3. Annual chest radiographic examinations per 100 000 population in Sivas dispensaries between 1989 and 2002 ( $r = -0.754$ ,  $p = 0.002$ ).

Employed doctor and total staff number correlations with dispensaries' applications in these 14 years were also evaluated. There were no significant correlations with doctor numbers and tuberculosis incidence, chemoprophylaxis, chest radiography and microscopy for AFB, or BCG vaccinations (data not shown). On the other hand, as total number of dispensaries personnel increased, chest radiographic examinations and microscopy for AFB numbers increased as well ( $r = 0.605$ ,  $p = 0.022$ , and  $r = 0.640$ ,  $p = 0.014$ , respectively).

The annual tuberculin skin test (TST) and Bacille Calmette-Guerin (BCG) vaccinations per 100 000 population performed by local dispensaries did not statistically differ within years ( $r = -0.451$ ,  $p = 0.106$  and,  $p = -0.152$ ,  $p = 0.605$ , respectively). The annual mean TST per 100 000 population was  $729 \pm 372$  and the mean annual BCG vaccination per 100 000 population was  $708 \pm 330$  in these fourteen years.

Annual contact tracing numbers throughout Sivas started to be mentioned in Statistical Year Books in 1995. According to the statistical infor-

mation gathered from these books, mean contact tracing per patient was  $3 \pm 0.54$  between 1995 and 2002. There was not any significant change in the number of annual contact tracing per patient registered from 1995 to 2002 ( $r = 0.218$ ,  $p = 0.604$ ). The average size of households is 5.0 in Sivas during this period.

Isoniazid chemoprophylaxis administered to 464 persons with latent tuberculosis infection (LTBI) throughout Sivas in 1989; however, only 64 persons with LTBI were commenced prophylaxis in the year 2002. Chemoprophylaxis administered to 1.34 patients per each registered TB patient in 1989 gradually declined, and in 2002, there were only 0.44 patient who commenced chemoprophylaxis ( $r = -0.603$ ,  $p = 0.022$ ). The contacts of the patients given chemoprophylaxis for LTBI per 100 000 population in the region between 1989 and 2002 are shown in Figure 4.

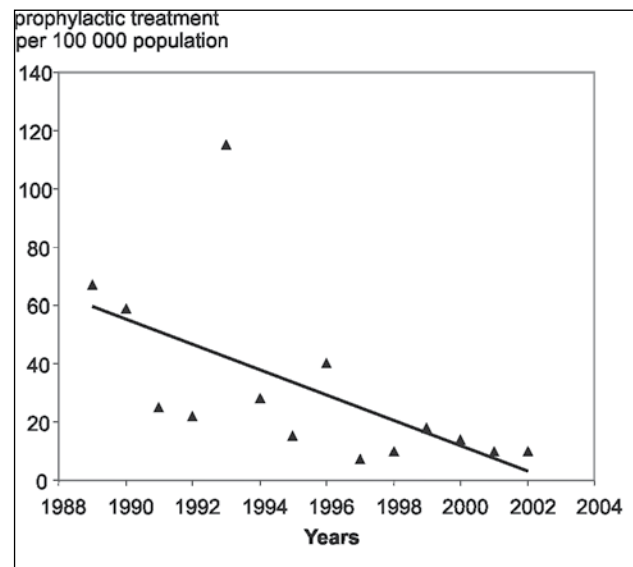


Figure 4. Chemoprophylaxis with isoniazid per 100 000 population in Sivas Province between 1989 and 2002 ( $r = -0.770$ ,  $p = 0.001$ ).

The annual TB incidence does not correlate with annually examined patient number ( $r = -0.501$ ,  $p = 0.68$ ), with microscopic evaluation for AFB ( $r = -0.208$ ,  $p = 0.476$ ) and chest radiography evaluation ( $r = -0.075$ ,  $p = 0.186$ ).

## Discussion

The 2003 WHO Report on Global TB Control identified primary constraints preventing WHO targets for TB control from being achieved, of which lack of qualified staff was the main constraint.<sup>3</sup> Staff of dispensaries of our region has decreased within years, from 50 people in 1989 to 37 people in 2002. Because staff have been chosen for dispensaries by Province Health Department (not by NTP officers), qualification of these personnel was not a subject of priority. In addition, there have no study about how many staff required for the central or peripheral units of NTP of Turkey.

This study clearly shows that TB incidence declined from 47.7 to 23.3 per 100.000 populations per year between 1989 and 2002, and this decline in the incidence significantly correlates with Turkey's national incidence data<sup>1</sup> for the same years. This mean annual reduction of 1.9% in TB incidences seems not to be related to the improvement of performance of TB dispensaries. While the number of the people applying to dispensaries decreased within years, annual chest radiographic and AFB microscopic evaluations declined as well. These findings suggest that the gradual decrease in official recordings of the patients with TB at local dispensaries' registry does not associate with any improvement in these institutions' practice. This situation may be associated with the improvement in the living standards of the country<sup>4</sup> or with lack of notification of patients who diagnosed other institutions or both. Ozsahin et al. reported that up to 50 % of the patients with TB were not notified to local dispensaries by the diagnosing hospitals in our region.<sup>5</sup> This may be a causal factor for the declining incidence of TB.

By the year 2004, tuberculosis control services were provided by 244 dispensaries which belong to the Ministry of Health, 31 dispensaries of which settled by voluntary organizations, 22 Chest Diseases Hospitals and 11 mobile tuberculosis groups in Turkey. These units examine approximately 2.5 million people each year and provide health care to 18 000 TB patients.<sup>2</sup> Although some idealist doctors try to implement DOT in their regions,<sup>6,7</sup> the overwhelming majority of the dispensaries all over the country have similar performance as

in our regional dispensaries. Therefore, we think that results obtained from our regional dispensaries' practices may reflect TB dispensary system of Turkey as a whole.

The first technical element of the WHO TB control strategy (DOTS) is case detection among symptomatic patients' self-reporting to health services using sputum smear microscopy. As inexpensive and simple to perform, smear microscopy remains the cornerstone for the diagnosis of pulmonary TB.<sup>8</sup> Many low-income countries TB units, with a passive case finding system, mostly without doctors, perform at least three sputum smear microscopy for acid-fast bacilli.<sup>9,10</sup> However, per each registered TB patient, the annual mean microscopy for AFB examination was less than five examinations in our region's dispensaries. Thus, approximately 10 000 persons admitted annually seem to be examined without microscopic evaluation. Our results suggest that smear microscopy was not a main diagnostic tool in our dispensaries, and this situation seems to get worse within years.

Patients with sputum smear-positive pulmonary TB should be monitored by sputum smear examination. These are the patients for whom bacteriological monitoring is possible. It is unnecessary, unreliable and waste of resources to monitor patients by chest radiography. For patients with sputum smear-negative pulmonary TB or extrapulmonary TB, clinical monitoring is the usual way of assessing the response to treatment. For smear-positive pulmonary patients, sputum smears should be performed at the end of the second month, during the fifth month and in the last month of the 6-month and 8-month treatment regimens.<sup>11</sup> It seems that, at dispensaries of Sivas, the patient follow-up examination has been performed through chest radiography rather than microscopy. If only the previously diagnosed patients had been examined through microscopy, it seems that microscopy had been performed for almost none of the newly admitted patients. The numbers of the radiography per registered patient also make us think that the patients have not been controlled through chest radiography.

One of the main results of our study shows that there is no change in annual TST and BCG vaccinations within years, in addition to contact tracing

and isoniazid chemoprophylaxis numbers were gradually declined from 1989 to 2002.

All these results mentioned above suggest that the practices at Sivas TB dispensaries seem not to be in accordance with the world-wide policy of WHO at least until the year 2002.

Unfortunately, some of the vital source of data, such as registered cases' ages, genders, and affiliated organs (pulmonary or extra-pulmonary) were not included in Statistical Year Books of Sivas Province Health Department. Therefore, we could not analyze and discuss such issues, and we think that this is the main limitation of this present study.

Our results suggest that TB dispensaries of our region's performances were not sufficient and getting worse within years in pre-DOTS era. On the other hand, a DOTS programme was implemented by our Province Health Department Communicable Diseases Section in the late 2003, we will able to compare DOTS performance with the pre-dots era applications in a few years later.

## References

1. World Health Organization. *Global tuberculosis Control: surveillance, planning, financing. WHO Report 2006. WHO/HTM/TB/2006.362. Geneva, Switzerland: WHO, 2006.*
2. Turkey's Statistical Yearbook, 2004. Health. [http://www.die.gov.tr/yillik/05\\_Saglik.pdf](http://www.die.gov.tr/yillik/05_Saglik.pdf) (Accessed December 2005)
3. World Health Organization. *WHO Report 2003. Global Tuberculosis Control, Surveillance, Planning, Financing. Communicable Diseases. WHO/CDS/TB/2003.316. Geneva, Switzerland: WHO, 2003.*
4. Turkish Statistical Institute. *Statistical Indicators 1923-2004. http://www.die.gov.tr/english/kiting\_1.pdf* (Accessed May 2006).
5. Ozsahin SL, Akkurt I, Ozdemir L, Dogan OT, Atalan A, Kocoglu F. *Are official reports sufficient? Official registration rates of patients with tuberculosis in Sivas, Middle Anatolia. Public Health 2006; 120(5): 434-440.*
6. Arpaz S, Keskin S, Sezgin N, Budin O. *Results of directly observed treatment applications for three years. (in Turkish) Toraks Dergisi 2005; 6(3):228-234.*
7. Ozkara S, Arpaz S, Ozkan S, Aktas Z, Orsel O, Ecevit H. *Directly observed Therapy (in Turkish). http://www.verem.org.tr/pdf/dgt1.pdf* (Accessed May 2006)
8. Tuberculosis Division, International Union Against Tuberculosis and Lung Disease, Paris, France. *Tuberculosis bacteriology-priorities and indications in high prevalence countries: position of the technical staff of the Tuberculosis Division of the International Union Against Tuberculosis and Lung Disease. Int J Tuberc Lung Dis 2005; 9(4): 355-361.*
9. Apers L, Wijarajah C, Mutsvangwa J, Chigara N, Mason P, van der Stuyft P. *Accuracy of routine diagnosis of pulmonary tuberculosis in an area of high HIV prevalence. Int J Tuberc Lung Dis 2004; 8(8): 945-951.*
10. Salim MAH, Declercq E, Van Deun A, Saki KAR. *Gender differences in tuberculosis: a prevalence survey done in Bangladesh. Int J Tuberc Lung Dis 2004; 8(8): 952-957.*
11. World Health Organization. *Treatment of tuberculosis. Guidelines for national programmes. 3rd ed. Geneva, Switzerland: WHO, 2003.*
12. World Health Organization. *Global Tuberculosis Control. WHO report 2005. WHO/HTM/TB/2005.349. Geneva, Switzerland: WHO, 2005.*

### Corresponding author:

Sulhattin ARSLAN  
Department of Chest diseases  
Faculty of Medicine, Cumhuriyet University  
Turkey  
e-mail:sulhattinaslan@mynet.com



# Effects of Plateau Environment on Social Interaction

Qun Yang<sup>1</sup>, Rui'an Wang<sup>2</sup>, Baoxin Shi<sup>3</sup>, Wenbin Zhang<sup>1</sup>, Tao Ke<sup>1</sup>, Yebing Yang<sup>1</sup>, Xiang jun Chen<sup>1</sup>, Baohua Cao<sup>1</sup>, Xiwen Liu<sup>1</sup>, Yinling Zhang<sup>1</sup>, Chunping Ni<sup>1</sup>, Xina Yuan<sup>1</sup>, Kaiyuan Li<sup>1</sup>, Yulong Ma<sup>1</sup>, Jingyuan Chen<sup>1</sup>, Wenjing Luo<sup>1</sup>

<sup>1</sup> Fourth Military Medical University, China

<sup>2</sup> Chang'an International Hospital, China

<sup>3</sup> Tianjin Medical University, China

## Abstract

**Objective:** The paper explored the change of social interaction of young people exposed in plateau environment.

**Methods:** 214 young people living in Tibet Plateau for more than one year (plateau group) and 190 young people living in inland (Inland group) completed the survey. Social avoidance and distress were used as the indexes of social interaction, with perceived social support, interpersonal trust and self-esteem as influencing factors.

**Results:** Plateau group had higher scores on social avoidance, distress, interpersonal trust and self-esteem, and lower scores on perceived social support. In Plateau group, both perceived social support and self-esteem had significant negative *betas* on social avoidance, and perceived social support, interpersonal trust and self-esteem all had significant negative *betas* on distress. In Inland group, only self-esteem had significant *beta* on distress.

**Conclusion:** Effects of Plateau environment on social interaction and people's mental health were discussed.

**Key Words:** plateau environment; self-esteem; perceived social support; social avoidance and distress; sociometer theory (SMT)

## Introduction

Sitting at the center of the Tibet Plateau (91°06E and 29°36N) of 3,650 meters (1,1975 feet) elevation with 29 and 16.5 Celsius of the yearly highest and lowest temperature respectively (7.4 yearly average temperature), the high-altitude Lhasa city

has low atmospheric pressure and showers over 3,000 hours every year distinguishing it as a "sun-lit city" with intensified sun radiation and dry air. Moreover, the remote and outlying geographic location makes the city a sparsely populated place (about 400,000 inhabitants) with inconvenient communications. These factors can infect people both physically and psychologically.

On the other hand, People have a fundamental need to belong that motivates them to seek out social interactions with close others (Baumeister R. & Leary M., 1995). And interpersonal ties with other people serve various important functions. They provide emotional, instrumental, and informational support and can be a source of satisfaction and fulfillment (Denissen J., et al., 2008). Especially under harsh conditions, where communication is not so convenient, social interactions must adapt to the environment.

The paper investigated the social interactions (using social avoidance and distress as indexes) and related factors, including perceived social support, self-esteem, interpersonal trust, of young people living on the Plateau (Lhasa of Tibet, Qinghai-Tibet Plateau) for more than one year and young ones living in inland. The aim was through studying the differences between the two groups and the relations between these factors and social avoidance and distress to find out the potential influences of the Plateau environment on young people's social interactions.



## 2. Methods

### 2.1. Sample and procedure

The study sample consisted of the plateau group including 214 people living and working in Lhasa, the capital of Tibet Autonomous Region for more than one year and the inland group with 190 people living in inland areas (9 provinces or cities around China). The two groups were all male people ranging from 16 to 23 years old (mean age 18.85 years, SD 1.02) for the Plateau group and from 16 to 22 years old (mean age 18.16 years, SD 1.32) for the inland group.

After excluding subjects who didn't complete the questionnaires as required, the study sample was made up of 211 people (mean age 18.83 years, SD 1.01) for the Plateau group from 9 provinces or cities before they were in Lhasa, with 20.1% from Sichuan, 6.1% from Heilongjiang, 12.2% from Chongqing, 8.2% from Guizhou, 10.2% from Hunan, 8.2% from Fujian, 15.6% from Zhejiang, 11.2% from Shaanxi, 8.2% from Yunnan, and 184 people (mean age 18.03 years, SD 1.53) for the inland group, with 22.3% from Sichuan, 10.3% from Jilin, 20.2% from Hunan, 15.1% from Fujian, 11.4% from Zhejiang, 11.2% from Shaanxi, 9.5% from Kunming. Other demographic data had been listed in table 1.

Table 1. The demographic data of the plateau group and the inland group

| Variable                  | Plateau group<br>(n=211)             | Inland group<br>(n=184)              |
|---------------------------|--------------------------------------|--------------------------------------|
| age(mean±SD)              | 18.85±1.02<br>(ranged from 16 to 23) | 18.03±1.53<br>(ranged from 16 to 22) |
| married                   | 0                                    | 0                                    |
| be in love                | 17.54 % (37)                         | 26.63 % (49)                         |
| coming from rural areas   | 55.45 % (117)                        | 78.80 % (145)                        |
| be in a one-parent family | 6.16 % (13)                          | 11.96 % (22)                         |
| be in a one-child family  | 28.10 % (59)                         | 32.61 % (60)                         |

Questionnaires were sent by post, and completed under the guidance of trained researchers or local research assistants. At first, the aim was intro-

duced to the subjects, and letters of consent were signed by every subject. Then they were directed to complete the set of questionnaires anonymously. And their information was numerically coded to hide their identities.

### 2.2. Measurements

The set of questionnaires included the Perceived Social Support (PSSS), the Rosenberg Self-esteem Scale (RSES), Interpersonal Trust Scale (ITS), and the Social Avoidance and Distress Scale (SADS).

#### 2.2.1. The Rosenberg Self-esteem Scale (RSES)

Self-esteem was assessed by the Rosenberg Self-esteem scale RSES (Rosenberg M., 1965), of which 10 items were used to evaluate one's worthiness as a human. The RSES was designed for a 4-point scale from 1 (*strongly disagree*) to 4 (*strongly agree*), with the sum score ranging from 10 to 40, and the higher of the score meant the higher of self-esteem. Cronbach's alpha was 0.80 and 0.81 for Plateau group and Inland group in this study respectively.

#### 2.2.2. Interpersonal Trust Scale (ITS)

Interpersonal trust was measured using Interpersonal Trust Scale, developed by Rotter J. (1967), including 25 items, with 5-point scale from 1 (*completely disagree*) to 5 (*completely agree*). The sum of the scores ranged from 25 to 125 and the higher of the score meant the higher of self-esteem. It had been proven to be an effective tool to measure interpersonal trust. In prisoner's dilemma games, people low in trust were more suspicious of peers (Schlencker B., Helm B., & Tedeschi J., 1973) and were perceived as less trustworthy themselves (Rotter B., 1967) relative to people high in trust. Cronbach's alpha was 0.67 and 0.65 for Plateau group and Inland group in this study respectively.

### 2.2.3. Perceived Social Support Scale (PSSS)

The degree of perceived social support was assessed by the Perceived Social Support Scale (PSSS) developed by Zimet et al. (1988) which was devised as a 7-point scale (from very strongly disagree to very strongly agree). Having been validated and commonly used in Chinese populations (e.g. Chou K., 2000; Cheng S., Chan A., 2004; Yu D., Lee D., & Woo J., 2004), the PSSS was a 12-item measure of three aspects of social networks: family network, friends network and others network (Lubben J., 1988). Its score was obtained through adding all the scores of each item together. Cronbach's alpha was 0.87 and 0.91 for Plateau group and Inland group in this study respectively.

### 2.2.4. Social Avoidance and Distress Scale (SADS)

Social Avoidance and Distress Scale (SADS) was used in this study as indexes of social interactions. The scale was developed by Watson and Friend in 1969. It mainly measured the tendency to avoid social interactions, and the bad feelings (like anxiety) during social interactions. It had 28 items, with 2 factors (social avoidance and distress), using 5-point scale from 1 (Strongly disagree) to 5 (Strongly agree). Scores of each factor range from 0 to 70, and higher scores meant more obvious tendency to avoid social interactions, or more serious distress. Cronbach's alpha for social avoidance was 0.70 and 0.79 for Plateau group and Inland group in this study respectively. Cronbach's alpha for distress was 0.68 and 0.83 for Plateau group and Inland group in this study respectively.

### 2.3. Statistical analysis

Descriptive analyses were performed for each scale for both groups, and based on which a series of independent sample *t*-tests were conducted to find out the differences between the two groups. Then multiple regressions were conducted using social avoidance and distress as dependant variable respectively and other variables as independent variables for both groups. All analyses were performed with Statistical Package for the Social Sciences (SPSS, version 14.0).

## 3. Results

### 3.1. Descriptive analysis and independent sample *t*-tests

Variables were computed based on each scale, including Perceived social support, Self-esteem, Interpersonal trust, Social avoidance and Distress. Each variable was analyzed and compared between the two groups. The results were shown in table 2. From these results, we could see that significant differences were found for all the variables studied. The scores of self-esteem, interpersonal trust, social avoidance and distress were significantly higher for plateau group, while the scores of perceived social support were significantly lower.

### 3.2. Multiple regression analyses

In order to study the influence of plateau environment on social interaction, multiple regression analyses were conducted. Social avoidance and distress were used as dependent variables respectively, and other variables were used as independent variables. The results were shown in table 3. From the

Table 2. Descriptive analyses and independent

| Variables                | plateau group (n=211) | inland group (n=184) | t     | p       |
|--------------------------|-----------------------|----------------------|-------|---------|
| Self-esteem              | 19.98±4.34            | 19.09±4.23           | 2.08  | 0.038*  |
| Interpersonal trust      | 77.00±7.38            | 71.37±7.71           | 7.46  | 0.000** |
| Perceived social support | 58.15±11.17           | 62.89±11.71          | -4.03 | 0.000** |
| Social avoidance         | 37.63±6.16            | 33.20±6.21           | 7.13  | 0.000** |
| Distress                 | 38.40±6.01            | 35.72±6.81           | 4.17  | 0.000** |

\* $p < 0.05$ , \*\* $p < 0.01$

Table 3. Results of multiple regression analysis for two groups (beta)

|                          | Social avoidance |              | Distress      |              |
|--------------------------|------------------|--------------|---------------|--------------|
|                          | Plateau group    | Inland group | Plateau group | Inland group |
| Perceived social support | -0.181**         | -0.004       | -0.133*       | -0.004       |
| Interpersonal trust      | -0.107           | -0.134       | -0.184**      | -0.112       |
| Self-esteem              | -0.364**         | -0.120       | -0.405**      | -0.163*      |
| R square                 | 0.222            | 0.028        | 0.253         | 0.034        |

\* $p < 0.05$ , \*\* $p < 0.01$

results, we could see that in different environment, the determining factors for social interactions were different. In Plateau group, both perceived social support and self-esteem had significant negative *betas* on social avoidance, and all the three variables studied had significant negative *betas* on distress. While in Inland group, none of the three variables had significant *beta* on social avoidance, and only self-esteem had significant *beta* on distress. Compared with Inland group, we could know that social interactions on plateau may have different mode. In this environment, perceived social support, interpersonal trust and self-esteem may be more important than in inland for social interaction.

#### 4. Discussion

This study was designed to find out how Plateau environment would influence social interaction. From the descriptive analyses and comparisons of the two groups, we could find that Plateau group differed from Inland group in all the variables studied. Social interaction was more avoided and the distress to interact was more serious, with lower perceived social support for Plateau group. However, people's interpersonal trust and self-esteem were higher in Plateau group. These results showed that Plateau environment had changed social interaction to some extent. And lower perceived social support may be because the population density was relatively smaller, and they were farther away from their families and friends.

And the results of multiple regressions were very interesting. Based on previous studies, perceived social support, self-esteem and interpersonal trust should be important factors for social interaction. But in this study, in the Inland group, all these

three variables had non-significant coefficients on social avoidance, and only self-esteem had significant coefficient on distress. While in Plateau group, only interpersonal trust had non-significant coefficient on social avoidance, and all these variables had significant coefficients on distress. From these results, we could see that the Plateau environment not only had changed people's tendency to interact with others, but also had changed the mode of social interactions. These results can be explained in the view of social exchange theory. For the Inland group there may be much more reasons for social interaction, common interests for example, and perceived social support, interpersonal trust, self-esteem may account for little variation. But on Plateau, social interaction is not so convenient, and reasons for social interaction are much less. Under this condition, they may expect more from social interaction, and are more easily to be disappointed. This may also be a reason for higher social avoidance and distress for plateau group. As a result, psychological factors, including perceived social support, interpersonal trust and self-esteem, become more important for social interaction. On this point, psychological intervention should be much more useful to adjust social interactions for Plateau group than Inland group.

According to Leary and Baumeisters's (2000) sociometer theory (SMT), at low levels of social inclusion, "the sociometer evokes emotional distress as an alarm signal and motivates behaviors to gain, maintain, and restore relational appreciation". And self-esteem serves as the sociometer. This may be the reason of lower self-esteem motivated higher distress for people in Inland group. On the other hand, people with higher self-esteem should like to communicate with others more. But in this study, the Plateau group had higher self-esteem and

higher social avoidance, which seemed to be paradoxical. This may be the other side of SMT, when social interaction become tough for objective conditions (such as Plateau environment), sociometer (self-esteem) may upgrade itself so as not to alarm. However, this is just a hypothesis needing further empirical study to support.

On the other hand, social interaction is the basic need of human beings. Factors influencing social interaction are also important to human's health. Many studies (e.g. Cohen S. & Wills T., 1985; House J., Landis K. & Umberson D., 1988; Schwarzer R. & Leppin A., 1989) had confirmed that the availability of social support was associated with a reduced risk of mental and physical illnesses, even the reduced mortality. And self-esteem is an essential feature of mental health, positive self-esteem was supposed to be a protective factor in the field of health and social behavior (Mann, et al., 2004). From this perspective, the plateau environment had complicated influence on peoples' mental health, with lower social support and higher self-esteem. And this effect should be further oriented in the future.

This study investigated the condition and special mode of social interaction on plateau environment, and found interesting results. But there were obvious limitations in this study. Firstly, all the results were derived on the basis of questionnaires, and there was no other strategy, which may cause common-method variation. Secondly, the number of subjects was relatively small, which had prevented the use of more advanced methods to analyze the data. On the basis of these results, future studies should pay more attention to using appropriate ways to regulate social interaction for people working and living on plateau.

## References

1. Baumeister, R., & Leary, M. (1995). *The need to belong: Desire for interpersonal attachments as a fundamental human motivation. Psychological Bulletin, 117*, 497–529.
2. Cheng S. & Chan A. (2004). *The multidimensional scale of perceived social support: dimensionality and age and gender differences in adolescents. Journal of Personality Individual Difference, 37*, 1359–1369.
3. Chou K. (2000). *Assessing Chinese adolescents' social support: the multidimensional scale of perceived social support. Journal of Personality Individual Difference, 28*, 299–307.
4. Cohen S, Wills T. (1985). *Stress, social support and the buffering hypothesis. Psychological Bulletin, 98*, 310-357.
5. Denissen J., Penke L., Schmitt D., and Marcel A. (2008). *Self-Esteem Reactions to Social Interactions: Evidence for Sociometer Mechanisms Across Days, People, and Nations. Journal of Personality and Social Psychology, 95*, 181-196.
6. House J., Landis K., Umberson D. (1988). *Social relationships and health. Science, 241*, 540-545.
7. Leary, M. R., & Baumeister, R. F. (2000). *The nature and function of self-esteem: sociometer theory. In M. P. Zanna (Ed.), Advances in experimental social psychology, 32*, 1–62. San Diego: Academic Press.
8. Lubben J. (1988). *Assessing social networks among elderly population, Journal of Family & Community Health, 11*, 42–52.
9. Mann M., Hosman C., Schaalma H. & de Vries N. (2004). *Self-esteem in a broad-spectrum approach for mental health promotion. Health Education Research, 19*, 357–372.
10. Rosenberg M. (1965). *Society and adolescent self-image, Princeton University Press, Princeton NJ.*
11. Rotter, J. (1967). *A new scale for the measurement of interpersonal trust. Journal of Personality, 35*, 651-665.
12. Schlenker, B., Helm, B., & Tedeschi, J. (1973). *Interpersonal trust, promise credibility, and behavioral trust. Journal of Personality and Social Psychology, 25*, 419–427.
13. Schwarzer R, Leppin A. *Social support and health: A meta-analysis. Psychol Hlh 1989;3:1-15.*
14. Watson, D., & Friend, R. (1969). *Measurement of social-evaluative anxiety. Journal of Consulting and Clinical Psychology, 33*, 448–457.
15. Yu D., Lee D. & Woo J. (2004). *Psychometric testing of the Chinese Version of the Medical Outcomes Study Social Support Survey (MOS-SSS-C). Research in Nursing & Health, 27*, 135–143.
16. Zimet G., Dehlem N., Zimet S. & Farley G. (1988). *The multidimensional scale of perceived social support. Journal of Personality Assessment, 52*, 30–41.

### Corresponding author:

Jingyuan Chen,  
Fourth Military Medical University,  
China,  
E-mail: Chenjy\_2009@yahoo.com.cn



# Risk factors of allergic rhinitis: a case-control study

Natasa Maksimovic<sup>1</sup>, Vesna Tomic-Spiric<sup>2</sup>, Slavenka Jankovic<sup>1</sup>, Aleksandra Jovic-Vranes<sup>3</sup>, Zorica Terzic-Supic<sup>3</sup>, Janko Jankovic<sup>3</sup>

<sup>1</sup> Institute of Epidemiology, Belgrade University School of Medicine, Serbia

<sup>2</sup> Institute of Allergology and Immunology, Clinical Center of Serbia, Belgrade University School of Medicine, Serbia

<sup>3</sup> Institute of Social Medicine, Belgrade University School of Medicine, Serbia

## Abstract

**Objective:** Allergic rhinitis is a major respiratory disorder and represents a global health concern. Its prevalence is increasing over the last decades and the reasons for this increase are not completely clear. The aim of this study was to investigate the factors associated with allergic rhinitis in the adult Serbian population.

**Method:** A case-control study of 100 outpatients with allergic rhinitis and 100 control subjects was conducted in Belgrade, Serbia in 2006. Cases and controls were matched by sex and age ( $\pm$  5 years). A detailed questionnaire based on the latest research results in this field was used to obtain information about known risk factors. Univariate and multivariate logistic regression analyses were used for statistical analysis.

**Results:** The following factors were independently associated with occurrence of allergic rhinitis: allergic asthma (Odds Ratio [OR], 3.00; 95% confidence interval [CI], 1.36-6.64) and atopic dermatitis (OR, 4.24; 95%CI, 1.35-13.31) among family members, allergic rhinitis (OR, 24.07; 95%CI, 3.15-183.89), allergic asthma (OR, 6.22; 95%CI, 1.34-28.87), infections of the airways (OR, 3.49; 95%CI, 1.82-6.72) and drug allergy (OR, 2.95; 95%CI, 1.01-8.61) in childhood, atopic dermatitis (OR, 3.02; 95%CI, 1.03-8.83), sinusitis (OR = 3.34; 95%CI, 1.52-7.35), and breastfeeding in the first months (OR, 2.86; 95%CI, 1.17-6.96). Asthma among family members was the only independent risk factor for allergic rhinitis (OR, 3.16; 95%CI, 1.04-9.59).

**Conclusion:** Our study indicates potentially important role of both genetic and environmental factors in the etiology of allergic rhinitis.

**Key words:** allergic rhinitis, case-control study, environmental factors, genetic factors, logistic regression analysis, risk factors.

## Introduction

Allergic rhinitis is a symptomatic disorder of the nose induced by an immunoglobulin E (IgE) mediated inflammation of the nasal membranes in response to allergen exposure. Predominant symptoms are rhinorrhea, nasal obstruction, nasal itching and sneezing. Allergic rhinitis affecting between 10% to 40% of the global population and its prevalence is increasing both in children and adults, over the last decades (1). Due to its high and increasing prevalence, its impact on quality of life, the association with multiple comorbidities (asthma, sinusitis, conjunctivitis) and the considerable socioeconomic burden, allergic rhinitis is a major respiratory disorder and represents a global health concern (2). The reasons for this increase are not completely clear but there is accumulating evidence that both genetic and environmental factors play an important role (3). Some aspects of "western" lifestyle and environment are thought to be responsible. During the last decade of the 20<sup>th</sup> century, much attention was paid to the "hygiene hypothesis" as the most plausible working hypothesis to explain both temporal changes and the regional differences in allergic rhinitis preva-



lence, but significance of hereditary factors cannot be ignored. Exposure to environmental allergens is the most significant environmental factor in development and exacerbation of allergic rhinitis. Atmospheric pollution, changes in diet, antibiotic use, immunisations and patterns of infection in childhood, leading to changes in numbers of people with T-helper (Th)<sub>2</sub>, rather than Th<sub>1</sub> immune responses, as well as number of people who produce IgE antibodies to inhaled allergens, also play an important role in the development of the allergic rhinitis (4). Another theory is that there has been a major change in the gene pool, predisposing more individuals to excessive IgE production and thus increased expression of allergic rhinitis (5).

In recent years, focus of our investigation has been on risk factors and health-related quality of life in allergic (atopic) diseases of the airways, primarily asthma (6,7) and rhinitis (8). The identification of risk factors is essential for the adaptation of preventive measures and optimization of rhinitis patient management.

The aim of this study was to assess the role of some suspected risk factors in development of allergic rhinitis in our environment.

## Patients and Methods

### Patients

This case-control study was conducted in 2006 year. The case group was composed of 100 adult allergic rhinitis patients who were consecutively recruited from the outpatient department of the Institute of Allergology and Immunology, Clinical Center of Serbia. All were clinically diagnosed as having allergic rhinitis on the basis of their medical history, clinical presentation (stuffy nose, runny nose, sneezing), skin prick testing with inhalant allergens and results of specific rhino provocation tests with allergens, following pertinent guidelines and recommendations (2).

All selected cases were interviewed – no one refused to participate. One control was chosen for each case. Controls (n = 100) were randomly selected outpatients from the other departments of the same Clinical Center. They were individually matched to cases by sex and age ( $\pm 5$  years). Af-

ter an appropriate medical work-up, the subjects of the control group were diagnosed with hypertension (20), gastritis (12), gastroduodenitis (10) nephrolithiasis (15), and orthopedic trauma (43).

### Questionnaire

All the subjects were interviewed by the same doctor using a detailed questionnaire based on the latest research results in this field. The questionnaire consisted of 61 items and included information about: (a) socio-demographic characteristics (age, sex, education, marital status, place of residence, birthplace, presence of air pollution in the place of residence); (b) associated upper respiratory illnesses: nonallergic rhinitis, sinusitis, nasal polyps; (c) associated allergic diseases: allergic asthma, atopic dermatitis (eczema), urticaria, food allergy, drug allergy; (d) history of already mentioned allergic diseases in childhood; (e) family history of allergic diseases; (f) history of respiratory tract infections /illnesses/ (pneumonia, bronchiolitis, bronchitis (wheeze), (g) history of prematurity, birth weight, maternal age of birth, breastfeeding, family size, various harmful behavioral factors (cigarette smoking, smoking during pregnancy, housing conditions, pets).

Items about associated respiratory and/or allergic illnesses refer to clinical diagnosis made by a physician and not only based on history, signs and symptoms of the disease.

### Statistical analysis

Univariate and multivariate logistic regression analyses were used for statistical analysis. The strength of the relationship between risk factors and allergic rhinitis was evaluated by calculating odds ratios (OR) and their 95% confidence intervals (CI) for all tested factors. Dependent variable was a "health status" (allergic rhinitis or absence of allergic rhinitis). All statistically significant variables from the univariate logistic regression analysis were put into the multivariate logistic regression model. Analysis was performed with the Statistical Package for the Social Sciences, SPSS, version 8.0 (SPSS Inc., Chicago, IL, USA), with significance set at  $P < 0.05$ .

## Results

The study included 100 patients with allergic rhinitis and 100 controls (57 males and 43 females), matched according to sex and age ( $\pm 5$  years). The mean age was  $33.79 \pm 12.44$  years in allergic rhinitis group and  $33.78 \pm 12.66$  years in the control group. There were not significant differences between cases and controls in educational level or in place of residence (Table 1).

Table 1. Socio-demographic characteristics of cases and controls

| Characteristics              | Cases (n = 100)   | Controls (n = 100) |
|------------------------------|-------------------|--------------------|
| Age in years (mean $\pm$ SD) | 33.79 $\pm$ 12.44 | 33.78 $\pm$ 12.66  |
| Sex (%)                      |                   |                    |
| Male                         | 57                | 57                 |
| Females                      | 43                | 43                 |
| Educational level (%)        |                   |                    |
| 0-8                          | 5                 | 5                  |
| 8-12                         | 55                | 56                 |
| >12                          | 40                | 39                 |
| Place of residence (%)       |                   |                    |
| Rural                        | 13                | 16                 |
| Urban                        | 87                | 84                 |

According to univariate logistic regression analysis (Table 2), air pollution in the place of residence, drug allergy, atopic dermatitis, sinusitis, respiratory infections in childhood, allergic asthma in childhood, allergic rhinitis in childhood, drug allergy in childhood, asthma among family members, allergic rhinitis among family members, atopic dermatitis among family members, and breastfeeding in the first months were significantly associated with allergic rhinitis.

In order to estimate an independent non-confounded effect of the potential risk factors, multivariate conditional logistic regression analysis was used. All statistically significant variables from univariate analysis were entered into the model. Significantly increased risk of allergic rhinitis was associated only with asthma among family members (OR, 3.16; 95%CI, 1.04-9.59;  $P = 0.042$ ) (Table 3).

Table 2. Factors related to allergic rhinitis – univariate logistic regression analysis

| Variable                               | B*   | SE <sup>†</sup> | P     | OR <sup>‡</sup> | 95% CI <sup>§</sup> |
|----------------------------------------|------|-----------------|-------|-----------------|---------------------|
| Air pollution near place of residence  | 1.15 | 0.31            | 0.000 | 3.17            | 1.72-5.85           |
| Drug allergy                           | 0.84 | 0.40            | 0.036 | 2.32            | 1.05-5.08           |
| Atopic dermatitis                      | 1.10 | 0.55            | 0.044 | 3.02            | 1.03-8.83           |
| Sinusitis                              | 1.21 | 0.40            | 0.003 | 3.34            | 1.52-7.35           |
| Respiratory infections in childhood    | 1.25 | 0.33            | 0.000 | 3.49            | 1.82-6.72           |
| Allergic asthma in childhood           | 1.83 | 0.78            | 0.020 | 6.22            | 1.34-28.87          |
| Allergic rhinitis in childhood         | 3.18 | 1.04            | 0.002 | 24.07           | 3.15-183.89         |
| Drug allergy in childhood              | 1.08 | 0.55            | 0.048 | 2.95            | 1.01-8.61           |
| Asthma among family members            | 1.09 | 0.41            | 0.007 | 3.00            | 1.36-6.64           |
| Allergic rhinitis among family members | 1.74 | 0.65            | 0.007 | 5.71            | 1.59-20.39          |
| Atopic dermatitis among family members | 1.44 | 0.58            | 0.013 | 4.24            | 1.35-13.31          |
| Breastfeeding in the first months      | 1.05 | 0.45            | 0.021 | 2.86            | 1.17-6.96           |

\*Coefficient, <sup>†</sup>Standard Error, <sup>‡</sup>Odds Ratio, <sup>§</sup>Confidence Interval.

Table 3. Factors related to allergic rhinitis – multivariate logistic regression analysis

| Variable                    | B*     | SE <sup>†</sup> | P     | OR <sup>‡</sup> | 95% CI <sup>§</sup> |
|-----------------------------|--------|-----------------|-------|-----------------|---------------------|
| Asthma among family members | 1.15   | 0.57            | 0.042 | 3.16            | 1.04-9.59           |
| Constant                    | -2.075 | 0.67            | 0.002 | 0.13            |                     |

\*Coefficient, <sup>†</sup>Standard Error, <sup>‡</sup>Odds Ratio, <sup>§</sup>Confidence Interval.

## Discussion

Allergic rhinitis represents a global health problem. Although often trivialised by patients and doctors allergic rhinitis is a significant cause of morbidity, in addition to its substantial economic impact (5). Much effort has been made to identify risk factors for the development of allergic rhinitis (4,9). It is likely that there is a multilevel interaction between genetic and environmental factors in the etiology of allergic rhinitis (3). Our study indicates potentially important role of allergic rhinitis, allergic asthma and atopic dermatitis among family members, allergic rhinitis, allergic asthma, infections of the airways and drug allergy in childhood, atopic dermatitis, sinusitis, air pollution and breastfeeding in the first months as dependent factors which increase the risk of allergic rhinitis. Asthma among family members was the only independent risk factor for allergic rhinitis.

Allergic rhinitis is most common atopic disease with strong links to asthma. Epidemiological studies have consistently shown that allergic rhinitis and asthma often co-exist in the same patients, suggesting the concept of "one airway, one disease" (2,10-12). According to our results, there was a strong association between allergic rhinitis and allergic asthma in childhood. Also, our study has shown that individuals with diagnosis of allergic rhinitis in childhood are about 24 times more likely to have allergic rhinitis as adults than negative controls.

Allergic rhinitis and asthma are frequently associated. It was shown that allergic rhinitis correlated to, and constituted a risk factor for the occurrence and severity of asthma (2). Approximately 80% of asthmatics have rhinitis and somewhere between 25% and 50% of patients with rhinitis have associated asthma (13). In patients with both disorders, rhinitis appears first in 45% of the cases (14). Recent studies show that the deposition of allergen into the lower respiratory tract leads to increased inflammation of the upper respiratory tract, even if the patients are only suffering from allergic rhinitis (15). It is now recognized that asthma and persistent allergic rhinitis do not constitute distinct disease categories but in fact represent the final common pathway of closely related pathological processes in the upper, lower or both parts of the respiratory tract (2,16).

Bugiani et al. (12) concluded that the risk of asthma in subjects with allergic rhinitis was significantly higher than the risk in subjects without allergic rhinitis, and that bronchial asthma and allergic rhinitis, when associated, seemed to share the same risk factors as allergic rhinitis alone, while asthma without allergic rhinitis seemed to be a different condition, at least with respect to some relevant risk factors.

A study that examined risk factors of onset and remission of allergic rhinitis and asthma in Swedish adults (17) showed that the onset of asthma was highly associated with allergic rhinitis among atopics (OR, 5.70), but also tended to be related to non-atopics (OR, 3.50). In the Copenhagen Allergy Study (18), more than 99% of subjects with allergic asthma also had allergic rhinitis.

According to hygiene hypothesis larger family size, exposure to respiratory infections, microbial exposure, and exposure to other bacterial components such as endotoxin have a protective effect against the development of allergic rhinitis and other allergic diseases (19). The hygiene hypothesis has been proposed as a possible explanation for the doubling of the incidence of allergic rhinitis in the past 20 years (20). Several cohort studies have demonstrated that the prevalence of allergic rhinitis is correlated with an increased number of older siblings (21), and with increased family size (22). The rationale of the protective effect of a large family is that older siblings transmit respiratory infections to younger siblings.

However, not all studies have found an inverse association between infections and allergic rhinitis. Recent study of Tamay et al (9) has shown that frequent respiratory infections in childhood significantly increase risk for allergic rhinitis (OR, 1.36). Our results are in concordance with this finding (OR, 3.49).

Different studies in North America (23), Europe (24) and South Australia (25) have shown that the prevalence of atopy (defined as positive skin tests to common aeroallergens) and allergic rhinitis is higher in urban than in rural areas. Recently, it has been found that farmers' children have less allergic rhinitis than other children, suggesting therefore that lifestyle in the countryside could protect children from the development of allergy (26). In the present study place of residence of the majority of subjects with allergic rhinitis was urban.

Our study was shown that air pollution near place of residence increase risk of allergic rhinitis. Recent findings suggest that exposure to outdoor air pollutants may increase the risk of allergic rhinitis in children (27-29). In a Taiwanese study (30) long-term exposure to outdoor air pollutants increases the risk of allergic rhinitis. In a British study the occurrence of general practice consultations due to allergic rhinitis was related to short term exposure to sulfur dioxide (SO<sub>2</sub>) and ozone (O<sub>3</sub>). The strongest association was found for daily levels during 3 to 4 days prior to consultation (27). Air pollution was significant environmental factor of allergic rhinitis in Singapore study (31). Traffic related air pollution may increase the risk of allergic development and exacerbate symptoms in particular susceptible subgroups (32). The study of von Mutius et al (24) has shown that air pollution is associated with increased rhinitis symptoms but is not necessarily associated with an increased incidence of allergic rhinitis or allergen sensitization.

The relationships between allergic rhinitis and chronic sinusitis are close and complex. They are often a continuum of disease (33). This relationship can be demonstrated not only in terms of anatomy, physiology and epidemiology, but also experimentally, by studying the sinus involvement following nasal allergen provocations and pathophysiologically, by phenotyping and comparing the inflammation present in both diseases. Results of such studies have confirmed the fundamental role of nasal inflammation in general and allergic inflammation in particular in the genesis of sinusitis (34). Sinusitis can increase risk for allergic rhinitis (OR, 1.29) (10). According to our results sinusitis was risk factor for allergic rhinitis (OR, 3.34), but not an independent one.

Atopic dermatitis frequently is the first manifestation of an atopic diathesis, which occurs in genetically predisposed individuals and also includes allergic rhinitis and asthma. Up to 80% of children with atopic dermatitis eventually develop allergic rhinitis later in childhood (35). In the present study subjects with atopic dermatitis were more frequently diagnosed as allergic rhinitis (OR, 3.02).

A genetic component in allergic rhinitis as well as in other allergic diseases has been shown and the best established risk factor for allergic rhinitis is a

family history of allergy, especially allergic rhinitis (36). Adults with a family history of asthma or rhinitis have a 3 to 4 fold risk of development asthma and a 2 to 6 fold risk of developing rhinitis, compared with adults with no family history (37). An increase risk of children developing allergic rhinitis was found when either parent had a positive history, and the risk is highest if both parents are affected (38,39). The risk of the second child developing allergic rhinitis when either parent or the eldest child, or both, had allergic rhinitis was found to increase in study of Lee et al (40). The German case-control study had determined that the correlation of allergic rhinitis between siblings is significantly higher than that between parent and child (41). In twin studies, concordance rates of allergic rhinitis and serum IgE levels in monozygotic twins are quite similar in twins raised apart, suggesting a strong familial/genetic component (39). Atopic individuals, with family history of rhinitis, first born children and immigrants are predisposed to develop allergic rhinitis (5). The risk of allergic rhinitis was also increased in a child with positive parental history of any atopic diseases (40).

In our study, family history of allergic rhinitis as well as history of asthma and atopic dermatitis are important risk factors of allergic rhinitis. Individuals who had family members affected with asthma had significantly higher risk of contracting the allergic rhinitis (OR, 3.16).

The effect of breastfeeding on the development of allergic rhinitis and other atopic conditions has been assessed in many studies but remains controversial with some researchers showing a protective effect of breastfeeding (42,43) and others showing no association (44,45) or even a positive association (46). In our study breastfeeding was the predisposing factor for allergic rhinitis, but not independent one.

Aspirin and other non-steroidal anti-inflammatory drugs commonly induce rhinitis and asthma. In a population-based random sample, aspirin intolerance was more frequent among subjects with allergic rhinitis than among those without (2.6% vs. 0.3%,  $P < 0.01$ ) (47). Drug allergy and drug allergy in childhood were risk factors for allergic rhinitis in our study.

Our results support the hypothesis that allergic rhinitis is a multifactorial disease related to



genetic and various environmental influences. It was shown that familial atopic background (allergic rhinitis, allergic asthma and atopic dermatitis among family members) have the important role in genesis of allergic rhinitis. According to our results, asthma among family members was the only independent risk factor for allergic rhinitis.

Recognizing the risk factors is important for the diagnosis and prevention of the disease. Patients with symptoms of allergic rhinitis should be evaluated for asthma. Allergic rhinitis patient have an increased risk of developing asthma and may form a suitable population for secondary intervention (early recognition and treatment of allergic rhinitis) to interrupt the “allergic march” in respiratory diseases, i.e. progression of allergic rhinitis to bronchial asthma, improve quality of life, and may also reduce the overall cost of care.

Family predisposition of allergy should be considered as simply and the most cheapest predictive risk factor for allergy development.

Further studies are required to evaluate the precise role of the environment. It would also be interesting to compare these results with those obtained in a population of asthmatic patient.

### Acknowledgement

This work was supported by the Ministry of Science and Technological Development of Serbia, contract No. 145037, 2006–2010.

### References

1. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol.* 2001; 108 (5 Suppl):S147-334.
2. Dahl R, Andersen PS, Chivato T, Valovirta E, de Monchy J. National prevalence of respiratory allergic disorders. *Respir Med.* 2004;98:398-403.
3. Kauffmann F, Dizier MH, Annesi-Maesano I, et al. EGEA (Epidemiological study on the genetics and environment of asthma, bronchial hyperresponsiveness and atopy) – descriptive characteristics. *Clin Exp Allergy.* 1999;29:17-21.
4. Wang DY. Risk factors of allergic rhinitis: genetic or environmental? *Ther Clin Risk Manag.* 2005;1:115-23.
5. Salib RJ, Drake-Lee A, Howarth PH. Allergic rhinitis: past, present and the future. *Clin Otolaryngol.* 2003;4:291-303.
6. Tomić Spirić V, Bogić M, Janković S, Maksimović N, Matović Miljanović S, Perić Popadić A, et al. Assessment of the Asthma Quality of Life Questionnaire (AQLQ), Serbian translation. *Croat Med J.* 2004;45:188-94.
7. Tomic Spiric V, Jankovic S, Bogic M, Maksimovic N, Djuric V, Bolpacic J. Atopic asthma and related risk factors (in German, Abstract in English). *Allergologie.* 2007;30:41-49.
8. Maksimovic N, Jankovic S, Tomic Spiric V, Bogic M, Marinkovic J. Measuring health-related quality of life in patients with allergic rhinitis (in German, Abstract in English). *Allergologie.* 2006;29:491-49.
9. Tamay Z, Akcay A, Ones U, Guler N, Kilic G, Zencir M. Prevalence and risk factors for allergic rhinitis in primary school children. *Int J Pediatr Otorhinolaryngol.* 2006;71:463-71.
10. Togias A. Mechanisms of nose-lung interaction. *Allergy.* 1999;54:94-105.
11. Bousquet J, Vignola AM, Demoly P. Links between rhinitis and asthma. *Allergy.* 2003;58:691-706.
12. Bugiani M, Carusso A, Migliore E, et al. ISAYA (ECRHS Italy) Study Group: Allergic rhinitis and asthma comorbidity in a survey of young adults in Italy. *Allergy.* 2005;60:165-70.

13. Scadding G. The coexistence of the upper and lower airways disease. *Respir Dis Pract.* 1994; 11:18-20.
14. Brydon M. Patient audit for allergy, asthma and rhinitis. *Asthma J.* 1995;32:29-32.
15. Virchow JC. Asthma, allergic rhinitis, sinusitis. Concept of the 'unified respiratory tracts'. *HNO.* 2005;53:S16-20.
16. Bolpatic J, Bogic M, Tadić J, Tomic Spiric V, Peric Popadic A, Sojic Rajcic J, et al. Pre-asthma stage in patients with allergic rhinitis (in German, Abstract in English). *Allergologie.* 2006;29:261-7.
17. Plaschke PP, Janson C, Norrman E, Bjornsson E, Ellbjar S, Jarvholm B. Onset and remission of allergic rhinitis and asthma and the relationship with atopic sensibilisation and smoking. *Am J Respir Crit Care Med.* 2000;162:920-924.
18. Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T. Secular trends of allergic asthma in Danish adults. The Copenhagen Allergy study. *Respir Med.* 2001;95:258-64.
19. Fuleihan RL. The hygiene hypothesis and atopic disease. *Curr Opin Pediatr.* 2002;14:676-7.
20. Maziak W, Behrens T, Brasky TM, Duhme H, Rzehak P, Weiland SK, et al. Are asthma and allergies in children and adolescents increasing? Results from ISAAC phase I and phase III surveys in Munster, Germany. *Allergy.* 2003;58:572-9.
21. Strachan DP. Epidemiology of hay fever: towards a community diagnosis. *Clin Exp Allergy.* 1995;25:296-303.
22. Ponsonby AL, Couper D, Dwyer T, Carmichael A. Cross sectional study of the relation between sibling number and asthma, hay fever, and eczema. *Arch Dis Child.* 1998;79:328-33.
23. Gergen PJ, Turkeltaub PC. The association of individual allergen reactivity with respiratory disease in a national sample: data from the second National Health and Nutrition Examination Survey, 1976-80 (NHANES II). *J Allergy Clin Immunol.* 1992;90:579-88.
24. von-Mutius E, Martinez FD, Fritzscher C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med.* 1994;149:358-64.
25. Crockett AJ, Cranston JM, Alpers JH. The changing prevalence of asthma-like respiratory symptoms in South Australian rural schoolchildren. *J Paediatr Child Health.* 1995;31:213-7.
26. Braun-Fahrlander C, Gassner M, Grize L, Neu U, Sennhauser FH, Varonier HS, et al. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution. *Clin Exp Allergy.* 1999;29:28-34.
27. Hajat S, Haines A, Atkinson RW, Bremner SA, Anderson HR, Emberlin J. Association between air pollution and daily consultations with general practitioners for allergic rhinitis in London, United Kingdom. *Am J Epidemiol.* 2001;153:704-14.
28. Ramadour M, Burel C, Lanteaume A, Vervloet D, Charpin D, Brisse F, et al. Prevalence of asthma and rhinitis in relation to long-term exposure to gaseous air pollutions. *Allergy.* 2000;55:1163-9.
29. Passali D, Lauriello M, Mezzedimi C, Bellussi L. Nasal allergy and atmospheric pollution. *Int J Pediatr Otorhinolaryngol.* 1999;49:257-60.
30. Bing-Fang H, Jouni JKJ, Yung-Ling L, Ying-Chu L, Yue-liang LG. Relation between air pollution and allergic rhinitis in Taiwanese schoolchildren. *Respir Res.* 2006;7:23.
31. Ng TP, Tan WC. Epidemiology of Allergic Rhinitis and its Associated Risk Factors in Singapore. *Int J Epidemiol.* 1994;23:553-8.
32. WHO. Health aspects of air pollution with particulate matter, ozone and nitrogen dioxide. Report on a WHO working Group. Bonn: WHO, 2003 [cited 2008 Jun 6] Available from URL: <http://www.euro.who.int/document/e79097.pdf>.
33. Annesi-Maesano I. Epidemiological evidence of the occurrence of rhinitis and sinusitis in asthmatics. *Allergy.* 1999;54(Suppl 57):7-13.
34. Demoly P, Bencherioua AM, Crampette L, Dhivert-Donnadieu H, Godard P, Michel FB. From allergic rhinitis to sinus diseases (sinusitis/nasal polyps): epidemiologic and experimental links. *Rev Mal Respir.* 2000;17:925-30.

35. Eichenfield LF, Hanifin JM, Beck LA, Lemanske RF, Sampson HA, Weiss ST, et al. Atopic Dermatitis and Asthma: Parallels in the Evolution of treatment. *Pediatrics*. 2003;111:608-16.
36. Halken S, Host A. The lessons of noninterventional and interventional prospective studies on the development of atopic disease during childhood. *Allergy*. 2000;55:793-802.
37. Lundback B. Epidemiology of rhinitis and asthma. *Clin Exp Allergy*. 1998;28(2 Suppl):3-10.
38. Illi S, von Mutius E, Lau S, Nickel R, Niggemann B, Sommerfeld C, et al. The pattern of atopic sensitization is associated with the development of asthma in childhood. *J Allergy Clin Immunol*. 2001;108:709-14.
39. Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD. Genetics of asthma and hay fever in Australian twins. *Am Rev Respir Dis*. 1990;142:1351-8.
40. Lee JT, Lam ZC, Lee WT, Kuo LC, Jayant V, Singh G, et al. Familial risk of allergic rhinitis and atopic dermatitis among Chinese families in Singapore. *Ann Acad Med Singapore*. 2004;33:71-4.
41. Diepgen TL, Blettner M. Analysis of familial aggregation of atopic eczema and other atopic diseases by odds ratio regression models. *J Invest Dermatol*. 1996;106:977-81.
42. Sarrinen UM, Kajosaari M. Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. *Lancet*. 1995;346:1065-69.
43. Oddy WH, Holt PG, Sly PD, Read AW, Landau LI, Stanley FJ, et al. Association between breast feeding and asthma in 6-year-old children: findings of a prospective birth cohort study. *BMJ*. 1999;319:815-9.
44. Gustafsson D, Lowhagen T, Andersson K. Risk of developing atopic disease after early feeding with cows' milk-based formula. *Arch Dis Child*. 1992;67:1008-10.
45. Stahlberg MR. Breast feeding, cow milk feeding, and allergy. *Allergy*. 1985;40:612-5.
46. Douchen K, Yo G, Bjorksten B. Atopic sensitization during the first year of life in relation to long chain polyunsaturated fatty acid levels in human milk. *Pediatr Res*. 1998;44:478-84.
47. Hedman J, Kaprio J, Poussa T, Nieminen MM. Prevalence of asthma, aspirin intolerance, nasal polyposis and chronic obstructive pulmonary disease in a population-based study. *Int J Epidemiol*. 1999;28:717-22.

Corresponding author:

Vesna Tomic Spiric,  
 Institute of Allergology and Immunology  
 Clinical Center of Serbia  
 Belgrade University School of Medicine  
 Serbia  
 spiric@drenik.net

# A Qualitative Evaluation of Nurses' Perception towards the Role of Pharmacist in Healthcare Setup in Pakistan

Azhar. S<sup>1</sup>, Hassali. MA<sup>1</sup>, Izham MI<sup>1</sup> & Khan .TM<sup>2</sup>

<sup>1</sup> Discipline of Social and Administrative pharmacy, Universiti Sains Malaysia, Malaysia

<sup>2</sup> School of Pharmaceutical Sciences, Universiti Sains Malaysia, Malaysia

## Abstract

This study aims to explore the nurses' perception toward the role of pharmacist in the health care setup. A qualitative study design was adopted. Face to face interviews were conducted using a semi-structure interview guide. A total of twelve nurses were the part of study. All the interviews were transcribed verbatim and thematically analyzed for its content. The main focus of interviews were on; 1) Perception regarding pharmacists' role; 2) Importance of pharmacists in health setting; 3) Seeking help from pharmacists; 4) Pharmacist - Nurses Collaboration. Findings demonstrated that some nurse has shared a thought that pharmacists have a vital role in the success of any healthcare system. However, in their opinion, pharmacists just provide medicine and having a kind of managerial job in hospital. When respondents were asked about their collaboration in patient care, some of the respondents showed positive perception and emphasized on the importance of the availability of pharmacists. They were willing to collaborate because it would definitely benefit the patients. Moreover respondents stressed that the pharmacists should be available around the clock for consultation. Although nurses considered pharmacists as drug expert who know more about medicines as compared to other healthcare professionals, they were more likely to believe that pharmacists' involvement would be an intrusion into their affairs. Pharmacists' services are focusing towards mana-

gement of pharmacies rather than patient care. A possible reason behind this behavior may be the minimal level of interaction among the two professions due to the unavailability of pharmacists.

**Key words:** Perception, Pakistan, Nurses, Pharmacist, Health care system;

## Introduction

For the last two decades a sudden increase in the demand for health care services is experienced worldwide (Sitzia, 2001). In response to this call the demand of health care professionals also has increased too many folds. Nowadays an average health care setup facing new challenges in the form of limited financial resources, rising healthcare cost, increasing health demand and heightened public expectations (Hagbaghery et al., 2004). A collaborative relationships among the multiple disciplinary health care professional will be an ideal strategy to cope with these challenges and use their expertise to improve the patient care as per consumers expectations (Alan. J et al., 2006). Moreover, collaborative care by the health care providers has the potential to improve patient care, enhance patient safety and reduce work load issues that cause burnout among healthcare professionals (Oandasan et al., 2006). It is cited that in the health care setup where pharmacist has collaboration in direct patient care has higher rate of positive outcomes (Dranitsaris et al., 2001,



Hanlon et al., 1996, Tsuyuki et al., 2002). Main focus of previous work was on the collaboration between pharmacist and the doctor (Adepu and Nagavi 2006; Nathan, A. and C.A. Sutters 1993). However, there is merely any effort that highlights the nurses and pharmacists interaction.

In developing countries especially in Pakistan, health seeking behavior and determinants of the use of health services is an important concern (Khowaja, 2009). Increase in the population is poor quality of life; pollution, improper diet and the low health literacy are the main challenges to provide effective health care to inhabitants (Babar, 2006). Moreover, the healthcare system in Pakistan has been confronted with problems of inequity, scarcity of resources, inefficient and untrained human resources, gender insensitivity and structural mismanagement (Babar, 2006). These issues are alarming for Ministry of Health Pakistan to take some positive steps to cope the shortage of human resource in health sector to provide affective health care to the community (Azhar et al., 2009). In Pakistani health care setup previously there was shortage of medical doctors only but with the initiative for the Punjab government to hire pharmacist as an essential member of the health care team has increased the demand of qualified pharmacist as well. On other hand if we see the paramedical staff then Nurses are the largest group serving health care system in Pakistan (WHO, 2002-2008). The role of nurses in primary care has developed rapidly (Hollis, 2005) and even some of the drug related issues are handled by the nursing staff or one can assume that there is a good collaborative working relationship of nurses with physicians in Pakistani health care setup (San Martin-Rodriguez et al., 2005, Ogbimi and Adebamowo., 2006). However, in current scenario the pharmacist demand in health care system is increasing, it is one of the future priorities of Ministry of Health Pakistan to incorporate the role pharmacist in Pakistani health care system. In other words pharmacist will collaborate with the physicians and nursing staff to provide affective care to the patients. Keeping in view this motivation, this study aims to explore the nurses' perception toward the role of pharmacist in the health care setup.

## Methods

Face to face interviews were conducted using a semi-structure interview guide. Qualitative approach was used to gain understanding on this issue, as it allows a flexible exploration of respondents' experiences (Berg, 2004).

## Study Location

This study was conducted among the nursing staff offering their services in health care units in Lahore. Lahore is one the third most populous city of Pakistan with the estimated population of 10 million approximately (NIOP, 2007). 18 public and 38 private hospitals at present serve in the city (Wikipedia, 2009). The time frame of this study was from April 2007 until June 2007.

## Study Design

Face to face interviews were conducted using a semi-structured interview guide, developed after extensive literature review was used for the interview. All the respondents were approached using their contact (Berg 2004). Through purposive sampling method, key informants were interviewed until saturation point was reached (Roy et al., 2007). Each interview lasted approximately 15 to 25 minutes. All the interviews were audio taped and transcribed verbatim. The transcripts were repeatedly and thematically analyzed line by line for its content (Mohamed et al., 2005). The main focus of interviews was on the nurse perception towards the role of pharmacists and their willingness to collaborate with pharmacists. Furthermore, information was also gathered regarding their experience working with pharmacists. Probing questions were used where necessary and respondents were given freedom to express their views.

## Ethical considerations

The study protocol was approved by the institutional research ethics committee school of pharmaceutical science Universiti Sains Malaysia.

Furthermore a written consent was obtained from the participants prior to the interview. It was assured to all the respondents that their personal information will be kept confidential.

## Results

A total of 12 respondents were identified through contacts and interviewed. All the respondents participated in this study were female with a working experience in nursing profession more than eight years. In terms of age 4(33.3%) of the respondents were aged less than thirty years. However, 5(42.6%) of respondents were from age group thirty to forty years and one fourth were aged over forty. The Thematic content analysis of the interviews yielded four major themes; perception regarding pharmacists' role, availability of pharmacists, seeking help from pharmacists and pharmacists-nurses collaboration. Of these twelve nurses only ten reported that pharmacist available at their hospital.

### **Theme one:** *Perception regarding pharmacists' role*

Nurses in Pakistan's healthcare system have the opinion that the pharmacists' role was limited to one who provides medicines and being the drug expert. Majority stated that;

Yes, we know about a pharmacist, the medicines supplied to ward and hospital are provided by Pharmacists. They are directly in contact with the suppliers and provide medicines which are required.

However very few shared a thought that;

*Pharmacist is the one having a good knowledge about medicine, it is the responsibility of pharmacist to check thoroughly about medicine, either the prescribed medicine is available at the store or not. If it is available then who is the manufacturer; is it the same name mentioned by the prescriber or it is the same drug with different name. Pharmacist is the one to make final decision about the use of medicine because they know more about the benefits and side effects as compared to other member of healthcare team.*

### **Theme Two:** *Importance of pharmacists in healthcare setting*

Regarding the importance of pharmacists in healthcare setting, all the interviewees agreed that with the presence of pharmacist in the health care setup we can provide better health care to the patients.

**Nurse one:** *Pharmacist is important member of health care team, if he doesn't provide medicine in time how can patient recover.*

**Nurse two:** *Pharmacists are the drug experts and seller who are trained properly.*

### Theme three: Seeking help from pharmacists

The theme three is one of the core elements of this study that evaluates the nurse attitude toward help seeking from the pharmacist. A negative attitude was found among the nurse regarding the help seeking from pharmacist regarding the patient care.

**Nurse one:** *among the health care professional our relation is limited to doctors only and after doctors we concern about the patient. Pharmacists are quite separate their job nature is different from the doctors and ours.*

**Nurse Two:** *We have nothing to do with Pharmacist. We have our own duty and they have their own. They come and check registers and medicines. We have to work within the wards. In foreign countries it happens that you can call pharmacist if needed but, here in our country we get medicines on weekly basis.*

### **Theme four:** *Nurses attitudes toward Pharmacist - Nurses collaboration*

Overall attitude was positive toward the Pharmacist - Nurses collaboration. Details are described as follow;

**Nurse five:** *Yes, we are willing to work in collaboration with pharmacist; they come and check what medicines are being used. They also check that the medicines which are being given to the patients are proper or not.*

**Nurse six:** *Yes, we will work with them in collaboration if they are available, this alliance would be definitely benefit for the patients.*

***Nurse seven:** Yes, it will be great if Pharmacist is the part of healthcare setting. The services provided by the pharmacist should be 24 hours; this will help the health care team to get pharmacist consultation at any time.*

## Discussion

This was a qualitative study that highlighted the nurses' perception and attitude toward pharmacists in Pakistani health care setup. Pharmacists in developing countries are still underutilized and under estimated for their professional skills. Moreover, their role as healthcare professionals is not deemed necessary by other healthcare provider. The findings of this study highlight a negative perception among nurse toward the capabilities of the pharmacist. It was revealed that majority has visualized the role of pharmacists as a drug provider and those who stay in contact with the suppliers for the availability of the medicine in the hospital. Although very few has considered pharmacists as drug expert who know more about medicines as compared to other healthcare team. However, still many perceive consider pharmacist role in the hospital for the managerial job at the main pharmacy (Azhar et al., 2009). This can be one of the possible factor that lying hindrance for the pharmacist to prove their capabilities in the patient care (Azhar et al., 2009).

If we see the health care setup at the developed countries the presence of pharmacist is essential in any health care team. Their importance was highlighted by almost all the interviewees and this was consistent with studies done in several countries which reported that pharmacists could make a great contribution to the provision of the primary health care, especially in developing countries (Smith, 2004, Jesson and Bissell, 2006) and that their role varies in different parts of the world. Some dealt with preparation and supply of medicines while some focus on sharing pharmaceutical expertise and knowledge with doctors, nurses and patients (Gilbert, 2001). Whilst questioning the respondents about the presence of pharmacists at the hospitals, more than half were unaware about the existence of pharmacists in their hospital. The main reason was the unavailability, which was

the common issue discussed in number of studies (Goel et al., 1996, FIP, 1998, FIP, 1997). Lack of human resources creates a significant difference between the health services availability. In many cases this was due to the fact that the number of pharmacists was less than required (Goel et al., 1996, FIP, 1998, FIP, 1997), or we can say that the numbers of post allocated for the pharmacist in Pakistan are less than the demand. In this case Ministry of Health Pakistan can play a vital role by taking some initiative to increase the number of post of pharmacist in government and private health care setup. Such initiatives will also help in presenting the role of pharmacist in better not only in terms of pharmacy management but also in the patient and community care. In this way the negative attitude toward the pharmacist among the health care professional can also be rectified as majority of the nurses they were not willing to seeking help from pharmacists in patient care. They have negative perception toward the role of pharmacist; they did not consider pharmacists as members of the health care team who can be involved in patient care. Their opinion regarding pharmacists was just to provide medicines and take care of their managerial job. The lack of interactions between the two professions and scarcity of pharmacist in the health care setup can be a possible reason for this behavior. These findings are some what consistent to the findings of Gillbert (1997) that report almost same attitude among the nurses toward help seeking form pharmacist.

However, surprisingly a very positive attitude was observed towards pharmacist-nurse collaboration. The respondents shared a thought that pharmacists' involvement is essential component to attain affective patient care. They understood that working together will improve patient outcomes. This finding complies with the findings of Makowsky et al, (2009) that reports the willingness by health care professionals to work together as a team. Moreover, nearly all agreed that collaboration with pharmacists can only be fulfilled if they were available in the hospital. These finding highlight that the availability of pharmacists is one of the major issue in developing countries especially in Pakistan (Azhar et al., 2009).

## Conclusion

Overall a negative perception was found toward the role of pharmacist in health care setup. Pharmacist was identified as a drug expert and his skill were only confounded only to the issues concerning pharmacy management. A possible factor for this behavior may be, Nurses believe that by incorporating the role of pharmacist in patient care may decrease their worth and can result intrusion into their duties. Other factors responsible for this behavior are the common belief in Pakistan's healthcare setting that only doctors and nurses' alliance can fulfill all the requirement of the patients. In a same way nurses in Pakistan visualized the role of pharmacists as a drug provider. Last but not the least the main cause behind this behavior may be was the minimal interaction between the two professions which are because of the less number of pharmacists that practice in patient care in Pakistan.

## Recommendation

Findings of this study highlight the need of more methodological strong studies that further focus on the identification of the factors responsible for this negative behavior among the nurses in Pakistan health care system. Furthermore, ministry of health should take initiative to increase the number of pharmacist in Pakistani health care setup. Such initiatives will be helpful in creating an environment for affective patient care and will be helpful in positive modification of the nurses' attitude toward the role of pharmacist.

## Competing interests

The authors declare that they have no competing interests.

## Acknowledgements

Saira Azhar is a Pakistani national PhD scholar at the Universiti Sains Malaysia (USM) and recipient of USM fellowships. Wish to thank USM for the financial support provided for their research.

## References

1. Adepun and Nagavi. (2006) *General practitioners perception about the extended role of the community pharmacist in the stste of Kamataka. Indian journal of pharmaceutical sciences.* (68): p. 36-40.
2. Alan. J, Z., Jessica. L, M. & Barry.L, C. (2006) *Utility of a questionnaire to measure physician pharmacist collaborative relationship Journal of American Pharmacist Association.*
3. Azhar, S., Hassali, M. A., Mohamed Ibrahim, M. I., Ahmad, M., Masood, I. & Shafie, A. A. (2009) *The role of pharmacists in developing countries: the current scenario in Pakistan. Human Resources for Health,* 7, 54.
4. Babar, Z. U. (2006) *Pakistan National University of Pharmaceutical Sciences. American Journal Of Pharmaceutical Education,* 70.
5. Berg (2004) *Qualitative research methods for the social sciences,, Allyn and Bacon,, Boston.*
6. Dranitsaris, G., Spizzirri, D., Pitre, M. & McGeer, A. (2001) *A randomized trial to measure the optimal role of the pharmacist in promoting evidence-based antibiotic use in acute care hospitals. . International Journal of Technology Assessment in Health Care,, 17, 171-180.*
7. FIP (1997) *Good pharmacy practice in developing country.*
8. FIP (1998) *Good Pharmacy practice in developing countries.*
9. Gilbert, L. (2001) *To Diagnose, Prescribe and Dispense: Whose Right Is It? The Ongoing Struggle Between Pharmacy and Medicine in South Africa. Current Sociology,* 49, 97-118.
10. Goel, P., Ross-Degnan, D., Berman, P. & Soumerai, S. (1996) *Retail pharmacies in developing countries: A behavior and intervention framework. Social Science & Medicine,* 42, 1155-1161.
11. Hagbaghery, M. A., Salsali, M. & Ahmadi, F. (2004) *The factors facilitating and inhibiting effective clinical decision-making in nursing: a qualitative study. BMC Nursing.*



12. Hanlon, J. T., Weinberger, M., Samsa, G. P., Sch-mader, K. E., Uttech, K. M. & Lewis, I. K. (1996) *A randomized, controlled trial of a clinical pharmacist intervention to improve inappropriate prescribing in elderly outpatients with polypharmacy. . The American Journal of Medicine, . 100, 428-437.*
13. Hollis, R. (2005) *The role of the specialist nurse in pediatric oncology in the United Kingdom. European Journal of Cancer, 41, 1758-1764.*
14. Jesson, J. & Bissell, P. (2006) *Public health and pharmacy: A critical review. Critical Public Health, 16, 159 - 169.*
15. Khowaja, K. (2009) *Healthcare Systems and Care Delivery in Pakistan. JONA, 39, 263-265.*
16. Mohamed Azmi Hassali, Kong, D. C. M. & Stewart, K. (2005) *Generic medicines: perceptions of consumers in Melbourne, Australia. International journal of Pharmacy Practice, 13, 257-264.*
17. Nathan, A. and C.A. Sutters. (1993) *A Comparison of Community Pharmacists' and General Practitioners' Opinions on Rational Prescribing, Formularies and other Prescribing Related Issues. The Journal of the Royal Society for the Promotion of Health. 113(6): p. 302-307.*
18. Oandasan, I., Baker, G. R., Barker, K., Bosco, C., D'Amour, D. & Jones, L. (2006) *Teamwork in healthcare: Promoting effective teamwork in healthcare in Canada.*
19. Ogbimi, R. I. & Adebamowo., C. A. (2006) *Questionnaire survey of working relationship between nurses and doctors in University Teaching Hospitals in Southern Nigeria. BMC Nursing, 5.*
20. Roy, N., Madhiwalla, N. & Pai, S. A. (2007) *Drug promotional practices in Mumbai: a qualitative study. Indian Journal of Medical Ethics IV, 57-61.*
21. San Martin-Rodriguez, L., Beaulieu, M. D., D'Amour, D. & Ferrada-Videla, M. (2005) *The determinants of successful collaboration: A review of theoretical and empirical studies. . Journal of Interprofessional Care, 19, 132-147.*
22. Sitzia, J. (2001) *Barriers to research utilization: the clinical setting and nurses themselves. European Journal of Oncology Nursing, 5, 154-164.*
23. Smith, F. (2004) *Community pharmacy in Ghana: enhancing the contribution to primary health care. Health Policy Plan., 19, 234-241.*
24. N. I. O. P Studies, (2007) *Pakistan: Demographic and Health Survey.*
25. Tsuyuki, R. T., Johnson, J. A., Teo, K. K., Simpson, S. H., Ackman, M. L. & Biggs, R. S. (2002) *A randomized trial of the effect of community pharmacist intervention on cholesterol risk management: the Study of Cardiovascular Risk Intervention by Pharmacists (SCRIP). . Archives of Internal Medicine., 162 1149-1155.*
26. WHO (2002-2008) *Nursing and Midwifery Services Strategic Directions.*
27. Wikipedia (2009) *List of hospitals in Pakistan.*

Corresponding author:

Tahir Mehmood Khan,  
School Of Pharmaceutical Sciences,  
Universiti Sains Malaysia,  
Malaysia,  
e-mail: tahir.pks@gmail.com

# An epidemiological study of physical and mental health of rescuing soldiers in 5.12 Wenchuan earthquake

Wei qiang Zhang<sup>1</sup>, Tian Sheng Sun<sup>2</sup>, Jing Zhao<sup>1</sup>, Ju qiang Han<sup>3</sup>, Yong Hong Yang<sup>4</sup>, Shu Jun Li<sup>5</sup>, Ya Qun Ma<sup>6</sup>,

<sup>1</sup> Department of Thoracic Surgery; The Medical Relief Team, Beijing Military General Hospital, China

<sup>2</sup> Department of osteological surgery; The Medical Relief Team, Beijing Military General Hospital, China

<sup>3</sup> Department of Hepatopathy; The Medical Relief Team, Beijing Military General Hospital, China

<sup>4</sup> Department of Nephrology; The Medical Relief Team, Beijing Military General Hospital, China

<sup>5</sup> Department of Gastroenterology; The Medical Relief Team, Beijing Military General Hospital, China

<sup>6</sup> Department of anesthesiology; The Medical Relief Team, Beijing Military General Hospital, China

## Abstract

**Introduction:** A powerful earthquake struck Wenchuan in China on 12 May 2008. The physical and mental health of rescuing soldiers was investigated in 5.12 powerful earthquake in Wenchuan.

**Methods:** The analysis of descriptive epidemiology was made in 1187 rescuing soldiers in the areas of Wenchuan county and Dujiangyan city. By the questionnaire survey method, the content involved the body general condition as well as the skin mucous membrane system, the respiratory system, the digesting system, the urinary system, the nervous system symptom and the psychological and mental health.

**Results:** The diseases in dermatological, psychological and respiratory systems were predominant, and the incidence was 50.35%, 48.80% and 37.93%, respectively. The diseases were testified to be lower in the incidence of digestive, neurological and urinary system, and the incidence rate was 29.14%, 21.93% and 5.08%, respectively.

**Conclusion:** The psychological and mental health of rescuing soldiers who experienced the earthquake disaster was harmed in various degrees, and the corresponding measures should be made in the medication and mental intervention.

**Key words:** epidemiology, Wenchuan earthquake, rescuing soldiers, physical and mental health

## Introduction

A violent earthquake may bring about the most devastating disasters that cause mass casualties. The severe bodily and psychological injuries in the survivors pose the most difficult problems with regards to emergency aid and treatment after the catastrophe.<sup>1,2,3</sup> Major disasters can be followed by an increase in incidence and outbreaks of infectious diseases<sup>4,5</sup> and a strong link exists between the extent of damage caused by catastrophic earthquakes and an increase in morbidity rates for acute illness.<sup>6</sup>

At 14:28:01.42 (Beijing time) on May 12, 2008 "Wenchuan" had the powerful earthquakes with a magnitude of 8.0 on the Richter scale in the province of Sichuan of China. The epicenter of the massive earthquake was located in Wenchuan, Sichuan. A report from the Chinese Ministry of Civil Affairs stated that the death toll from this devastating earthquake rose to 69,196, 374,176 people were injured and 18,381 people were missing within a seriously devastated area of 100,000 km<sup>2</sup>. Hundreds and thousands of buildings, including hospitals and health-care centers were destroyed. Almost all the roads in western Sichuan were blocked. The estimation of the economic loss was higher than US \$140 billion, making the earthquake the costliest natural disaster in Chinese history by far.

The People’s Liberation Army rapidly reached, rescuing the life from the ruins, processing massive victim remains, searching crashed aircraft and transporting the grains to the isolated mountainous area and played the very important role in the earthquake relief work process. The serious earthquake caused mass casualties, bringing about the physical and mental illness in the survivors and rescuers. Previous studies have suggested that major disasters can be followed by an increase in the incidence of complex injuries and outbreaks of infectious diseases in victims.<sup>7,8</sup> The earthquake will also have the very tremendous influence to the disaster relief personnel’s physical and mental health. The previous research paid great attention the disaster relief personnel’s psychological question.<sup>9, 10, 11, 12</sup>

The epidemic studies to the relief personnel diseases were little reported.<sup>13, 14, 15</sup>

In order to understand earthquake relief work officers and soldiers’ psychology and health situation, our Beijing Military Region General hospital “Hua Yiwei the earthquake relief work medical team” had carried on the sample investigation of relief army officers and soldiers in the earthquake areas of Wenchuan and the Dujiangyan county and identified the types of illness, which will provide the basis for the concerned departments to draw up the related measures.

### Subjects and Methods

**Subjects:** Investigation subjects were the rescue army officers and soldiers in 5.12 serious earthquake in Wenchuan. We distributed 1187 copies of questionnaires one month after the earthquake, reclaimed 1187 copies of questionnaires.

**Methods:** The method was the overall general survey and the time was two months after the disaster. By the questionnaire survey method, the content involved the body general condition as well as the skin mucous membrane system, the respiratory system, the digesting system, the urinary system, the nervous system symptom and the stress disorder symptom. All investigators accepted unified training, using the unified quantification investigation standard. The personnel included: (1) one person was totally in charge of spot investigation and the quality control. He was responsible to dis-

tribute individual ID number for the surveyor and to carry on the quality inspection of the returned survey forms. (2) eight persons were responsible for the questionnaire survey specifically.

**Statistical analysis:** After all questionnaire completed, the data entry was carried on. One staff entered the data 2 times using the Excel software and another staff carried on the data checkup. The categorical variables are presented as percentages. A statistical significance was deemed present when the p-value was equal to or less than 0.01. All statistical calculations were performed using the SPSS, version 11.0, software for Windows.

### Results

**Demographics:** There were 1187 army officers and soldiers participating in the investigation. Of these, 1173 were male and 14 were female (range: 17 years to 35 years, average age 22.09 years). Of the patients, 28 were younger than 18 years, 1100 were aged between 18 and 30 years and 59 were older than 30 years (Table. 1). They mainly undertook the duties of dealing with project emergency, searching and rescuing the disaster victims, shipping the casualty and searching the crashed aircraft.

*Table 1. Demographic characteristics of rescuing soldiers in earthquake areas. Variables category number rate (%)*

|           |                 |      |       |
|-----------|-----------------|------|-------|
| sex       | male            | 1173 | 98.80 |
|           | female          | 14   | 0.18  |
| age range | ≤18 years old   | 28   | 0.36  |
|           | 18-30 years old | 1100 | 92.67 |
|           | ≥30 years old   | 59   | 4.97  |

According to the survey result, diseases were classified according to the body system. The incidence rate ranging from high to low was that of skin disease, the psychological illness, the respiratory disease, digestive tract disease, the nervous system disease and the urinary disease, respectively. The corresponding disease incidence rate was 50.35%, 48.80%, 37.93%, 29.14%, 21.93% and 5.08%, respectively (Table 2). The incidence rates of skin disease, the psychological illness, respiratory disease were higher than those of digestive tract disease, the nervous system disease and the urologic disease (p<0.01)

Table 2. The epidemiological study of illnesses of earthquake relief armies in Dujiangyan district

| Category              | Total number | Statistical number | Patient number (person-time) | Incidence rate (%) |
|-----------------------|--------------|--------------------|------------------------------|--------------------|
| Dermatological system | 1187         | 1134               | 571                          | 50.35              |
| Respiratory system    | 1187         | 1181               | 448                          | 37.93              |
| Digestive system      | 1187         | 1177               | 343                          | 29.14              |
| Nervous system        | 1187         | 1172               | 257                          | 21.93              |
| Mental status         | 680          | 625                | 305                          | 48.80              |
| Urinary system        | 1187         | 669                | 34                           | 5.08               |

Note: The subject having one symptom was calculated 1 person-time.

Table 3. Distribution of symptoms of dermatological system

| Symptom       | Statistical number | Patient number (person-time) | Incidence rate (%) |
|---------------|--------------------|------------------------------|--------------------|
| Skin rash     | 1134               | 362                          | 31.92              |
| Tinea of feet | 1134               | 256                          | 22.57              |

Note: The subject having one symptom was calculated 1 person-time.

Table 4. Distribution of symptoms of mental status

| Symptom                   | Statistical number | Patient number (person-time) | Incidence rate (%) |
|---------------------------|--------------------|------------------------------|--------------------|
| Vigilance                 | 625                | 64                           | 10.24              |
| Irritability-restlessness | 625                | 78                           | 12.48              |
| Irritability              | 625                | 60                           | 9.60               |
| Sadness                   | 625                | 63                           | 10.08              |
| Un-relaxation             | 625                | 48                           | 7.68               |
| Worry                     | 625                | 47                           | 7.52               |
| Tension                   | 625                | 57                           | 9.12               |
| Depression                | 625                | 45                           | 7.20               |
| Anxiety                   | 625                | 41                           | 6.56               |

Note: The subject having one symptom was calculated 1 person-time.

Table 5. Distribution of symptoms of respiratory system

| Symptom            | Statistical number | Patient number (person-time) | Incidence rate (%) |
|--------------------|--------------------|------------------------------|--------------------|
| Muscular stiffness | 1181               | 162                          | 13.72              |
| Cough              | 1181               | 209                          | 17.70              |
| Expectoration      | 1181               | 211                          | 17.87              |
| Hemoptysis         | 1181               | 39                           | 3.30               |
| Nasal discharge    | 1181               | 171                          | 14.48              |
| Dyspnea            | 1181               | 88                           | 7.45               |
| Pharyngalgia       | 1181               | 153                          | 12.96              |

Note: The subject having one symptom was calculated 1 person-time.

The survey results showed that the cardinal symptoms of skin and mucous membrane system were skin rashes such as papule nettle rash and eczema, and the fungus infectious athlete's foot in the army officers and soldiers (Table 3). The incidence rate of was 31.92% (362/1134) and 22.57% (256/1134), respectively. The main performances of psychological illness were irritability-restle-

ssness, the vigilance, sadness, testiness, intensity and so on (Table 4). The corresponding disease incidence rate was 12.48% (78/625) 10.24% (64/625) 10.08% (63/625) 9.60% (60/625) and 9.12% (57/625). 45 cases suffered from depression (7.20%), whereas 6.56% had anxiety (n=41)

The results showed that cardinal symptoms of respiratory system were the cough, the expectora-



Table 6. Distribution of symptoms of digestive system

| Symptom              | Statistical number | Patient number (person-time) | Incidence rate (%) |
|----------------------|--------------------|------------------------------|--------------------|
| Stomachache          | 1177               | 184                          | 15.63              |
| Vomiting             | 1177               | 42                           | 3.57               |
| Abdominal distension | 1177               | 54                           | 4.59               |
| Anepithymia          | 1177               | 88                           | 7.48               |
| Constipation         | 1177               | 72                           | 6.12               |
| Diarrhea             | 1177               | 125                          | 10.62              |

Note: The subject having one symptom was calculated 1 person-time.

Table 7. Distribution of symptoms of nervous system

| Symptom   | Statistical number | Patient number (person-time) | Incidence rate (%) |
|-----------|--------------------|------------------------------|--------------------|
| Headache  | 1172               | 128                          | 10.92              |
| Dizziness | 1172               | 128                          | 10.92              |
| Insomnia  | 1172               | 116                          | 9.89               |
| Vertigo   | 1172               | 87                           | 7.42               |

Note: The subject having one symptom was calculated 1 person-time.

tion and nasal discharge (Table 5) and the incidence rate was 17.70% (209/1181) 17.87% (211/1181) and 14.48% (171/1181), respectively. The serious respiratory symptoms such as hemoptysis and dyspnea were rare, and the incidence rate was 3.3% (39/1181), 7.45% (88/1181), respectively.

The cardinal symptoms of digestive tract symptom were gastroenteritis with the symptoms of abdominal pain and diarrhea (Table 6). 15.63% (n=184) had abdominal pain and 10.62% (125/1177) suffered diarrhea.

The main performances of nervous system were headache, dizziness, insomnia and vertigo (Table 7), the incidence rate respectively was 10.92% (128/1172), 10.92% (128/1172), 9.89% (116/1172) and 7.42% (87/1172), respectively.

## Discussion

Serious natural disaster had great influence on physical and mental health of disaster victims and relief staffs. It is necessary to investigate and analyze the changes and know the rule and the reason, which will contribute to the recovery and treatment of victims, and have great significance to relief work.

Our survey showed that the incidence rates of skin disease and the psychological illness were the highest in the earthquake relief work process

officers and soldiers. Skin diseases' main manifestations were papule nettle rash, eczema, dermatitis and fungus infectious athlete's foot. During the Chi-Chi earthquake of Taiwan the most commonly illnesses in disaster refugees were acute respiratory infection (50.1%), musculoskeletal pain (17.4%), trauma (12.5%), acute gastroenteritis (7.2%), and skin rash (7.1%). The incidence rates of diseases in relief staffs were different from those in disaster refugees.<sup>13</sup> The trauma was the common disease in refugees while not in relief staffs. Han<sup>14</sup> reported that the diseases were found mainly in psychological symptoms (62.16%) and respiratory system (51.78%) in the inhabitants in "Wenchuan earthquake", which were similar to our results.

The reasons were as follows: the climate of earthquake disaster area was moist, sultry and suitable for mosquito growth and reproduction. After the disaster, the destructed building, the bad lodging environment and the bad sanitary condition were advantageous to the occurring of skin disease. Because the army officers and soldiers lived together in the tents set up roadside, the incidence rate of allergic disease (papule nettle rash) caused by mosquito biting was higher, and the incidence rates of athlete's foot, the ringworm of the body and eczema and dermatitis's disease caused by fungal infections were also quite higher. In addition, it was the reason of higher incidence rate of

skin disease during the early time of disaster relief that the corresponding medicines were quite deficient and treats were not prompt.

The investigation showed that earthquake relief work officers and soldiers had the varying degree's psychological illness and the main performances were agitation, the vigilance, sadness, intensity and so on.

The reasons were as follows:

1. The major part of army soldiers were born in the 1980s, and their psychology growth had not been mature. Their former living experiences were quite simple, and had not withstood the quite obvious psychological discipline. The casualties this big earthquake created were huge. The disaster relief scene was frigid, which was an obvious direct-viewing psychological impact on relief soldiers, and their psychological bearing capacity was insufficient.
2. Usual trains lacked the pointed psychological training and the psychological education, and self-adjustment was insufficient under the emergency mode;
3. After the earthquake occurred, the psychological medicine workers were relatively deficient, and psychological unblocking measures weak. The above factors can cause the psychological illness of relief army officers and soldiers. The incidence rates of psychological illnesses such as depression and anxiety were lower than that of the disaster victims.<sup>14</sup> On the one hand because the very severe earthquake scene and the family member lose caused the enormous damage at heart, and the psychological wound was serious. On the other hand because disaster victims cannot estimate the future life condition, they easily had obviously sorrowful, despondent, anxious moods. Moreover the society and the government's positive propaganda, the care and the salute to rescue groups were helpful to enhance confidence, increase the value feeling, the sense of honor, and improve officers' and soldiers' condition at heart in a large degree.

In addition, we discovered that respiratory symptoms were mainly common cold performan-

ces such as cough, expectorating, pharyngalgia and nasal discharge. The reasons were as follows:

1. The earthquake relief work load was heavy physical ability consumes in a big way and the body was weary.
2. The seismic region environment was disorderly and the air pollution was heavy.
3. Do not adapt to the moist sultry climate and climate changes after the disaster such as the high temperature and rains.
4. The living in the open country, the centralized personnel and impeded ventilates created respiratory disease's dissemination easily. All above factors caused the increase of probability of the respiratory infections, but the serious breath diseases of hemoptysis, the scant of breath were seen seldom.

The digestive tract symptoms were mainly abdominal pain, diarrhea, distension of the abdomen, poor appetite and so on. The reasons were as follows: The food habit changed suddenly and the living conditions rapid changed, causing the function disorder of the stomach and intestines. The intense psychological stimulation caused the digestive stress response. After earthquake the country and the local government took the advantageous preventive measures such as strengthening the protection and the control of tap water, preventing the water source pollution, Using of the bottled water and paying attention to the health and disinfection, the serious intestinal infectious diseases were unpopular and the incidence rates of the digestive disease were lower. In view of the above survey results and the army reality, we suggest that we may strengthen the intensity of training and take the preventive action as following:

1. We should improve the army officers' and soldiers' daily life condition under emergency situation as far as possible and amend the emergency equipment including the clothing, the diet and the drugs. In this survey that the incidence rate of skin disease is extremely high in the army officers and soldiers, because soldiers cannot take a bath regularly, cannot change the clothes and lack the corresponding medicine. Similarly the respiratory diseases with higher incidence rate were related with weary body and the

dropped body resistance. Therefore the improvement of wartime living conditions and jury rig are imperative.

2. It is very important to pay great attention to the mental hygiene construction in army officers and soldiers usually, adopt the practical efficacious device and the measure to carry on the scientific psychological exercise and improve the mental hygiene quality. During the emergency we should strengthen psychological counseling and the opening, rich and optimize the methods to reduce the pressure fast. The concerned department must detail some psychological workers thorough disaster area and the army basic unit, carrying on specialized psychological unblocking to the soldiers and the disaster victims. The daily medical worker must grasp some mental hygiene general knowledge, while on duty discovering promptly and carrying on essential psychological unblocking and the treatment to the patients. 16
3. We should usually strengthen the pointed adaptation training in the adverse circumstance, trying to find out effective, suitable wartime demand rule of thumb and enhancing the adaptive ness to the adverse circumstance.17 We should further strengthen individual and the environmental sanitation management under the emergency mode. It is necessary that eliminating the vectors of mosquito, fly, mouse and preventing infectious diseases' popularity.
4. We must reasonably equip and assign the medical service resources and safeguard medical service way is unobstructed. It must be avoided that the lack of medical service resources with the waste coexisted in different area

## References

1. Ligon BL. *Infectious diseases that pose specific challenges after natural disasters: a review. Semin Pediatr Infect Dis* 2006;17(1):36–45.
2. Watson JT, Gayer M, Connolly MA. *Epidemics after natural disasters. Emerg Infect Dis* 2007;13(1):1–5.
3. Noji EK. *Earthquake. In Noji EK, editor. The public health consequences of disasters. New York: Oxford University Press* 1997: 135-178.
4. Aghababian RV, Teuscher J. *Infectious diseases following major disasters. Ann Emerg Med* 1992;21:362-367.
5. Schneider E, Hajjeh RA, Spiegel RA, Jibson RW, Harp EL, Marshall GA, et al. *A coccidioidomycosis outbreak following the Northridge, Calif, earthquake. JAMA* 1997;277:904-908.
6. Matsuoka T, Yoshioka T, Oda J, Tanaka H, Kuwagata Y, Sugimoto H, et al. *The impact of a catastrophic earthquake on morbidity rates for various illnesses. Public Health* 2000;114:249-253.
7. Benca J, Kalavsky E, Miklosko J, Rudinsky B, Taziarova M, McKenzie F. *Infectious diseases in disaster areas/ catastrophes. Neuro Endocrinol Lett.* 2007;28:40–43.
8. Toole MJ. *Communicable disease epidemiology following disasters. Ann Emerg Med* 1992;214:418-20
9. Chang CM, Lee LC, Connor KM, Davidson JR, Jeffries K, Lai TJ. *Posttraumatic Distress and Coping Strategies among Rescue Workers after an Earthquake. The journal of nervous and mental disease* 2003; 191: 391-398.
10. Hagh-Shenas H, Goodarzi MA, Dehbozorgi G, Farashbandi H. *Psychological Consequences of the Bam Earthquake on Professional and Nonprofessional Helpers. Journal of Traumatic Stress* 2005; 18: 477–483
11. Collins S. Long, A. *Working with the psychological effects of trauma: Consequences for mental health-care workers: A literature review. Journal of psychiatric and mental health nursing* 2003;10: 417–424.
12. Galea S, Nandi A, Vlahov D. *The epidemiology of post-traumatic stress disorder after disasters. Epidemiol Rev* 2005; 27:78-81.

13. Chen KT, Chen WJ, Malilay J, Twu SJ. *The Public Health Response to the Chi-Chi Earthquake in Taiwan, 1999. Public Health Reports 2003; 118: 493-499*
14. Han JQ, Li SJ, Yang YH, Ma YQ, Feng J, Xia WH, et al. *An epidemiological study on physical and mental health of inhabitants who experienced a catastrophic event of "5.12 serious earthquake in Wenchuan county". Natl Med J China 2008; 88: 3329-3331.*
15. Yang C, Wang HY, Zhong HJ, Zhou L, Jiang DM, Du DY, et al. *The epidemiological analyses of trauma patients in Chongqing teaching hospitals following the Wenchuan earthquake. Injury 2009, doi:10.1016/j.injury.2009.01.102.*
16. Eric K Noji. *Public health in the aftermath of disasters. BMJ 2005; 330: 1379-1381.*
17. Dai J, Zhao Y, Li G. *Wenchuan earthquake: response of Chinese dental professionals. Br Dent J 200; 206:273-276*

Corresponding author:

Weiqliang Zhang,  
Department of Thoracic Surgery,  
Beijing Military General Hospital,  
China,  
e-mail: zhangwq0715@yahoo.cn



# Lessons from an IV Drug abuser: Reform the blood safety surveillance measures

Mehrdad Jalalian Hosseini<sup>1,2</sup>, Reyhaneh Bazargani<sup>1</sup>, Sadegh Shokri<sup>3</sup>, Latiffah Latiff<sup>2</sup>, Syed Tajuddin Syed Hassan<sup>2</sup>, Mohammad Hosseinpour<sup>4</sup>

<sup>1</sup> Research center of Iranian Blood Transfusion Organization, Khorasan-e Razavi Blood Center, Iran

<sup>2</sup> Department of Community Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia

<sup>3</sup> Vice chancellery for treatment affairs, Mashhad University of Medical Sciences, Iran

<sup>4</sup> HIV Sector, Center for Disease Control, Vice chancellery for Health affairs, Mashhad University of Medical Sciences, Iran

## Abstract

An IV drug abuser donated his blood to check his HIV status for free. He concealed his past drug abuse, but appropriately chose the Confidential Unit Exclusion (CUE) option. Since the screening for transfusion transmissible infections tested negative, he tried to donate blood for a second time following a consultation session. This time, he declared his past drug behavior and was permanently rejected from further blood donation. Apart from the factors on the donor side, the health interview, donor notification, and post-CUE consultation procedures should also be sufficient for making such donors feel safe about stating their past history of risky behaviors.

## Case presentation

This report details how a blood center learned from an IV drug abuser of the need to improve the blood safety surveillance efficacy at the donor interview, consultation, and Confidential Unit Exclusion (CUE) system levels. A young married man in his mid 30s with a university degree, living in an urban area of Mashhad and working as a governmental employee, volunteered to donate blood in April 2007. The donor was determined to be emotionally stable during the health interview session. After successfully completing the health interview

and prior to blood donation, he chose the CUE option, designating that his blood should not be used for transfusion. Following the successful donation, his blood tested negative for routine screening tests. Due to the lack of post-CUE consultation and donor notification system for self-rejected donors, no feedback was collected from the donor. Three months later, the same donor contacted the blood center to ask about the donor testing results. Since any information regarding blood donors and their donation is kept confidential and the donor identity had to be verified in a face-to-face manner, he was referred to the consultation department.

At the consultation session, he indicated that an unsafe sex practice seven months prior to the donation was the main reason for his decision to choose CUE. The consultant was a female physician who had not been especially trained for counseling the blood donors infected with common transfusion transmissible infections; moreover, no psychologist was present for the consultation. Based on the routine screening test results, the physician informed the donor of his eligibility to donate blood one year after the last unsafe sex experience. Nine months later, in June 2008, the donor volunteered to donate his blood for a second time. He declared his IV drug abuse as the real reason for choosing the CUE option in his previous donation; consequently, the donor was permanently rejected from blood donation.

## Discussion

The donor serves as an overt example of those who volunteer to donate blood just to check their HIV status for free [1]. In the Mashhad blood center, as in other regional blood centers under the Iranian Blood Transfusion Organization, the task of screening and interviewing donors is done by physicians who have the required knowledge of blood safety and transfusion medicine and have been specially trained for counseling and interviewing blood donors. Based on the data from the digital databank of Mashhad Blood Center, 3.54% of all cases of permanent deferral of blood donation in the most recent two years were IV drug abusers. In addition, unpublished data from the Vice Chancellor of the treatment office of Mashhad University of Medical Sciences indicate that 24% of the customers of withdrawal clinics for opiate addiction treatment are IV drug abusers.

The donor in question was also an example of those who properly choose the CUE option. The application of the CUE system began following a 1986 FDA recommendation about providing the opportunity for self-exclusion for donors who think their blood is not safe for blood transfusion [2]. The history of introducing CUE at the Mashhad blood center dates back to the 1990s.

A good reason why the donor concealed his past history of IV drug abuse would be the mistrust of the blood center's privacy policy regarding the confidentiality of his private information. This is especially significant when sharing information regarding the infection or drug addiction with others, which may threaten the social position or job of donors. In addition, people sometimes volunteer to donate blood in groups. In such situations, the donor may feel pressured by others in the group to donate blood despite concerns of probable rejection by the physician during the health interview session because of a past history of high risk behaviors [3, 4]. A physician of the same sex might provide better communication to a donor to convince him or her to declare the past history of drug abuse, unsafe sex, or other common causes of donor rejection. Indeed, three months after the initial donation, the donor in question failed to correctly state the real reason of his CUE during the consultation session. A standard consultation

with a physician and a psychologist who are especially trained to counsel donors infected with common transfusion transmissible infections would be appropriate for convincing such donors not to conceal their past history of risky behaviors.

The good news from this particular donor was that he properly chose the CUE option during the first blood donation and ultimately stated his drug abuse, which resulted in being permanently rejected for blood donation. Maybe the factors that were evident in the donor (e.g., living in an urban area, possessing a university degree, and having a governmental job) are predictors of donors' understanding and appropriate use of CUE. The bad news could be his concealment of the drug abuse in the second blood donation, which could have subsequently increased the rate of "Remained Risk of Transfusion Transmissible Infections".

## Conclusion

The significance of this case was that it demonstrated the need to pay more attention to the factors affecting outcomes of both health interviews with blood donors and consultations with infected blood donors. It is also essential to provide an atmosphere in which donors—especially first-time donors who are not still familiar with blood centers—realize that the center's employees respect their privacy and confidentiality. A post-CUE consultation should be a routine procedure as well as employing trained physicians and psychologists in consultation department, which has proved to be helpful. Additional noteworthiness of the case was that the in-depth analysis of it shed light on future research. Based on the experience of this case, several hypotheses were developed regarding the factors associated with the appropriate use of CUE.

## References

1. Lau JT, Thomas J, Lin CK: *HIV-related behaviors among voluntary blood donors in Hong Kong. AIDS CARE 2002; 14(4):481–492*
2. *Department of Health and Human Services, Food and Drug Administration: Additional recommendation for reducing further the number of units of blood and plasma donated for transfusion or for further manufacture by persons at increased risk of HTLV-3/LAV infection. Memorandum to all registered blood establishments, October 30, 1986.*
3. Sally V. Rudman: *Textbook of Blood Banking and Transfusion medicine. Second edition, 2005*
4. Jeanne V. Linden, Celso Bianco: *Blood Safety and surveillance, 2001*

*Corresponding Author:*  
*Mehrdad Jalalian Hosseini*  
*Research center of Iranian Blood Transfusion Organization,*  
*Khorasan-e Razavi Blood Center,*  
*Iran*  
*e-mail: mehrdad.medic@gmail.com*

# Dexamethasone / human chorionic gonadotrophin (hCG) and dexamethasone / nafarelin as a valid diagnostic tests in reproductive aged women with polycystic ovarian syndrome (PCOS)

## DEKSAMETASON /HUMANI HORIONIŠKI GONADOTROPIN (HCG) AND DEKSAMETASON/NAFARELIN SU VALIDNI DIJAGNOSTIČKI TESTOVI U REPRODUKCIJSKOJ DOBI ŽENE SA POLICISTIČNIM OVARIJSKIM SINDROMOM (PCOS)

Hajder E.<sup>1</sup>, Hajder M.<sup>2</sup>

<sup>1</sup> University Clinical Center Tuzla, Gynecology and Obstetrics clinic, Bosnia and Herzegovina.

<sup>2</sup> University Clinical Center Tuzla, Internal clinic, department of Endocrinology, Bosnia and Herzegovina,

### Abstract

**Introduction.** Hyperandrogenism is a functional, diagnostic and therapeutic problem of women in reproductive age. The aim of this study was to determine the validity of dexamethasone-human chorion gonadotrophin (DEXA/hCG) and dexamethasone-nafarelin (DEXA/N) testing in the differential hyperandrogenism diagnosis in differentiating polycystic ovary syndrome (PCOS) from other forms of hyperandrogenism.

**Subjects and methods.** Prospective study included 30 women of reproductive age with PCOS, selected by diagnostic Rotterdam criteria (group A) and 12 women of reproductive age who are not PCOS (group B). Subgroup A1 (n = 15) and B1 (n = 6) were treated with DEXA/hCG test, subgroups A2 (n = 15) and B2 (n = 6) were treated with

DEXA/N test. All respondents had the same parameters: cortisol, dehydroepiandrosterone sulphate (DHEAS), total testosterone, SHBG, free androgen index (FAI) and 17-hydroxyprogesterone (17-OHP) basal, during and after testing.

**Results:** The suppression with dexamethasone during (DEXA /hCG) test in subgroups A1 and B1, the fourth day was followed by a significant decline in average values ( $p < 0.05$ ) DHEAS, testosterone, SHBG, free androgen index (FAI) and 17-OHP in relation to the basal value. After the 5000 IU hCG subgroup A1 was followed by a significant increase 17-OHP ( $p < 0.001$ ), greater than 7 nmol / l, while in subgroup B1 there was significant increase in value ( $p < 0.01$ ) less than 5 nmol/l in relation to the value before giving hCG. In DEXA / N after the suppression test in subgroups A2 and B2 there was a significant decline



in average values of ( $p < 0.05$ ) DHEAS, testosterone, SHBG, FAI and 17-OHP on the fourth day of the test when compared to the basal value. After the injection of nafarelin ampoules of 100 ug in subgroup A2 there was a significant increase in the value of 17-OHP ( $p < 0.001$ ), greater than 7 nmol/l, while the significant increase in subgroup B2 ( $p < 0.01$ ) was 5 nmol /l in relation to the value before injection of nafarelin. Average value of 17-OHP in the DEXA /N test was significantly higher ( $p < 0.001$ ) when compared to the average value of 17-OHP in the DEXA /hCG test in subjects with PCOS.

**Conclusion:** The results of this study showed that DEXA /hCG and DEXA/N are valid tests for the diagnosis of PCOS, but the nafarelin test is more significant.

**Key words:** PCOS, dexamethasone/hCG test, dexamethasone/nafarelin test.

## Sažetak

**Uvod.** Hiperandrogenizam je veliki funkcionalni, dijagnostički i terapijski problem u reproduktivnoj dobi žene.

**Cilj** ove studije bio je da se utvrdi validnost dexamethason/humani horionski gonadotropin (DEXA/hCG) i dexamethason/nafarelin (DEXA/N) testova u diferencijalnoj dijagnozi hiperandrogenemije u razlikovanju policističnog ovarijskog sindroma (PCOS) od drugih oblika hiperandrogenizma.

**Ispitanici i metode.** Prospektivnom studijom obuhvaćeno je 30 žena reproduktivne dobi sa PCOS, odabranih po Rotterdam dijagnostičkim kriterijima (grupa A) i 12 žena reproduktivne dobi koje nemaju PCOS (grupa B). Podgrupa A1 ( $n=15$ ) i B1 ( $n=6$ ) su tretirane DEXA/hCG testom, podgrupe A2 ( $n=15$ ) i B2 ( $n=6$ ) su tretirane DEXA/N testom. Svim ispitanicama su određeni isti parametri: kortizol, dehidroepiandrosteron sulfat (DHEAS), ukupni testosteron, SHBG, slobodni androgeni indeks (FAI) i 17-hidroksiprogesteron (17-OHP) bazalno, u toku i nakon testiranja.

**Rezultati:** Nakon supresije dexamethasonom tokom (DEXA/hCG) testa u podgrupama A1 i B1, četvrtog dana uslijedio je signifikantan pad srednjih vrijednosti ( $p < 0.05$ ) DHEAS, testoste-

rona, SHBG, slobodni androgeni indeks (FAI) i 17-OHP u odnosu na bazalne vrijednosti. Nakon davanja 5000 i.j. hCG u podgrupi A1 uslijedio je signifikantan porast vrijednosti 17-OHP ( $p < 0.001$ ), veći od 7 nmol/l, dok je u podgrupi B1 signifikantni porast vrijednosti ( $p < 0.01$ ) bio manji od 5 nmol/l u odnosu na vrijednosti prije davanja hCG. U (DEX/N) testu nakon supresije, u podgrupama A2 i B2 uslijedio je signifikantan pad srednjih vrijednosti ( $p < 0.003$ ) DHEAS, testosterone, SHBG, FAI i 17-OHP četvrtog dana testa u odnosu na bazalne vrijednosti. Nakon injiciranja ampule nafarelina od 100 ug, u podgrupi A2 uslijedio je signifikantan porast vrijednosti 17-OHP ( $p < 0.001$ ), veći od 7 nmol/l, dok je u podgrupi B2 signifikantni porast ( $p < 0.01$ ) bio manji od 5 nmol/l u odnosu na vrijednosti prije injiciranja nafarelina. Srednja vrijednost 17-OHP u DEX/N testu bila je signifikantno viša ( $p < 0.001$ ) u odnosu na srednju vrijednost 17-OHP u DEX/hCG testu u ispitanica sa PCOS.

**Zaključak:** Rezultati ove studije su pokazali da su DEXA/hCG i DEXA/N testovi validni za dijagnozu PCOS, ali je nafarelin test signifikantniji.

**Ključne riječi:** PCOS, dexamethason-hCG test, dexamethason-nafarelin test.

## Introduction

Clinical hyperandrogenism (hirsutism, acne, alopecia, seborrhea, menstrual disorder, infertility) is a problem in reproductive age women with androgen hypersecretion. Androgens can stimulate the ovaries, adrenal glands, or both together, or arise from peripheral conversion of androstenedione. Androgen found in the following breakdown diseases: androgen active tumors, non-classical congenital adrenal hyperplasia (NCAH), hyperandrogenism with insulin resistance and acanthosis nigricans (HAIRAN), idiopathic hirsutism (IH), hyperandrogenemia with hirsutism in PCOS. About 82% of the total hyperandrogenism is result of PCOS (1) The various forms of hyperandrogenism, use functional endocrine tests for their diagnosis. Low doses of dexamethasone suppressive test (48h/2mg LDDST) is a method to distinguish between screening tumor and functional hyperandrogenism (2). Role of adrenal gland

in PCOS is known, because the abnormal regulation of the cytochrome P450c17-alpha, cause abnormal function of enzymes in the ovary and adrenal glands in sintesy of androgen 17-OHP (3). Disregulation of adrenocortical cytochrome P450c17-alpha causes the increase of 17-OHP after ACTH stimulation. Hyperandrogenemia ih about 40-60% of women has elevated 17-OHP response to ACTH stimulation (4). DHEAS is in 95% synthesized in the adrenal cortex, but is often elevated in PCOS for more than 50% (5). Also, women with PCOS have elevated 17-OHP after GnRH stimulation (6). Good hormonal response to GnRH agonists injected nafarelin proved to be a good diagnostic test in testing shafts hypothalamus-pituitary-gonads. Numerous studies showed that the response to other GnRH agonists were similar to response to nafarelin (7,8,9,10). Significant difference in response was found after the test of nafarelin and leuprolid of 10ug/kg, except that the same doses of nafarelin gave 10 times stronger response (11). Good growth of 17-OHP values after injection of 5000 IU HCG showed a good diagnostic test for the diagnosis of PCOS. (12) The aim of this study was to determine the validity of the functional tests DEXA /hCG and DEXA / N for the differential diagnosis in functional ovarian hyperandrogenism (FOH).

### Subjects and methods.

Prospective study included 30 women of reproductive age under suspicion of PCOS, selected by Rotterdam criteria (13), 18-35 years old, with signs of hyperandrogenism, menstrual disorders and infertility. The study covers treatment of infertile patients during the period from 2007th to 2009th. Excluded from the study were patients who had NCAH, hyperprolactinemia, Sy Cushing, androgen active ovarian tumors and adrenal glands. Patients with PCOS were divided into two subgroups: 15 were tested with DEXA / hCG test (group A1), 15 was tested with DEXA / N test (group B1). The control group consisted of 12 healthy women of reproductive age between 18-35 years of age, of which six were treated with DEXA /hCG test (subgroup A2) and six of them with DEXA / N test (subgroup B2). Respondents with PCOS and

those in the control group had the same parameters. With DEXA/hCG test in high follicular phase of the menstrual cycle, we analyzed the basal levels of hormones and hormone levels during the fourth day of therapy with dexamethasone (cortisol, DHEAS, testosterone, SHBG, 17-OHP). The subjects were treated daily with 4x1 tablets of 0.5 mg of dexamethasone (dexametazon, Krka, Novo Mesto, Slovenia) during the five days. On the fourth day of testing, we analyzed the levels of hormones, then injected 5000 IU of hCG (Chorionom, IBSA, Lugano, EU)ni them, and the fifth day of the test the level of 17-0Hp were determined.

### Dexamethasone-Nafarelin test

Same procedure was then carried out, as in DEXA /hCG test, with the 100 ug of Nafarelin, instead of hCG.

**Definition:** Hirsutism is defined based on the Ferriman-Gallwey (FG) score greater than 8 (14), ovulatory dysfunction was defined by less than 8 menstrual cycles per year or luteal progesterone less than 9.54 nmol / l, menstrual cycle dysfunction more than 34 days and less than 21 days (15). Metabolic syndrome is defined by the recommendation of International Diabetes Federation (IDF) (16). Biochemical hyperandrogenism is defined if the total testosterone is greater than 2.08 nmmol / L, DHEAS greater than 7.8 umol / L. If the total testosterone (UT) is greater than 7.2 nmol / L and greater than DHEAS 20.8 umol / l there was doubt on the existence of active androgenic tumors, and elevated serum 17-OHP over 9.1 nmmol / L and elevated peak higher after ACTH of 30 .3 nmmol / L is considered NCAH (17). HOMA-IR greater than 2.16 and QUICKI less 0.34 than they were a sign of insulin resistance (IR) and reduced insulin sensitivity, (IS), counted by the formulas (18). Free androgen index (FAI) was counted by the formula = (total testosterone (nmmol/L) x 100 / SHBG (nmmol / L) (19). Transvaginal color Doppler (TVCD) criterion was: more than 12 follicles in the ovary, the size of 2-9 ml, ovarian volume greater than 10 ml, increased ovary Stroma (formula =  $p / 6 (DB1 \times DB2 \times DB3)$  (21).

**Laboratory assays:** Taking blood for determination of insulin and fasting glucose. Insulin

( $\mu\text{IU} / \text{ml}$ ) was determined by RIA (direct radio-immunoassay) on Wallec automatic counter (Wizard) Turku Finald company. INSI-CKIT Irma was used, firm DiaSorin, Italy. Insulin sensitivity was calculated by formula:  $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{IU} / \text{ml}) \times \text{fasting glucose } (\text{mmol} / \text{L}) / 22.5$ , higher finding greater than 2.16.  $\text{QUICK} = 1 / \log \text{fasting inzulinan } (\mu\text{IU} / \text{ml}) + \log \text{fasting glucose } (\text{mg} / \text{dl})$ . DHEAS, 17-OHP, total T, 17-OHP, SHBG, as determined by RIA (direct radio-immunoassay) on Wallec automatic counter (Wizard) Turku Finald company. The original IRMA kits for individual hormone IMMUNOTHEC a Beckman Coulter Company, France were used.

Oestradiole, Pg, FSH, LH, prolactin, testosterone were determined by the method of the apparatus Fluroimmunoassay Wallace, DELFIA FLUROMETER. The original DELFIA KITS for individual hormone Turku, Finald were used. . Glucose in the hospital conditions is measured by enzym colorimetrics method (Glucose GOG-PAP) on the unit VP Super System, diagnosis Division, USA. Estimation of free testosterone was calculated by the formula  $\text{FAI} = \text{total testosterone } (\text{nmol/L}) \times 100 / \text{SHBG } (\text{nmol} / \text{L})$  (ref.  $\leq 3$ )<sup>(15)</sup>.

**Statistical analysis:** Collected data were entered in a specially created database on personal computer. Statistical processing is carried out under

Table 1. Clinical and basal hormonal characteristics of polycystic ovary syndrome (PCOS) and control subjects

|                                                   | PCOS<br>N=30    | CONTROL<br>n=15 | PCOS vs.<br>control |
|---------------------------------------------------|-----------------|-----------------|---------------------|
| Age                                               | 29.2 (17-37)    | 29 (18-35)      | p > 0.4             |
| Age of menarche                                   | 12.5 (11-14)    | 11.6 (11-15)    | p > 0.9             |
| BMI (kg/m <sup>2</sup> )                          | 29.7 $\pm$ 9.6  | 26.1 $\pm$ 4.1  | p < 0.03            |
| Vaist circumference (cm)                          | 89 (76-104)     | 76 (64-88)      | p < 0.001           |
| Obesity (%)                                       | 17 (58%)        | 5 (30%)         | p < 0.03            |
| Metabolic Syndrome (%)                            | 8 (31.1%)       | 2 (20%)         | p < 0.05            |
| Acne/ seborrhoea (%)                              | 12 (41.2%)      | 4 (30.4%)       | p > 0.6             |
| Hirsute (%) F-G score                             | 22 (75.7%)      | 4 (40%)         | p < 0.05            |
| Infertile (%)                                     | (82.7%)         | 1 (10%)         | p < 0.001           |
| PCOM, 10 ml (%)                                   | (75.9%)         | (40%)           | p < 0.004           |
| Insulin fasting ( $\mu\text{U}/\text{mL}$ )       | 14.4 (11-21)    | 9.8 (7.2-15)    | p < 0.03            |
| Insulin 120 min. OGTT ( $\mu\text{U}/\text{mL}$ ) | 74 (25.-80.6)   | 35.2 (21-75)    | p < 0.007           |
| Glucose fasting (mmol/L)                          | 4.7 (4.1-5.4)   | 4.6 (4.3-4.9)   | p > 0.09            |
| Glucose in 120 min. OGTT                          | 5.81 (4.6-6.7)  | 5.8 (5.1-6.2)   | p > 0.7             |
| HOMA-IR (ref. < 2.16)                             | 3.2 (2.4-5.7)   | 2.0 (1.9-2.5)   | p < 0.003           |
| QUICKI (ref. > 0.34)                              | 0.36 (0.2-0.5)  | 0.47 (0.3-0.6)  | p < 0.05            |
| LH/FSH ratio                                      | 1.6 (0.8-1.9)   | 0.9 (0.7-1.4)   | p < 0.04            |
| Estradiol (pmol/L)                                | 161 (112.-245)  | 218.4 (180-272) | p < 0.006           |
| Pg -luteal (nmol/L)                               | 4.6 (2.3-18.1)  | 38.6 (16.5-53)  | p < 0.001           |
| Prolactin (ng/ml)                                 | 10.6 (7.6-13.1) | 9.5 (6.2-13.1)  | p > 0.08            |
| Total testosterone (nmol/L)                       | 2.8 (1.4-2.5)   | 1.52 (1.3-1.9)  | p < 0.03            |
| SHBG (nmol/L)                                     | 28.3 (15.1-54)  | 51.6 (37.-72.8) | p < 0.006           |
| FAI (range 0-3)                                   | 10.7 (8.2-24.6) | 2.9 (1.5-4.6)   | p < 0.001           |
| DEHEAS (nmol/L)                                   | 7.3 (3.9-8.9)   | 6.5 (2.7-9.5)   | p > 0.81            |
| 17-OHP (nmol/L)                                   | 3.1 (2.6-6.1)   | 3.4 (2.4-4.6)   | p > 0.061           |

Note: Parametri su izraženi kao srednja vrijednost  $\pm$  SD, kao median (99% confidence interval), FAI= free androgen indx, HOMA=homeostasis model assessment, QUICKI=quantative insulin sensitivity index, DHEAS-dehydroapiandrosterone sulphate, OHP-17 hydroxy progesterone. SHBG=sex hormone-binding hormone. LH=luteinizing hormone, FSH=follicle stimulating hormone. Significant values (\*) p < 0.05 for PCOS vs. control, NS=no significant

application software SPSS for Windows version 12<sup>th</sup> Numerical data are presented as mean  $\pm$ SD, the median number is expressed in %. To test the hypothesis within groups t-test, Fisher exact test was used, with significance level of  $p < 0.05$ .

## Results

Comparison between PCOS group vs. control group (Table 1).

When comparing women with PCOS and control groups, the first had significantly higher BMI ( $p < 0.03$ ), the waist circumference (OS) ( $p < 0.001$ ), and greater representation of the metabolic syndrome ( $p < 0.05$ ), PCOM ( $p < 0.004$ ), fasting insulin ( $p < 0.03$ ), 2h after OGTT insulin ( $p < 0.007$ ), HOMA-IR ( $p < 0.003$ ) and significantly lower QUICK index ( $p < 0.05$ ). Women with PCOS had significantly high hirsutism prevalence ( $p < 0.05$ ), and a higher rate of infertility ( $p < 0.001$ ) in comparison to healthy control group. Total testosterone ( $p < 0.03$ ) and FAI ( $p < 0.001$ ) were significantly higher, whereas SHBG ( $p < 0.006$ ) was significantly lower in women with PCOS when compared to control group. Women with PCOS had significantly elevated LH / FSH ratio ( $p < 0.04$ ), low luteal Pg ( $p < 0.001$ ) and lower follicular oestradiol ( $p < 0.006$ ) in comparison to the control group of healthy women.

After the suppression of DEXA /hCG test in women with PCOS there was a significant drop in cortisol ( $p < 0.01$ ), DHEAS ( $p < 0.01$ ), testosterone ( $p < 0.05$ ), FAI ( $p < 0.01$ ), 17-OHP ( $p < 0.05$ ) in comparison to basal values before therapy with dexamethasone and a significant increase in 17-OHP ( $p < 0.001$ ) in the HCG test, greater than 7 nmol / L compared to the value prior to HCG (table 2). After the suppression of DEXA /hCG test in healthy women there was a significant drop in cortisol ( $p < 0.01$ ), DHEAS ( $p < 0.01$ ), testosterone ( $p < 0.01$ ), FAI ( $p < 0.05$ ), 17-OHP ( $p < 0.05$ ), when compared to the basal values before dexamethasone therapy and significant increase in 17-OHP ( $p < 0.01$ ) in the HCG test, less than 5 nmol / l in relation to the value prior to HCG. (Table 2)

After the suppression of DEXA / N test in women with PCOS there was a significant drop in cortisol ( $p < 0.01$ ), DHEAS ( $p < 0.01$ ), 17-OHP ( $p < 0.01$ ) testosterone ( $p < 0.05$ ), FAI ( $p < 0.01$ ) when compared to the basal dexamethasone values before therapy and a significant increase in 17-OHP ( $p < 0.001$ ) in the nafarelin test, higher than 7 nmol / L when compared to values before nafarelin therapy (Table 3). After the suppression of DEXA / N test in healthy women there was a significant drop in cortisol ( $p < 0.01$ ).

TABLE 2. Dexamethasone /human chorionic gonadotrophin (hCG) test

| Parameters           | Before Dexamethasone |                 | After Dexamethasone          |                           | HCG test                   |                             |
|----------------------|----------------------|-----------------|------------------------------|---------------------------|----------------------------|-----------------------------|
|                      | PCOS                 | CONT.           | PCOS                         | CONT.                     | PCOS                       | CONT.                       |
| Cortisol (nmol/L)    | 412 $\pm$ 150        | 414 $\pm$       | <50 $\diamond^b$             | <50 $\Delta^b$            |                            |                             |
| DHEAS ( $\mu$ mol/L) | 9,3 $\pm$ 4,2        | 7,6 $\pm$       | 3,2 $\pm$ 1,2 $\diamond^b$   | 1,2 $\pm$ $\Delta^b$      |                            |                             |
| UT (nmol/L)          | 2,9 $\pm$            | 1,9 $\pm$       | 1,4 $\pm$ 0,5 $\diamond^a$   | 0,9 $\pm$ $\Delta^b$      |                            |                             |
| FAI (nmol/L)         | 9,1 $\pm$ 3,6        | 2,1 $\pm$ 1,4   | 4,6 $\pm$ 0,8 $\diamond^b$   | 0,8 $\pm$ 0,12 $\Delta^a$ |                            |                             |
| SHBG (nmol/L)        | 31 $\pm$ 5,2         | 64 $\pm$ 38     | 33 $\pm$ 4,2                 | 65 $\pm$ 3,6              |                            |                             |
| 17-OHP (nmol/L)      | 5,42 $\pm$ 0,32      | 4,70 $\pm$ 0,32 | 2,14 $\pm$ 0,34 $\diamond^a$ | 0,9 $\pm$ 0,10 $\Delta^a$ | 8,9 $\pm$ 1,2 $\ddagger^c$ | 4,20 $\pm$ 0,56 $\dagger^b$ |

<sup>a</sup> $P < 0,05$ ; <sup>b</sup> $P < 0,01$ ; <sup>c</sup> $P < 0,001$ ; PCOS  $\diamond$  Dexamethasone before vs.after; Control  $\Delta$  Dexamethasone before vs.after; PCOS  $\diamond \ddagger$  hCG before vs.after; control  $\Delta \dagger$  hCG before vs.after, DHEAS dehydroepiandrosterone; UT, ukupni testosteron; FAI, free androgen indeks; SHBG, seks hormone-binding globulin; 17-OHP, 17-hydroxyprogesterone.



**TABLE 3. Dexamerhasone /nafarelin test**

| PARAMETRI         | Before dexamethasone |           | After dexamethasone   |                         | NAFARELIN              |                         |
|-------------------|----------------------|-----------|-----------------------|-------------------------|------------------------|-------------------------|
|                   | PCOS                 | CONT.     | PCOS                  | CONT.                   | PCOS                   | CONT.                   |
| Cortisol (nmol/L) | 410±120              | 398±120   | <50 ∅ <sup>b</sup>    | <50Δ <sup>b</sup>       |                        |                         |
| DHEAS (μmol/L)    | 10,1±3,6             | 7,2±1,6   | 3,1±1,4∅ <sup>b</sup> | 1,4±0,9Δ <sup>b</sup>   |                        |                         |
| UT (nmol/L)       | 3,1±0,13             | 1,36±0,15 | 1,3±0,6∅ <sup>a</sup> | 0,8±0,4Δ <sup>b</sup>   |                        |                         |
| FAI (nmol/L)      | 9,4±4,2              | 2±2,2     | 4,1±0,9∅ <sup>b</sup> | 0,9±0,10Δ <sup>b</sup>  |                        |                         |
| SHBG (nmol/L)     | 32±4,3               | 62±48     | 34±3,1                | 63±3,8                  |                        |                         |
| 17-OHP (nmol/L)   | 5,62±0,2             | 4,60±0,38 | 2,1±0,3∅ <sup>b</sup> | 0,72±0,11Δ <sup>b</sup> | 16,5±0,8 <sup>‡c</sup> | 4,52±0,30 <sup>†b</sup> |

<sup>a</sup>P<0,05; <sup>b</sup>P<0,01; <sup>c</sup>P<0,01; PCOS ∅ Dexamethasone before vs.after; Control Δ Dexamethasone before vs.after; PCOS ∅<sup>‡</sup> nafarelin before vs.after ; Control Δ<sup>†</sup> .nafarelin before vs. after;

DHEAS dehydroepiandrosterone; UT, totali testosteron; FAI, free androgen indeks; SHBG, sex hormone-binding globulin;

17-OHP, 17-hydroxyprogesterone.

DHEAS (p< 0.01), 17-OHP (p<0.01) testosterone (p< 0.01), FAI (p< 0.01) when compared to the basal values before dexamethasone therapy and significant increase in 17-OHP (p< 0.01) in the nafarelin test, less than 5 nmol / l in relation to the value before nafarelin (Table3).

Average value of 17-OHP in DEXA / N test was significantly higher (p< 0.001) compared to the average value of 17-OHP in DEXA /hCG test in women with PCOS, while the control group did not have any significant difference in the value of 17-OHP (NS). In comparison to the control group, value 17-OHP in women with PCOS was significantly higher in DEXA / N (p < 0.001) and DEXA /hCG test (p< 0.01) (Figure 1).

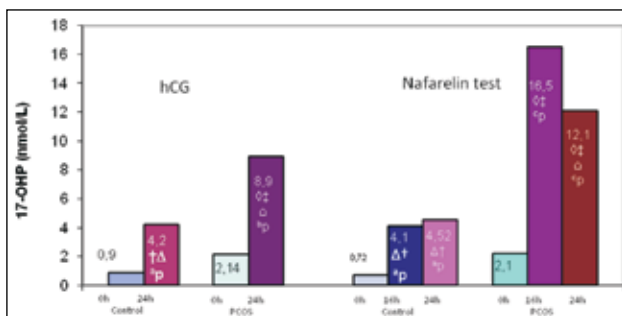


Figure 1. 17-OHP concentration after hCG and nafarelin stimulation (mean ±SEM) in women with Polycystic ovary syndrome (PCOS). †Δ control before vs. after; ∅<sup>‡</sup> PCOS before vs. after; ∅ PCOS 24 h hCG vs. nafarelin; <sup>a</sup>P<0,05; <sup>b</sup>P<0,01; <sup>c</sup>P<0,001;

**Discussion**

Hyperandrogenism is a functional problem in women of reproductive age, manifested through hirsutism, acne / seborrhoea, oligo-anovulatory disorders, obesity,infertility. Approximately 85% of the total hypeandrogenism belongs to PCOS. (1). Low dose of dexamethasone suppressive test (2mg/48h LDDST) is the possibility to differentiate the functional tumor hyperandrogenism. Good suppression of cortisol, with values of less than 50 nmol / l excludes cancer, ovarian cancer and adenoma of adrenal gland. If suppression of UT is less than 40% of basal values, the suppression of cortisol greater than 50 nmol / L, this indicates the existence of malignant ovarian secretory tumors and adrenal glands that secrete androgens. LDDST shows 100% sensitivity and 88% specificity in identifying patients with androgen secretory tumors. Suppression of cortisol less than 50 nmol / l, with suppression of testosterone less than 40% of basal value indicates the existence of a benign ovarian tumors and adrenal cortex. Good suppression of cortisol levels (less than 50 nmol / l) and DHEAS, with the weak suppression of testosterone, requires additional nafarelin or hCG test, to be separated from other forms of PCOS FOH (2). The results of this study showed that DEXA / hCG and DEXA / N are valid tests in distinguishing PCOS from other forms of FOH. 17-OHP in-



crease in both the test above 7 nmol / l confirmed the diagnosis of PCOS. Adrenal gland under the control of ACTH has an important role in the pathogenesis of PCOS. Cytochrome P450c17-alpha is a key enzyme in steroidogenesis androgens in the ovary and adrenal gland. Abnormal regulation of cytochrome P450c17-alpha in the ovary and adrenal glands causes a rise in 17-OHP, a forerunner of the synthesis of cortisol and other androgens (3,4). Rosenfield et al. indicate that abnormal regulation of cytochrome P450c17-alpha causes a hyperandrogenism originated from the ovary and adrenal cortex (22). Approximately 40-60% of women with hyperandrogenemia have excessive adrenal androgen response to ACTH stimulation (3). Androgen DHEAS is encouraging 95% of adrenal glands in healthy women, but in women with PCOS the origin of 50% DHEAS is ovarian (5). Therefore, it is necessary to suppress the function of adrenal gland with dexamethasone, and then GnRH agonist and hCG stimulates steroidogenesis in the ovaries and increase 17-OHP after stimulation of ovarian origin. Women with PCOS have elevated 17-OHP response to nafarelin stimulation with GnRH agonist (6,23). Results of other studies have showed similar results, obtained with different values, depending on the GnRH agonist used, with and without using dexamethasone test. Sahin et al. indicates that the 17-OHP response after ACTH was significantly higher in women with PCOS when compared to control group (3). Fulghesu et al. indicate that women with PCOS with increased ovarian stroma cancer have an increased response to LH, 17-OHP, testosterone, androstenedione and after stimulation with GnRH agonist nafarelin when compared to a control group of healthy women and women with PCOS with normal ovarian stroma (24). Levrant et al. indicate that the increase in 17-OHP was significant in dexamethasone and nafarelin and HCG test in comparison to a control group of healthy women, and HCG test can be used as a diagnostic test for FOH (12). In conclusion, the combined five-day DEXA /hCG and DEXA / N tests were significant in the differentiation of tumor hyperandrogenism FOH, and are also significant in distinguishing PCOS from other forms of FOH.

## References

1. Azziz R, Sanchez LA, Knochelhauer MC, Lazenby J, Stefens KC, Taylor K and Boots LR. Androgen excess in women: Experience with over 1000 consecutive patients. *The J Clin Endocrinol Metab* 2000;89(2):453-462.
2. Kaltsas GA, Isidori AM, et al. The value of the low-dose dexamethasone suppression test in the differential diagnosis of hyperandrogenism in women. *J Clin Endocrinol Metab* 2003;86(6):2634-43.
3. Sahin Y, Kelestimur F. 17-Hydroxyprogesterone response to gonadotrophin releasing hormone agonist busorelin and adrenocorticotrophin in polycystic ovary syndrome: investigation of adrenal and ovarian cytochrome P450c17-alfa dysregulation. *Human Reproduction* 1997;12:910-913.
4. Auchus RJ, Leedh et Miller WL. The regulation of human P450c17 activity: relationship to premature adrenarche and polycystic ovarian syndrome. *Trends in Endocrinology and Metabolism* 1998 ; 9 : 47-50.
5. Barnes RB. Hyperandrogenism, polycystic ovary syndrome, and hirsutism. *Curr Opin Endocrinol Diab* 1994; 1: 200-205.
6. Barnes RB, Rosenfield RL et al. Pituitary-ovarian responses to nafarelin testing in the polycystic ovary syndrome. *N Eng J Med* 1989; 320: 559-565.
7. Rosenfield RL, Barnes RB and Ehrmann DA. Studies of the nature of 17-hydroxyprogesterone hyperresponsiveness to gonadotrophin releasing hormone agonist challenge in functional ovarian hyperandrogenism. *J Clin Endocrinol Metab* 1994; 79: 1686-1692.
8. Sahin Y, and Kelestimur F. 17-hydroxyprogesterone response to busorelin testing in the PCOS. *J Clin Endocrinol* 1993; 39: 151-155.
9. Chang PL, Lindheim SR, et al. Normal ovulatory women with polycystic ovaries have hyperandrogenic pituitary ovarian responses to GnRH agonist testing. *Journal of Clinical Endocrinology and Metabolism* 2000;85:995-1000.
10. White D, Leigh A, Wilson C. et al. Gonadotropin and gonadal steroid response to a dose of long-acting agonist of gonadotropin-releasing hormone in ovulatory and anovulatory women with polycystic ovary syndrome. *Clin Endocrinol(Oxf)* 1995; 42:475-481.

11. Rosenfield RL, Perovic N, et al. Acute hormonal responses to the gonadotropin releasing hormone agonist leuprolide: dose –response studies and comparison to nafarelin –a clinical research center study. *Journal of Clinical Endocrinology and Metabolism* 1996; 81:3408-34..
12. Levrant SG, Barnes RB and Rosenfield RL. A pilot study of the human chorionic gonadotrophin test or ovarian hyperandrogenism. *Human Reproduction* 1997; 12: 1416-1420.
13. The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Work-shop Group 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Human Reproduction* 2004; 19: 41-47.
14. Legro RS, Myers ER, Barnhart HX, et al. The Pregnancy in Polycystic Ovary Syndrome Study: baseline characteristics of the randomized cohort including racial effect. *Fertil Steril* 2006;86:914.
15. Marco CA, Aldo G, et. Al. The evolution of metabolic parameters and insulin sensitivity for a more robust diagnosis of the polycystic ovary syndrome. *Clinic Endocrinology* 2008;69:52-60.
16. Alberti KG, Zimmet P, Shaw J. IDF, Epidemiology Task Force Consensus Group. The metabolic syndrome-a new worldwide definition. *Lancet* 2005; 336:1059-62.
17. Carmina E, Lobo RA. Ovarian suppression reduces clinical and endocrine expression late onset congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Fertil Steril* 1994;62:738-743.
18. Belli SH, Graffigna MN, et al. Effect of rosiglitazone, growth factors and reproductive disturbances in women with polycystic ovary syndrome. *Fertil Steril* 2004;81: 624-629.
19. Legro RS, Myers ER, Barnhart HX, et al. The Pregnancy in Polycystic Ovary Syndrome Study: baseline characteristics of the randomized cohort including racial effect. *Fertil Steril* 2006;86:914-33.
20. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22:1462-1470.
21. Orsini LF, Venturoli S, et al. Ultrasonic findings in polycystic ovarian disease. *Fertility and Sterility* 1985;43:709-714.
22. Rosenfield RL, Bornes RB, et al. Disregulation of cytochrome P450c17alpha as a cause of polycystic ovary syndrome. *Fertil Steril* 1990;53:785-791.
23. Ghai K, Cara J and Rosenfield R. Gonadotropin releasing hormone agonist (nafarelin) test to differentiate gonadotropin deficiency from constitutionally delayed puberty in teen-age boys a clinical research center study. *J Clin Endocrinol Metab* 1995;80: 2980-2986.
24. Fulgesu AM, Angioni S, Beleosi C, Apa R, Selvaggi L, Ciampelli M, Luculano A, Melis BG, and Lanzone A. Pituitary – ovarian response to the gonadotrophin-releasing hormone-agonist test in anovulatory patients with polycystic ovary syndrome: predictive role of ovarian stroma. *Clinical Endocrinology* 2006;65:396-401.

Corresponding author:

Hajder E.  
 University Clinical Center Tuzla,  
 Gynecology and Obstetrics clinic,  
 Bosnia and Herzegovina,  
 e-mail: ensarh@bih.net.ba

# Results of treatment of simple unstable uncomplicated lower leg shaft fractures treated operatively and nonoperatively

Fuad Dzankovic<sup>1</sup>, Dijana Avdic<sup>2</sup>, Amra Macic-Dzankovic<sup>3</sup>

<sup>1</sup> Clinic for ortopaedic and traumatology, University of Sarajevo, Clinical Centre, Bosnia and Herzegovina

<sup>2</sup> Department for physiotherapy, Clinic for orthopaedic and traumatology, University and Clinical Centre, Bosnia and Herzegovina

<sup>3</sup> Department of Cardiology, General Hospital "Prim.dr Abdulah Nakaš", Bosnia and Herzegovina

## Abstract

For the purpose of this research, 60 patients were observed with simple unstable uncomplicated fracture of lower leg, divided into two groups: group treated nonoperatively and group treated operatively subdivided on subgroup of patients treated with compression plate and screw and subgroup of patients treated with intramedullary nails and secure screws. After eight months period the results of maior complication (neurovascular complications, non union, osteomyelitis, amputation) and deformities (varus-valgus, antecurvatum-recurvatum, shortening, rotation) were measured and compared. The differences between the groups in all parametries were not significant what leads to conclusion that nooperative treatment of this fractures should not be abandoned.

## Introduction

Lower leg bones are the most frequently injured among the other bone injuries. According to data of National Centre for Health statistic in USA, 492000 fractures of tibia, fibula and ankle joint were reported per year.<sup>1</sup>

Therefore it would be advisable that doctrine about the treatment of this fracture is harmonized. But, the therapy of fractures of this region, besides the femoral neck fractures, are still the object of the most conflicting opinions.<sup>(2)</sup>

Tibia is, because of its location, very exposed to injuries, mostly because that one third of its surface is located subcutaneously. In addition to, the blood supply is more uncertain than the other bones which are surrounded with muscles. The ankle is, as the knee joint, ginglymus type of joint and there is no capability for accommodation on rotatory deformations after fracture of tibia. So, the special care is necessary during reduction of the fracture i.e. correction of such deformities. Delayed union, non union and infections are relatively often complications of the lower leg bones.<sup>(2)</sup>

What is the optimal treatment of the simple unstable uncomplicated lower leg fracture?

Maybe, the best answer on this questions gave Nicol who emphasises importance of good judgement in treatment of this fracture: "Every fracture is particularly problem and decision to treat it with internal osteosynthesis or nonoperatively should be done by particularly judgement of benefits and risks of all methods and particularly circumstances of every case. This is the invitation for the best judgement but that is more difficult to acquire than virtuosity in the operation room."<sup>(1)</sup>

Today, the majority of authors who are occupied with this problems agree that doing with classification is necessary to describe very precisely anatomic location, pattern of fracture line, associated injury of fibula, position and number of fragments and amount of soft tissue damage.<sup>(1,2,3,4,5)</sup>

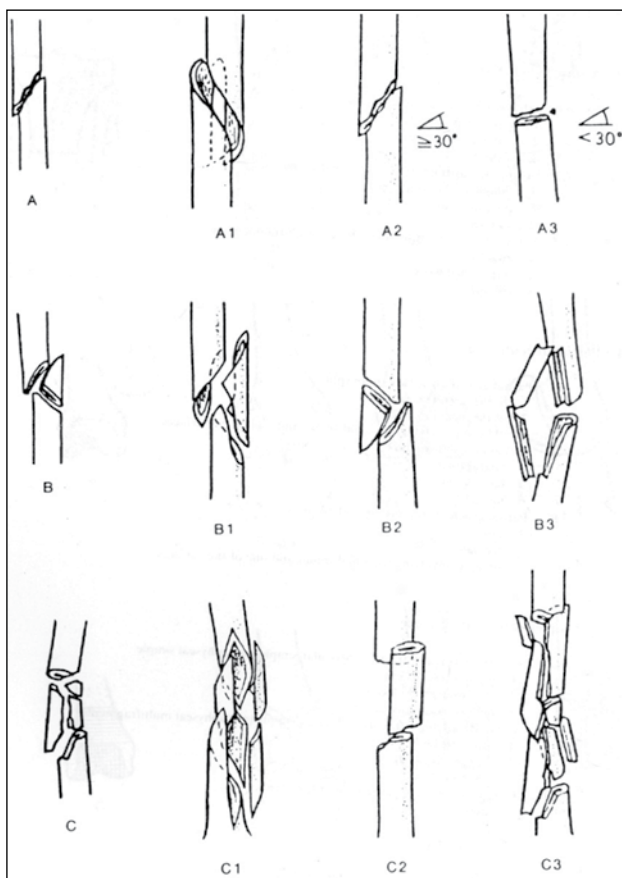
Very detailed classification system was suggested by AO ASIF group Muller and all. (1990) and, with amendment, accepted by Orthopedic Trauma Association (OTA)

Tibial shaft fractures, according of this classification are divided into:

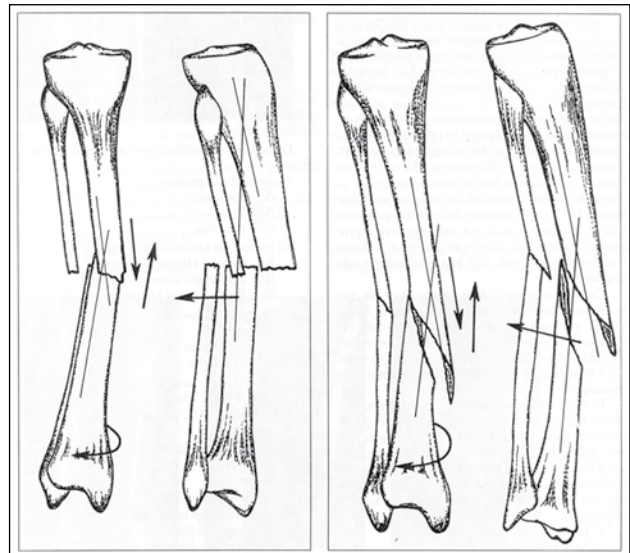
- A simple
- B with wedge fragment
- C complex

Further, simple tibial shaft fracture are divided:

- A/1 spiral
- A/2 oblique
- A/3 transverse



Picture 1. Division of lower leg fracture according AO group<sup>5</sup>



Picture 2. Transverse dislocated fracture

Picture 3. Oblique unstable fracture

### Patients and methods

#### Patients

In this research was included 60 patients with lower leg fractures, treated on Clinic for orthopaedic and traumatology of Clinic Centre in Sarajevo, in period from 2001 to 2004 operatively and nonoperatively:

- 20 patients was treated with skeletal traction through tibial tuberosity, upper knee cast immobilisation than with Patella Tendon Bearing (PTB) cast according Sarmiento
- 20 patients were treated with rigid osteosynthesis with plates and screws
- 20 patients were treated with intramedullary nail and secure screws

This research was included:

- patients of different ages, both gender, with lower leg shaft fractures: simple, unstable, uncomplicated
- the patients without signs of cardiac, pulmonary, hepatal, renal or other insufficiency or other conditions that could be interfered on fracture healing



This research was excluded next conditions:

- uncooperated patients which were not keeping adequate postoperative regime
- patients which were not appeared at first or control examinations

The result of treatment were estimated after eight months of starting treatment w based on anamnesis, clinical examination and radiogram of lower leg with knee and ankle joint

### Method of research

At admission of every patient on Clinic for orthopaedic and traumatology, anamnesis, clinical exam, standard laboratory analyses, chest radiogram, lower leg radiogram with ankle and knee joint in two directions, ECG and exam by internist were done.

Patients included in this research were divided into two groups:

- a) group of patients treated nonoperatively (skeletal traction, upper knee cast, PTB cast according Sarmiento ... 20 patients
- b) group of patients treated operatively:
  - rigid osteosynthesis with compressive plates and screws...20 patients
  - intramedullary nail and secure screws...20 patients

The goal of this research was to measure and to compare finally results of nonoperative and operative treatment of the patients with simple unstable uncomplicated lower leg shaft fracture using the parameters from this table:

From the Table 1. in this research, when treatment of the patients with simple, unstable, uncomplicated lower leg shaft fracture were finished, the results of major complications (nonunion, osteitis, amputation and neurovascular disorders) and deformities (varus-valgus, antecurvatum-recurvatum, shortening and rotation) were measured and compared.

Table 1. (Johner R, Whrus O.): Classification of tibial shaft fractures and correlation results)<sup>6</sup>

|                                | Excellent | Good      | Fair     | Poor       |
|--------------------------------|-----------|-----------|----------|------------|
| non union osteitis, amputation | no        | no        | no       | yes        |
| neurovascular disorders        | no        | minimally | moderate | serious    |
| deformity:                     |           |           |          |            |
| varus/valgus                   | no        | 2-5       | 6-10     | over 10    |
| antecurvatum/recurvatum        | 0-5       | 6-10      | 11-20    | over 20    |
| rotation                       | 0-5       | 6-10      | 11-20    | over 20    |
| shortening                     | 0-5mm     | 6-10mm    | 11-20mm  | over 20 mm |

Table 2. Table of frequencies neurovascular disturbances

|                                                                                           | Excellent | Good    | Fair     | Poor   |
|-------------------------------------------------------------------------------------------|-----------|---------|----------|--------|
| Neurovascular disturbances                                                                | none      | minimal | moderate | severe |
| Patients treated with transosseal extension-upper knee cast -PTB cast according Sarmiento | 20        | 0       | 0        | 0      |
| Patients treated i.e. operated with intramedullary nail and secure screws                 | 20        | 0       | 0        | 0      |
| Patients treated i.e. operated with DC plate and screws                                   | 20        | 0       | 0        | 0      |

$$df=(3-1) \times (4-1)=2 \times 3=6; \alpha=0,05; \chi^2_{2\alpha}=12,6; \chi^2=0$$

Theoretical value of  $\chi^2$  test in this case is 12,6. Empirical value is 0. There are no significant differences between the groups

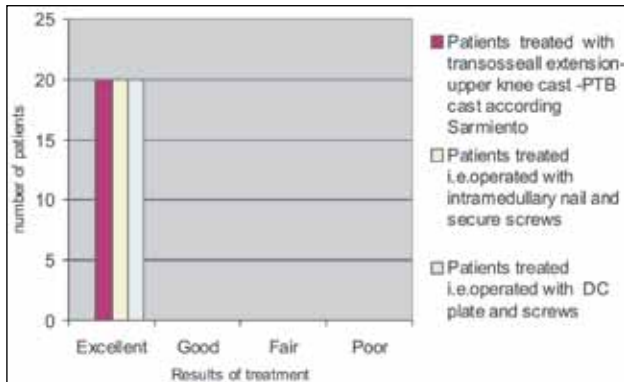


**Statistic processing of finally results**

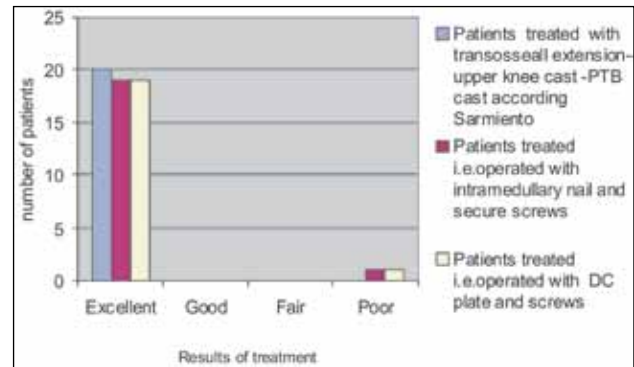
The results were processed by hi - square test with degree of freedom 6.

Theoretical value is 12,6 .Empirical value is 0,001 that means the differenses between the groups are not significant.

**Result of research**



Graph 1. Graphic representation frequencies of neurovascular disorders



Graph 2. Graphic representation frequencies of nonunion, osteitis and amputation

Table 3. Table of frequencies nonunion,osteitis and amputation after treatment,  $\chi^2=0,001$

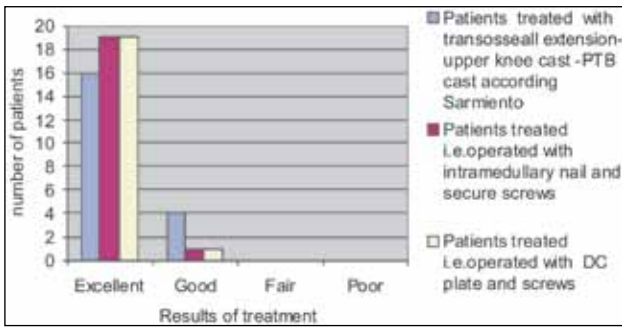
|                                                                                           | Excellent | Good | Fair | Poor         |
|-------------------------------------------------------------------------------------------|-----------|------|------|--------------|
| Nonunion                                                                                  | no        | no   | no   | yes          |
| Osteitis                                                                                  | no        | no   | no   | yes          |
| Amputation (maior complication)                                                           | no        | no   | no   | yes          |
| Patients treated with transosseal extension-upper knee cast -PTB cast according Sarmiento | 20        | 0    | 0    | 0            |
| Patients treated i.e.operated with intramedullary nail and secure screws                  | 19        | 0    | 0    | 1 (osteitis) |
| Patients treated i.e.operated with DC plate and screws                                    | 19        | 0    | 0    | 1 (nonunion) |

Table 4. Table of frequencies varus-valgus deformities after treatment,  $\chi^2=0,125$ .

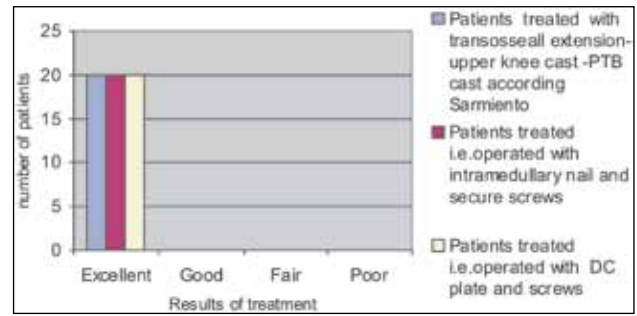
|                                                                                           | Excellent | Good       | Fair         | Poor           |
|-------------------------------------------------------------------------------------------|-----------|------------|--------------|----------------|
| Deformity Varus-valgus                                                                    | none      | 2-5 degree | 6- 10 degree | over 10 degree |
| Patients treated with transosseal extension-upper knee cast -PTB cast according Sarmiento | 16        | 4          | 0            | 0              |
| Patients treated i.e.operated with intramedullary nail and secure screws                  | 19        | 1          | 0            | 0              |
| Patients treated i.e.operated with DC plate and screws                                    | 19        | 1          | 0            | 0              |

Theoretical value is 12,6.

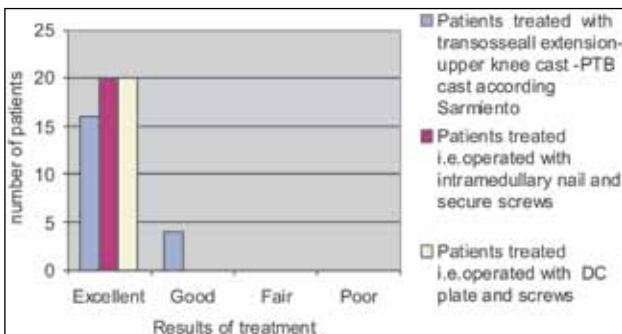
Empirical value is 0,125 that means the differensies between the groups are not significant.



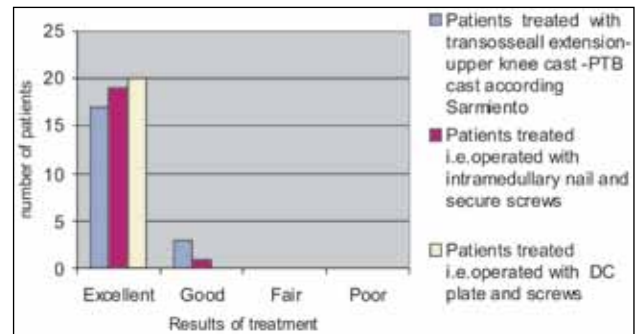
Graph 3. Graphic representation frequencies varus-valgus deformity



Graph 5. Graphic representation frequencies of rotation deformity



Graph 4. Graphic representation frequencies antecurvatum- recurvatum deformity



Graph 6. Graphic representation frequencies shortening deformity

Table 5. Table of frequencies antecurvatum-recurvatum deformities,  $\chi^2=0,251$

|                                                                                           | Excellent  | Good        | Fair         | Poor           |
|-------------------------------------------------------------------------------------------|------------|-------------|--------------|----------------|
| Deformity<br>Antecurvatum-recurvatum                                                      | 0-5 degree | 6-10 degree | 11-20 degree | over 20 degree |
| Patients treated with transosseal extension-upper knee cast -PTB cast according Sarmiento | 16         | 4           | 0            | 0              |
| Patients treated i.e.operated with intramedullary nail and secure screws                  | 20         | 0           | 0            | 0              |
| Patients treated i.e.operated with DC plate and screws                                    | 20         | 0           | 0            | 0              |

Theoretical value is 12,6.

Empirical value is 0,251 that means the differensies between the groups are not significant.

Table 6. Table of frequencies deformity rotation of fragments,  $\chi^2=0$

|                                                                                           | Excellent  | Good        | Fair         | Poor           |
|-------------------------------------------------------------------------------------------|------------|-------------|--------------|----------------|
| Deformity<br>Rotation of fragments                                                        | 0-5 degree | 6-10 degree | 11-20 degree | over 20 degree |
| Patients treated with transosseal extension-upper knee cast -PTB cast according Sarmiento | 20         | 0           | 0            | 0              |
| Patients treated i.e.operated with intramedullary nail and secure screws                  | 20         | 0           | 0            | 0              |
| Patients treated i.e.operated with DC plate and screws                                    | 20         | 0           | 0            | 0              |

Theoretical value is 12,6.

Empirical value is 0 that means the differensies between the groups are not significant.

Table 7. Table of frequencies deformity of shortening after treatment,  $\chi^2=0,073$ 

|                                                                                           | Excellent | Good    | Fair     | Poor       |
|-------------------------------------------------------------------------------------------|-----------|---------|----------|------------|
| Deformity shortening                                                                      | 0-5 mm    | 6-10 mm | 11-20 mm | over 20 mm |
| Patients treated with transosseal extension-upper knee cast -PTB cast according Sarmiento | 17        | 3       | 0        | 0          |
| Patients treated i.e.operated with intramedullary nail and secure screws                  | 19        | 1       | 0        | 0          |
| Patients treated i.e.operated with DC plate and screws                                    | 20        | 0       | 0        | 0          |

*Theoretical value is 12,6.*

*Empirical value is 0,073 that means the differences between the groups are not significant.*

## Discussion

There were no neurovascular disturbances in group nonoperatively neither operatively treated patients in this research ( Table 2, graph 1). Sarmiento <sup>(7,8)</sup> reports few cases (1-4) nonoperatively treated patients but in series of 231 - 761 patients. Muller<sup>(5)</sup>, Tausch<sup>(9)</sup>, Roman<sup>(10)</sup> report 2-6 cases of neurovascular disturbances operatively treated patients but in series of over 300 patients. In literature, neurovascular complications are more often in operatively treated patients.

There are no statistically significant differences in neurovascular disturbances between operatively and nonoperatively treated patients in this research.

In the group of operatively treated patients, in this research were two cases of major complications: one case of osteomyelitis and one case of nonunion ( Graph 2, Table 3).

In the group of nonoperatively treated patients there are no cases of nonunion, osteomyelitis and amputation. In the research of Matusu <sup>(10)</sup>, Karaharua et al<sup>(11)</sup>, Jensen<sup>(12)</sup>, te Mullera <sup>(5)</sup> the cases of nonunion and osteomyelitis were reported

(6-21) but in series of 123-286 operatively treated patients. In researches of Sarmiento <sup>(7,8)</sup> in series over than 700 patients there are no nonunion and osteomyelitis of nonoperatively treated patients. There are no significant differences between operatively and nonoperatively treated patients. In literature the number of frequencies of nonunion and osteomyelitis are more numerical in groups of operatively treated patients what was shown in this research too.

Although, number of frequencies of varus - valgus deformities is more numerical in group of nonoperatively treated patients that differences are not significant between the groups in this research. ( Table 4., graph 3.). In researches of Infanger<sup>(9)</sup> and Matusu<sup>(10)</sup> percentage of angular deformities are less numerous in groups of operatively treated patients. In these researches authors suggested operative treatment i.e. intramedullary fixation.

There are no statistically significant differences frequencies of antecurvatum- recurvatum deformity between the groups operatively and non operatively treated patients in this research although the frequencies of this deformities are more numerous in the group of nonoperatively treated patients ( Table 5., graph 4.). There are a lot of researches with suggestion for operative treatment because of less number of angular deformities frequencies <sup>(9,10,11,12)</sup>. In researches of Dehne, Nicol and Sarmiento with suggestion for nonoperative treatment. These authors admit more numerous frequencies of angular deformities and shortening deformities but comparing other important parameters they still suggests nonoperative treatment.

Neither one patients in this research had rotation deformity in both groups so there are no significant differences between the groups in this research ( Table 6., graph 5.).

There are no significant differences frequencies of shortening deformities between groups although frequencies of shortening deformities are more numerous in the groups of nonoperatively treated patients. Empirical value of hi-square test is 0,073 but theoretical value is 12,6, so, there are no significant differences between the groups (Table 7, graph 6.).

Sarmiento<sup>(7,8)</sup> reports average length of shortening of 10 mm in series over 400 nonoperatively patients so the length of shortening in this research is less than 10 mm. In the researches of Muller, Van Lindena et al.<sup>(5,11,12)</sup> the operative treatment is preferable.

### Conclusion

There are no statistical differences between operatively and nonoperatively treated group of patients with simple unstable uncomplicated lower leg shaft fracture. This research leads to conclusion that nonoperative method with skeletal traction than with upper knee cast and finally with Patelae Tendon Bearing cast according Sarmiento should not be abundant in the treatment of simple unstable uncomplicated lower leg shaft fracture because, according to the results, there are no significant differences between this groups and operatively treated group but there are no operative risks.

### References

1. Crenshaw AH. *Cambell's Operative Orthopaedic: Vol. 2. 8<sup>th</sup> ed. St. Louis, etc.: Mosby, 1992.*
2. Butković IJ. *Prelomi potkoljenice. U: Banović DM. i saradnici. Traumatologija koštano-zglobnog sistema. 2. dopunjeno izd. Beograd: Zavod za udžbenike i nastavna sredstva, 1998: 751- 797.*
3. Charnley J. *Closed Treatment of Common Fractures. 3.ed. Edinburgh: Livingstone, 1961.*
4. Muller ME, Allgover M, Schneider R, Willeneger H. *Manual of internal fixation. 3<sup>rd</sup> ed. Wurzburg: Universitatdruckeri, 1992.*
5. Littenberg B, Weinstein LP, Mc Carren M, et al. *Closed fracture of the tibial shaft .A meta analysis of three method of treatment. J Bone Joint Surg. Am 1998 Feb; 80 (12): 174-83*
6. Sarmiento A. *Displaced isolated fractures of the tibial shaft treated with either a cast or intramedullary nailing. An outcome analysis of matched pairs of the patients J. Bone Joint Surg Am. 1998 Jul; 80 (7): 1084-5 PMID: 9698014 / Pub Med – indexed for MEDLINE/*
7. Sarmineto A, Gersten LM, Sobol PA , Shankwiler JA, Vangress CT. *Tibial shaft fracture treated with functional braces. Expirience with 780 fractures PMID : 2768307/ Pub Med – indexed for MEDLINE/*
8. Infanger K, Fenner A, Cadalbert M, Schauman M,: *Results in the surgical treatment of lower leg diaphyseal fracture based on 230 cases. Helv Chir Acta. 1971 May 38 (3): 311-6 German .PMID : 5163119 / Pub Med – indexed for MEDLINE/*
9. Matusu I, Wondrak E: *Results and sequelae in surgically treated diaphyseal fractures of the lower leg, Rozhl Chir 1989 Aug; 60 (8); 529-33 Czech, PMID: 7313868 (Pub Med – indexed for MEDLINE)*
10. Karaharyu E, Alho A, Nieminen J; *The result of operative and nonoperative management of tibial fracture . Injury 1975 Aug; 7(1): 47- 52*
11. Jensen JS, Hansen FW, Johansen J: *Tibial shaft fractures. A comparison of conservative treatment and internal fixation with conventional plates or AO compresion plates , Acta Orthop Scan1977; 48(2) 204*

#### Corresponding author:

Fuad Dzankovic  
Clinic for ortopaedic and traumatology,  
University of Sarajevo,  
Clinical Centre,  
Bosnia and Herzegovina  
e-mail: ifsa@bih.net.ba

# Comparison in Mycobacterium Tuberculosis Positivity between Bronchoalveolar Lavage and Sputum Examination - the Importance of Bronchoalveolar Lavage in Diagnostic of Pulmonary Tuberculosis

## KOMPARACIJA U POZITIVNOSTI NA MYCOBACTERIUM TUBERCULOSIS IZMEĐU BRONHOALVEOLARNE LAVAŽE I SPUTUMA - ZNAČAJ BRONHOALVEOLARNE LAVAŽE U DIJAGNOSTICI PLUĆNE TUBERKULOZE

Vesna Cukic

Clinic for Pulmonary Diseases and TB, Clinical Center of Sarajevo University, Bosnia and Herzegovina

### Abstract

**AIM** of this research is to show the importance of mycobacteriological examination of bronchoalveolar lavage (BAL) in diagnostic of pulmonary tuberculosis.

**Material and methods:** Retrospective study was carried out to show the number of patients suspected to be ill with pulmonary tuberculosis (TB) whose bronchoalveolar lavage (BAL) samples were examined in Mycobacteriological laboratory of Clinic for Pulmonary diseases and TB "Podhrastovi" in a six-year period (2003. - 2008.) and it was compared *Mycobacterium tuberculosis* (BK- Bacillus Koch) positivity of BAL with sputum examination findings: microscopic (smear) and cultural.

**Results:** In six-year period (2003. - 2008.) BAL was done in 115 patients suspected to be ill

with pulmonary TB. In 33,04 % of them BAL was microscopically and in all of them (100%) cultural *Mycobacterium tuberculosis* (Bacillus Koch -BK) positive. In the same patients sputum examination findings were microscopically (smear) *Mycobacterim tuberculosis* (Bacillus Koch -BK) positive in 16,52 % and cultural positive in 75,65 % cases.

**Conclusion:** By this research it has been shown the importance of bronchoalveolar lavage in obtaining a sample of a better quality for finding *Mycobacterium tuberculosis* (Bacillus Koch) in respiratory system and its value in diagnostic of pulmonary tuberculosis.

**Key words:** *Mycobacterium tuberculosis*, sputum, BAL.



## Sažetak

**Cilj:** pokazati značaj mikobakteriološkog ispitivanja bronhoalveolarnog lavata u dijagnostici plućne tuberkuloze.

**Materijal i metode:** Ovo je retrospektivna analiza broja pacijenata koji su bili suspektni da boluju od plućne tuberkuloze (TBC) čiji su uzorci bronhoalveolarne lavaže (BAL) ispitivani u Mikobakteriološkom laboratoriju Klinike za plućne bolesti i TBC "Podhrastovi" u šestogodišnjem periodu (od 2003.- 2008.) i upoređivana je pozitivnost na *Mycobacterium tuberculosis* (Bacillus Koch- BK) sa nalazima ispitivanja sputuma : mikroskopski ( razmaz) i u kulturi.

**Rezultati:** U šestogodišnjem periodu (2003-2008.) urađen je BAL kod 115 pacijenata sumnjivih da boluju od plućne tuberkuloze. Kod njih 33,04 % BAL je bio mikroskopski i kod svih (100%) kulturelno pozitivan na *Mycobacterium tuberculosis* (Bacillus Koch- BK). Kod istih pacijenata ispitivanja sputuma su bila *Mycobacterium tuberculosis* (Bacillus Koch- BK ) mikroskopski ( razmaz) pozitivna u 16,52%, a u kulturi u 75,65 % slučajeva .

**Zaključak:** Ovim istraživanjem je pokazan značaj bronhoalveolarne lavaže u dobijanju uzorka većeg kvaliteta za otkrivanje *Mycobacterium tuberculosis* (Bacillus Koch) u respiratornom sistemu i njen značaj u dijagnostici plućne tuberkuloze.

**Ključne riječi:** *Mycobacterium tuberculosis*, BAL, Sputum

## Introduction

The most important step in diagnostic of pulmonary tuberculosis is the finding of *Mycobacterium tuberculosis* in sputum, either by microscopic examination or by sputum cultivation (1,2,3). Although all clinical and chest x-ray parameters indicate pulmonary tuberculosis, sputum finding is often smear and cultural *Mycobacterium tuberculosis* ( Bacillus Koch-BK) negative(1). Bronchoalveolar lavage is endoscopic method in which, by fiber-optic bronchoscopy, lower airways are washed by instillation and aspiration of isotonic salt solution, and so obtained material is examined in the same way as sputum.

## AIM

Aim of this research is to show the importance of mycobacteriological examination of bronchoalveolar lavage (BAL) in dignostic of pulmonary tuberculosis.

## Material and methods

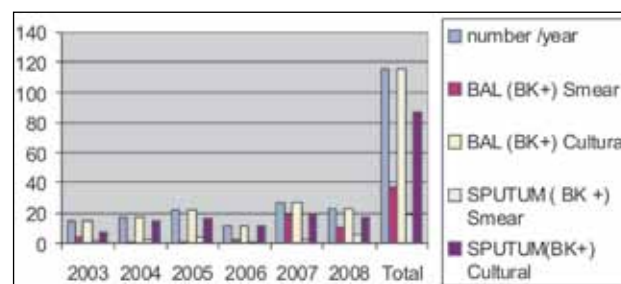
This is the retrospective analysis of the number of patients suspected to be ill with pulmonary TB whose bronchoalveolar lavage samples were examined in Mycobacteriological laboratory of Clinic for Pulmonary diseases and TB "Podhrastovi" in a six-year period ( 2003. - 2008.) and it was compared *Mycobacterium tuberculosis* ( Bacillus Koch-BK) positivity of BAL with sputum examination findings : microscopic and cultural.

## Results

Results are shown on the Table 1., Table 2., and Grafic1.

Table 1. Mycobacteriological examination of BALs per year sputum examination in the same cases suspected of pulmonary TB

| Year  | number /year | BAL (BK+) Smear | BAL (BK+) Cultural | SPUTUM ( BK +) Smear | SPUTUM(BK+) Cultural |
|-------|--------------|-----------------|--------------------|----------------------|----------------------|
| 2003  | 15           | 4               | 15                 | 2                    | 8                    |
| 2004  | 17           | 1               | 17                 | 3                    | 15                   |
| 2005  | 22           | 1               | 22                 | 4                    | 16                   |
| 2006  | 11           | 3               | 11                 | 1                    | 11                   |
| 2007  | 27           | 19              | 27                 | 3                    | 20                   |
| 2008  | 23           | 10              | 23                 | 6                    | 17                   |
| Total | 115          | 38              | 115                | 19                   | 87                   |



Graphic 1. Mycobacteriological examination of BALs per year and sputum examination in the same cases suspected of pulmonary TB

Table 2. Comparison in mycobacteriological positivity between BALs and sputum examination in per cents

| Year         | Number per year | BAL BK+            |            | SPUTUM BK +         |              |
|--------------|-----------------|--------------------|------------|---------------------|--------------|
|              |                 | microsc. (smear) % | cultural % | microsc. (.smear) % | cultural %   |
| 2003.        | 15              | 26,67              | 100        | 13,33               | 53,33        |
| 2004.        | 17              | 5,88               | 100        | 17,65               | 88,24        |
| 2005.        | 22              | 4,54               | 100        | 18,18               | 72,73        |
| 2006.        | 11              | 27,27              | 100        | 9,09                | 100          |
| 2007.        | 27              | 70,37              | 100        | 11,11               | 74,07        |
| 2008.        | 23              | 43,48              | 100        | 26,09               | 73,91        |
| <b>Total</b> | <b>115</b>      | <b>33,04</b>       | <b>100</b> | <b>16,52</b>        | <b>75,65</b> |

In 2003. we did 15 BAL. 4 ( 26,67%) of them were microscopically, but all 15 were cultural positive. Sputum examination: 2 microscopically (13,33%) , and 8 (53,33%) cultural positive. In 2004. we did 17 BAL . One was microscopically(5,88%) ,and all were cultural positive. Sputum examination: 3 microscopically (17,65%) ,and 15 (88,24%) cultural positive. In 2005. we did 22 BAL. One was microscopically (4,54%) ,and all cultural positive. Sputum examination : 4 were microscopically(18,18%) ,and 16(72,73%) cultural positive. In 2006 we did 11 BAL. 3 were microscopically (27,27%) ,and all cultural positive. Sputum examination: 1 microscopically (9,09%),and all cultural positive. In 2007. we did 27 BAL. 19 were microscopically (70,37%) , but all of them were cultural positive. Sputum examination : 3 microscopically( 11,11%), and 20 (74,07%) cultural positive. In 2008. we did 23 BAL. 10 were microscopically(43,48%) and all of them were cultural positive. Sputum examination: 6 microscopically (26,09%)and 17 (73,91%) cultural positive. In all 115 patients in 6-year period BAL was microscopically positive in 33,04 % and cultural 100%. With sputum examination there were 16,52 % microscopically and 75,65 % cultural *Mycobacterium tuberculosis* ( Bacillus Koch-BK) positivity .

The higher level of positivity was in samples obtained by BAL either microscopically or by cultural examination.

## Discussion

In Clinic for pulmonary diseases and TB “ Podhrastovi” we do mycobacteriological examination of BAL in each case of pulmonary disease which is suspected to be pulmonary TB ,but it is smear or cultural *Mycobacterium tuberculosis* ( Bacillus Koch-BK) negative,or in other suspicious cases where there is doubt is it pulmonary TB or other pulmonary disease with similar chest x-ray or clinical findings ,whether there is association of pulmonary TB and other pulmonary disease first of all lung cancer. This retrospective examination shows a greater number of *Mycobacterium tuberculosis* positivity of BAL in comparison with sputum examination. Ranasinha C.D. (4) in Shri Lanka shows the results of prospective study of smear- negative pulmonary TB using BAL in 281 individuals with pulmonary TB suspected clinically or on chest radiography and 23, 1% of smear negative individuals had microscopically BK positive BAL. Safianovska A. and al. (5) did BAL in 642 patients in Warsaw ,Poland during 7 years( 1999.-to 2006.) with different chest x-ray and in 61 of them the *Mycobacterium tuberculosis* was isolated ; in 24 of them isolated mycobacteria were clinical significant : *Mycobacterium tuberculosis* in 17 and *Mycobacterium cansasi* in 7 cases. Triller N. and al. ( 6) in 1469 patients who were underwent bronchoscopy examination in 2006. year with 40 of them pulmonary TB was suspected and in 6 of them *Mycobacterium tuberculosis* was isolated in culture of bronchial

washing. They suggest that bronchoscopy in suspected pulmonary TB should be performed only in high suspicious patients where sputum smear examination before bronchoscopy is negative.

### Conclusion

By this research it has been shown the importance of bronchoalveolar lavage in obtaining a sample of a better quality for finding *Mycobacterium tuberculosis* in respiratory system and its value in diagnostic of pulmonary tuberculosis.

### References

1. Crofton J., Horne N. , Miller F.: *Tuberculosis control programmes in: Clinical Tuberculosis . Mac Millan Education LTD London, 1992; 15-27*
2. Crofton J., Horne N. , Miller F : *Pulmonary tuberculosis in adults in: Clinical Tuberculosis . Mac Millan Education LTD London, 1992; 89-115*
3. *Centres for Disease Control .Prevention Tuberculosis Control Laws – United States: Recommendations of the Advisory Council for the Elimination of Tuberculosis (ACET) MMWR 1993; 42 (No RR -15): 1-13*
4. *Ranasinha C.D. : Prospective study of smear -negative pumonary TB using bronchoalveolar lavage. 5<sup>th</sup> Congress of the International Union Against Tuberculosis and Lung Diseses, The Union, Europe Region, Dubrovnik, Croatia 27<sup>th</sup>- 30<sup>th</sup> May 2009. Abstract Book: P 18*
5. *Safianowska A., Walkiewicz M. , Novacka J. and al.: How often Mycobacteria isolated from bronchoalveolar lavage indicate a lung disease? 5<sup>th</sup> Congress of the International Union Against Tuberculosis and Lung Diseses, The Union, Europe Region, Dubrovnik, Croatia 27<sup>th</sup>- 30<sup>th</sup> May 2009. Abstract Book: P 20*
6. *Triller N., Rozman A., Žolnar- Dović M.: Diagnostic yield of bronchoscopy in smear negative pulmonary disease. 5<sup>th</sup> Congress of the International Union Against Tuberculosis and Lung Diseses, The Union ,Europe Region, Dubrovnik, Croatia 27<sup>th</sup>- 30<sup>th</sup> May 2009. Abstract Book : P 26*

Corresponding author:

Cukic Vesna

Clinic for Pulmonary Diseases and TB,

Clinical Center of Sarajevo University,

Bosnia and Herzegovina,

e-mail:

# The influences of traumatic brain injuries in largeness of fussion convergency

*Raif Serdarevic, Emina Alimanović-Halilovic*

Ophthalmology Clinic, Clinical Centar University of Sarajevo, Bosnia and Herzegovina

## Abstract

Traumatic brain injuries had a main reason to depend upon with deficit of motor fusion. We aim to investigate whether the patients with traumatic brain injuries had deficit motoric fusion. The study of populations contains 50 patients with many subjectively problems because can not explain objectively analyses. Deficit of motoric fusion indicated that disturbances had to be motoric and following disability of patients to hold superposition of view to fixation object. In our study the influences of traumatic brain injuries in largeness of fusion convergence was significantly higher after 6 months of the medical treatment  $p < 0.01$ ,  $t = 0.914$  and  $r = 0.881$  and  $Hi^2$  t-test 6.81. The explanations about difficulties of near vision after traumatic brain injuries the visual disturbances must to be observed in all symptoms because that it is only conducted for check really diagnosis and control of visual motor system.

**Key words:** traumatic brain injuries, motor fusion.

## Sažetak

Traumatske povrede mozga su u uzročnoj zavisnosti sa gubitkom motorne fuzije što se mora posmatrati u odnosu na ukupnu simptomatologiju. Osnovni cilj ovog istraživanja je da se ustanovi da li postoji deficit motorne fuzije kod pacijenata sa traumatskom povredom mozga. Ispitali smo 50 pacijenata sa traumatskom povredom mozga koji su imali niz subjektivnih poteškoća a koje nisu bile otkrivene objektivnim analizama. Gubitak motorne fuzije pokazuje da je ovaj poremećaj

čisto motorni i praćen je nesposobnošću pacijenta da se održi superpozicija pogleda na fiksirani predmet. U našem istraživanju uticaj traumatskih povreda mozga na veličinu fuzione konvergencije dobili smo signifikantno veću vrijednost poslije tretmana od 6 mjeseci  $p < 0.01$ ,  $t = 0.914$ ,  $r = 0.881$  i  $Hi^2$  t-test 6.8. Objašnjenje teškoća blizinskog gledanja nakon traumatskih povreda mozga moraju se posmatrati u cijeloj simptomatologiji jer je to jedini način da se dođe do realne dijagnoze i kontrole visuo motornog sistema.

**Ključne riječi:** traumatska povreda mozga, motorna fuzija.

## Introduction

Traumatic brain injuries using vibration waves, stroke power and without visible damage causing differences cerebral disorders and between those disorder fusion convergence. In physiological condition fusional convergence develop disparities pictures at the retina. The disparities causing fusion mechanism those showed as convergence. The pictures of the objects started on temporal side both retina because the object saw like double at the near. Corrected reflex of fusion convergence and double vision disappear.

## AIM

We aim to investigate whether the patients with traumatic brain injuries had deficit motoric fusion.

## Materials and method

Our research was based on 50 patients with traumatic brain injuries at the Ophthalmology Clinic, Clinical University Sarajevo. All the patients were sent to this clinic from 2000 to 2003. The group of patients was between from 10 to 40 years old. The study group had traumatic brain injuries and subjectively disturbances at the near vision. The authors have tried to do comparative study and compare results those with of other fifty patients with similar problems who's had not traumatic brain injuries. Following the study authors was done in both groups of patients: anamnesis,

determining vision acuity in near and far vision monocular and binocularly, examination of the anterior and retinal segment, skiascopy, eye motility and oculomotor balance of external eye muscles, cover-uncover test, measuring angle with prisms, neurological examination, brain computer tomography (CT) and statistic examination.

## Results

In this study examination 50 patients with traumatic brain injuries, 40 (80 %) were males and 10 (20%) femail.

Table 1. Vision capabilities of our patient on near vision

| Near vision  | Binocular                           | Number of patients | %            |
|--------------|-------------------------------------|--------------------|--------------|
| VOU          | reading J1 at the distance of 75 cm | 27                 | 54 %         |
| VOU          | reading J1 at the distance of 40 cm | 14                 | 28 %         |
| VOU          | Reading J1 at the distance of 35 cm | 9                  | 18 %         |
| <b>TOTAL</b> |                                     | <b>50</b>          | <b>100 %</b> |

Table 2. Size angle of exophory measurement with prisms dioptries before treatment

| Ortophory at the far | Number of patients | %            | Size angle in prisms dioptries (pd) |
|----------------------|--------------------|--------------|-------------------------------------|
| Egsohory at the near | 9                  | 18 %         | 2 - 4 Pd                            |
|                      | 15                 | 30 %         | 4 - 6 Pd                            |
|                      | 26                 | 52 %         | 6 - 8 Pd                            |
| <b>TOTAL</b>         | <b>50</b>          | <b>100 %</b> |                                     |

Table 3. Middle values of convergent fusion before treatment

| Size of fusion (pd)     | Number of patients | %            | S. D.         | Hi <sup>2</sup> t-test |
|-------------------------|--------------------|--------------|---------------|------------------------|
| To 2 Pd                 | 25                 | 50 %         | 14. 61        | 9. 61                  |
| Unstable between 0-6 Pd | 15                 | 30 %         | 18. 6         | 11. 22                 |
| To 12 Pd                | 10                 | 20 %         | 14. 18        | 6. 84                  |
| <b>TOTAL</b>            | <b>50</b>          | <b>100 %</b> | <b>17. 11</b> | <b>9. 41</b>           |

Table 4. Values of convergent fusion 2 months after treatment

| Size of fusion (pd)                              | Number of patients | %            | S. D.         | Hi <sup>2</sup> t-test |
|--------------------------------------------------|--------------------|--------------|---------------|------------------------|
| unstable from 0 to 6 Pd                          | 13                 | 26 %         | 18. 41        | 8. 11                  |
| to 10 Pd                                         | 15                 | 30 %         | 18. 6         | 9. 02                  |
| to 18 Pd                                         | 10                 | 20 %         | 14. 18        | 7. 84                  |
| Unstable from 1 do 2<br>(without of improvement) | 12                 | 24 %         | 18. 86        | 9. 12                  |
| <b>TOTAL</b>                                     | <b>50</b>          | <b>100 %</b> | <b>16. 44</b> | <b>12. 1</b>           |



Table 5. Values of convergent fusion 6 months after treatment

| Size of fusion (pd)                  | Number of patients | %            | S. D.        | Hi <sup>2</sup> t-test |
|--------------------------------------|--------------------|--------------|--------------|------------------------|
| Stabile from 0 to 10 Pd              | 9                  |              | 17.61        | 5.41                   |
| Stabile from 0 to 4 Pd<br>about 1 Pd | 8                  | 50 %         | 16.41        | 5.13                   |
|                                      | 8                  |              | 15.01        | 5.18                   |
| From 0 to 16 Pd                      | 15                 | 30 %         | 18.6         | 7.22                   |
| From 0 to 22 Pd                      | 10                 | 20 %         | 14.18        | 6.84                   |
| <b>TOTAL</b>                         | <b>50</b>          | <b>100 %</b> | <b>21.44</b> | <b>6.81</b>            |

The results of examination anterior and retinal segments were normal. The examination of eye motility finding orthophoria at the far and exophoria at the near. Size angle of exophoria measurement with prisms dioptries.

### Discussion

The authors have worked sample 50 patients with traumatic brain injuries who's have subjectively disturbances at the near vision. The convergent fusion after 2 months of medical treatment of the total number where convergent fusion shows the values 10 patients (20 %) where convergent fusion shows the value 18 Pd, were S. D. 14,18

and Hi<sup>2</sup> t-test 7.84, 15 patients (30%) where convergent fusion shows the value 10 Pd where standard deviation shows the value 18.6 and Hi<sup>2</sup> t-test 9.02, 13 patients (26 %) where convergent fusion shows the unstable value from 0 to 6 Pd where standard deviation shows the value 18.41 and Hi<sup>2</sup> t-test 8.11, 12 patients (24 %) where unstable values from 1 to 2 without improvement standard deviation shows value 18.6 and Hi<sup>2</sup> t-test 9.12.

The value of convergent fusion after 6 months medical treatment of the total number of 50 patients. We had 15 patients (30 %) shows values from 0 to 16 Pd, S. D. shows values 18.6 and Hi<sup>2</sup> t-test 7.22., 9 patients (18 %) shows stabil values from 0 to 10 Pd, S. D. shows values 17.61 and Hi<sup>2</sup> t-test 5.41., 10 patients (20 %) shows values of convergent fusion from 0 to 22 Pd, S. D. shows values 14.18 and Hi<sup>2</sup> t-test 6.84., 8 patients (16 %) shows stabile values of convergent fusion from 0 to 4 Pd, S. D. shows values 16.41 and Hi<sup>2</sup> t-test 5.18 and 8 patients (16 %) shows values of convergent fusion about 1 Pd, S. D. shows values 15.01 and Hi<sup>2</sup> t-test 5.18.

Base acquired data patient's shows signification improvement of near vision in relation of statistic values of convergent fusion before treatment that we had Hi<sup>2</sup> t-test 9.41., after 2 months of the medical treatment Hi<sup>2</sup> t-test 12.10 and best statistic data we had after 6 months of the medical treatment  $p < 0.01$ ,  $t = 0.914$  and  $r = 0.881$  and Hi<sup>2</sup> t-test 6.81.

We had compared our data with data of other authors. Gianutsos R. (1) and Rizzo M. (2, 3) shows that 50% of patients with traumatic brain injuries had visual systematic changes, like as disturbances of central visual at near vision without of disturbances of visual motor balance. This authors in their study of 314 patients showed that the 70 % of patients had parafoveal fixations of -5° and less. Kerkhoff and co-authors (4, 5) shows that the visual disturbances after trauma brain injuries had disability of visual system made differences between normal objects at the differences brighten so that more of objects appeared like as static's or moves objects.

Many effects of the disturbances fusion and visual motor changes are very important for long-time work at near. Kerkoff G. (6, 7, 8). The disturbances of visual motor fusion after traumatic brain injuries, the patients had blurred and doubled visual acuity and sometimes had to lose ability of recognize known objects at the mesopic and scotopic brighten Zihl J (9, 10, 11, 12), Kerkoff G (13, 14, 15, 16, 17), some of them wanted stronger brightness at the near work. All of this subjectively disturbances had our patients. That the authors can to explained this disturbances, we had to remind some of the events in physiological visual acuity at near, when we had to unit three cerebral fenomens: accommodation, convergence and myosis whose had to use clear foveolar binoculars visual acuity at the nears objects. When this physiological process changed, than both of eye had to

limited accommodation, less convergence or without of convergent and we had not myosis. After deficiency of fusion convergence the patients with traumatic brain injuries had exophoria at the near vision. First authors who's had to write that this disturbance of visual near acuity after traumatic brain injuries with his study of small number patients was Jaensch (18). Some of them patients with dysfunction of motility had to be diagnostics like as paralytic squint and whose had to diplopies and had not harmonize with objectively analysis of visual motility.

The patients with traumatic brain injuries had disturbances of visual motor fusions and disturbances of fusions convergence had to lead in exophories at the near vision. The posttraumatic insufficiency of fusion convergence had to difficult prognosis at the near vision but in long times these symptoms to disappear or became tolerable after medical treatment.

## Conclusion

- The study with 50 patients was conducted at the Ophthalmology Clinic, Clinical University Center in Sarajevo. All of the patients had to mentioned problems at the near vision and fast tired.
- The brain CT and neurological examination was normal.
- After following and consideration exanimate the authors had to find defect of visual motor fusion that's had to hard diagnostics as a clinical units.
- The explanations about difficulties of near vision after traumatic brain injuries the visual disturbances must to observed in all simpomatology's because that it is only conduct for check really diagnosis and control of visual motor system.
- All patient shows increased after prismatic adaptation treatment for long time all those different visual responds.

## References

1. Gianutsos R. Vision rehabilitation following acquired brain injury. In: Gentile M, ed. *Functional Visual Behavior. A Therapists Guide to Evaluation and Treatment Options*. Bethesda, MD. AOTO. 1997;267-294.
2. Rizzo M., Boller F., Grafman J. *Handbook of neuropsychology*, Amsterdam. Elsevier. 1989;415-427.
3. Rizzo M., Robin D. Bilateral affects of unilateral visual cortex lesions in human. *Brain*. 1996;119:951-96.
4. Kerkhoff G., et al. Treatment of fusional disorders in patients with brain damage. *Klin Monatsbl Augenheilkd*. 1994;205(2):70-5.
5. Kerkhoff G. Rehabilitation of Visuospatial Cognition and Visual Exploration in Neglect: a Cross-over Study. *Rest Neurol Neurosci*. 1998;12(1):27-40.
6. Kerkhoff G. Restorative and compensatory therapy approaches in cerebral blindness. *Rest Neural Neurosci*. 1998;8:15-22.
7. Kerkhoff G., et al. Anamnesis of brain-originated vision disorders. *Nervenartz*. 1990;61(12):711-8.
8. Kerkhoff G., Zoelch C. Disorders of visuospatial orientation in the frontal plane in patients with neglect following right or left parietal lesions. *Exp Brain Res*. 1998;122(1):108-20.
9. Zihl J. Eye movement patterns in hemianoptic dyslexia. *Brain*. 1995;118:891-912.
10. Zihl J. Visual Scanning Behavior in Patients With Homonymous Hemianopia. *Neurophysiologia*. 1995; 33:288-303.
11. Zihl J., Kennard C. Disorders of higher visual functions in Neurological Disorders. *Course and Treatment*. Academic Press. 1998;201-212.
12. Zihl J., Kerkhoff G. Foveal photopic and scotopic adaptation in patients with brain damage. *Clin Vision Sci*. 1999;2:185-195.
13. Kerkhoff G. Restorative and compensatory therapy approaches in cerebral blindness. *Restor Neurol Neurosci*. 1999;15(2-3):255-71.
14. Kerkhoff G., Artinger F., Ziegler W. Contrasting spatial hearing deficits in hemianopia and spatial neglect. *Neuro Report*. 1999;10(17):3555-60.
15. Kerhoff G., Stogerer E. Recovery of fusional convergence after systematic practice. *Brain Inj*. 1994;8(1):15-22.
16. Kerhoff G., Schindler I. *Neurovisuelle Storungen* Blackwell, Oxford. 1999;S:313-336.
17. Kerhoff G. *Cognitive neurovisual Rehabilitation: Recent Developments and Future Directions*. JNNP, London. 2000;20:1-25.
18. Jaensch PA. Fusionsstorungen. Horror fusions und Konvergenzspasmen. *Klinische Monatsblätter für Augenheilkunde und für Augenärztliche Fortbildung*. 1946; 111:142-149.
19. Serdarevic R., Alimanovic-Halilovic E. The disturbances of near vision after traumatic head Injuries, *HealthMED*, 2009; 3(4):. 435-441

### Corresponding author:

Raif Serdarevic,  
Ophthalmology Clinic,  
Clinical Centar University of Sarajevo  
Bosnia and Herzegovina  
e-mail: dr.raifserdarevic@yahoo.com

# Bioavailability of antioxidants from tea infusions with honey addition

## BIORASPOLOŽIVOST ANTIOKSIDANASA IZ RASTVORA ČAJA SA DODATKOM MEDA

*Aldina Kesic, Zorica Hodzic, Aida Crnkic, Mirzeta Saletovic, Benjamin Catovic*

University in Tuzla, Faculty of Science, Department of Chemistry, Bosnia and Herzegovina

### Abstract

It is often assumed that antioxidant nutrients contribute to the protection afforded by fruits, vegetables, red wine, tea and honey against diseases of aging. In this study the antioxidant activities of fourteen tea samples and three types of Bosnian honey were evaluated. Total antioxidant activity was determined using Ferric Reducing Ability of Plasma (FRAP) assay. Antioxidant activities of evaluated tea varied from 1276 -11710  $\mu\text{M/L}$ , and honey diluted samples from 182,5 – 4612,5  $\mu\text{M/L}$ . Honey contains more antioxidants and minerals than sugar (the darker, the more). In this research we would like to establish benefits of adding honey instead of sugar in tea. Our results strongly suggest that honey addition in tea plays important role in their antioxidant activity. These role depend on botanical origin of honey.

**Key words:** Antioxidant activity, FRAP, tea, honey.

### Sažetak

Česte su pretpostavke da antioksidansi sadržani u voću, povrću, crvenom vinu, čaju i medu učestvuju u zaštiti organizma od bolesti i starenja. U ovom istraživanju izmjerena je antioksidacijska aktivnost četrnaest uzoraka čaja i petnaest uzoraka meda, koji su svrstani u tri različite vrste. Svi uzorci porijeklom su iz Bosne i Hercegovine. Mjerenje ukupne antioksidacijske aktivnosti uzoraka čaja i meda vršeno je spektrofotometrijskom FRAP (Ferric Reducing Ability of Plasma) metodom.

Ukupna antioksidacijska aktivnost čaja kreće se od 1276 do 11710  $\mu\text{M/L}$ , a uzoraka rastvora meda od 182,5 do 4612,5  $\mu\text{M/L}$ . Sadržaj antioksidanasa i minerala u rastvoru meda veći je nego njihov sadržaj u rastvoru šećera. Tamniji uzorci meda imaju veću antioksidacijsku aktivnost u odnosu na svjetlije uzorke. Analize vršene u ovom istraživanju dokazuju prednost dodatka meda umjesto šećera u čaj. Rezultati dobijeni ovim istraživanjem ukazuju na činjenicu da se dodatkom meda u čaj značajno povećava njegova antioksidacijska aktivnost. Antioksidacijska aktivnost meda zavisi od njegovog botaničkog porijekla.

**Ključne riječi:** Antioksidacijska aktivnost, FRAP, čaj, med.

### Introduction

Antioxidant components are microconstituents present in the diet that can delay or inhibit lipid oxidation, by inhibiting the initiation or propagation of oxidizing chain reactions, and are also involved in scavenging free radicals (1).

Free radical reactions occur in the human body as well as in food systems. Reactive oxygen and nitrogen radical species (ROS/RNS) are an integral part of normal physiology. The over-production of these reactive species due to oxidative stress can cause damage to biomolecules and cause cellular injury and death, which may lead to various chronic diseases such as cancers, cardio- and cerebrovascular diseases (2,3).

In addition, naturally occurring antioxidants, when consumed, can also act as nutraceuticals

which can help protect one from oxidative damage in the body (4,5). In western medicine, the balance between antioxidation and oxidation is believed to be a critical concept maintaining a healthy biological system (6,7,8).

Tea is one of the most commonly consumed beverages in the world (9). The chemical composition of tea leaves consists of: tanning substances, flavonols, proteins and amino-acids enzymes, aroma substances, vitamins, minerals and trace elements (10). It is of interest to investigate the antioxidant properties of medicinal plant extracts especially those traditionally used in folk medicine.

Honey is a remarkable product from the hive prepared by honeybees from the nectar and other sugary substances derived from many plants. (11) All over the world, honey is considered a part of traditional medicine. (12). Honey contains a variety of phytochemicals (as well as other substances such as organic acids, vitamins and enzymes) that may serve as sources of dietary antioxidants (13). The amount and type of these antioxidant compounds depends largely upon the floral source/ variety of the honey (13).

Honey has been reported to be effective in the healing of wounds and burns (14) and as an antimicrobial agent (15) and in providing gastric protection against acute and chronic gastric lesions (15).

The aim of the present study was to compare antioxidant activity levels in the extracts different types of tea and honey, from the north-east area of Bosnia and Hercegovina. Total antioxidant activity has been determined using ferric reducing ability of plasma assay - FRAP (16).

## Methods and materials

All chemicals and reagents were of analytical grade and were purchased from: Fluka-Switzerland: (2,4,6-tri[2-pyridyl]-s-triazine) and Semikem-Sarajevo: (chloric acid; ferrous sulphate heptahydrate; ferric chloride hexahydrate; sodium acetate trihydrate; acetic acid). Spectrophotometric measurements were performed by Cecil CE 2021 UV-VIS spectrophotometer.

Samples of tea and honey were prepared according to a standard protocol. To 1 g of plant material was added 200 mL deionised water (tempera-

ture of added water was 98 °C), and left to stay 15 min, without additional heating. The extracts were filtered and the liquid portions were analyzed for their antioxidant activity.

To 5g of honey was added 20 mL deionised water. Honey solution quantitative added in volumetric tasks (50 mL) and fill with deionised water. Liquid portions of 200 µL were analyzed for their antioxidant activity.

FRAP assay measures the change in absorbance at 593 nm owing to the formation of blue colored Fe<sup>II</sup>-tripyridyltriazine compound from colorless oxidized Fe<sup>III</sup> form by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (100-1000 µmol/L) FeSO<sub>4</sub>×7H<sub>2</sub>O. All solutions were used on the day of preparation. In the FRAP assay the antioxidant efficiency of the antioxidant under the test was calculated with reference to the reaction signal given by an Fe<sup>II</sup>-solution of known concentration, this representing a one electron exchange reaction. The results were expressed in µmol Fe<sup>II</sup>/L of extract. Data presented are average of three replications.

## Results

Antioxidant activities of evaluated diluted honey samples varied from 182,5 – 4612,5 µM/L.

Antioxidant activities of evaluated tea varied from 1276 -11710 µM/L.

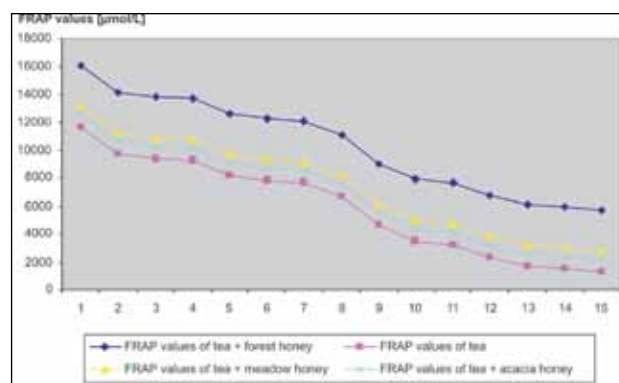


Figure 1. Influence of adding different botanical origin honey in tea

Figure 1. shows an influence of adding honey with different botanical origin in examined types of tea. It is understood that forest honey, which con-



Table 1. The total antioxidant activity of the medicinal plant extracts

| Plant material (Medicinal name) | Location   | Antioxidant activity ( $\mu\text{mol/L}$ ekstrakt) |
|---------------------------------|------------|----------------------------------------------------|
| Melissa folium                  | Tuzla area | 11710,0                                            |
| Salviae folium                  | Tuzla area | 9757,5                                             |
| Rosae pseudogructus             | Banovici   | 9352,5                                             |
| Valeriana herba                 | Banovici   | 9297,5                                             |
| Sanbuci flos                    | Banovici   | 8225,0                                             |
| Crataegi flos                   | Banovici   | 7890,0                                             |
| Hyperici herba                  | Tuzla area | 7645,0                                             |
| Mentha piperita folium          | Zivinice   | 6680,0                                             |
| Betulae folium                  | Banovici   | 4657,5                                             |
| Achillea millefolium folium     | Banovici   | 3520,0                                             |
| Achillea millefolium folium     | Tuzla area | 3250,0                                             |
| Teucarii herba                  | Banovici   | 2300,0                                             |
| Calendulae flos                 | Tuzla area | 1685,0                                             |
| Tiliae flos                     | Banovici   | 1517,5                                             |
| Calendulae flos                 | Banovici   | 1275,0                                             |

Table 2. The total antioxidant activity analyzed honey samples

| Honey sample | Location      | Antioxidant activity ( $\mu\text{mol/L}$ ekstrakt) |
|--------------|---------------|----------------------------------------------------|
| Forest       | Kladanj       | 4612,5                                             |
| Forest       | Kladanj       | 4240,0                                             |
| Meadow       | Kladanj       | 2705,0                                             |
| Meadow       | Mramor        | 2607,5                                             |
| Meadow       | Srebrenik     | 2357,5                                             |
| Meadow       | Čelić         | 1720,0                                             |
| Meadow       | Tuzla – Plane | 1687,5                                             |
| Meadow       | Gradačac      | 1570,0                                             |
| Meadow       | Tuzla         | 1490,0                                             |
| Acacia       | Tuzla         | 1162,5                                             |
| Meadow       | Miričina      | 1075,0                                             |
| Meadow       | Tuzla I       | 775,0                                              |
| Acacia       | Suha-Živinice | 597,5                                              |
| Meadow       | Tuzla III     | 525,0                                              |
| Meadow       | Tuzla         | 182,5                                              |

tains the most antioxidants has the biggest impact on the antioxidative tea activity. The least impact on the antioxidative tea activity has acacia honey, because it has the least content of antioxidants.

Honey antioxidant activity values show concentration within 182,5  $\mu\text{M/L}$  and 4612,5  $\mu\text{M/L}$ . Average antioxidant activity of forest honey is 4426,25  $\mu\text{M/L}$ , meadow 1517,727  $\mu\text{M/L}$  and acacia 880  $\mu\text{M/L}$ .

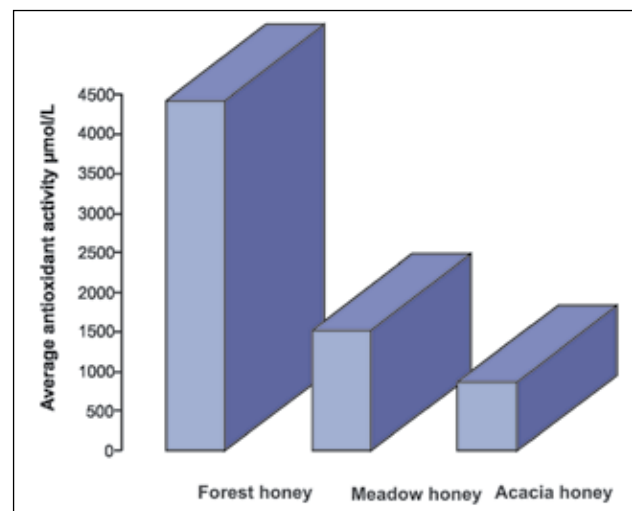


Figure 2. Effect of different botanical origin of honey on antioxidant activity

When we studied the effect of honey botanical origin on antioxidant activity, it was found that the highest antioxidant activity shows forest honey samples. In contrast, acacia honey samples show the lowest antioxidant activity (Fig. 2).

These data show relationship between botanical origin of honey and their antioxidant activity.

## Discussion

The results of the 4 researches show that medicinal plants are very rich source of antioxidants. In this work, antioxidative activity was measured in the extracts of medicinal plants that were prepared as teas. Based on the values of antioxidative activity, we have classified medicinal plants in four groups. Extract *Melissa folium* has very high FRAP (>10 mM/L); high FRAP (8 - 10 mM/L)  $n = 4$ ; good FRAP (5 - 8 mM/L)  $n = 3$ ; low FRAP (1 - 5 mM/L)  $n = 7$ . The researches of antioxidative activity in medicinal plants show very high value in extract *Melissa officinalis* L (17,18,19,20). It is also proved high antioxidative activity in plants from Labiatae family (21).

Honey has a phytochemical profile which includes polyphenols and other substances that can act as antioxidants. Antioxidants perform the role of eliminating free radicals, which are reactive compounds in the body. Free radicals are created through the normal process of metabolism and contribute to many serious diseases.

Investigations of Slovenian honey show that antioxidant activity was the lowest in the brightest acacia and lime honeys and the highest in darker honeys, namely fir, spruce and forest (22). These results are in correlation with ours. Our results show that antioxidant activity was the lowest in the brightest acacia honeys and the highest in darker forest honey. The colour of the analysed honey samples in this study was very variable and ranged from pale yellow to dark brown.

Some investigations in USA show that antioxidant activity of honey is 0.2 to 0.7 mmol FRAP/100 g. These investigations confirm that honey shows higher antioxidant activity than refined sugar (23). The results of antioxidant activity of honey obtained by using FRAP method are shown in Table 2.

According to their reducing ability/antioxidant power, 15 honey samples can be divided in three groups: a) low FRAP (<1 mM/L),  $n = 4$ ; b) good FRAP (1-2 mM/L)  $n = 6$ ; high FRAP (>2 mM/L)  $n = 5$ .

Figure 1. shows an influence of adding honey with different botanical origin in examined types of tea. It is understood that forest honey, which contains the most antioxidants has the biggest impact on the antioxidative tea activity. The least impact on the antioxidative tea activity has acacia honey, because it has the least content of antioxidants.

Taken together, our results strongly suggest that honey addition in tea plays an important role in their antioxidant activity. These roles depend on botanical origin of honey.

In this research we would like to establish benefits of adding honey instead of sugar in tea. Sugar is the quintessential source of energy and most foods, when digested, are metabolized by the body as basic sugar (glucose). It is a major source of calories in the diet. The body will save the excess energy in sugar as fat. While some stored fat is necessary, too much is undesirable and poses several health concerns especially for diabetics. Sugar also supports the growth of the bacteria that causes tooth decay.

A healthier alternative is honey. Honey is one of the oldest sweeteners used by man and was highly valued by ancient Egyptians for its medicinal and healing properties.

Honey contains more antioxidants and minerals than sugar (the darker, the more). Unlike table sugar, it is made up of inverted sugars. This makes it an ideal "instant energy" source. Regular sugar is made up of sucrose. Honey is made up of varying proportions of fructose and glucose, which means it's much sweeter.

## Acknowledgment

We gratefully acknowledge the sponsorship from the Federal Ministry of Education and Science, the Republic of Bosnia and Herzegovina, for the support of the research work.

## References

1. Azizah Othman Amin Ismail, Nawalyah Abdul Ghani, Ilham Adenan. (2007) „Antioxidant capacity and phenolic content of cocoa beans“, *Food Chemistry*, 100: 1523-1530.
2. Gutteridge, J., M., C., Halliwell, B. (1994) *Antioxidants in nutrition, health and disease*. Oxford University Press, Oxford. pp. 143.
3. Papas, A., M. (1999) *Antioxidant status, diet, nutrition and health*. London CRC Press, pp.66-68.
4. Halliwell, B. (1999). *Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept*. *Nutrition Reviews*. 57, 104-113.
5. Zheng, W., Wang, S. Y. (2001) *Antioxidant activity and phenolic compounds in selected herbs*. *Journal of Agricultural and Food Chemistry*. 49: 5165-5170.
6. Dreosti, I. E. (1991) *Trace elements, micronutrients, and free radicals*. Totowa: Human Press, pp. 149-199.
7. Ahmad, S. (1995) *Oxidative stress and antioxidant defenses in biology*. New York: Chapman & Hall.
8. Davies, K. J. A. (2000) *Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems*. *IUBMB Life*, 50, 279-289.
9. Lie C., Xie B. (2000) *Evaluation of the antioxidant pro-oxidant effects of tea catechin oxypolymers*. *Journal of Agricultural and Food Chemistry*. 48: 4290-4293.
10. Jha A, Mann R S, Balachandran R. (1996) *Tea: A refreshing beverage*. *Indian Food Industry*, 15: 22-29.
11. White J V and Landis W D. *Beekeeping in the United States Agriculture Handbook Number 335. Honey Composition and Properties*. 1980;
12. Salem S N. *Honey regimen in Gastrointestinal Disorders*. *Bulletin of Islamic Medicine*. 1982; 2(5): 422-425.
13. Gheldof N, Wang X and I Engeseth N. *Identification and Quantification of Antioxidant Components of Honey from Various Floral Sources*. *J Agric and Food Chem*. 2002; 50(21):5870-5877.
14. Subrahmanyam M. *A Prospective Randomised Clinical and Histological Study of Superficial Burn Wound Healing with Honey and Silver Sulfadiazine*. *Burns*. 1998; 14: 91-99.
15. Allen K L, Molan P C and Reid G M. *A Survey of the Antibacterial Activity of Some New Zealand Honeys*. *J Pharm Pharmacol*. 1991; 43: 817-822.
16. Benzie, I., F., Strain, J., J. (1996) “The Ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP Assay. *Analytical Biochemistry*, 239: 70-76.
17. Katalinić V., Milos M., Kulisić T., Jukić, M. (2006) *Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols*. *Food Chemistry*. 94: 550-557.
18. Pourmorad F, Hosseinimehr S J, Shahabimajd N (2006) *Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants*. *African Journal of Biotechnology*. 5 (11): 1142-1145.
19. Apak R, Guclu K, Ozyurek M, Esin Karademir S, Ercag E (2006) *Int Food Sci Nutr*. 57 (5-6): 292-304.
20. Halvorsen B L, et all. (2006) *A Systematic Screening of Total Antioxidants in Dietary Plants*. *Journal of the American College of Nutrition*. 132: 461-471.
21. Szollosi R, Szollosi Varga I. (2002) *Total antioxidant power in some species of Labiate*. *Acta Biologica Szegediensis*. 46 (3-4): 125-127.
22. Jasna Bartoncelj, Urška Doberšek, Mojca Jamnik and Terezija Golob (2007). *Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey*.
23. Katherine M. Philips, Monica H. Carlsen, Rune Blomhoff, (2008). „Total Antioxidant Content of Alternatives of Refined Sugar“. *Journal of the American Dietetic Association, Volume 109, Issue 1*, 64-71.
24. Benjamin Catovic, Almir Sestan, *Identification and quantification of microbiogen elements ferum, cuprum and zinc, TTEM*, 2008; 3(2): 46-50

### Corresponding author:

Aldina Kesic,  
University in Tuzla  
Univerzitetska 4, 75000 Tuzla  
Bosnia and Herzegovina  
e-mail: aldina.kesic@untz.ba

# Metabolic syndrome: comparative analysis of dermatological, biochemical and electroneurographic parameters of n.medianus and n.ulnaris in patients of both sexes

## METABOLIČKI SINDROM: KOMPARATIVNA ANALIZA DERMATOLOŠKIH, BIOHEMIJSKIH I ELEKTRONEUROGRAFSKIH PARAMETARA N. MEDIANUSA I N. ULNARISA KOD PACIJENATA OBA SPOLA

*Edin Suljagic<sup>1</sup>, Nermina Hadzigrabic<sup>2</sup>, Emir Tupkovic<sup>3</sup>*

<sup>1</sup> Dermatology Center „Farah“ Tuzla, Bosnia and Herzegovina,

<sup>2</sup> Dept of Dermatology, Tuzla University, Bosnia and Herzegovina,

<sup>3</sup> Department of Neurophysiology, Primary Health Centre Tuzla, Bosnia and Herzegovina

### Abstract

This study has proved differences in aspects of metabolic syndrome (MS) among sexes, as well connection between MS and neuropathies. The aim of our research was to compare blood sugar levels before and after GTT as well as neurophysiological parameters of n.medianus and n.ulnaris in persons of both sexes with newly discovered MS. All subjects were dermatologically examined. The analysis comprised of 36 subjects of both sexes each with found MS. Men's age was in average  $52.75 \pm 7.5$  (40-65), women  $52.1 \pm 7.7$  (38-67). Average blood sugar level in women was  $5.86 \pm 0.87$  (4.5-8) mmol/L and in men a little higher ( $p=0.0969$ ), or  $6.19 \pm 0.8$  (4.7-8) mmol/L. Average values of blood sugar after 120 minutes were not significantly different ( $p=0.7052$ ), or  $5.41 \pm 1.63$  (3.3-9.7) mmol/L in women, and  $5.27 \pm 1.52$  (2.7-9.8) mmol/L in males. Motoric velocity medians were

higher in women for n.medianus sin and n.ulnaris sin ( $p=0.0081$ ) and n.ulnaris dex ( $p=0.0293$ ), while terminal motoric latency medians were significantly longer for n.ulnaris sin ( $p=0.0349$ ) and n.ulnaris dex ( $p=0.011$ ). There were no significant differences in sensory conduction velocities of n.medianus and n.ulnaris among groups, but the amplitude of the highest peak f sensory response was higher for n.medianus sin and n.ulnaris sin ( $p=0.0009$ ) in females. The results point to the fact that there are differences in neurophysiological parameters for the examined nerves between sexes, meaning the nerve structures during MS are more prominent in men. There are no significant differences in skin changes between sexes. There was no significant difference among blood sugar value, sensory symptoms of neuropathy and examined skin pigmented lesions ( $p=0.8564$ ). Also, there were no significant difference between blood sugar value and skin types ( $p=0.81$ ).

**Key words:** Metabolic syndrome ( MS), Bool sugar level, GTT, Electroneurography, N. medianus, N. ulnaris, Skin changes

### Sazetak

Ova studija je pokazala aspekte metaboličkog sindroma (MS), spolnu zastupljenost, udruženost MS i neuropatija, povezanost MS i pigmentnih promjena na koži, kao i tipovima kože. Cilja studije je bio da se istraži nivo glukoze prije i poslije GTT testa, kao i neurofiziološke parametre nervus medianus i nervus ulnarisa kod osoba oba spola sa tek otkrivenim MS. Analizom je obuhvaćeno 36 ispitanika oba spola sa utvrđenim MS. Starosna dob muškaraca je bila  $52.75 \pm 7.5$  (40-65), a kod žena  $52.1 \pm 7.7$  (38-67). Prosječna vrijednost šećera u krvi kod ženskih ispitanika iznosila je  $5.86 \pm 0.87$  (4.5-8) mmol/L, a kod muških ispitanika nešto viša ( $p=0.0969$ ), ili  $6.19 \pm 0.8$  (4.7-8) mmol/L. Nakon 120 minuta nije ustanovljena signifikantna razlika u prosječnoj vrijednosti šećera u krvi između muških  $5.27 \pm 1.52$  (2.7-9.8) mmol/ i ženskih ispitanika  $5.41 \pm 1.63$  (3.3-9.7) mmol/L ( $p=0.7052$ ). Motorička provodljivost n. medianus l.sin. i n. ulnaris l.sin. je bila viša kod žena ( $p=0.008$ ) i n. ulnaris l. dex. ( $p=0.0293$ ), dok je terminalna motorička latencija bila signifikantno duža za n. ulnaris- sin ( $p=0.0349$ ) i ulnaris dex ( $p=0.011$ ). Nije ustanovljena signifikantna razlika u senzornoj kondukcionoj provodljivosti n. medianusa i n. ulnarisa unutar grupe, ali je najviši pik amplitude senzornog odgovora ustanovljen kod žena na n. medianus sin. ( $p=0.0269$ ) i n. ulnaris dex. ( $p=0.011$ ). Rezultati pokazuju da postoje signifikantne razlike između polova nakon analize neurofizioloških parametara, sa većom zastupljenošću kod muškaraca sa MS. Nije ustanovljena signifikantna razlika u promjenama na koži između ispitanika oba spola. Nije utvrđena signifikantna razlika između vrijednosti šećera u krvi, simptoma senzorne neuropatije i ispitivanih pigmentnih promjena na koži ( $p=0.8564$ ). Takođe, nije ustanovljena signifikantna razlika između vrijednosti šećera u krvi i tipova kože ( $p=0.81$ ).

**Ključne riječi:** Metabolički sindrom ( MS ), Glikemija, GTT, Elektromiografija, N. medianus, N. ulnaris, Kožne promjene

### Introduction

The first component of metabolic syndrome (MS) is central obesity, defined by waist length, combined with at least two of four elements: elevated triglycerides (TGL), lowered HDL cholesterol, elevated blood pressure and elevated blood sugar on an empty stomach.

There is evidence that incidence of MS and distribution of its components in men and women differ. Research of the working population in Spain and France show the prevalence of MS 11.5% in men and 4.1% in women, with tendency of growth in older age, which actually varies in different categories of professional activity (1).

Among adults in Turkey, MS was found present in 26.9% subjects, 31.3% women and 21.7% men (2). The data that in persons with acute ischemic syndromes MS is found in 70.2% women and 52.6% men shows the possible consequences of this syndrome (3).

From the very adolescence, accumulation of intra-abdominal fat tissue can cause the onset of MS, including metabolic and inflammatory components, which has a more serious effect on blood pressure in men, which can then be added to magnification of sympathetic activity noticed also only in men (4). Obesity is in strong connection with MS, a non-obesity has a stronger association with the risk of insulin resistance in women (5).

There is an inverse connection of adiponectin in plasma and insulin resistance. Subclinical inflammation is present in MS with proved difference in levels and expression of different sensitive biomarkers of inflammation between sexes. In, for example, non-diabetic population, smoking is related to the fall in the level of adiponectin, rise in high-sensitivity C-reactive protein (hs-CRP) and higher level of interleukin-1 receptor antagonist (il-1Ra) in women (6,7,8).

In persons with acute ischaemic syndromes and MS, men had a more frequent rise in TGL concentration, and women fall of HDL concentration (3). As a confirmation of genetic predisposition in women, there is a rise in the risk for low HDL-C, dyslipidaemia and MS, which is combined with alleles of the gene apolipoprotein A5 (APOA5), more precisely, APOA5 SNPS (9).



So, according to a study in Sweden, women with MS had a higher Body Mass Index (BMI) and the level C-reactive protein, and stood a higher chance of fulfilling the waist length criteria, and the syndrome was more prevalent and characterized by obesity, and low-level inflammation (10).

In the pathogenesis of MS there is a great interest about the role of free radicals oxidation stress. Research in Japan has shown cellular anti-oxidant enzymes such as glutathione peroxidase 1 (GPX1), play the central role in the control of reactive oxygen species, and GPX1 polymorphism (Pro198Leu) is combined with MS in men, but not in women (11).

MS is in fact often present in patients with neuropathy, and also impaired glucose intolerance (IGT), as well as normal glucose tolerance (NGT) compared to the prevalence within general population (12). It is well known that the polyneuropathy of longer nerves is common complication of diabetes mellitus. However, intermittent hyperglycemia or insulin resistance combined with prediabetes could be sufficient to damage distal parts of nerves. Preferential damage of small non-myelinated nerves is suggested by the existence of prominent neuropathic pain, predominant sensory damage and early autonomic dysfunction (13).

Studies confirm certain differences in aspects of MS between sexes, as well as connection of MS and neuropathies, but relatively small number of them is concerned with this syndrome and differences of electroneurographic parameters between the sexes. Therefore, the aim of our research is to attempt to at least partly shed light on this problem.

### **Aim of research**

In persons with newly discovered MS (both sexes) we will compare the levels of blood sugar on empty stomach and after GTT, pigmented skin changes, as well as neurophysiological parameters of n.medianus and n.ulnaris.

### **Materials and methods**

Analysis encompassed 36 subjects of each sex, with newly-discovered MS. Men were aged in average  $52.75 \pm 7.5$  (40 – 65), and women  $52.1 \pm 7.7$

(38 – 67). 11 female subjects and 9 male subjects had sensory symptoms of neuropathy in the hand. All patients with MS were tested for blood glucose levels and oral glucose tolerance test (OGTT) was performed. Skin and visible mucous were examined. Pigmented skin changes were tested by dermatoscopy (Visiomed system).

Neurophysiological testing was conducted in room temperature, and 'physiological' skin temperature with female subjects in the lying position. Neurographic parameters were measured with the machine EMNG Medelec Synergy (EMG and EP Systems – Oxford Instruments, 2004). Superficial stimulatory registration bipolar electrodes were used (so called, large touch-proof). Sensory conduction velocity n.medianus and n.ulnaris was measured by stimulation of the wrist and registration on index and little fingers, between the first and second interphalangeal joint. Terminal motoric latency was measured by stimulating the wrist and registration on thenar and hypothenar, more precisely 6cm proximally from the registration electrode on the stomach, m.abductor pollicis brevis for n.medianus and m.adductor digiti minimi for n.ulnaris. Proximal measurement for both nerves was conducted in the segment just above the elbow. During electroneurographic processing (ENG) sensory nerve conduction velocity (from the stimulating artefact to primary deviation of the isoelectric line) was analyzed, with an amplitude of the sensory nerve action potential (SNAP); terminal motoric latency of n. medianus and n. ulnaris on both sides, with the highest amplitude of compound muscle action potential (CMAP), measured from the location of the primary deviation from the isoelectric line upon stimulatory artefact up to the final return of the needle to the isoelectric line. While determining motoric and sensory response, stimulation was performed until the CMAP and SNAP amplitudes ceased to grow. The levels of blood sugar and glucose tolerance after 120 minutes were shown as median with standard deviation, and during the statistical analysis of differences between sexes T-test and HiSquare test were used. During the statistical analysis of neurophysiological parameters, were estimated values of median and non-parameter Mann-Whitney U test, and differences were valued as significant for  $p < 0.005$ .

## Results and discussion

The average blood sugar value in women was  $5.86 \pm 0.87$  (4.5-8.0) mmol/L, and in men the value was not significantly higher ( $p=0.0969$ ), that is,  $6.19 \pm 0.8$  (4.7 – 8 mmol/L). Majority of 27 female subjects and 20 male subjects had the blood sugar values within standard (3.9 – 6.1 mmol/L), while values above this were shown in 9 women and 16 men, which was a significantly different distribution among sexes ( $p=0.0189$ ).

The average blood sugar levels after 120 minutes during OGTT test were not significantly different ( $p=0.7052$ ),  $5.41 \pm 1.63$  (3.3 – 9.7) mmol/L in women and  $5.27 \pm 1.52$  (2.7 – 9.8) mmol/L in men. 23 persons of each sex had blood sugar values within standard, 8 females and 9 males had above that, and 5 females and 4 males had lowered values, so there was no important difference in the distribution of these values among groups ( $p=0.8348$ ).

Skin examination showed 22 patients with skin type Fitzpatrick 2, 12 patients with skin type Fitzpatrick 3, and two patients with Fitzpatrick 4. There were ten patients with seborrheic warts, six patients with compound naevi (Visiomed score - between 2 and 4) , and 3 patients (female) with lentiginos and melasma. In one patient we found naevus dysplasticus (Visiomed score 8.4) . There was no significant difference among blood sugar value, sensory symptoms of neuropathy and examined skin pigmented lesions ( $p=0.8564$ ). Also, there were no significant difference between blood sugar value and skin types (  $p= 0.81$ ).

Parameters of motoric and sensory electroneurographic analysis of n.medianus and n.ulnaris in male and female subjects with MS are shown in tables 1-4. Motoric conductory velocity medians usually have higher values in women, terminal motoric latencies are lower.

Statistical analysis has shown that motoric velocity medians of n.medianus sin n.ulnaris sin and n.ulnaris dex are significantly higher in women while terminal motoric latency medians of both n.ulnaris in women are longer (Table 5). CMAP surfaces acquired upon proximal and distant stimulation did not show important differences among sexes (Table 6).

There were no significant differences in sensory conduction velocities of n.mwsianus and n.ulnaris among the groups. However, the amplitude of the highest peak of the sensory response was significantly higher in n.medianus sin and n.ulnaris sin in females (Table 7). One patient did not show sensory response of n.medianus sin and one in n.medianus dex.

*Table 1. Parameters of motoric neurographic analysis of n.medianus and n.ulnaris in 36 female subjects with metabolic syndrome.*

| Motoric velocity (m/s) | Mediana            |
|------------------------|--------------------|
|                        | Percentile (25-75) |
| n.medianus sin.        | Min.               |
|                        | Max.               |
|                        | 57,95              |
|                        | 55,6-61,35         |
| n.medianus dex.        | 51,6               |
|                        | 66,8               |
|                        | 57,2               |
|                        | 53,75-59,7         |
| n.ulnaris sin.         | 49                 |
|                        | 65,3               |
|                        | 53,2               |
|                        | 51-55,1            |
| n.ulnaris dex.         | 46,6               |
|                        | 63,2               |
|                        | 55,85              |
|                        | 53,65-58,025       |
| Motoric latency (msec) | 50                 |
|                        | 63,4               |
|                        | Mediana            |
|                        | Percentile (25-75) |
| n.medianus sin.        | Min.               |
|                        | Max.               |
|                        | 3,4                |
|                        | 3,15-3,675         |
| n.medianus dex.        | 2,6                |
|                        | 4,9                |
|                        | 3,275              |
|                        | 2,85-3,66          |
|                        | 2,6                |
|                        | 5,8                |

|                        |                                                                           |                                                                                                                                     |                                                                           |
|------------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| <b>n.ulnaris sin.</b>  | 2,75<br>2,4875-3,0125<br>2,1<br>3,7                                       | <b>n.ulnaris dex.</b>                                                                                                               | 14,5<br>10,9-16,45<br>3,1<br>24,6                                         |
| <b>n.ulnaris dex.</b>  | 2,65<br>2,3875-3,1<br>1,9<br>3,75                                         | CMAP: complex muscle action potential:<br>CMAP I upon distal stimulation;<br>CMAP II upon proxima stimulation                       |                                                                           |
| <b>CMAPI (mV)</b>      | <b>Mediana</b><br><b>Percentile (25-75)</b><br><b>Min.</b><br><b>Max.</b> | <i>Table 2. Parameters of motoric neurographic analysis of n.medianus and n.ulnaris in 36 male subjects with metabolic syndrome</i> |                                                                           |
| <b>n.medianus sin.</b> | 20,7<br>14,925-24,825<br>8<br>40,2                                        | <b>Motoric velocity (m/s)</b>                                                                                                       | <b>Mediana</b><br><b>Percentile (25-75)</b><br><b>Min.</b><br><b>Max.</b> |
| <b>n.medianus dex.</b> | 19,5<br>16,5-32,325<br>6,9<br>40,8                                        | <b>n.medianus sin.</b>                                                                                                              | 54,6<br>51,325-58,825<br>44<br>62,9                                       |
| <b>n.ulnaris sin.</b>  | 14,2<br>10,95-19,65<br>5,2<br>37,1                                        | <b>n.medianus dex.</b>                                                                                                              | 55,6<br>52,05-58<br>34<br>62,4                                            |
| <b>n.ulnaris dex.</b>  | 14,9<br>10,55-18,8<br>4,1<br>25,8                                         | <b>n.ulnaris sin.</b>                                                                                                               | 50,9<br>48,05-53,975<br>32,4<br>61,3                                      |
| <b>CMAPII (mV)</b>     | <b>Mediana</b><br><b>Percentile (25-75)</b><br><b>Min.</b><br><b>Max.</b> | <b>n.ulnaris dex.</b>                                                                                                               | 52,8<br>50,7-56,7<br>46,8<br>61,5                                         |
| <b>n.medianus sin.</b> | 18,9<br>15,05-23,2<br>6,3<br>38,6                                         | <b>Motoric latency (msec)</b>                                                                                                       | <b>Mediana</b><br><b>Percentile (25-75)</b><br><b>Min.</b><br><b>Max.</b> |
| <b>n.medianus dex.</b> | 18<br>14-28,525<br>5,8<br>42,9                                            | <b>n.medianus sin.</b>                                                                                                              | 3,55<br>3,35-3,912<br>2,85<br>4,9                                         |
| <b>n.ulnaris sin.</b>  | 13,45<br>10,175-16,025<br>3,7<br>35,7                                     | <b>n.medianus dex.</b>                                                                                                              | 3,525<br>3,2375-3,825<br>2,6<br>5,2                                       |

| <b>n.ulnaris sin.</b>  |                           | <b>n.ulnaris dex.</b>                                                                                                                 |                           |
|------------------------|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
|                        | 2,925                     |                                                                                                                                       | 15,1                      |
|                        | 2,8-3,05                  |                                                                                                                                       | 10,35-19                  |
|                        | 2,3                       |                                                                                                                                       | 4                         |
|                        | 3,65                      |                                                                                                                                       | 28,8                      |
| <b>n.ulnaris dex.</b>  | 3,05                      | CMAP: complex muscle action potential:<br>CMAP I upon distal stimulation;<br>CMAP II upon proxima stimulation                         |                           |
|                        | 2,7-3,25                  |                                                                                                                                       |                           |
|                        | 2,05                      |                                                                                                                                       |                           |
|                        | 4,1                       |                                                                                                                                       |                           |
| <b>CMAPI (mV)</b>      | <b>Mediana</b>            | <i>Table 3. Parameters of sensory neurographic analysis of n.medianus and n.ulnaris in 36 female subjects with metabolic syndrome</i> |                           |
|                        | <b>Percentile (25-75)</b> | <b>Sensory velocity (m/s)</b>                                                                                                         |                           |
|                        | <b>Min.</b>               |                                                                                                                                       | <b>Median</b>             |
|                        | <b>Max.</b>               |                                                                                                                                       | <b>Percentile (25-75)</b> |
| <b>n.medianus sin.</b> | 21,5                      |                                                                                                                                       | <b>Min.</b>               |
|                        | 17,3-28,475               |                                                                                                                                       | <b>Max.</b>               |
|                        | 6                         | <b>n.medianus sin.</b>                                                                                                                | 49,45                     |
|                        | 43,9                      |                                                                                                                                       | 45,975-53,8               |
| <b>n.medianus dex.</b> | 23,1                      |                                                                                                                                       | 0                         |
|                        | 16,475-30,9               |                                                                                                                                       | 57,7                      |
|                        | 6,6                       | <b>n.medianus dex.</b>                                                                                                                | 49,1                      |
|                        | 47,6                      |                                                                                                                                       | 43,675-52,225             |
| <b>n.ulnaris sin.</b>  | 18,15                     |                                                                                                                                       | 0                         |
|                        | 12,675-21,475             |                                                                                                                                       | 58,9                      |
|                        | 5,1                       | <b>n.ulnaris sin.</b>                                                                                                                 | 48,9                      |
|                        | 39,3                      |                                                                                                                                       | 45,2-51,45                |
| <b>n.ulnaris dex.</b>  | 16,95                     |                                                                                                                                       | 39,7                      |
|                        | 12-20,925                 |                                                                                                                                       | 54,9                      |
|                        | 4,7                       | <b>n.ulnaris dex.</b>                                                                                                                 | 49,05                     |
|                        | 37,9                      |                                                                                                                                       | 46,475-51,025             |
| <b>CMAPII (mV)</b>     | <b>Mediana</b>            |                                                                                                                                       | 41,5                      |
|                        | <b>Percentile (25-75)</b> |                                                                                                                                       | 56,2                      |
|                        | <b>Min.</b>               | <b>Amplitude SNAP (µV)</b>                                                                                                            | <b>Median</b>             |
|                        | <b>Max.</b>               |                                                                                                                                       | <b>Percentile (25-75)</b> |
| <b>n.medianus sin.</b> | 19,9                      |                                                                                                                                       | <b>Min.</b>               |
|                        | 16,15-27,675              |                                                                                                                                       | <b>Max.</b>               |
|                        | 6,5                       | <b>n.medianus sin.</b>                                                                                                                | 16,9                      |
|                        | 38,4                      |                                                                                                                                       | 11,55-23,4                |
| <b>n.medianus dex.</b> | 21,15                     |                                                                                                                                       | 0                         |
|                        | 14,5-28,075               |                                                                                                                                       | 35                        |
|                        | 3,7                       | <b>n.medianus dex.</b>                                                                                                                | 14,65                     |
|                        | 46,8                      |                                                                                                                                       | 10,675-20,05              |
| <b>n.ulnaris sin.</b>  | 15                        |                                                                                                                                       | 0                         |
|                        | 11,425-19,725             |                                                                                                                                       | 49,7                      |
|                        | 3,8                       |                                                                                                                                       |                           |
|                        | 35,9                      |                                                                                                                                       |                           |

|                                                                                                     |                                     |                        |                                   |
|-----------------------------------------------------------------------------------------------------|-------------------------------------|------------------------|-----------------------------------|
| <b>n.ulnaris sin.</b>                                                                               | 19,05<br>15,3-28,075<br>8,8<br>41   | <b>n.medianus dex.</b> | 11,65<br>8,15-17,15<br>4,7<br>30  |
| <b>n.ulnaris dex.</b>                                                                               | 18,35<br>11,25-26,2<br>2,25<br>46,1 | <b>n.ulnaris sin.</b>  | 13,4<br>8,075-21,2<br>4,3<br>33,9 |
| SD: standard deviation;<br>Min.: minimal;<br>Max.: maximal;<br>SNAP: sensory nerve action potential |                                     | <b>n.ulnaris dex.</b>  | 16,1<br>10,9-18,65<br>4,2<br>30,5 |

*Table 4. Parameters of sensory neurographic analysis of n.medianus and n.ulnaris in 36 male subjects with metabolic syndrome*

**Sensory velocity (m/s)**

|                            | <b>Mediana</b><br><b>Percentile (25-75)</b><br><b>Min.</b><br><b>Max.</b> |
|----------------------------|---------------------------------------------------------------------------|
| <b>n.medianus sin.</b>     | 49,55<br>45,525-52,975<br>37,1<br>62,2                                    |
| <b>n.medianus dex.</b>     | 49<br>44,35-52,325<br>29,5<br>60                                          |
| <b>n.ulnaris sin.</b>      | 48,1<br>44,625-50,35<br>19,8<br>55                                        |
| <b>n.ulnaris dex.</b>      | 47,2<br>45,15-50<br>40,7<br>59,5                                          |
| <b>Amplitude SNAP (µV)</b> |                                                                           |
|                            | <b>Mediana</b><br><b>Percentile (25-75)</b><br><b>Min.</b><br><b>Max.</b> |
| <b>n.medianus sin.</b>     | 12,05<br>9,2-16,85<br>2,7<br>28,9                                         |

SD: standard deviation;  
Min.: minimal;  
Max.: maximal;  
SNAP: sensory nerve action potential

*Table 5. Significance of differences in motoric conduction velocities of n.medianus and ulnaris in male and female subjects with MS*

**MOTORIC NERVE**  
**PARAMETER**  
**P...**

|                                                                                          |
|------------------------------------------------------------------------------------------|
| <b>n.medianus sin</b><br>Velocity<br><b><u>0,0024</u></b>                                |
| TML<br>0,0545<br><b>n. medianus dex.</b><br>Velocity<br>0,0733                           |
| TML<br>0,0673<br><b>n.ulnaris sin.</b><br>Velocity<br><b><u>0,0081</u></b>               |
| TML<br><b><u>0,0349</u></b><br><b>n.ulnaris dex.</b><br>Velocity<br><b><u>0,0293</u></b> |
| TML<br><b><u>0,011</u></b>                                                               |
| TML: terminal motoric latency                                                            |



Table 6. Significance of differences in CMAP surfaces of n.medianus and n.ulnaris in male and female subjects with MS

**MOTORIC NERVE**

**CMAP**

**P...**

**n.medianus sin**

I

0,2437

II

0,3384

**n. medianus dex.**

I

0,7018

II

0,4305

**n.ulnaris sin.**

I

0,0989

II

0,1712

**n.ulnaris dex.**

I

0,2648

II

0,4404

CMAP: complex muscle action potential:

CMAP I upon distal stimulation;

CMAP II upon proxima stimulation

Table 7. Differences in sensory conductivity velocities and amplitude SNAP of n.medianus and n.ulnaris in male and female subjects with MS

**SENSORY NERVE**

**PARAMETER**

**P...**

**n.medianus sin**

Velocity

0,9865

Amplitude SNAP

**0,0269**

**n. medianus dex.**

Velocity

0,8614

Amplitude SNAP

0,1122

**n.ulnaris sin.**

Velocity

0,3412

Amplitude SNAP

**0,0009**

**n.ulnaris dex.**

Velocity

0,1414

Amplitude SNAP

0,1161

SNAP: sensory nerve action potential.

Our results show that there is no significant difference in the age of onset and diagnose of MS among males and females. According to Zeliunas and Co (2008) study of patients with acute ischemic syndrome, women with MS are significantly older than man (68.1 and 60.2) (3).

Even though in our study males have higher blood sugar levels, there is no difference between sexes after GTT after 120 minutes. The interesting research is that, for example, low level of total testosterone and sexual/hormone bounding globuline (SHBG) independently represent a risk factor for the development MS and diabetes mellitus in middle-aged men. Hypo-androgenism is early marker of disrupted insulin and glucose metabolism, which can progress to MS or diabetes and can contribute to pathogenesis. However, study with late adolescences and early adults has shown that men are more obese, have hypertension and hyper triglicerydemia than women, but all cases of glucose intolerance were in women (15).

Men and women displayed differences in neurophysiological parameters of n.medianus and n.ulnaris. central values of motoric conduction velocities in our study are lower for the most part, and terminal motoric latences are higher in men. On the other hand, amplitudes of sensory response for the examined nerves on the left side are lower in men. These observations suggest that the researched nerve structures during MS are somewhat more prominently noticed in men.

## Conclusion

Motoric velocities of n.medianus sin and n.ulnaris sin and dex are significantly higher, terminal motoric latencies of both n.ulnaris in women are longer, and amplitudes of the highest peak of sensory response are higher in n.medianus sin and n.ulnaris sin in females, which suggests that the examined nerve structures during metabolic syndrome are more prominent in men.

## References

1. Sánchez-Chaparro MA, Calvo-Bonacho E, González-Quintela A, Fernández-Labandera C, Cabrera M, Sáinz JC, Fernández-Meseguer A, Banezas JR, Ruilope LM, Valdivielso P, Román-García J; Occupation-related differences in the prevalence of metabolic syndrome. *Diabetes Care* 2008;31(9):1884-1885.
2. Erem C, Hacıhasanoglu A, Deger O, Topbaş M, Hosver I, Ersoz HO, Can G. Prevalence of metabolic syndrome and associated risk factors among Turkish adults: Trabzon MetS study. *Endocrine* 2008;33(1):9-20.
3. Zaliūnas R, Slapikas R, Babarskiene R, Slapikienė B, Luksiene D, Milvidaitė I, Laukaitiene J. The prevalence of the metabolic syndrome components and their combinations in men and women with acute ischemic syndromes. *Medicina (Kaunas)* 2008;44(7):521-8.
4. Syme C, Abrahamowicz M, Leonard GT, Perron M, Pitiot A, Qiu X, Richer L, Totman J, Veillette S, Xiao Y, Gaudet D, Paus T, Pausova Z. Intra-abdominal adiposity and individual components of the metabolic syndrome in adolescence: sex differences and underlying mechanisms. *Arch Pediatr Adolesc Med* 2008; 162(5):453-61.
5. Cordero A, León M, Andrés E, Ordoñez B, Laclaustra M, Grima A, Pascual I, Luengo E, Civeira F, Pocovi M, Alegria E, Casasnovas JA; on behalf of the MESYAS Registry investigators Gender differences in obesity related cardiovascular risk factors in Spain. *Prev Med* 2008 Nov 7 [In press]
6. Ahonen TM, Kautiainen HJ, Keinänen-Kiukkaanniemi SM, Kumpusalo EA, Vanhala MJ. Gender difference among smoking, adiponectin, and high-sensitivity C-reactive protein. *Am J Prev Med* 2008; 35(6):598-601.
7. Rogowski O, Shapira I, Berliner S. Exploring the usefulness of inflammation-sensitive biomarkers to reveal potential sex differences in relation to low-grade inflammation in individuals with the metabolic syndrome. *Metabolism* 200; 57(9):1221-6.
8. Saltevo J, Vanhala M, Kautiainen H, Kumpusalo E, Laakso M. Gender differences in C-reactive protein, interleukin-1 receptor antagonist and adiponectin levels in the metabolic syndrome: a population-based study. *Diabet Med.* 2008; 25(6):747-50.
9. Komurcu-Bayrak E, Onat A, Poda M, Humphries SE, Palmen J, Guclu F, Can G, Erginel-Unaltuna N. Gender-modulated impact of apolipoprotein A5 gene (APOA5) -1131T>C and c.56C>G polymorphisms on lipids, dyslipidemia and metabolic syndrome in Turkish adults. *Clin Chem Lab Med* 2008;46(6):778-84.
10. Tonstad S, Sandvik E, Larsen PG, Thelle D. Gender differences in the prevalence and determinants of the metabolic syndrome in screened subjects at risk for coronary heart disease. *Metab Syndr Relat Disord.* 2007;5(2):174-82.
11. Kuzuya M, Ando F, Iguchi A, Shimokata H. Glutathione peroxidase 1 Pro198Leu variant contributes to the metabolic syndrome in men in a large Japanese cohort. *Am J Clin Nutr* 2008;87(6):1939-44.
12. Smith AG, Rose K, Singleton JR. Idiopathic neuropathy patients are at high risk for metabolic syndrome. *J Neurol Sci* 2008;273(1-2):25-8.
13. Singleton JR, Smith AG. Neuropathy associated with prediabetes: what is new in 2007? *Curr Diab Rep* 2007;7(6):420-4
14. Laaksonen D E, Niskanen L, Punnonen K, Nyysönen K, Tuomainen TP, Valkonen VP, Salonen R, Salonen JT. Testosterone and Sex Hormone-Binding Globulin Predict the Metabolic Syndrome and Diabetes in Middle-Aged Men. *Diabetes Care* 27:1036-1041, 2004.
15. Huang TT, Shmel A, Lee RE, Delancey W, Strotter ML. Metabolic Risks among College Students: Prevalence and Gender Differences. *Metab Syndr Relat Disord* 2007;5(4):365-72.

### Corresponding author:

Edin Suljagic,  
Dermatology Center „Farah“ Tuzla,  
Bosnia and Herzegovina,  
e-mail:

# Determination of mean glandular dose from routine mammography for two age groups of patients

Suad Kunosic<sup>1</sup>, Denis Ceke<sup>2</sup>, Mustafa Kopric<sup>1</sup>, Lidija Lincender<sup>3</sup>

<sup>1</sup> University of Tuzla, Faculty of Natural Sciences and Mathematics, Bosnia and Herzegovina

<sup>2</sup> University of Tuzla, University Center for Distant Education, Bosnia and Herzegovina

<sup>3</sup> University of Sarajevo, Faculty of Health Studies, Bosnia and Herzegovina

## Abstract

An objective of mammographic screening is an early detection of breast cancer. At this moment there is no any known or published data about doses for women included into mammography screening in Bosnia and Herzegovina. Hence, a potential risk of cancerogenesis caused by radiation in this procedure is increasing. The object of this research was to define a mean glandular dose (MGD) at mediolateral (MLO) and craniocaudal (CC) projection for every individual breast and a total dose for a whole mammographic examination at the Radiology Clinic (of the University of Sarajevo Clinics Centre) for two different age groups (age: 40 – 49 and 50 - 64). Dose estimates were made for 63 patients who were subduced to a routine mammographic examination and they involved corrections in regard to variations of age, breast thickness and applied clinical spectra. A mean MGD for women between 40 and 49 was 1,64 mGy for a MLO screen and 1,36 mGy for a CC screen. For a group between 50 and 64 mean MGD was 1,74 mGy for a MLO screen and 1,45 mGy for a CC screen. Differences of MGD at MLO and CC examination are caused by a huger thickness of a compressed breast at MLO projection, which is 9 - 11 % huger than on CC projection. According to a performed correlation analysis one defined a remarkable significance between MGD and thickness of a compressed breast for MLO and CC screens; the first age group (MLO:  $r=0,852$ ,  $p < 0,01$ ; CC :  $r = 0,817$ ,  $p < 0,01$ ) and the second age group (MLO :  $r = 0,721$ ,  $p < 0,01$ ; CC :  $r = 0,674$ ,  $p < 0,01$ ). MGD for the whole mammographic examination

was 3,11 mGy and it was significantly connected to breast thickness ( $r= 0,77$ ,  $p < 0,01$ ).

**Key words:** mammography, mean glandular dose (MGD), dosimetry on mammography, compressed breast thickness (CBT).

## Introduction

There is a risk of cancerogenesis caused by radiation and connected to examination of female breast with X – rays. This risk is small if a modern equipment and technology are used, while a benefit from the examination is significant (1). Numerous authors assessed the risk of radiation and the benefit from mammography in regard to breast screening (2, 3, 4, 5, 6). A working unit of a Board for Protection from Radiation and NHSB (The National Health Service Breast Screening Program in the UK) program of breast screening recently surveyed radiation risks from mammography and concluded that the radiation risk that caused breast cancer was about 1 on 100 000 per mGy with women that were screened in the United Kingdom (7). Although this risk was relatively small, it is important to use appropriate equipment and techniques so that an optimum quality of screens can be achieved with the smallest possible dose that corresponds to quality of the screen. Measuring a dose received by a breast is an important part of the program which ensures quality of mammographic screening. Early detection of breast cancer is a key for a long – term control and a good prognosis of the disease and a high quality mammography is a prerequisite for that (8).

All women who belong to the age group 40 – 64 should be included in a regular screening program every year. This research included women between 40 and 64. On the first mammographic examination one screens mediolateral and cranio-caudal mammograms.

One of responses which need to be found during a mammographic examination of a woman is a risk and a benefit brought with a patient's dose. A very few data about patient's dose are available during mammography of younger women. It is expected that patient's doses for mammography for younger women are somewhat bigger since their breast contain more glandular tissue. Law et al (9) assessed a relative possibility to introduce early detection of the breast cancer in screening of younger women, but without any concrete data about patient's doses. Young and Burche (10) undertook a similar research and assessed patient's doses for younger women in the UK from 40 to 48. The mentioned study gave somewhat bigger percentage of doses for women from 40 to 48 than for women from 50 to 64, but deviations are not significant. A set of mammographic studies (10, 11, 12) showed that a basis for an assessment of the radiation risk is defining a mean glandular dose (MGD).

## Materials and methods

### Data collection

All experimental measuring of the patient's dose during diagnostic mammographic examinations of patients were done at the Department for Thoracic Diagnostic with Breast of the Radiology Clinic (of the University of Sarajevo Clinics Centre). Siemens Mammomat 1000 (Mo/Mo) was used for diagnostic examinations of patients. Measuring was done in the period between July 2007 and July 2008. Data about patient's doses were collected for 63 patients (234 screens) from 40 to 64 years during a routine mammographic control.

The following data were recorded during the diagnostic examination:

- (1) Age of patients
- (2) Used clinical spectra

- (3) Compressed breast thickness (CBT) and type of projection
- (4) Exposition factors and charge (mAs), anode voltage (kVp), clinical spectra (target / filter) for each screen.
- (5) Size of used film

Patient's doses were calculated according to the recorded data.

### Quality control

During the period of data collection the value of anode voltage, reproducibility of doses and filter half value (HVL) were measured without returnable radiation and compression board was checked for different settings of kVp and a target / filter combination following recommendations of the European Protocol (13), which recommends a frequency and a methodology for measuring. Accuracy of reading of thickness of a compressed breast was checked according to recommendations of the mentioned Protocol. All of quality control tests and dosimetry in diagnostic radiology were done with Barracuda instrument.

### Dosimetry

MGD for each mammogram is defined on a basis of conversion factors calculated by Dance et al (14) and a calculated ESAK (entering air kerma measured freely in air without backscatter), using the following relation:

$$MGD = g \cdot c \cdot s \cdot ESAK$$

ESAK for each individual exposition is calculated with exit voltage and charge (mAs) used in an exposition field. Conversion factors were calculated by Dance, for a different clinical spectrum (target/filter combination), HVL, compressed breast thickness and breast glandularity. Factor  $g$  defined by Dance et al (14) corresponds to glandularity of 50 % while factor  $c$  includes every change in breast glandularity of 50 %. Factor  $c$  was defined by Dance et al (14) for a normal composition and for various thickness of a compressed breast (20 – 110 mm) and HVL.  $C$  factor was defined by Dance for a normal breast of a woman of age 40



- 49 and 50 - 64. Estimation of glandular changes of a breast and a composition of both age groups was defined by Beckett and Kotre according to experimental results (15). Finally, factor *s* includes a correction for used type of the clinical spectrum (12), and all screens were made using the same clinical spectra Mo/Mo.

### Statistical analysis

The data were statistically processed in SPSS and they were shown as standard deviation and confidence interval. Pearson's coefficient was used for statistical significance of correlation between MGD and CBT. A value of  $p < 0.05$  was considered as indicative of significance.

## Results and discussion

### Age and compressed breast thickness

Age of examined population varied from 40 to 64 years. Distribution of thickness of a compressed breast was symmetrical, and varied from 23 to 81 mm. Accuracy of defining of thickness of a compressed breast was  $\pm 1$  mm. There was a good correlation between the age and the thickness of a compressed breast. Mean value of the thickness of a compressed breast was 52,76 mm (SD : 10,88). It is known that compressed breast thickness shows a tendency of increasing before the age of 60 and then a tendency of decreasing with older women (9), which was the case in our sample of a mammographic analysis.

Mean thickness of a compressed breast with a mediolateral projection was about 9 - 11 % higher than with craniocaudal. This information is important for understanding an explanation of results achieved for a mean glandular dose of a breast from Table 1.

### Quality control

Accuracy of measured voltage in a X-ray tube (maximal deviation) was 0.7 kV for a volume of 23 and 31 kVp. Reproducibility was better for

0.14 kV. Exit radiation (mGy/mAs) was measured every 3 months and it was  $\pm 5$  % from an initial value. The most frequently used voltage at exposition was 30 kVp.

Voltage of 29 kVp (11 %) was applied in most cases when thickness of compressed breast was from 25 to 43 mm, and voltage of 31 kVp (26,50 %) was mainly applied in situations when thickness of a compressed breast varied from 59 to 71 mm and an appropriate compromise between values should have been made in order to achieve the best possible screening. Voltage of 30 kVp (53,85 %) was most frequently used for a compressed breast thickness of 44 to 58 mm, which indicates that most patients were within those values on the examinations. Rarely used voltage of 32 kVp (8,55 %) was applied with extremely thick compressed breast which varied from 72 to 81 mm.

### X-ray technique

The hugest number of screens from examinations was two for MLO and two for CC projection. A total number of screens for a complete examination was 4. The same combination of target/filter (same clinical spectra) Mo/Mo was used for all measuring. There were 234 films made for 63 patients, out of which for the whole mammographic examination of 54 (85,71 %) patients there were 212 films

(two for each projection). In that way both breast were completely examined. With the rest of 9 (14,29 %) patients we did a regular mammographic control of one breast.

Percentage of 14,29 % patients for whom we examined with two screens shows a need for routine controls of patients and mammography because it represents a percentage of patients to whom a breast cancer was detected in time with a mammography. It is common in all developed countries in the world with developed programs of early detection of breast cancer that most patients are examined with 4 screens in order to get a complete clinical picture. This information is a result of routine examinations that are obligatory every three years in developed countries and as a consequence there is a huge number of patients with early detection of breast cancer.



**Patient's doses**

The most frequent procedure in the routine mammography imposes 2 screens for every breast, craniocaudal and mediolateral oblique screen. Even though there is anomaly at only one breast, a screen has to be done for both breasts. This procedure enables us to compare breasts and check possible anomalies in details (16). In the last 15 years several studies have been done in Europe and their objective was to define MGD (10, 11, 15, 17, 18). A similar study was done in Thailand (19). There is no any results about defining MGD, exposition factors applied in mammography and sizes of compressed breasts population have been published regarding Bosnian population.

Table 1. shows results achieved for mean glandular doses per every individual projection and all sizes on which it depends for the complete sample during MLO and CC projection.

MGD and CBT values were defined for a sample of 234 screens (117 for MLO and CC projection). A mean dose for CC screen was 1,41 mGy (SD : 0,43) and 1,70 mGy (SD : 0,51) for MLO screen. Defined significant difference between doses (according to achieved values) which is caused by a compressed breast thickness is about 9,05 %. A similar situation was noted in works of other authors (10, 19). A possible cause of the tendency are doses that are somewhat bigger with MLO than with CC projection, which can be explained with the fact that the pectoral muscle overlying in the MLO projection causes greater attenuation and therefore higher exposure. A total dose for the

complete mammographic examination was 3,11 mGy (SD : 0,49), which is something lower than 3,2 mGy which on the other hand is prescribed by international authorities. Over 77 % of all mammographic examinations were done with a dose smaller than 3 mGy as recommended by the American College of Radiology (20, 21). According to a correlation analysis (Figure 1.) one defined a remarkable significance between MGD and CBT ( $r=0,77$ ,  $p < 0,01$ ).

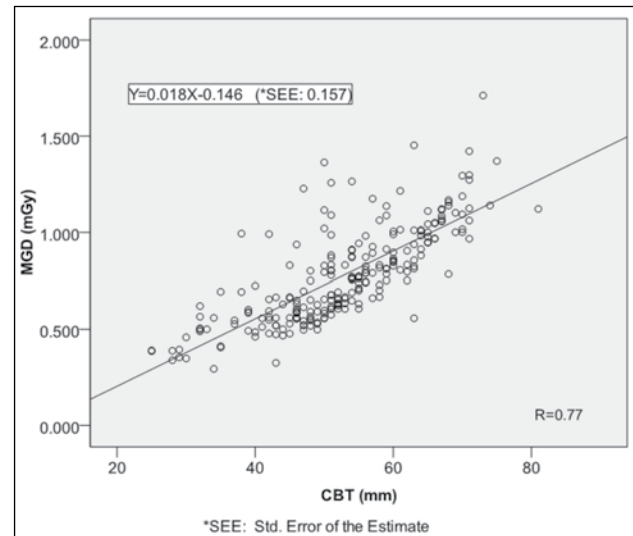


Figure 1. Correlation between MGD and CBT Patient's doses for two different age groups

It was especially important to examine a connection between a position during screening (mediolateral and craniocaudal position), a compressed breast thickness and a mean glandular dose received by a patient during one exposition and the whole examination for two different age

Table 1. Summary of the statistics for kV, compressed breast thickness, ESAK, and MGD for the total sample and for the two views (CC, MLO)

|                  | Number of images | kVp ± SD <sup>c</sup> | CBT (mm)               | ESAK(mGy)              | MGD per exposure (mGy) |                |
|------------------|------------------|-----------------------|------------------------|------------------------|------------------------|----------------|
|                  |                  |                       | Mean ± SD <sup>c</sup> | Mean ± CI <sup>d</sup> | Mean ± CI <sup>d</sup> | Third quartile |
| Total            | 234              | 30,30 ± 0,72          | 52,76 ± 10,88          | 8,56 ± 0,23            | 1,55 ± 0,03            | 1,90           |
| CC <sup>b</sup>  | 117              | 30,29 ± 0,77          | 50,26 ± 9,91           | 7,50 ± 0,27            | 1,41 ± 0,04            | 1,65           |
| MLO <sup>a</sup> | 117              | 30,31 ± 0,68          | 55,26 ± 11,27          | 9,63 ± 0,35            | 1,70 ± 0,05            | 2,02           |

<sup>a</sup>MLO : Mediolateral oblique view.

<sup>b</sup>CC : Craniocaudal view.

<sup>c</sup>SD : Standard deviation.

<sup>d</sup>CI : Confidence interval for the mean of 95 %

CBT : Compressed breast thickness

Table 2. Summary of the statistics for kV, compressed breast thickness, ESAK, and MGD for the two age groups and for the two views (CC, MLO).

| Age range (years) |                  | Number of images | kVp ± SD <sup>c</sup>  |                        | CBT (mm)               | ESAK (mGy)             | MGD per exposure (mGy) |  |
|-------------------|------------------|------------------|------------------------|------------------------|------------------------|------------------------|------------------------|--|
|                   |                  |                  | Mean ± SD <sup>c</sup> | Mean ± SD <sup>c</sup> | Mean ± CI <sup>d</sup> | Mean ± CI <sup>d</sup> | Third quartile         |  |
|                   | Total            | 102              | 30,25 ± 0,69           | 51,70 ± 12,12          | 8,41 ± 0,35            | 1,50 ± 0,05            | 1,80                   |  |
| 40 - 49           | CC <sup>b</sup>  | 51               | 30,22 ± 0,67           | 49,02 ± 12,09          | 7,41 ± 0,41            | 1,36 ± 0,05            | 1,60                   |  |
|                   | MLO <sup>a</sup> | 51               | 30,29 ± 0,70           | 54,39 ± 12,68          | 9,42 ± 0,53            | 1,64 ± 0,07            | 1,98                   |  |
|                   | Total            | 132              | 30,35 ± 0,83           | 53,57 ± 9,75           | 8,68 ± 0,31            | 1,60 ± 0,04            | 1,98                   |  |
| 50 - 64           | CC <sup>b</sup>  | 66               | 30,35 ± 0,83           | 51,21 ± 8,86           | 7,57 ± 0,36            | 1,45 ± 0,06            | 1,77                   |  |
|                   | MLO <sup>a</sup> | 66               | 30,35 ± 0,83           | 55,92 ± 10,09          | 9,78 ± 0,46            | 1,74 ± 0,06            | 2,02                   |  |

<sup>a</sup>MLO : Mediolateral oblique view.  
<sup>b</sup>CC : Craniocaudal view.  
<sup>c</sup>SD : Standard deviation.  
<sup>d</sup>CI : Confidence interval for the mean of 95 %.  
 CBT : Compressed breast thickness

groups. Patients were grouped into a group of younger patients (40- 49) and a group of older patients (50-64) according to recommendations of the European Protocol on dosimetry in mammography (22). Table 2. Shows results achieved for MGD per every individual projection (MLO or CC) and for the whole screening for the given projection.

The first group of age 40 - 49 consisted of 27 patients and 102 screens were made for their routine examination. Average thickness of a compressed breast was 51,70 mm (SD : 12,12 ). We noted a tendency of increasing of thickness of a compressed breast with age in this group (13,18). A compressed breast thickness on MLO projection was 10,95 % bigger than on CC projection. Obtained patient's

doses on MLO and CC projection of 1, 64 mGy (SD : 0,37) and 1,36 mGy (SD : 0,52) are within results promoted by the NHSB (The National Health Service Breast Screening Program in the UK) and K.C.Young (10) for age group 40 – 48. In NHSBA's recommendations a mean glandular dose for MLO screening is 1,73 mGy and for CC screening 1,40 mGy. In its work from 2002 K.C.Young (10) analyzed the mean glandular doses for the age group 40 – 49 and he found that the MGD for MLO screen was 1,76 mGy and 1,48 mGy for CC projection. Results of correlation analysis for both projections of mammographic screens are presented in Figure 2. with a regression line (MLO:  $r = 0,852$  ,  $p < 0,01$ ; CC:  $r = 0,817$  ,  $p < 0,01$ ).

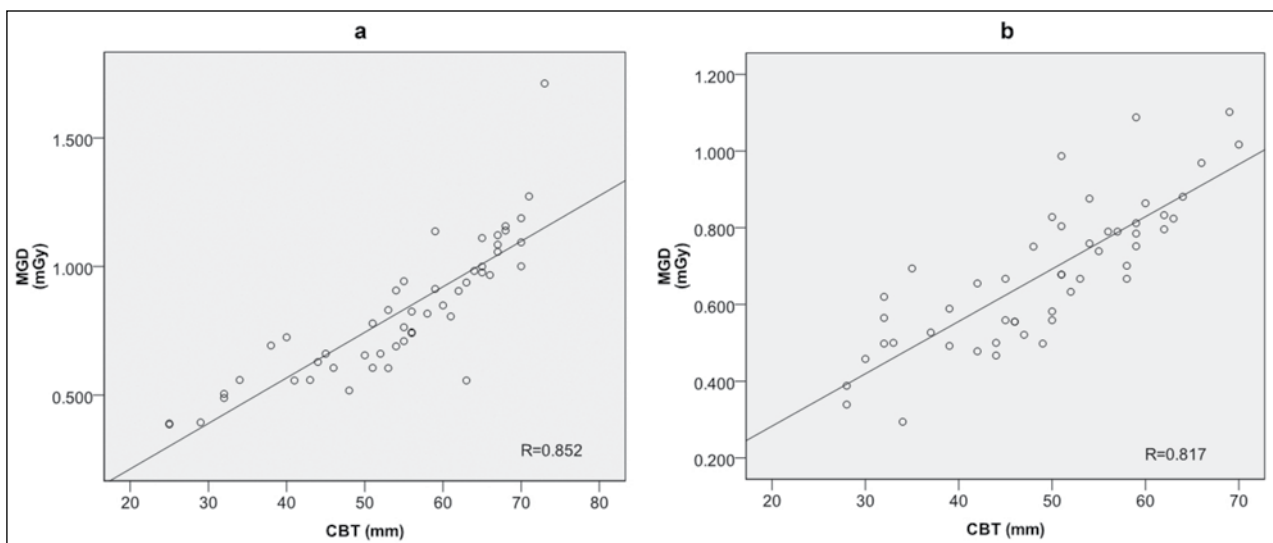


Figure 2. Correlation between MGD and CBT, MLO projection (a) and CC projection (b).

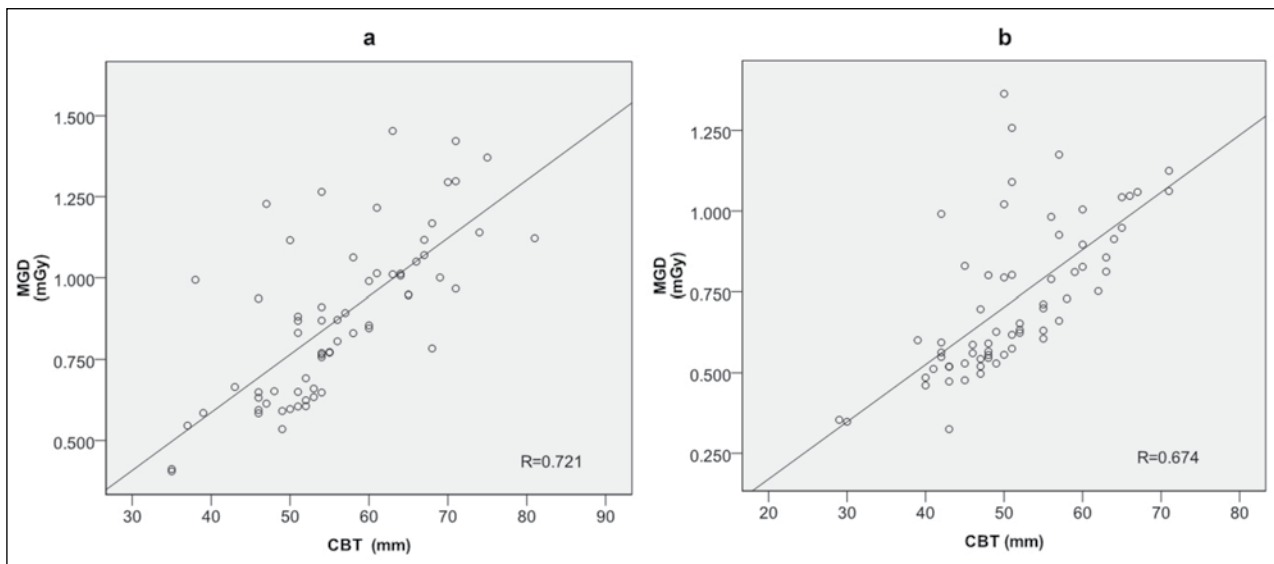


Figure 3. Correlation between MGD and CBT, MLO projection (a) and CC projection (b).

The second age group of people from 50 to 64 consisted of 36 patients and 132 screens were made for their routine examination. A mean compressed breast thickness was 53,57 mm. We noted a tendency of growth of a compressed breast thickness up to 60 years of age in this group (15, 18), and then a slight tendency of decreasing of the breast thickness. Thickness of a compressed breast with MLO projection was 9,20 % bigger than with CC projection. Patient's doses obtained during MLO and CC projections of 1,74 mGy (SD: 0,50 ) and 1,45 mGy (SD : 0,47), respectively, are within a frame of results of a study undertaken by Burch & Goodman (23). The study offered doses of 1,70 mGy for MLO projection (mean thickness 57 mm) and 1,40 mGy for CC projection (mean thickness 52 mm). It is indicative that in the age group of 50 to 64 years MGD value was decreasing with an increase of age, probably due to decreasing of breast glandularity. Correlation analysis for this age group (Figure 3.) showed a remarkable significance for both mammographic projections between MGD and CBT. (MLO:  $r=0,721$ ,  $p < 0,01$  ; CC :  $r = 0,674$  ,  $p < 0,01$  ).

### Conclusion

A total dose for the complete mammographic examination was 3,11 mGy, and over 77 % of all mammographic examinations were done with

doses less than 3 mGy. We noted a tendency of growth of compressed breast thickness with age for patients from 40 to 49 years of age. This is easily explained since a breast is a very dynamic organ (24) and its evolution does not end so early (sometimes at the age of 40) and it passes through a range of dynamic changes during its growth and development. Patient's doses obtained for this age group are 1,64 mGy for MLO and 1,36 mGy for CC projection. For patients from 50 to 64 one noted a tendency of growth of a compressed breast up to 60 years of age followed by a slight tendency of decreasing of breast thickness. MGD measured for the mentioned age group was 1,74 mGy for MLO and 1,45 mGy for CC projection. MGD for MLO and CC screens was significantly connected to compressed breast thickness. According to obtained results we can define that mammographic diagnostics at the Radiology Clinic (Clinical Center University of Sarajevo) is done with patient's doses which are in accordance with European standards.

### Acknowledgments

This study was supported by the University Clinical Centre, Clinical for Radiology and Department for Thoracal Diagnostic with Breast in Sarajevo.

## List of abbreviations

MGD - mean glandular dose  
 MLO - mediolateral projection  
 CC - craniocaudal projection  
 CBT - compressed breast thickness  
 ESAK - entering air kerma measured freely in air without backscatter

## References

1. National Radiological Protection Board, *Estimates of Late Radiation Risks to the UK Population, Documents of the NRPB 4 (4)*, Chilton, Didcot, Oxon, 1993.
2. Beckett J., Kotre C.J., Michaelson J.S. *Analysis of benefit: risk ratio and mortality reduction for the UK Breast Screening*. *Br. J. Radiol.* 2003; 76: 309-320.
3. Law J. *Risk and benefit associated with radiation dose in breast screening programmes*. *Br. J. Radiol.* 1995; 68: 870 – 876.
4. Law J. *Cancer detected and induced in mammographic screening: new screening schedules and younger women with family history*. *Br. J. Radiol.* 1997; 70: 62 – 69.
5. Law J., Faulkner K. *Cancer detected and induced, and associated risk and benefit in a breast screening programme*. *Br. J. Radiol.* 2001; 74: 1121 – 1127.
6. Law J., Faulkner K. *Concerning the relationship between benefit and radiation risk, and cancers detected and induced, in a breast screening programme*. *Br. J. Radiol.* 2002; 75: 678 -684.
7. Young K.C., Faulkner K., Wall B., Muirhead C., *Review of Radiation Risks in Breast Screening, NHSBSP Publication No.54.*, Sheffield, 2003.
8. Miller A. B. *Screening for breast cancer – is there an alternative to mammography?* *Asian Pac. J. Cancer Prev.* 2005; 6: 83 – 86.
9. Law J., Dance DR., Faulkner K., Fitzgerald MC., Ramsdale ML., Robinson A., *Commissioning and routine testing of mammographic X – ray systems. (IPSM Report 59/2)* York, UK: Institute of Physical Sciences in Medicine. 1994; 67 -73.
10. Young K.C. and Burche A. *Radiation doses in the UK of breast screening in women aged 40 – 48 years*. *Br. J. Radiol.* 2002; 75: 362 – 370.
11. Adlien D., Adlys G., Cerapaite R., Jonaitiene E., Cibulskaitė I. *Optimisation of X – ray examinations in Lithuania: start of implementation in Mammography*. *Radiat. Prot. Dosimetry.* 2005; 114: 399 – 402.
12. Faulkner K., Law J., Robson K. J. *Assessment of mean glandular dose in mammography*. *Br. J. Radiol.* 1995; 75: 877 – 881.
13. Moore A.C., Dance D. R., Evans D. S., Lawinski C. P., Pitcher E. M., Rust A., *The Commissioning and Routine Testing of Mammographic X-Ray Systems, The Institute of Physics and Engineering in Medicine, York, 2005.*
14. Dance D. R., Skinner C. L., Young K. C., Beckett J. R. and Kotre C.J. *Additional factors for the estimation of mean glandular dose using the UK mammography protocol*. *Phys. Med. Biol.* 2000a; 45: 3225 – 3240.
15. Beckett J. R., Kotre C. J. *Dosimetric implications of age related glandular changes in screening mammography*. *Phys. Med. Biol.* 2000; 45: 801 – 813.
16. Hackshaw A. K., Wald N. J., Michell M. J. *An investigation into why two - view mammography is better than one – view in breast cancer screening*. *Clin. Radiol.* 2000; 55: 454 – 458.
17. Wall M.A., Roberts P.J. *Radiation dose in relation to compressed breast thickness for screening mammography*. *Radiat. Prot. Dosimetry.* 1992; 43: 253 – 255.
18. Klein R., Aichinger H., Dierker J. *Determination of average glandular dose with modern Mammography units for two large groups of patients*. *Phys. Med. Biol.* 1997; 42: 651 – 671.
19. Sookpeng. S., Kettled P. *Mean glandular dose from routine mammography*. *NU Journal.* 2006; 14: 19-26.
20. American College of Radiology. *Mammography quality control manual, U.S. American College of Radiology, Washington D.C., 1999.*
21. Suleiman O. H., Splice D.C., McCrohan J. L., Symonds G. R., Houn F. *Mammography in the 1990s*. *Radiology* 1999; 210: 345 – 351.
22. Pery N., Broeders M., C. De Wolf and Tornberg S. *Commissions of the European Communities, European Guidelines for Quality Assurance in Mammography Screening, Report EUR 14821, Luxemburg, 2001.*
23. Burch A., Goodman D. A. *A pilot survey of radiation doses received in the United Kingdom breast screening program*. *Br. J. Radiol.* 1998; 71: 517 – 527.
24. Kopans D.B., Rafferty E., Georgian-Smith D., et al. *A simple model of breast cancer growth may provide explanations for observations of apparently complex phenomena*. *Cancer* 2003; 97: 2951 – 2955.

### Corresponding author:

Suad Kunosic,  
 Faculty of Natural Sciences and Mathematics,  
 University of Tuzla,  
 Bosnia and Herzegovina,  
 e-mail: suad.kunosic@untz.ba

# The serum fructosamine concentration at patients with diabetes mellitus

## SERUMSKA KONCENTRACIJA FRUKTOZAMINA KOD PACIJENATA SA DIABETES MELLITUS

Serdarevic N.<sup>1</sup>, Pepic E.<sup>2</sup>, Musanovic J.<sup>3</sup>, Pleho-Kapic A.<sup>2</sup>

<sup>1</sup> Institute for Clinical Chemistry and Biochemistry, Clinics Center University of Sarajevo, Bosnia and Herzegovina

<sup>2</sup> Department of Pathophysiology, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina

<sup>3</sup> Department of Biology and Human Genetic, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina

### Abstract

**Introduction:** Hyperglycaemia allows glucose to react with proteins in the plasma and tissues, resulting in the accumulation of glycated products. In clinical practice the term „fructosamine“ is usually reserved for glycated serum proteins. Albumin, the principal protein in plasma, and the other plasma proteins are glycated when exposed to hyperglycaemia, producing fructosamine residues. Since the half-life of albumin is 19 days, measurement of fructosamine gives estimation of average glycaemic control over the previous 2-3 weeks.

**The aims of study:** The study was to investigate serum lipids and glucose concentration at patients with diabetes mellitus depending on their fructosamine levels were classified as good, satisfactory or poor glycaemic controls. The second aim of study was to find correlation between fructosamine, levels of lipids and glucose.

**Material and methods:** The measurement of fructosamine was done using Hitachi (Boehringer Mannheim) 904 Automatic Analyser at 530 nm wave length. The lipids, lipoproteins and glucose was measured using Dade Behring Dimension Pand plus. The retrospective study included one hundred of patients with diabetes mellitus who were analysed blood on Institute for Clinical

Chemistry and Biochemistry, Clinics Center University of Sarajevo, in a period of June 2006. to May 2007. The study completed 53 men and 57 women (age 55-70 years). At all subjects we did the following analysis: serum level of fructosamine, glucose, total cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) .

**Results:** The patients classified as good glycaemic control has lower concentration of glucose and lipids in comparing with patient classified as satisfactory and poor glycaemic controls. Our results have shown statistically significant correlation between fructosamine and glucose level. We found significant correlation between fructosamine and level of total cholesterol, triglyceride and HDL. It was not found correlation with LDL and VLDL.

**Key words:** fructosamine, lipids, glucose, diabetes mellitus.

### Sažetak

**Uvod:** Hiperglikemija omogućuje da glukoza reaguje sa proteinima, prisutnim u plazmi i tkivima što dovodi do nakupljanja glikiranih produkata. U kliničkoj praksi termin „fruktozamin“ obuhvata serumske glikirane proteine. Albumin,



glavni protein plazme i ostali plazmatski proteini se glikiraju pri izlaganju hiperglikemiji, pri čemu nastaje fruktozamin. S obzirom da je polu-život albumina 19 dana, mjerenje fruktozamina predstavlja procjenu prosjeka glikemične kontrole u protekle 2-3 sedmice.

**Ciljevi rada:** Studija je obuhvatala istraživanje serumske koncentracije lipida i glukoze kod pacijenata sa dijabetes mellitus ovisno od koncentracije fruktozamina i koji su klasificirani kao dobra, zadovoljavajuća i loša kontrola. Drugi cilj studije je bilo pronalaženje korelacije između fruktozamina te vrijednosti lipida i glukoze.

**Materijal i metode:** Za određivanje fruktozamina koristio se Hitachi (Boehringer Mannheim) 904 automatski analizator na talasnoj dužini od 530 nm. Lipidi, lipoproteini i glukoza su određeni na analizatoru Dade Behring Dimension Pand plus. Retrospektivna studija je sadržavala sto pacijenata sa dijabetes mellitusom kojima je analizirana krv na Institutu za kliničku kemiju i biokemiju, Kliničkoga centra Univerziteta u Sarajevu, u periodu juni 2006. do maja 2007. Studija je uključivala 53 muškarca i 57 žena (godine 55-70). Svim pacijentima su određene sljedeće analize: serumska koncentracija fruktozamina, glukoze, ukupnoga holesterola, triglicerida, lipoprotein visoke gustoće (HDL), lipoprotein niske gustoće (LDL) i lipoprotein veoma niske gustoće (VLDL).

**Rezultati:** Pacijenti koji su klasificirani kao dobra glikemična kontrola su imali nižu koncentraciju glukoze i lipida u poređenju sa pacijentima klasificiranim kao srednja i loša kontrola. Naši rezultati su pokazali izrazito statistički signifikantnu korelaciju između fruktozamina i glukoze. Utvrđena je signifikantna korelacija između fruktozamina i vrijednosti ukupnoga holesterola, triglicerida i HDL-a. Nije utvrđena korelacija sa LDL i VLDL.

**Ključne riječi:** fruktozamin, lipidi, glukoza, dijabetes mellitus.

## Introduction

Determination of glucose in the blood only is the current image to assess metabolic disorders in diabetic patients which provides information on the status of a few minutes before removing the

blood. Glucose is able to bind to a variety of structures, including proteins, in a non-enzymatic irreversible reaction, a process called glycation. Proteins are glycated by a non-enzymic post-translational reaction to form an intermediate Schiff base, which is then transformed by Amadorri rearrangement into stabile ketoamine. This ketoamine end-product of protein glycation is fructosamine, the trivial name for 1-amino-1-deoxyfructose. (1,2). Fructosamine is the common name for ketoamine a serum protein, is derived from the name of the product structure ketoamine arising interaction of glucose with N-terminal  $\alpha$ -amino groups or the  $\epsilon$ -amino group of lysyl residues in proteins. In healthy people non-enzymatic glycation of plasma proteins is less than 2% and in diabetics patients about up to 20%.

Fructosamine testing has been available since the 1980s as a monitoring tool to help diabetics control their blood sugar. The test for serum fructosamine is simpler and less costly than that for hemoglobin A1C, but at present is less frequently used. The level of fructosamine correlates well with fasting glucose and with hemoglobin A1C levels (3, 4). The measurement of fructosamine is useful to monitor the average concentration of blood glucose for an extended period of time (2-3 weeks) in individuals with diabetes mellitus. Therefore, fructosamine is an indicator of the blood glucose concentration over a longer period of time than a single blood glucose measurement (1,2). The American Diabetes Association (ADA) recognizes the utility of both tests, and says that fructosamine may be a better choice when A1C cannot be reliably measured. These situations include: 1) The evaluation of changes in diabetic treatment, since the effects of adjustment can be evaluated after a couple of weeks rather than months. 2) In pregnancy, since the glucose and insulin needs of the mother and fetus change rapidly during gestation. 3) Any condition that affects the average age of red blood cells, such as hemolytic anemia, sickle cell anemia, or blood loss (5).

Fructosamine test is useful for screening patients with a confirmed diagnosis of diabetes and the test can not be used in controlling glucose levels healthy population. However, it can be used in patients who have acute and systemic diseases that could alter glucose and insulin values and do

not have confirmed diagnosis of diabetes (4,6). The study was to investigate whether the serum lipids in type 2 diabetes mellitus was different between groups of patients classified as good, satisfactory or poor glycemic controls, depending on their serum fructosamine levels. Determination of lipids is of great importance in patients with diabetes mellitus because it increases the risk of disease atherosclerosis and cardiovascular diseases (7,8).

## Methods and material

The measurement of fructosamine was done using Hitachi (Boehringer Mannheim) 904 at 530 nm wave length. The fructosamine reagent set is based on the ability of ketoamines to reduce nitroblue tetrazolium (NBT) to formazan dye under alkaline conditions. The rate of formazan formation is directly proportional to fructosamine concentration (9). The lipids, lipoproteins and glucose were measured using Dimension LxR (Dade Behring). The glucose method is an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method, presented as a general clinical laboratory method by Kunst (10). The cholesterol method is based on the principle first described by Stadman (11). The triglycerides method is based on enzymatic procedure in which a combination of enzymes are employed for the measurement of serum or plasma triglycerides. The sample is incubated with lipoprotein lipase (LPL) enzyme reagent that converts triglycerides into free glycerol and fatty acids. Glycerol kinase (GK) catalyzes the phosphorylation of glycerol by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate. Glycerol-3-phosphate-oxidase oxidizes glycerol-3-phosphate to dihydroxyacetone phosphate and hydrogen peroxide ( $H_2O_2$ ). The catalytic action of peroxidase (POD) forms quinonimine from  $H_2O_2$ , aminoantipyrine and 4-chlorophenol. The change in absorbance due to the formation of quinonimine is directly proportional to the total amount of glycerol, using measurement is bichromatic (510,700 nm). The high-density lipoprotein (HDL) is measured directly. This method is based on acceleration the reaction of cholesterol oxidase (CO) with HDL unesterified cholesterol and dissolving HDL selectively using a specific detergent. The second reagent consist of a detergent capable to solubili-

zing HDL specifically, cholesterol esterase (CE) and then forming  $\Delta^4$ cholestenone and  $H_2O_2$ . The  $H_2O_2$  react with chromagenic coupler to develop color for quantitative determination of HDL (10). The VLDL and LDL concentrations is calculated by Friedwald equation (13). The referent values are: fructosamine 2-2.8 mmol/L, glucose 3.3-6.1 mmol/L, cholesterol 3.1-5.2 mmol/L, triglycerides 0.11-1.70 mmol/L HDL-a 1.06-1.94 mmol/L, LDL 2.00-4.30 mmol/L and VLDL (0,13-090 mmol/L).

## Patients

The patient samples of blood were collected in serum separation Vacutainer test tubes (Beckton Dickinson, Rutherford, NJ 07,070 U.S.) in volume of 3.5 mL. The anticoagulant sodium - heparin or EDTA was used. Serum samples were obtained by centrifugation at 3000 rpm using centrifuge (Sigma 4-10). After centrifuging, serum concentration of fructosamine, cholesterol, triglycerides and high-density lipoprotein were determined. The investigation was done respecting ethical standards in the Helsinki Declaration. The retrospective study included patients (n=100) with type 2 diabetes mellitus and who has analysed blood at Institute for clinical chemistry and biochemistry at Clinical centre University in Sarajevo. Laboratory analyses were performed on blood samples collected after a 12-h after fast.

## Statistic

The results were statistically analyzed using NCSS and statistical software SPSS version 12.0 software. Determined by the average value ( $\bar{x}$ ), standard deviation (SD), Pearson correlation coefficient (r), equations of linear regression and Student t test with statistical significance level of 0.05 and 0.01 ( $p < 0.05$ ;  $p < 0.01$ ).

## Results

The results of serum concentrations fructosamine, glucose, cholesterol, triglycerides, HDL, VLDL and LDL at patients with diabetes mellitus (53 men

and 57 women) are shown in Table 1. The patients were classified depending on their fructosamine levels as good, satisfactory or poor glycemic controls. *Table 1. Serum concentration of biochemical parameters at patients with diabetes mellitus*

| Mean serum concentration of biochemical parameters in group with good glycemic control fructosamine level < 2.8 mmol/ (n=25) ( $\bar{x} \pm SD$ )             |               |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| Fructosamine (mmol/L)                                                                                                                                         | 2.43 +/- 0.28 |
| Glucose (mmol/L)                                                                                                                                              | 5.27 +/- 1.33 |
| Cholesterol (mmol/L)                                                                                                                                          | 4.84 +/- 1.35 |
| Tryglicerides (mmol/L)                                                                                                                                        | 1.45 +/- 0.59 |
| HDL(mmol/L)                                                                                                                                                   | 1.18 +/- 0.50 |
| VLDL(mmol/L)                                                                                                                                                  | 0.71 +/- 0.36 |
| LDL(mmol/L)                                                                                                                                                   | 2.99 +/- 1.32 |
| Mean serum concentration of biochemical parameters in group with satisfactory glycemic control fructosamine level 2.8 – 4.0 mmol/ (n=37) ( $\bar{x} \pm SD$ ) |               |
| Fructosamine (mmol/L)                                                                                                                                         | 3.50 +/- 0.35 |
| Glucose (mmol/L)                                                                                                                                              | 9.67 +/- 4.08 |
| Cholesterol (mmol/L)                                                                                                                                          | 6.58 +/- 0.75 |
| Tryglicerides (mmol/L)                                                                                                                                        | 1.99 +/- 0.92 |
| HDL(mmol/L)                                                                                                                                                   | 1.11 +/- 0.34 |
| VLDL(mmol/L)                                                                                                                                                  | 0.90 +/- 0.42 |
| LDL(mmol/L)                                                                                                                                                   | 3.42 +/- 0.94 |
| Mean serum concentration of biochemical parameters in group with poor glycemic control fructosamine level > 4.0 mmol/ (n=38) ( $\bar{x} \pm SD$ )             |               |
| Fructosamine (mmol/L)                                                                                                                                         | 4.86 +/- 0.71 |
| Glucose (mmol/L)                                                                                                                                              | 10.6 +/- 1.98 |
| Cholesterol (mmol/L)                                                                                                                                          | 6.93 +/- 0.75 |
| Tryglicerides (mmol/L)                                                                                                                                        | 2.41 +/- 0.67 |
| HDL(mmol/L)                                                                                                                                                   | 0.84 +/- 0.40 |
| VLDL(mmol/L)                                                                                                                                                  | 1.01 +/- 0.32 |
| LDL(mmol/L)                                                                                                                                                   | 3.48 +/- 0.34 |

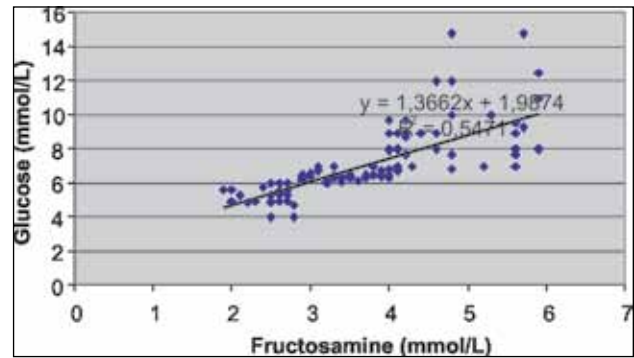
Increasing concentrations fructosamine followed an increase concentration of glucose, cholesterol, triglycerides and VLDL. The concentration of HDL cholesterol was lower in all three groups. The results of correlation between fructosamine and glucose, lipids using Pearson coefficient of correlation are shown at Table 2.

*Table 2. Correlation between fructosamine glucose and lipids*

|   | Glucose | Cholesterol | Tryglicerides | HDL    | LDL   | VLDL  |
|---|---------|-------------|---------------|--------|-------|-------|
| r | 0.740   | 0.605       | 0.409         | -0.242 | 0.012 | 0.470 |
| p | 0.000** | 0.000**     | 0.000**       | 0.015* | 0.906 | 0.640 |

statistically significant for  $p < 0.01$  \*\*;  $p < 0.05$  \*

It is determined a significant difference between the average concentration fructosamine and glucose ( $p < 0.01$ ) with Pearson correlation coefficient ( $r = 0.740$ ). Using the same test identified significant differences between the average concentration fructosamine the average concentration of cholesterol and triglycerides ( $p < 0.01$ ). According to our results identified a significant difference between the average concentration fructosamine and HDL cholesterol ( $p < 0.05$ ) with negative Pearson correlation coefficient ( $r = - 0.242$ ). The current study has not been established fructosamine statistically significant correlation with LDL and VLDL cholesterol. A comparison of fructosamine and glucose at patiens with diabetes are shown at Figure 1.



*Figure 1. Correlation between fructosamine and glucose at patients with diabetes mellitus*

The correlation coefficient was 0.740 and regression line had slope of 1.3662 and y axis intercept of 1.9874. The mean difference between fructosamine and glucose was statistically significant  $p < 0.01$  using Student t-test. Increasing concentrations fructosamine follows an increase of glucose in the blood so that allows us to track changes over a longer period of time. Therefore, with a linear regression equation can be predict the concentration of glucose in the blood based on the value fructosamine.

## Discussion

Increasing concentrations of glucose increases the rate glycation of serum proteins. Fructosamine considered medium parameter control of diabetes, although the method of determining a cheaper and more accessible than those used for the determination of HbA1c. On the basis of a recent European Diabetes Study Group recommendation on targets for a diabetic control, we defined good metabolic control as serum fructosamine less than or equal to 2.8 mmol/L, satisfactory 2.8-4.0 mmol/L and poor fructosamine level more or equal to 4.0 mmol/L (15). Results of our study have shown that increasing fructosamine and glucose concentrations rise cholesterol, triglycerides and VLDL. The concentration of VLDL with regard to the groups with good, satisfactory and poor control was not changed. The results of our study showed a lower concentration of HDL in all these groups. With good glycemic control and fructosamine values 2.0-2.8 mmol / L, it is achieved that the lipids are in the reference area. The high levels of fructosamine is associated with an increased risk of death from cardiovascular causes. The association between fructosamine and cardiovascular mortality was confirmed at women patients with elevated fructosamine levels > 2.8 mmol/L. The diabetes is a risk factor for cardiovascular disease and other measurements of glucose control, such as hyperglycemia or glycosylated hemoglobin levels, correlate with the risk of cardiovascular disease with known diabetes (16).

The results of our study showed a significant correlation between glucose and fructosamine the coefficient of correlation  $r = 0.740$  ( $p < 0.01$ ). The results of other groups show that blood glucose concentration were positively correlated with serum fructosamine ( $r = 0.42$ ) (15). Baker and investigators have found that correlation of fructosamine and mean plasma glucose was significant with Pearson's correlation coefficient 0.75 ( $p < 0.01$ ) (15). Our results have showed that increasing concentrations of cholesterol and triglyceride levels paralleled the increase in the concentration and fructosamine statistically significant difference ( $p < 0.01$ ). These changes, which manifest as an increase in plasma triglyceride and cholesterol are exacerbated by poor metabolic control (16). It

is positive correlation between triglycerides and fructosamine at patients with diabetes (17).

The high density cholesterol was low in all groups. According to our results identified a significant negative correlation between the concentration fructosamine and HDL cholesterol ( $p < 0.05$ ). Fructosamine and increase of glucose concentration is accompanied by declining concentrations of HDL. The primary biochemical abnormality is an decrease in high-density lipoprotein cholesterol. Values of LDL are usually within normal limits (18,19). The products from of lipid peroxidation and from fructosamine oxidation are associated with pronounced dislipidemic disorders and reduction of HDL cholesterol (20,21). The other investigators have found similar results of lipids and lipoproteins in comparison with fructosamines level (22).

Today, more fructosamine associated with various cardiovascular diseases and even. Therefore it is consider possible future parameter for patients with cardiovascular diseases such are stroke and cardiac shock. Good control at diabetes has a direct impact on the reduction of lipids and it is one of the major risk for coronary artery disease in patients with type 2 diabetes.

## Conclusion

The fructosamine, glycated serum protein in control of type 2 diabetes mellitus show significantly significant correlation with diabetic related dyslipidemia and possible reduced risk of atherosclerosis. Over periods of months and years, these form of glycation products are resulting in dysfunction and the pathogenesis of diabetic complications such as vascular stiffening, hypertension, nephropathy and retinopathy. It is high specific test in monitoring of diabetic patients in regard of antidiabetes therapy and lipids control. The serum fructosamine measure gives a good index for the glycemic control, and its value can reflect the profile of serum lipids. The patients with diabetes mellitus have an increased risk of developing cardiovascular disease and atherosclerosis, so maintenance fructosamine at referent scope is important.



## References

1. Pettiti D.B., Contreas R. *Randomized trial fructosamine home monitoring in patients with diabetes. Effective Clinical Practice* 2001; 4(1):18-23
2. Lothar T.: *Labor und Diagnose TH – Books, Frankfurt/Main* 2000, pp 123-152.
3. Guillausseau P-J, Charles M-A, Godard V, Timsit J, Chanson P, Paolaggi F et al. *Comparison of fructosamine with glycated hemoglobin as an index of glycemic control in diabetic patients. Diabetes Research*, 1990. 13:127-31
4. Lothar T.: *Labor und Diagnose TH – Books, Frankfurt/Main* 1992, pp 177-184.
5. Salway J.G.: *Medical Biochemistry at a Glance, Blackwell Publishing Ltd, Massachusetts 02148-5020, USA* 2008: pp 76-77.
6. [www.personalmd.com/news/a1999030402.shtml](http://www.personalmd.com/news/a1999030402.shtml) : Fructosamine Levels Linked To Heart Diseases. SOURCE: *American Journal of Epidemiology* 1999; 149:471-75
7. Pleho–Kapic A, Beslagic R, Pepic E, Fajkic A. *Level of cholesterol and lipoprotein fractions in cardiovascular diseases, HealthMED*, 2008; 2(3): 154-161
8. Pepic E, Fajkic A, Pleho-Kapic A, Dzubur A, Musanovic J. *Plasma level of high-density lipoprotein-HDL and low-density lipoprotein - LDL in patients with ischemic stroke, HealthMED*, 2009; 2 (4): 467-472
9. *Operator's Manuel, Boehringer Mannheim*, 2004.
10. Kunst A., Draeger B., Ziegenhorn J., *UV- methods with hexokinase and glucose-6-phosphate dehydrogenase, Methods of Enzymatic Analysis Vol VI, Bergmeyer, HU, Eu, Verlag Chemie, Deerfield, FL* 1983, pp 163-172
11. Stadtman TC, *Methods in Enzymology, Vol III, Colowick, SP, and Caplan, NO, (Eds.), Academy Press, New York, NY*, 1957, pp 392-394, 678-81.
12. *Operator's Manuel, Dimension System Dade Behring*, 2000.
13. *FAQs – Genera*. 2008. [www.icgp.ie/go/courses/heartwatch/heartwatch\\_faqs/FB2EF29C-A12D-9EDA-52E4FE182C061591.html](http://www.icgp.ie/go/courses/heartwatch/heartwatch_faqs/FB2EF29C-A12D-9EDA-52E4FE182C061591.html) (dostupno 16. mart 2009)
14. *Risk Factors*. 2006. [http://www.apotex.com/ca/en/healthcareprofessionals/ce/web/dys/PDFFiles/types\\_and\\_causespdf](http://www.apotex.com/ca/en/healthcareprofessionals/ce/web/dys/PDFFiles/types_and_causespdf) (dostupno 16. mart 2009)
15. Lim TO, Selvan A, Suppiah A, Khan N, Ismail F. *Comparison of serum fructosamine and blood glucose concentrations as indices of glycaemic control in non-insulin dependent diabetic out patients. Singapore Med J* 1992; 33:287-89.
16. Browner WS, Pressman AR, Lui LY, Cummings SR. *Association between serum fructosamine and mortality in elderly women. Am J Epidemiol* 1999; 149(5):471-75.
17. Baker JR, Metcalf PA, Holdaway IM, Johnson RN. *Serum fructosamine concentration as measure of blood glucose control in type I (insulin dependent) diabetes mellitus. Br Med J* 1985; 290:352-55.
18. *American Diabetes Association Position Statement: Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. J Am Diet Assoc* 2002; 102(1):109-18.
19. Tuomilehto J, Lindstrom J, Eriksson JG. *Finnish diabetes prevention study group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med* 2001; 344:1343-50.
20. Gerhard GT, Ahmann AA, Meeuws K, McMurry MP, Duell B, Connor WE. *Effects of low-fat diet compared with those of a high-monounsaturated fat diet on body weight, plasma lipids and lipoproteins, and glycemic control in type 2 diabetes. Am J Clin Nutr*. 2004; 80(3):668-73.
21. Volchegorskii A, Kharchenkova NV. *Fructosamine, high density lipoproteins and degree of dyslipidemia in patients with vascular complications of diabetes mellitus type I. Klin Lab Diagn* 2003; 1:14-7.
22. Sathiyapriya V, Nandeesh H, Bobby Z, Pavithran P, Selvaraj N, Rattina Dasse N. *Insulin resistance and enhanced protein glycation in men with prehypertension. Clin Chem Lab Med* 2006; 44(12):1457-61.

### Corresponding author:

Serdarevic N.

Institute for Clinical Chemistry and Biochemistry,  
Clinics Center University of Sarajevo,  
Bosnia and Herzegovina,

e-mail: healthmed\_bih@yahoo.com



# Dosage of Erythropoietin and iron-replacement therapy in patients on chronic hemodialysis

## DOZIRANJE ERITROPOETINA I NADOMJESNA TERAPIJA ŽELJEZOM U BOLESNIKA NA HRONIČNOJ HEMODIJALIZI

Edin Ostrvica<sup>1</sup>, Dzenana Ostrvica<sup>2</sup>, Vahidin Katica<sup>3</sup>

<sup>1</sup> Health Center „Dr. Mustafa Sehović” Tuzla, Bosnia and Herzegovina

<sup>2</sup> Clinic for Pediatrics, University Clinical Center of Tuzla, Bosnia and Herzegovina

<sup>3</sup> Clinic for Gynecology and Obstetrics, Clinical Center of University of Sarajevo, Bosnia and Herzegovina

### Abstract

Anemias are diseases of decreased number of erythrocytes or hemoglobin in a volume blood unit with hypoxia of muscles as a result. The objective of the study was to establish iron-replacement therapies to achieve target values of hemoglobin and hematocrit in renal anemia treated by erythropoietin in chronic hemodialyzed patients. The study involved 60 patients, both sexes, randomly chosen and divided into two groups. The first (experimental) group numbered 20 examinees administered epoetin alpha intravenously, while the other group numbered 40 patients administered epoetin beta (control group). In this group 20 patients were administered epoetin beta intravenously, 20 of them subcutaneously. All the patients were administered epoetin alpha or beta weekly after hemodialysis. Erythropoietin dose was determined and adjusted according to the blood picture findings. Comparison of the mean doses of erythropoietin between experimental and control groups showed no statistically significant difference ( $p > 0,05$ ). The highest dose of iron-replacement was administered to the patients on subcutaneous therapy by epoetin beta, but no significant difference in intravenous iron-doses was found ( $p > 0,05$ ). We found that increased levels of hemoglobin result in increased need for iron,

while decreased dose of intravenous iron results in fall of hemoglobin level. Insufficient replacement therapy of iron resulted in weaker response to the erythropoietin therapy in the patients with renal anemia who were administered erythropoietin alpha and beta intravenously.

**Key words:** renal anemia, erythropoietin, hemodialysis

### Sažetak

Anemije su bolesti sa smanjenom količinom eritrocita ili hemoglobina u volumnoj jedinici krvi s posljedičnom hipoksijom tkiva. Cilj studije je bio utvrditi uticaj nadomjesne terapije željezom na postizanje ciljnih vrijednosti hemoglobina i hematokrita u liječenju renalne anemije eritropoetinom kod hroničnih hemodijaliziranih bolesnika. Istraživanjem je obuhvaćeno 60 ispitanika, oba pola, odabranih metodom slučajnog odabira i podijeljenih u dvije grupe. Prva (eksperimentalna) grupa se sastojala od 20 ispitanika koji su primali epoetin alfa intravenski, dok je u drugoj grupi bilo 40 ispitanika koji su primali epoetin beta (kontrolna grupa). U ovoj grupi 20 ispitanika je primalo epoetin beta intravenski, a 20 bolesnika epoetin beta subkutano. Svi ispitanici su primali epoetin alfa ili beta tri puta sedmično nakon hemodijalize. Doza eri-

tropoetina određivala se i prilagođavala prema nalazu krvne slike. Upoređivanjem vrijednosti srednjih doza eritropoetina između eksperimentalne i kontrolnih grupa nije nađena statistički značajna razlika ( $p > 0,05$ ). Najveću dozu nadomjesnog željeza dobili su bolesnici na subkutanoj terapiji epoetinom beta, ali u dozama intravenskog željeza nije uočena značajna razlika ( $p > 0,05$ ). Našli smo da viši nivoi hemoglobina vode povećanoj potrebi za željezom, te da smanjenjem doze intravenskog željeza dolazi do pada nivoa hemoglobina. Nedovoljna supstitucionna terapija željezom bila je razlog slabijeg odgovora na terapiju eritropoetinom u bolesnika sa renalnom anemijom koji su liječeni intravenskim eritropoetinom alfa i beta.

**Ključne riječi:** renalna anemija, eritropoetin, hemodijaliza

## Introduction

Anemias are diseases of decreased number of erythrocytes or hemoglobin in a volume blood unit with hypoxia of muscles as a result. After long-years unsuccessful treatment of renal anemia, introduction of recombinant human erythropoietin (rHuEPO) brought revolutionary progress in therapeutical approach. Two forms of erythropoietin should be distinguished, erythropoietin alpha and erythropoietin beta. Safely and successfully rHuEPO has been used nearly 20 years in the treatment of renal disease, chronic renal damage and renal anemia induced by hemotherapy in the case of malignants (1). It is particularly effective in the anemia treatment of the patients in the course of renal disease terminal phase (2). Presently, a majority of patients are not anemic and have greater quality of life than before (3). European Best Practice Guidelines (EBPG) recommends maintaining hemoglobin values over 11g/dL and hematocrit over 33% in all chronic renal patients. In fact, erythropoietin dose should be harmonized with hemoglobin level. Increased needs for iron in hemodialyzed patients are the result of great loss of iron through gastrointestinal tract, decreased absorption of iron, frequent laboratory check-ups, and loss of blood in the course of hemodialysis. Adequate amount of the iron available increases erythropoiesis and decreases needs for therapy by

the erythropoiesis stimulating agents (ESA) (4). To achieve target hemoglobin levels it is necessary to maintain serum ferritin between 200 and 500  $\mu\text{g/L}$ , and the percentage of hypochromic erythrocytes below 2,5%. Suggested upper limit of the serum ferritin for all the patients is 800  $\mu\text{g/L}$ . At the time before ESA, dialysed patients often had access of iron (with ferritin over 1000 mg/L) as a result of polytransfusions (5). In the case of absolute or functional iron deficit identified or suspected, it is necessary to introduce iron-replacement, in order to achieve maximal effects of rHuEPO-therapy (6). General recommendation of rHuEPO is to administer 25-150 mg of iron intravenously in the course of the first 6 months of the ESA-therapy. Extra good results were achieved when that dose was administered after „filling dose“ of 400-600 mg of iron through two weeks (4).

## Objectives

The objective of the research was to establish the effects of the iron-replacement therapy in order to achieve target values of hemoglobin and hematocrit in the treatment of renal anemia by erythropoietin in the chronic hemodialyzed patients.

## Patients and methods

The study involved 60 patients, both sexes, randomly chosen, divided into two groups. The first one (experimental) consisted of 20 patients being administered epoietin alpha intravenously, while the other one consisted of 40 patients being administered epoietin beta (control group). The control group was divided into two subgroups: one consisting of 20 patients treated by epoietin beta intravenously, the other one consisting of 20 patients treated by epoietin beta subcutaneously. Before the research all the examinees, who were longer than three months on hemodialysis treatment, were treated by epoietin beta subcutaneously. When introduced into research, the examinees had a stable hemoglobin level between 9 and 11 g/dL at least during two successive measurements. Patients with malignant disease were not included into research. The study was prospective and performed in the

Clinic for Internal Diseases, University Clinical Center of Tuzla, lasting six months. Before the research, complete blood picture (check-up), iron and ferritin, were done before being dialyzed, for each examinee. All the said parameters were measured for all the patients in the course of the first month every week, then after the third and sixth month. Erythropoietin dose has been determined and adjusted according to the blood picture findings according to the following principles:

- in the case of hemoglobin level fall for 1-1,9 mmol/L, erythropoietin dose increased for 25%
- in the case of hemoglobin level fall for 2 mmol/L, erythropoietin dose increased for 50%
- in the case of hemoglobin level rise for 1-1,9 mmol/L, erythropoietin dose decreased for 25%
- in the case of hemoglobin level rise for 2 mmol/L, erythropoietin dose decreased for 50%.

All the examinees were administered epoietin alpha or beta three times a week after hemodialysis, intravenously, or subcutaneously. The majority of the patients were treated by iron replacement therapy intravenously (generic name Venofer) in order to maintain ferritin level between 300 and 500 ug/L.

Statistical significance of the mean values of the parameters measured were tested by the Student's t-test. For all the calculations we used significance level of  $p \leq 0,05$ .

## Results

Out of 60 patients analyzed, 29 (48,3%) were males, 31 (51,7%) females. Average age was  $57,6 \pm 13,18$  (27-80) years in experimental, and  $54,2 \pm 13,5$  (27-79) years in control group ( $p=0,66$ ). Comparison of the mean values of erythropoietin doses through six-month period of therapy, no statistically significant difference was found ( $p=0,64$ ).

Average dose of epoietin alpha in experimental group of patients was  $3314,6 \pm 619,79$  i.u. Patients on intravenous epoietin beta therapy received approximately  $3242,6 \pm 792,61$  i.u. of epoietin, and patients on subcutaneous epoietin beta received approximately  $3341,9 \pm 829,13$  i.u. of epoietin. Mean dose for all the examinees in the experimental period was  $3291,9 \pm 962,92$  i.u. of epoietin (Figure 1).

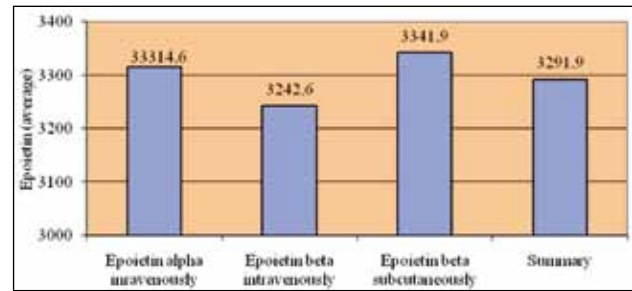


Figure 1. Average values of epoietin alpha and beta doses in the tested groups

Examinees on intravenous epoietin beta therapy received approximately some smaller dose or erythropoietin in relation to the other two groups of examinees. Average doses of epoietin between the examined groups are minimally different. Comparison of the values of weekly doses of epoietin/kg of body weight no significant difference was found ( $p > 0,05$ ). No difference was found in the weekly doses among examinees on intravenous epoietin alpha or subcutaneous epoietin beta. Namely, average weekly dose for these two groups of patients amounted 40 i.u./kg of body weight, while intravenous weekly epoietin beta dose was some higher, 45 i.u./kg of body weight.

Out of total 60 patients 46 (76, 7%) of them received iron-replacement therapy during the experiment. In experimental group 12 (60%) patients received iron-replacement in average dose of  $578,6 \pm 392,58$  mg (100-1600 mg). Out of total of 20 patients 17 (85%) of them were administered iron-replacement in average dose of  $473,3 \pm 334,80$  mg (100-1500 mg). In the group of patients on the subcutaneous epoietin therapy, iron-replacement therapy was applied for 16 patients (80%), and the mean dose amounted of  $700 \pm 413,11$  mg (100-1500 mg) of intravenous iron. Average dose of intravenous iron, during six-months therapy by epoietin, amounted  $586,7 \pm 385,29$  mg (Figure 2) for all the patients in total.

So, the patients on subcutaneous epoietin beta therapy received the highest dose of iron-replacement, then come the patients on intravenous epoietin alpha and beta therapy. Testing the statistical significance of difference among the mean values of iron doses in the tested groups showed no statistical difference ( $p=0,27$ ). Comparison of the mean values of ferritin, a month before including

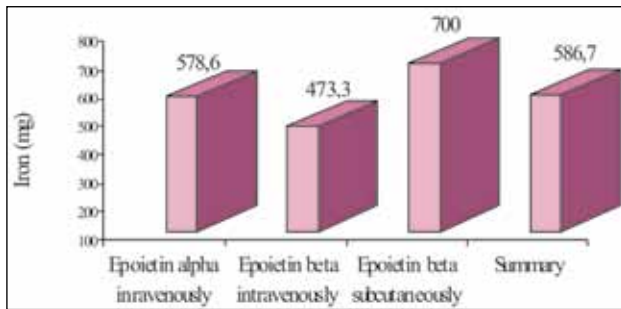


Figure 2. Relation of the mean values of iron doses in the tested patients

the patients into research with ferritin measured after three and sixth months of research, no significant difference was found in the testing groups ( $p > 0,05$ ). Relation of average values of ferritin is shown in the Figure 3.

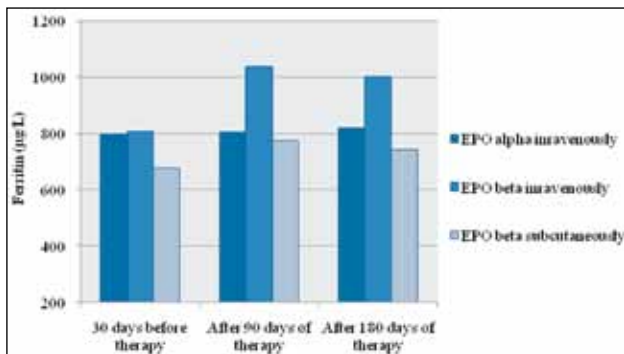


Figure 3. Average values of ferritin in the tested patients

Values of ferritin in the experimental group of patients in the course of six month-therapy by epoietin alpha increased from 796,3 to 820,3 µg/L. After three months of intravenous epoietin beta and iron-replacement therapy, average value of ferritin increased from 806,7 to 1037,5 µg/L. Decreased dose of iron-replacement, ferritin fell to 1003,7 µg/L. Average values of ferritin in the patients on subcutaneous epoietin beta therapy were increasing from the initial 675,3 µg/L (start of the experiment) to 743,4 µg/L after six months (Figure 3).

## Discussion

Results of different studies (7, 8) show that subcutaneous epoietin is equally effective as well as the intravenous one in chronic renal patients who are on hemodialysis, but to achieve target values of hemo-

globin and hematocrit, smaller doses of subcutaneous epoietin could be used, and that way to low the treatment cost. Vanrenterhem et al., found that needed dose of subcutaneous epoietin (alpha and beta) is 22% lower in comparison to intravenous epoietin (9).

The highest dose of epoietin (though it is slightly increased) in correction of renal anemia, received the patients on subcutaneous epoietin beta therapy ( $p > 0,05$ ). Average dose of subcutaneous epoietin beta was higher for 0,8% than the average dose of intravenous epoietin alpha and for 2,9% in comparison to intravenous epoietin beta. Dose of epoietin alpha was approximately higher for 2,2% in comparison to average dose of intravenous epoietin beta.

However, there was no difference (40 i.u./kg of body weight), in weekly dose of erythropoietin among the patients on intravenous therapy by epoietin alpha and subcutaneous epoietin, while weekly dose of intravenous epoietin beta was approximately higher for 5 i.u./kg of body weight.

Beside that, no statistically significant difference was found in hemoglobin, hematocrit, and biochemical parameters levels depending on a remedy dose, in the tested groups, what shows that in our study epoietin alpha and beta were equally effective.

As there was no difference in weekly doses of erythropoietin, we could speculate with the reasons of better therapeutical respond in the patients treated by subcutaneous epoietin beta, concerning to the prevalence of males in comparison to females in this group, or differences in isomorphous composition of epoietina alfa and beta (caused by glycolisation form), that is most possible reason of variations in the potential of remedy (10).

Our study results also show that doses of epoietin were slightly smaller than in recommendations of EBPg on the treatment of renal anemia. Concerning the results cited, except in erythropoietin dose, explanations could be found in differences in iron-replacement doses in the tested groups.

Titration of iron administration was followed by measuring serum ferritin level. In our research 76,7% (60% of patients in experimental, and 85% and 80% in control groups) were administered replacing iron. The patients on subcutaneous epoietin beta therapy received approximately the highest dose of intravenous iron, what is in accordance with the study showing that higher levels of hemoglobin cause higher need for iron (11). Slightly smaller dose



of replacing iron was administered the patients on epoetin alpha therapy. It should be mentioned that these patients had lower level of hemoglobin, either. On the contrary, the patients on intravenous epoetin beta therapy after three months had increased level of ferritin over suggested upper limit of 800 µg/L (1037,55 ±473,32 µg/L), that remained of the same values till the end of the study (1003,7 ±424,66 µg/L). Considering the safety measures pointing to the fact that high doses of iron can cause organ's damage because of iron deposit in such patients (12) iron dose was decreased in the course of the last three months of therapy. The lowest dose of replacing iron in these patients is probably one of the reasons of hemoglobin levels fall in the course of the last three months of therapy.

As none of the patients in this group was transfused in this period, our results are not in accordance to the findings in Locatelli's et al. study (5) that shows that such high values of iron deposit (>1000 µg/L) should be caused by frequent blood transfusions. All the cited above bring a number of dilemmas. Firstly, it is possible that combination of erythropoietin beta and intravenous iron in higher doses had better effect, and that replacing epoetin beta with epoetin alpha in experimental group resulted in a weak hematopoietic respond, while continued therapy by beta epoetin resulted in achieving target values of hemoglobin and hematocrit (hemoglobin >11 g/dL and hematocrit >33%).

## Conclusion

Insufficient substitutional iron therapy was the reason of a weaker respond to the erythropoietin therapy in the patients with renal anemia who were treated by intravenous epoetin alpha and beta.

## References

1. Puri V. Detection of circulating anti-erythropoietin antibodies in patient on recombinant erythropoietin therapy. *Indian J Nephrol* 2004; 14: 187-191.
2. Abbas EE, Afioni N, Al Wakeel J et al. The new rHuEPO alpha (epoetin) in the management of anemia of end-stage renal disease in patients on maintenance hemodialysis. *Transplant Proc* 2004; 36(6): 1805-1811.

3. Ng T, Marx G, Littlewood T, Macdougall I. Recombinant erythropoietin in clinical practice. *Postgrad Med J* 2003; 79: 367-376.
4. Besarab A, Amin N, Ahsan M et al. Optimization of epoetin therapy with intravenous iron therapy in hemodialysis patients. *J Am Soc Nephrol* 2000; 11: 530-538.
5. Locatelli F, Baldamus CA, Villa G, Ganeva A, Martin de Francisco AL. Once weekly compared with three times weekly subcutaneous epoetin beta: results from a randomized, multicenter, therapeutic-equivalence study. *Am J Kid Dis* 2002; 40: 119-125.
6. Radman I, Slipac J, Golubić-Čepuljić B. Može li kombinirani humani eritropoetin zamijeniti transfuziju eritrocita. *Paediatr Croat* 2005; 49(1): 271-274.
7. Albitar S, Meulders Q, Hammoud H, Soutif C, Bouvier P, Pollini J. Subcutaneous versus intravenous administration of erythropoietin improves its efficiency for the treatment of anemia in hemodialysis patients. *Nephrol Dial Transplant* 1995; 10(6): 40-43.
8. Kaufman JS, Reda DJ, Fye CL et al. Subcutaneous compared with intravenous epoetin in patients receiving hemodialysis. *N Engl J Med* 1998; 339: 578-583.
9. Vanrenterghem Y, Barany P, Mann JF et al. Randomized trial of darbepoetin alfa for treatment of renal anemia at a reduced dose frequency compared with rHuEpo in dialysis patients. *Kidney Int* 2002; 62: 2167-2175.
10. Storring PL, Tiplady RJ, Gaines Das RE et al. Epoetin alfa and beta differ in their erythropoietin isoform compositions and biological properties. *Br J Haematol* 1998; 100: 79-89.
11. Besarab A, Bolton WK, Browne JK et al. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. *N Engl J Med* 1998; 339: 584-590.
12. Sutton HC. Efficiency of chelated iron compounds as catalysis for the Haber-Weiss reaction. *Free Radic Biol Med* 1985; 1: 195-202.

Corresponding author:

Edin Ostrvica,  
Health Center „Dr. Mustafa Šehović” Tuzla  
Bosnia and Herzegovina  
e-mail: edinostrvica@yahoo.com



# Application of the PowerPlex® S5 MINISTR System in Forensic DNA Analysis of Human Telogenic Hair

## PRIMJENA POWERPLEX® S5 MINISTR SISTEMA U FORENZIČNOJ DNK ANALIZI HUMANIH TELOGENIH DLAKA

Lejla Kovacevic<sup>1</sup>, Dzenisa Buljugic<sup>1</sup>, Mirela Dzehverovic<sup>1</sup>, Adaleta Durmic-Pasic<sup>1</sup>, Damir Marjanovic<sup>1,2</sup>

<sup>1</sup> Institute for Genetic Engineering and Biotechnology, University of Sarajevo, Bosnia and Herzegovina

<sup>2</sup> Genos, doo, Zagreb, Croatia

### Abstract

The primary value of DNA typing has been significantly increased over the last fifteen years due to introduction of short tandem repeat (STR) loci in paternity testing, as well as in forensic cases and mass disaster human identification. Nowadays, several commercial multiplex kits are available, allowing routine use of this powerful forensic-genetic tool. In addition, it is well known that even extremely highly degraded nuclear DNA may be successfully analyzed using PCR primers that are close to the STR repeat. This modification of STR molecular markers is described as miniSTR approach. In this study we have examined possible application of nuclear miniSTR loci in forensic DNA analysis of 65 telogenic hair that collected from various locations within the facility of Institute for Genetic Engineering and Biotechnology. All samples were briefly washed in absolute ethanol and extracted using Qiagen DNA Dnaeasy™ Tissue Kit. DNA concentration was determined using Quantifiler Human DNA Quantification Kit as described previously. The reaction was carried out in AB 7300 Real-Time PCR System according to the manufacturer's recommendations. The PowerPlex S5® system has been used to amplify 4 miniSTR loci in 10µl total reaction volume. PCR amplification was carried out in AB GeneAmp

PCR System Thermal Cycler. Electrophoresis of the amplification products was performed on an ABI PRISM 310 Genetic Analyzer. Raw data have been compiled, analyzed and numerical allele designations of the profiles were obtained using the accessory software: ABI PRISM® Data Collection Software v3.0 and GeneMapper™ ID Software v3.1. As the result of these analyses, 19 telogenic hair were completely or partially profiled and for 46 of them no profiles were detected. The most stable results were detected at TH01 locus. These facts, together with the other results of this study, imply that *PowerPlex® S5 System* may be useful in forensic analysis of the samples with very low amount of nuclear DNA such as telogenic hair. However, its application has certain limitations in the field that should also be considered and overcome in the future application of miniSTR approach in the field of forensic genetic.

**Key words:** telogen hair, miniSTR, forensic DNA analysis

### Sažetak

Primarna vrijednost DNK analize je značajno porasla u posljednjih petnaest godina primjenom STR (*Short Tandem Repeats*) markera u DNK analizama kako u sklopu testiranja spornog

paterniteta i širokom spektru forenzičnih slučajeva, tako i u identifikacijama humanih skeletnih ostataka. Danas je na tržištu dostupno nekoliko komercijalnih multipleksnih kitova koji su uvijetovali da primjena forenzične DNK analize postane doslovno jednostavan rutinski postupak. Također, primjenom savremenih metoda kao što je PCR procedura u kojoj su prajmeri locirani bliže polimorfnom STR regionu, danas čak i visoko degradirana nuklearna DNK može biti uspješno analizirana. Ovak novi pristup u analizi već postojećih STR markera u naučnoj literaturi se naziva miniSTR model.

U ovoj studiji istražili smo mogućnost primjene miniSTR lokusa u forenzičnoj DNK analizi 65 dlaka koje se nalaze u telogenoj fazi rasta, i koje smo prikupili sa različitih lokacija. Prikupljeni uzorci dlaka su pažljivo oprani apsolutnim etanolom i vodom, a potom je primjenjen komercijalni set, tj. Qiagen Dnaeasy™ Tissue Kit za DNK izolaciju iz prikupljenih tragova. Koncentracija izolirane DNK determinirana je primjenom Quantifiler Human DNA Quantification kita, po protokolu koji je ranije opisan, te AB 7300 Real-Time PCR mašine, također prema preporukama proizvođača. PowerPlex S5® amplifikacijski kit je korišten za amplifikaciju četiri miniSTR lokusa. Ukupni volumen reakcije iznosio je 10 µl. Amplifikacija je realizirana u AB GeneAMP PCR 9700 mašini, a elektroforetska separacija odabranih markera primjenom ABI PRISM 310 genetičkog analizatora. Generirani DNK profili dobijeni su upotrebom softverskih paketa: ABI PRISM® Data Collection Software v3.0 i GeneMapper™ ID Software v3.1

Rezultati su pokazali da je za 19 telogenih dlaka detektovan parcijalni ili potpuni miniSTR profil, dok kod preostalih 46 to nije bilo moguće. Locus TH01 se pokazao kao najdetektibilniji. Ove činjenice, zajedno sa preostalim rezultatima ove studije, impliciraju na mogućnost primjene PowerPlex S5® amplifikacijski kita u forenzičnoj DNK analizi veoma malih količina nuklearne DNK, koja je upravo karakteristična za telogene dlake.

Ipak postoje određeni nedostaci primjene miniSTR amplifikacijskih sistema u analizi telogenih dlaka, koje bi trebalo razmotriti i savladati, u svrhu što efikasnije buduće primjene miniSTR pristupa u oblasti forenzične genetike.

## Introduction

Hair is one of the most common types of physical evidence that could be found at the crime scene. Hairs can provide investigators with valuable information for potential leads. Also, hair shaft is desirable forensic evidence, because it is sturdy and can maintain its essential distinctiveness for years. They carry plenty of biological information and they are easy to examine (1). Until recently, the comparison by microscope was considered the only reliable tool for hair identification. Today, DNA testing, both nuclear (nuDNA) and mitochondrial (mtDNA), frequently provides valuable information about biological source of the examined evidence (1).

The primary component of hair is protein keratin. It is defined as slender outgrowth in the skin of mammals (3). Its growth is characterized by three phases: anagen, telogen and catagen. Anagen is the phase of active growth when follicle produces new cells and pushes them up the hair shaft (4). Because of massive presence of surrounding bulb cells, this phase of hair growth is preferable for forensic nuclear DNA analysis. Catagen is transitional phase. During this phase of growth, the follicle begins to shut down production of cells (1). Almost 80% of human hairs found at the crime scene are in telogen phase, the terminal, degenerative phase of the hair's development cycle (5). Telogenic hairs are characterized by the presence of club roots. They contain few bulb cells, which produce extremely low amount of highly degraded nuDNA, hardly suitable for this kind of DNA typing. Until recently, catagen hair shafts were used only for mtDNA analysis.

Considerable distinction exists among the types of hairs originating from different regions of the human body, such as head, pubic region, arms, legs etc. The region of the body where a hair originated can be determined with considerable accuracy by its gross appearance and microscopic characteristics. Length, shape, size, color, hardness, curliness, and microscopic appearance all contribute to the body area determination (2). The first step in examination of hair routinely involves the identification of hairs and their visual (microscopical) comparison with the reference. Primarily, the origin of the hair is confirmed (human or animal). Afterwards, comparison of specific features

may reveal whether particular individual should be considered as a possible biological source of the physical evidence.

However, hairs that have been matched or associated by microscopic examination should also be examined by DNA analysis. In fact, nuclear DNA testing provides the most relevant information in the sense of individualization of the possible origin of the hair (5). However, the amount and condition of recovered nuDNA vary depending on the phase of hair growth.

The primary value of DNA typing has been significantly increased in the last fifteen years due to introduction of short tandem repeat (STR) loci in routine paternity testing, as well as in forensic cases and mass disaster human identification (6). Nevertheless, the ability to obtain DNA profiles from very small amounts of sample still presents certain type of challenge in forensic casework (7). As it is already known, low-copy number (LCN) DNA testing typically refers to examination of less than 100 pg of input DNA (5, 8). In such cases, improvement of the result is frequently attempted by increasing the number of PCR cycles. Nevertheless, application of LCN results should be approached with caution due to the possibilities of allele dropout, allele drop-in, and increased risks of collection-based and laboratory based contamination. Therefore, additional DNA analysis of these samples with an available miniSTR system is sometimes required (7, 9).

At the beginning of the 21<sup>st</sup> century, it was demonstrated that highly degraded DNA as well as very low amounts of DNA could be more successfully typed using new redesigned PCR primers positioned closer to the STR repeat (10). Many authors consider that it is likely that miniSTRs will play major role in the future of degraded DNA analysis found in physical evidence such as human telogen hair. Several commercial miniSTR multiplex were released over the last few years. PowerPlex S5 (Promega corporation, Madison, USA) is one of them and its potential was already described in our previous work (7,9,11).

### Aim/objectives

The aim of this study was to examine and optimize the application of miniSTR multiplex sy-

stem, and consequently, to analyze the capacity of this forensic genetic tool within forensic DNA analysis of human biological traces that yield small amounts of highly degraded DNA.

Also, the goal was to estimate the level of success of miniSTR multiplex system, particularly PowerPlex S5 (Promega corporation, Madison, USA) applied to human telogenic hairs, and potentially optimize nuDNA analysis process of human telogenic hairs.

### Material and methods

Hair samples were collected using sterile tweezers from various locations within the facility of Institute for Genetic Engineering and Biotechnology (INGEB). In total, 100 of hair samples were collected and stored separately in paper envelopes labeled with unique lab code. The collected samples were processed in the Laboratory of Forensic Genetics at INGEB, University of Sarajevo. The hairs were visually analyzed using light microscope. Accurate detection of the hair growth phase was performed using binocular light microscope, type Novex B-range, Euromex Holland. Telogenic hairs, which were the study target, were separated from anagenic and catagenic hair specimens.

Hair specimens were transferred to Petri-dish and washed first with absolute ethanol and, after that with DNA-free ddH<sub>2</sub>O. The cut was made approximately one centimeter from root-region, to avoid melanin containing keratinized fragment. With the aid of one glycerol drop, hairs were easily placed on glass slide and then covered with slips. Microscopic examination was performed using 10 x magnification. All hair specimens were photographed using *Olympus BX51* camera and viewed using *Studio Lite* and *Viewfinder Lite* software. Hairs identified as telogenic were selected for further DNA analysis.

Each selected telogenic hair was washed successively in absolute ethanol, and DNA free ultrapure water, and dried in 1,5ml sterile tubes. DNA extraction was performed using QIAGEN *DNeasy<sup>TM</sup> tissue kit* (12). Extraction procedure was additionally optimized for telogenic hair specimens by adding 1M DTT solution. Some samples required additional purification and concen-

tration step applying Centricon-100 centrifugal filter units (Millipore, Billerica, MA, USA). The concentrates were transferred to 1,5ml centrifuge tubes and diluted with DNA free ultra-pure water to a final volume of 50µl. DNA concentration was determined using Quantifiler Human DNA Quantification kit (Applied Biosystems) (13). The reaction was carried out in AB 7300 Real-Time PCR System (ABI, Foster City, CA, USA) according to manufacture’s recommendations. Amplification of STR loci was performed using PowerPlex S5 miniSTR amplification system (Promega corporation), that contain 4 STR loci: D18S51, D8S1179, TH01 and FGA, and sex determining marker, amelogenin. The total reaction volume was 10 µl. PCR amplification was carried out in an AB Gene Amp PCR System 9700. Electrophoresis of the PCR products was performed on an ABI PRISM 310 Genetic Analyzer. Raw data were compiled and analyzed, and numerical allele designations of the profiles were obtained using the accessory software: ABI PRISM® Data Collection Software v3.0 and GeneMapper™ ID Software v3.1.



Figure 1. Photomicrograph of telogenic hair root (HS 27)

**Results**

**Microscopic analysis of the collected hair specimens**

Microscopic analysis identified all the collected hair specimens as human, originating from head (Table 1). In addition, the same analysis focused on root appearance implied that most of hairs, 65 of them exactly, were in telogen phase of growth, (Figure 1, Table 2).

**Quantitative analysis of nuDNA extracted from telogenic hair samples**

Real-time based quantification using *Quantifiler® DNA Identification* kit (Applied Biosystems) detected nuDNA in 14 (22%) DNA extracts from telogenic hairs. For the remaining 51 telogen hairs extract, either no nuDNA was detectible or PCR inhibitors were present (6 samples; Table 3). In

Table 1. The origin of hair specimens as determined by microscopic analysis

| Collected hairs - total | Hairs of human origin |                  | Hairs of animal origin |
|-------------------------|-----------------------|------------------|------------------------|
|                         | Head                  | Other body areas |                        |
| 100                     | 100                   | 0                | 0                      |

Table 2. Microscopic identification of hair growth phase

| Collected hairs - total | Anagen phase of growth | Catagen phase of growth | Telogen phase of growth |
|-------------------------|------------------------|-------------------------|-------------------------|
| 100                     | 23                     | 12                      | 65                      |

samples with detectable DNA, nuDNA concentration ranged from  $8,69 \times 10^{-4}$  ng/ $\mu$ l to  $9.50 \times 10^{-2}$  ng/ $\mu$ l. (Table 4).

### The analysis of the obtained miniSTR profiles

The profiles obtained using miniSTR markers are presented in Table 5. DNA profiles were obtained for 19 (29%) telogenic hair specimens. Number of the detected loci varies from one to five per

Table 3. Overall results of DNA quantification

| Total number of processed hairs | Samples with determined nudna concentration | Samples with non-detectable nudna | Samples with pcr inhibitors |
|---------------------------------|---------------------------------------------|-----------------------------------|-----------------------------|
| 65                              | 14                                          | 45                                | 6                           |

Table 4. The results of DNA quantification presented per processed sample

| Hair Sample | Concentration (ng/ $\mu$ l) | Hair Sample | Concentration (ng/ $\mu$ l) |
|-------------|-----------------------------|-------------|-----------------------------|
| HS 1        | -                           | HS 34       | -                           |
| HS 2        | $5.57 \times 10^{-3}$       | HS 35       | $1.39 \times 10^{-2}$       |
| HS 3        | -                           | HS 36       | -                           |
| HS 4        | -                           | HS 37       | $3.07 \times 10^{-3}$       |
| HS 5        | -                           | HS 38       | -                           |
| HS 6        | -                           | HS 39       | -                           |
| HS 7        | -                           | HS 40       | $7.53 \times 10^{-3}$       |
| HS 8        | -                           | HS 41       | -                           |
| HS 9        | -                           | HS 42       | -                           |
| HS 10       | $8.59 \times 10^{-4}$       | HS 43       | -                           |
| HS 11       | -                           | HS 44       | -                           |
| HS 12       | -                           | HS 45       | -                           |
| HS 13       | -                           | HS 46       | -                           |
| HS 14       | -                           | HS 47       | $1.39 \times 10^{-2}$       |
| HS 15       | -                           | HS 48       | -                           |
| HS 16       | $7.11 \times 10^{-3}$       | HS 49       | $2.53 \times 10^{-2}$       |
| HS 17       | -                           | HS 50       | -                           |
| HS 18       | -                           | HS 51       | -                           |
| HS 19       | $1.19 \times 10^{-3}$       | HS 52       | $4.04 \times 10^{-2}$       |
| HS 20       | -                           | HS 53       | -                           |
| HS 21       | -                           | HS 54       | -                           |
| HS 22       | -                           | HS 55       | -                           |
| HS 23       | $1.42 \times 10^{-3}$       | HS 56       | -                           |
| HS 24       | -                           | HS 57       | -                           |
| HS 25       | -                           | HS 58       | -                           |
| HS 26       | -                           | HS 59       | -                           |
| HS 27       | $3.55 \times 10^{-3}$       | HS 60       | -                           |
| HS 28       | -                           | HS 61       | -                           |
| HS 29       | -                           | HS 62       | -                           |
| HS 30       | -                           | HS 63       | -                           |
| HS 31       | -                           | HS 64       | $9.50 \times 10^{-2}$       |
| HS 32       | $1.49 \times 10^{-2}$       | HS 65       | -                           |
| HS 33       | -                           |             |                             |



Table 5. The profiles obtained by miniSTR typing of the analyzed telogenic hairs

| HS | AMEL | D18S51   | D8S1179 | TH01  | FGA   |
|----|------|----------|---------|-------|-------|
| 1  | -    | -        | -       | -     | -     |
| 2  | -    | -        | 13;16   | 6     | -     |
| 3  | X    | -        | -       | 9     | -     |
| 4  | -    | 14       | 11;13   | -     | -     |
| 5  | X;Y  | -        | -       | 6;9   | 19    |
| 6  | -    | -        | -       | -     | -     |
| 7  | -    | -        | -       | 9     | -     |
| 8  | X;Y  | 8;17     | -       | 6     | -     |
| 9  | X    | 13;17;20 | 10      | -     | -     |
| 10 | Y    | -        | 12;13   | 6;9   | 22.2  |
| 11 | -    | -        | -       | -     | -     |
| 12 | X    | 20       | -       | 6     | -     |
| 13 | -    | -        | -       | -     | -     |
| 14 | -    | -        | -       | -     | -     |
| 15 | -    | -        | -       | -     | -     |
| 16 | -    | -        | -       | -     | -     |
| 17 | -    | -        | -       | -     | -     |
| 18 | -    | -        | -       | -     | -     |
| 19 | -    | -        | -       | -     | 6     |
| 20 | -    | -        | -       | -     | -     |
| 21 | -    | -        | -       | -     | -     |
| 22 | -    | -        | -       | -     | -     |
| 23 | -    | -        | -       | -     | -     |
| 24 | -    | -        | -       | -     | -     |
| 25 | -    | -        | -       | -     | -     |
| 26 | -    | -        | -       | -     | -     |
| 27 | -    | -        | -       | -     | -     |
| 28 | -    | -        | -       | -     | -     |
| 29 | -    | -        | -       | -     | -     |
| 30 | -    | -        | -       | -     | -     |
| 31 | -    | -        | -       | -     | -     |
| 32 | -    | -        | -       | -     | -     |
| 33 | -    | -        | -       | -     | -     |
| 34 | -    | -        | -       | -     | -     |
| 35 | -    | -        | -       | 9.3   | 20;25 |
| 36 | -    | 15.2     | -       | -     | -     |
| 37 | -    | -        | -       | -     | -     |
| 38 | -    | -        | -       | -     | -     |
| 39 | -    | -        | -       | -     | -     |
| 40 | X    | 14       | 12      | 9;9.3 | 22    |
| 41 | -    | -        | -       | 9     | -     |
| 42 | -    | -        | -       | -     | -     |
| 43 | -    | -        | -       | -     | -     |
| 44 | -    | -        | -       | -     | -     |
| 45 | -    | -        | -       | -     | -     |
| 46 | -    | -        | 12      | -     | -     |
| 47 | X;Y  | 12       | 12;16   | 9     | 20;23 |
| 48 | -    | -        | -       | -     | -     |
| 49 | X;Y  | -        | -       | 9     | 23    |
| 50 | -    | -        | -       | -     | -     |
| 51 | -    | -        | -       | -     | -     |
| 52 | X    | 13       | 10;12   | 7;10  | 21    |
| 53 | -    | -        | -       | -     | -     |
| 54 | -    | -        | -       | -     | -     |
| 55 | -    | -        | -       | -     | -     |
| 56 | -    | -        | -       | -     | -     |
| 57 | -    | -        | -       | -     | -     |
| 58 | -    | -        | -       | -     | -     |
| 59 | -    | -        | -       | -     | -     |
| 60 | -    | -        | -       | -     | -     |
| 61 | -    | -        | -       | -     | -     |
| 62 | -    | -        | -       | -     | -     |
| 63 | -    | -        | -       | -     | -     |
| 64 | X    | 12;14    | 13;14   | 9;9.3 | 20;25 |
| 65 | -    | -        | -       | -     | -     |

profile. Complete miniSTR profiles were obtained for four hair specimens only. Figure 2 presents one of the full DNA profiles. Other fifteen miniSTR profiles were partial (Figure 3). MiniSTR profiling did not provide any results for 46 processed hair samples. Finally, TH01 locus was the most frequently detected locus in this study. On the other hand, FGA locus provided the weakest results in the typing of the telogen hair samples.

### Discussion

As reported previously, microscopic analysis identified all the collected samples as human, originating from the head area. Absence of animal specimens was expected since the hair samples were collected from the various locations within the INGEB facility, where animals are not allowed. In addition, the same analysis focused on root appear-

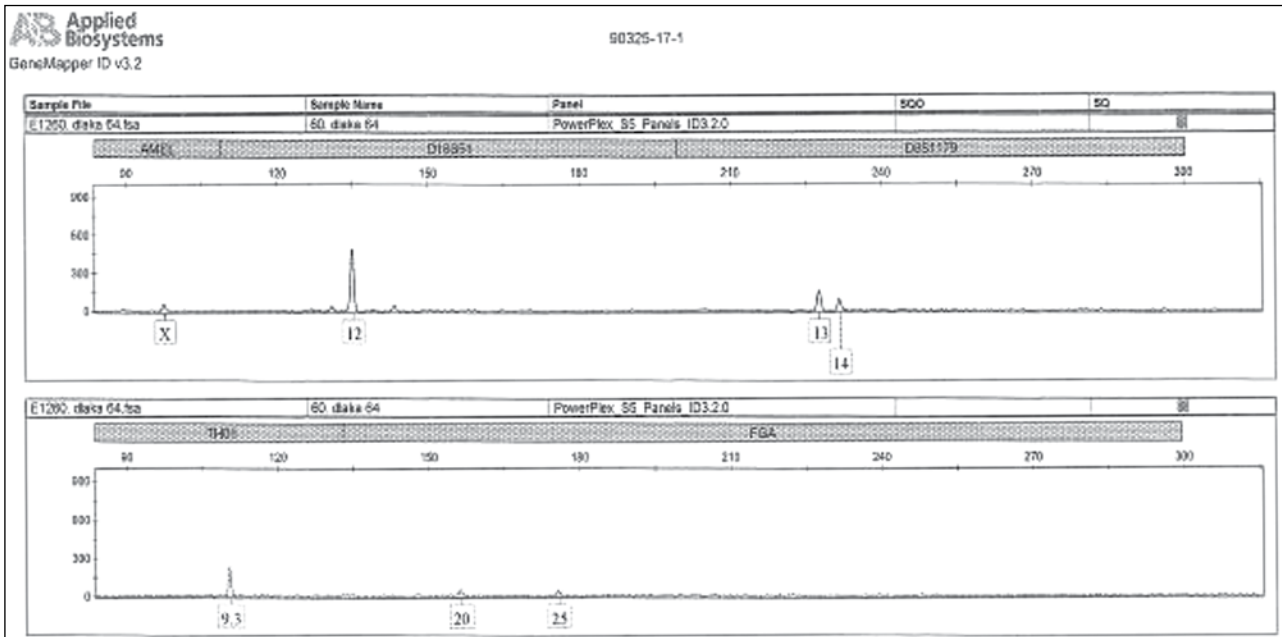


Figure 2. Electropherogram of full miniSTR profile obtained from HS 64.

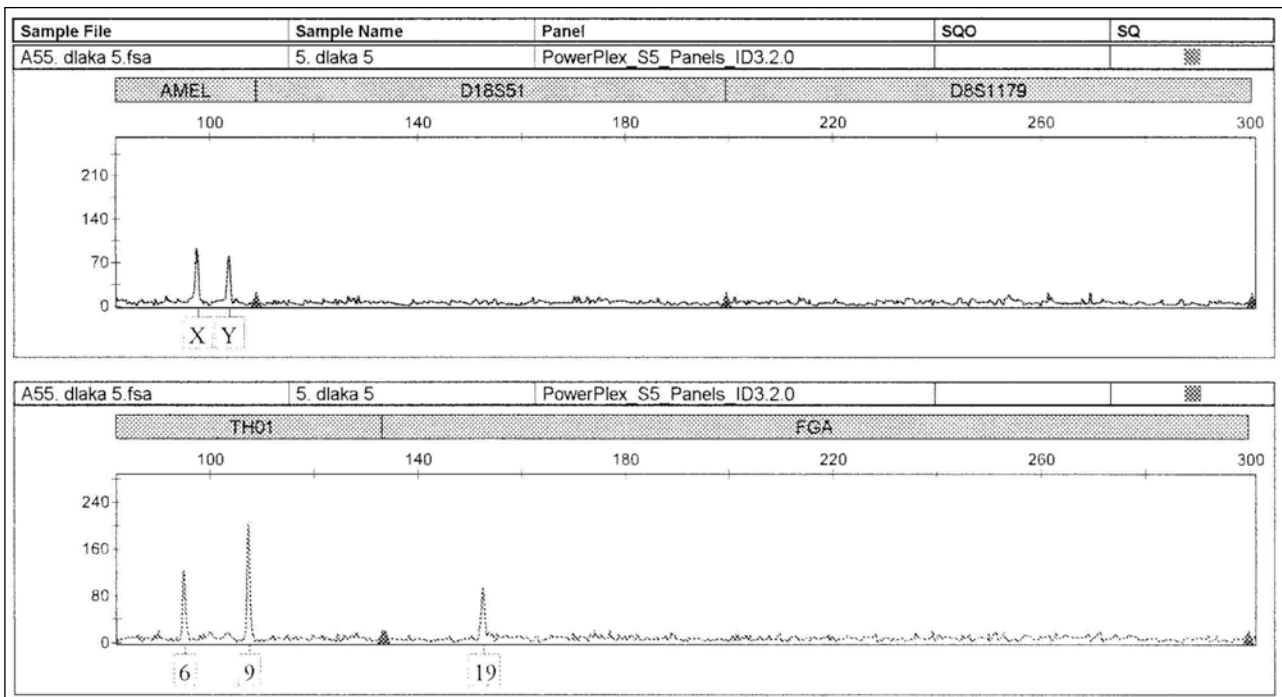


Figure 3. Electropherogram of partial miniSTR profile obtained from HS 5.

rance implied that 65% of the collected hairs were in telogen phase of growth. This result mainly concurs with previously published information, which reports 80% of human hairs found on crime scenes to be in telogen phase of growth (5). Slightly increased number of the recovered anagen hair samples may be explained by the fact that some of the specimens were collected from the personal INGEB employees' clothing, where this type of specimens could be expected with higher frequency.

The quantification categorized all telogenic hair samples processed in this study as, so called, LCN (low copy number) DNA evidence, since the detected amounts of extracted nuDNA were well below 100pg/ $\mu$ l (8). Similar study was conducted before (14). Previous study has shown that the concentration of DNA extracted from hair samples collected from various combs was below 100 pg/ $\mu$ l in more than 30% of the specimens. Of course, there is a marked difference in the modality of specimens' collection between these two studies. Therefore, the detection of higher amounts of DNA in almost 70% specimens from the combs was expected.

Interestingly, higher mean DNA concentrations were obtained from 60 years old skeletal remains' samples (15) than from telogen hair samples processed in this study. That also serves to prove how challenging the DNA analysis of telogen hair evidence may be. Therefore, the selection of the most suitable DNA extraction protocol is a crucial step in successful profiling of telogenic hair samples. Guided by the available data from literature, (16, 17) and earlier gathered experience from our laboratory, we have chosen alcohol pretreatment, which significantly increases the possibility of successful isolation of DNA from telogen hair specimens. Also, based on the same premises, we selected DNA extraction procedure based on the use of silica membrane (12), with the addition of dithiothreitol (DTT), which prevents renaturation of disulphide bonds in keratin that prevents successful isolation of DNA from the hair specimens (18). Although, several studies indicate that DTT reduces the concentration of extracted DNA (19), our previous experience shows significantly better results in its presence.

Finally, considering four full and fifteen partial miniSTR profiles obtained from hair specimens in this initial study, we tend to maintain positive

attitude towards usefulness of this genetic tool in forensic DNA analysis of these challenging physical evidences. Of course, it is already well known that miniSTR assays can help recover information from degraded DNA samples that typically result in partial profiles and total loss of information using regular STR amplicons (7). This approach has already been used in the analysis of highly degraded samples like those processed within the identification of victims from the World Trade Center terrorist attacks (7), in identification of World War II victims (7), but it could also be used within case studies in the analysis of telogenic hair samples (11). It is noteworthy that in even though RealTime-PCR quantification assay was unable to detect DNA, in some cases, miniSTR provided useful profile. The same observation was also recorded in previous study (7). Considering all the relevant information, it is expected that the most recent concept of miniSTR kits should certainly upgrade the analysis of LCN DNA biological evidence, including human telogenic hair samples.

## Conclusion

In latest years, DNA forensic analysis of hair shafts has significantly improved both in terms of selecting the most useful genetic markers, as well as, in terms of the optimization of the analysis procedures. It is likely that miniSTR assay will certainly play a role in the future of degraded DNA analysis. The results of this initial study indicate that miniSTR PowerPlex S5 System, as well as mini STR concept in general, has the potential in DNA analysis of telogenic hair specimens.

Until recently, usage of miniSTRs had several disadvantages, such as differences in heterozygous allele call detected in comparison with standard STR markers, or low discriminatory power within individualization of miniSTR profiles due to low number of miniSTR loci within existing multiplex kits, which have restricted the power of its usage in the field of forensic genetics.

Well planned concordance studies as well new concept of the joint STR and miniSTR multiplex kit promoted by Promega Corporation successfully work towards solving these items. All these joint efforts, together with the new solution in specimen

pretreatment procedures and optimized DNA extraction protocol will certainly significantly improve usage of miniSTR concept in DNA profiling of telogenic hair shafts.

## References

1. Houck MM, Siegel JA. *Fundamentals of Forensic science*. Elsevier Academic Press, London, 2006.
2. Deedrick DW, Koch LS. *Microscopy of Hair part I: A Practical Guide and Manual for Human Hairs*. *Forensic Sci. Com.*, January 2004; 6 (1).
3. Bojanić N, Milosavljević M. *Praktikum za kriminalistiku 5 (forenziku)*. Fakultet Kriminalističkih nauka, Sarajevo, 2008.
4. Harding H, Rogers G. *Physiology and growth of human hair*. In: *Forensic examination of hair*. Edited by Robertson J. Tayler and Francis Forensic Science series, London, 1999; 1-77.
5. Marjanović D, Primorac D. *Molekularna forenzična genetika*. Institut za genetičko inženjerstvo i biotehnologiju, Sarajevo, 2009
6. Butler JM. *Forensic DNA typing, biology, technology and genetics of STR markers*. Second edition, Elsevier (USA) Academic Press, 2005.
7. Marjanović D, Durmić-Pašić A, Kovačević L, Avdić J, Džehverović M, Haverić S, Ramić J, Kalamujić B, Lukić-Bilela L, Škaro V, Projić P, Bajrović K, Drobnić K, Davoren J, Primorac D. *DNA Identification of Skeletal Remains of Communist Armed Forces Victims During and After World War II: Combined Y-Chromosome Short Tandem Repeat (STR) and MiniSTR Approach*. *Cro. Med. J*, 2009; 50 (3): 296-304.
8. Hill C.R, Kline C.M, Coble M.D, Butler J.M. *Characterization of 26 MiniSTR Loci for Improved Analysis of Degraded DNA Samples*. *J. Forensic Sci*, 2007; 50(3): 73-80
9. Marjanovic D, Kovacevic L, Durmic-Pasic A, Avdic J, Hindija J, Skaro V, Projic P, Primorac D. *Usage of the PowerPlex® S5 Mini-STR System in LCN DNA Testing*. *19th International Symposium in Human Identification, Hollywood, USA, (2008)*, Abstract book 26
10. Wiegand P, Kleiber M. *Less is more – length reduction of STR amplicons using redesigned primers*. *Int J Legal Med*, 2001; 114:285–287
11. Marjanovic D, Kovacevic L, Durmic-Pasic A, Avdic J, Hindija J, Skaro V, Projic P, Primorac D. *Usage of PowerPlex® S5 Mini-STR System in DNA Identification of Skeletal Remains from the Second World War: 19th International Symposium in Human Identification, Hollywood, USA, (2008)*, Abstract book 106.
12. *Qiamp DNA Micro Handbook*, Qiagen GmbH, Hilden, Germany, 2007
13. Applied Biosystems. *Quantifiler™ Kits, Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification. User's Manual*, Applied Biosystems, 2006.
14. Opel KL, Fleishaker EL, Nicklas JA, Buel E, McCord BR. *Evaluation and Quantification of Nuclear DNA from Human telogen Hairs*. *J. Forensic Sci*. 2008; 53 (4).
15. Marjanovic D, Durmic-Pasic A, Bakal N, Haveric S, Kalamujic B, Kovacevic L, Ramic J, Pojskic N, Skaro V, Projic P, Bajrovic K, Hadziselimovic R, Drobnic K, Huffine E, Davoren J, Primorac D. *DNA Identification of Skeletal Remains from the Second World War Mass Graves Uncovered in Slovenia*. *Croatian Medical Journal*, 2007; 48: 4: 513-519
16. Schreiber A, Amtmann E, Storch V, Sauer G. *The extraction of high-molecular- mass DNA from hair shafts*. *FEBS Lett*, 1988; 230(1/2): 209-211.
17. Han CY, Lin BK, Lin HJ. *Methanol for preparing hair bulbs for PCR*. *Nucleic Acids Res*, 1992; 20 (23): 6419-6420.
18. Ackerman AB, de Viragh PA, Chongehitnant N. *Anatomic, histologic and biological aspects in: Ackerman AB, de Viragh PA, Chongehitnant N(Eds), Neoplasms with follicular differentiation (Ackerman's Histologic Diagnosis of Neoplastic Skin Diseases: A Method by Pattern Analysis)*. Lea and Febiger. Philadelphia, 1993; 29-90.
19. Pizzamiglio M, Virgili A, Vespi G, D'Errico G, Veechio C, Garofano L. *DNA typing on single hair- recent possibilities based on new extraction method*. In: *Advances in Forensic Haemogenetics*. Edited by: Baer W, Fiori A Rossi U, Springer-Verlag, Berlin, 1994; 333-335.

### Corresponding author:

Lejla Kovacevic,  
Institute for Genetic Engineering and Biotechnology,  
University of Sarajevo,  
Bosnia and Herzegovina,  
e-mail: lejla.kovacevic@ingeb.ba

# Evaluation of Serologic tests for diagnosis of Brucellosis

Sukrija Zvizdic<sup>1</sup>\*, Ramiz Velic<sup>2</sup>, Tarik Bajrovic<sup>2</sup>, Lejla Velic<sup>2</sup>, Sadeta Hamzic<sup>1</sup>, Salih Tandir<sup>3</sup>, Jasmin Musanovic<sup>1</sup>, Azra Kudumovic<sup>1</sup>

<sup>1</sup> Medical Faculty, University of Sarajevo, Bosnia and Herzegovina

<sup>2</sup> Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina

<sup>3</sup> Faculty of Health, University of Zenica, Bosnia and Herzegovina

## Abstract

In the past fifteen years, Bosnia and Herzegovina has been among the countries with endemic form of brucellosis, i.e. with high incidence of the disease in animals and humans. Brucellosis has become a significant public-health problem in a major part of the area of Bosnia and Herzegovina, due to this fact. Since both the causal agent of the disease and the disease belong to the group of zoonoses, the infection in humans is narrowly related to the infection in animals. Diagnosis of the disease implies isolation of microorganisms from biological material samples or detection of specific antibodies in serum. Results of the laboratory confirmation of brucellosis suspicion, based on determining of the presence of specific antibodies in the sera of 43 hospitalized individuals, are presented in this paper. The presence of specific antibodies was confirmed in 16 (37.2%) individuals using the method of Rose Bengal agglutination, while positive finding was obtained for 10 (23.2%) individuals using the CBR method. Result of the Rose Bengal test was indicated as positive or negative. There were 12 male and 4 female individuals, out of a total of 16 seropositive patients. Concerning the age of individuals, it was determined that there had been 3 (18.75%) positive individuals in the group aged up to 30, and 13 (81.25%) in the group aged older than 30. Concerning the obtained results of antibody titer levels determined by CBR, it is evident that they varied between 1:8 and  $\geq$  1:312.

**Key words:** brucellosis, Bosnia and Herzegovina, Rose Bengal, CFT.

## Sažetak

Bosna i Hercegovina se u zadnjih petnaest godine ubraja u jednu od država sa endemskim oblikom bruceloze, odnosno sa visokom incidencom oboljevanja životinja i ljudi. Zbog ove činjenice, bruceloza je postala značajan javnozdravstveni problem na velikom dijelu teritorije Bosne i Hercegovine. Pošto uzročnik oboljenja, kao i oboljenje spada u skupinu zoonoza, to je i oboljevanje ljudi usko povezano sa oboljevanjem životinja. Dijagnoza oboljenja podrazumijeva izolaciju mikroorganizama iz uzoraka bioloških materijala ili nalaz specifičnih antitijela u serumu. U ovom radu, prikazani su rezultati laboratorijske potvrde sumnje na brucelozu ispitivanjem prisustva specifičnih antitijela u serumu 43 hospitalizirana ispitanika. Metodom aglutinacije Rose Bengal je kod 16 (37,2%) ispitanika dokazano prisustvo specifičnih antitijela, dok je metodom RVK, pozitivan nalaz dobiven kod 10 (23,2%) ispitanika. Rezultat Rose Bengal testa se iskazivao kao pozitivan ili negativan. Od ukupno 16 seropozitivnih pacijenata, na muški spol otpada 12, a na ženski 4 ispitanika. U odnosu na dob ispitanika, ustanovljeno je da je u dobnoj skupini do 30 godina bilo 3 (18,75 %) pozitivna ispitanika, a u dobnoj skupini preko 30 godina 13 (81,25 %). U odnosu na dobivene rezultate visine titra antitijela dobivenih RVK, vidljivo je da su se kretali od 1: 8 do  $\geq$  1:312.

**Ključne riječi:** brucele, Bosna i Hercegovina, Rose Bengal, CFT.



## 1. Introduction

In the past fifteen years, Bosnia and Herzegovina (BiH) has been among underdeveloped countries with endemic form of brucellosis and Q fever, i.e. with high incidence of the disease in animals and humans, caused by related infectious agents (1, 2, 3, 4, 5, ). In the analyzed areas in BiH, infections are registered in cattle, sheep, goats, and other animals, as well as in humans. Human infections are diagnosed clinically, epidemiologically, and serologically, using adequate methods. In some cases, the attempt of isolation of brucellas results in isolation from blood samples or other biological materials, when suspicion of the disease is confirmed by isolation of the causal agent as well.

Small, non-motile, Gram-negative aerobic coccobacilli are causal agents of brucellosis. They belong to the group of aerobic bacteria that are oxidase- and catalase-positive, do not reduce nitrates, do not produce indole, and do not degrade gelatin. They are intracellular parasites, which are difficult to cultivate in standard nutritional media. The most reliable method of isolation of these bacteria is their isolation from blood, by cultivation of hemoculture that lasts up to one month. Reliable method of confirmation of brucellosis includes observing the titer of specific antibodies, or detection of occurrence of specific IgM antibodies, and then IgG as well, in the sera of infected patients. Specific anti-Brucella antibodies or increase in the overall antibody titers is searched for in serum samples in acute phase of the disease, which are collected after the infection. Quality of detected antibodies is determined in even-numbered and each subsequent serum sample, collected in the interval of 14 to 21 days, i.e. increase or decrease in the titer of specific antibodies is observed.

Brucellosis, as well as Q fever, represents an infectious disease in the group of zoonoses. It has acquired the features of endemic disease in the Mediterranean area, as well as in certain regions in BiH. Brucellas (*B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, etc) primarily infect domestic animals (goats, sheep, cattle, pigs), causing unapparent or manifest diseases, or abortion in gravid animals. Cattles represent a natural reservoir and they are carriers of *B. abortus*, goats and sheep of

*B. melitensis*, and pigs and dogs of *B. suis* and *B. canis*, respectively. Brucellas are transmitted from animals to humans by direct contact, via damaged skin or mucous, by consuming contaminated milk and dairy products, or via the aerogenic route. Frequency of the infection in humans is narrowly related to their occupation, as well as to the presence of animal infections. The infection and disease are more frequent in cattle-breeders, veterinarians, technicians, as well as those who handle the dairy and meat products and animal products. Interhuman infections are rare. Brucellosis is also one of the possible laboratory infections. The infection is likely to occur in working conditions that include unprotected environment or working without protective wear (gloves, masks, protective glasses), mouth pipetting, i.e. exposing the skin and mucous of eyes, nose or mouth to contaminated aerosol.

Brucellas cause chronic infections in animals, since they are located within the cells of various organs and systems, cells of mammary glands or reproductive organs. Depending on infection of organs or organ systems, various forms of clinical features develop.

Brucellosis in humans is a multisystemic disease, which is clinically manifested depending on organs and systems that are infected. Clinical symptoms of brucellosis can be flu-alike, with the occurrence of high temperature, headache, pain in muscles and joints, hepatosplenomegaly, feel of cold, anorexia, and general weakness. Brucellosis is characterized by the occurrence of undulant, low temperature in the morning and high temperature in the evening. Immunity remains solid after the recovery. Complications occur in certain number of infected individuals. These bacteria have been found to inhabit the cells of reticuloendothelial system, liver, spleen, bones, joints, urogenital tract, central nervous system, skin, lungs, and heart. As a consequence of the infection and complications, organ damages, such as endocarditis, or damages of various localizations are likely to occur in a long period of time (6, 7, 8). Human brucellosis is difficult to treat; the treatment requires long-term application of certain antibiotics, with the possibility of their penetration into an infected cell (for adults - doxycycline combined with streptomycin, for children – gentamicin and sulfametoxazol+trimethoprim, throughout six

weeks). The therapy is strictly prescribed (doses and duration), aimed at eradication of the causal agents from organism, by avoiding the occurrence of complications or relapse of the disease. Modified therapeutic regimen is recommended for children younger than 8, pregnant women, and other groups at risk (9, 10).

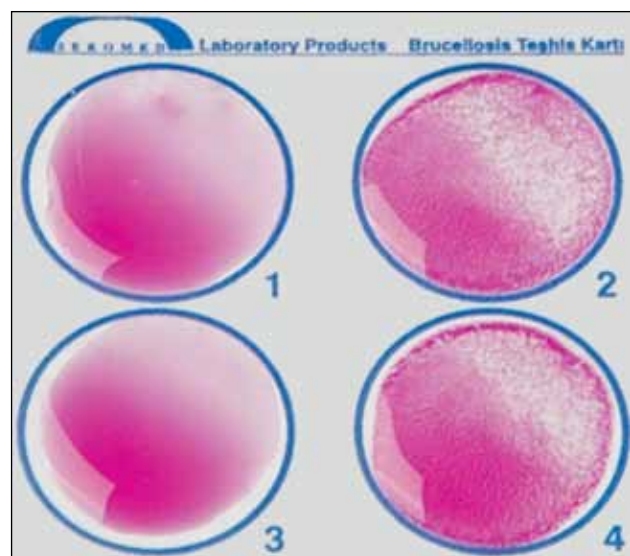
Specific and unspecific protection measures are available, aimed at prevention of the occurrence and spreading of this disease. Live attenuated vaccine has been produced for certain animal species (Rev 1), while an adequate vaccine is intended for persons exposed to the risk of infection. The most important unspecific protection measures include the available veterinary-sanitary measures, since veterinarian, as well as human medicine, is responsible for spreading of the causal agent and disease in humans. Constant veterinary control of the animals is necessary, as well as to vaccinate healthy animals, to sacrifice infected animals, and to initiate eradication of focal points of the infection. It is also necessary to pasteurize milk and dairy products. Veterinarians and other medical staff in the field must regularly apply personal protective wear. In the area of occurrence of the infection, it is necessary to educate the population, to apply certain procedures of disinfection, to recommend boiling or pasteurization of milk, to test animals, and to initiate their immunization (9, 10).



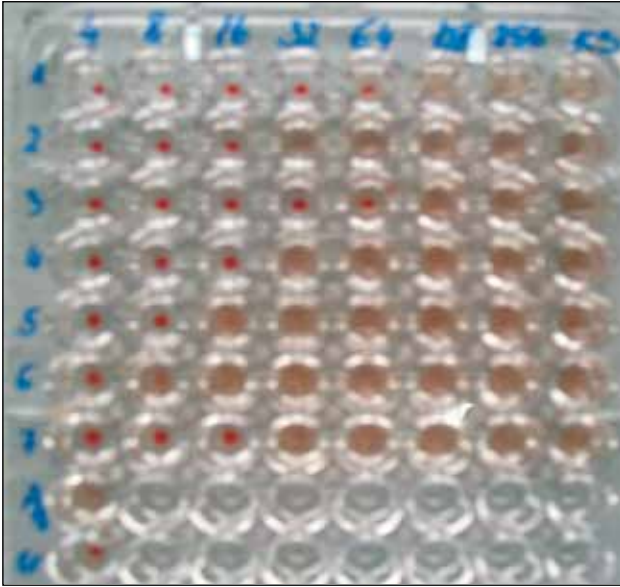
Picture 1. Euthanasia and safe removal of animals, in accordance with the legislation in force

## 2. Material and methods

A total of 53 sera of 43 patients with clinical suspicion of brucellosis were tested in the period from October 1<sup>st</sup> to 31<sup>st</sup> 2009. Blood samples were collected by venepuncture; a part of the blood was used for isolation of bacteria and the other was centrifuged, i.e. serum was separated from the blood, in order for certain serological reactions to be carried out. The sera collected were kept at +4°C or -20°C until initiation of certain tests. Clinically suspicious cases of brucellosis were confirmed by examination of sera to the presence of specific antibodies, using the qualitative Rose Bengal agglutination test (screening method) and the complement-binding reaction (CBR) (7, 9). Rose Bengal agglutination test (Pourquier) and CBR (Pourquier) were carried out in accordance with the original, i.e. standard procedures and recommendations of the test producers. The antigen in both tests was represented by the bacteria *Brucellae abortus*. CBR test result was indicated as antibody titer, i.e. increase or decrease in the titer of specific antibodies was observed in even-numbered serum samples. Rose Bengal test result was indicated as positive or negative.



Picture 2. R results of the sera examined by the Rose Bengal agglutination test



Picture 3. Demonstration of results of complement-binding reaction test

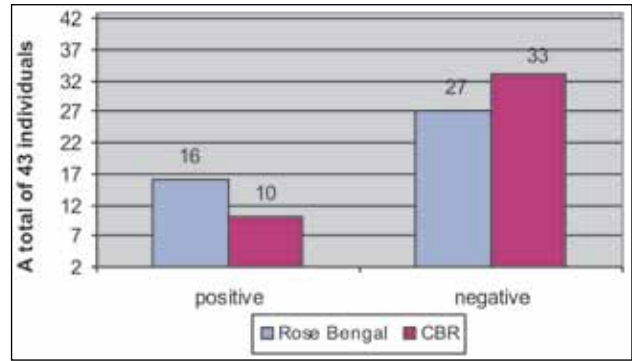


Chart 1. Results of testing the sera using Rose Bengal and CBR, presented in absolute numbers

### 3. Results

The presence of anti-Brucella antibodies was confirmed in 16 (37.2%) individuals, out of a total of 53 tested sera of 43 patients with clinically manifested suspicion of brucellosis, using the screening method, i.e. the Rose Bengal agglutination test, in the period from October 1<sup>st</sup> to 31<sup>st</sup> 2009. Significant increase in the titer of anti-Brucella antibodies in even-numbered serum samples was confirmed in 10 (23.2%) individuals, out of 43 tested patients, using the CBR method. If the significant finding of antibodies obtained by the CBR method is compared to the overall number of positive anti-Brucella antibodies in the patients examined, it is evident that the result did not match in 6 (37.5%) individuals, i.e. significant difference ( $p=0.001$ ) is evident (Chart 1).

There were 12 (75.0%) male and 4 (25.0%) positive female patients, out of 16 seropositive patients, using one of the two applied methods, in the period from October 1<sup>st</sup> to 31<sup>st</sup> 2009. There were 7 (70.0%) male and 3 (30%) female individuals, out of 10 individuals with significant finding of anti-Brucella antibodies, obtained using the CBR method (Table 1).

Concerning the age of individuals, it was determined that there had been 3 (18.75%) positive individuals in the group aged up to 30, and 13 (81.25%) individuals in the group aged older than 30, out of 16 individuals with positive finding of anti-Brucella antibodies, obtained by both methods. There were 4 (40.0%) individuals in the group aged up to 30, and 6 (60.0%) in the group older than 30, out of 10 (100.0%) patients with significant finding of anti-Brucella antibodies, obtained by the CBR method. Level of the antibody titer obtained by CBR varied from 1:8 to 1:312 and more.

### 4. Discussion

It is well-known that the occurrence of human brucellosis is related to the presence of its causal agent in certain natural hosts, from which it is

Table 1. The overall number of individuals, as well as the number and percentage of the presence of positive male and female individuals

| Method      | Examined individuals | Positive, with % of the presence | Positive males, with percentage of the presence | Positive females, with % of the presence |
|-------------|----------------------|----------------------------------|-------------------------------------------------|------------------------------------------|
| Rose Bengal | 43                   | 16 (37.2)                        | 12 (75.0%)                                      | 4 (25.0)                                 |
| CBR         | 43                   | 10 (23.2)                        | 7 (70.0%)                                       | 3 (30.0)                                 |



transmitted to humans as well, via certain routes. Since brucellosis is a zoonosis that is significantly present in the Mediterranean Basin, in the surrounding and developing countries, it is important, from epidemiological aspect, to reveal its reservoirs on time, to eradicate focal points, to confirm suspicion of the disease in laboratory, and to initiate the accurate treatment of infected individuals (11, 12). Brucellosis occurred sporadically in the area of BiH in the pre-war period (until 1992). It was registered for the first time in 1952. It occurred in individual cases in the following period. The number of infected animals, as well as humans, has progressively increased in the post-war period (since 1995). In the post-war period, the disease has been registered in certain regions of BiH; in 2009, its distribution was registered in the entire area of BiH (1, 4, 5, 13, 14). According to reports of the cantonal public-health institutes in the Federation of BiH, only four cases of brucellosis were registered during the year of 2001. The number of infected individuals was constantly increasing during the subsequent years. In the period from 2006 to September 2009, the number of infected individuals in the area of the Federation of BiH was 1741. The same trend of increase in the number of infected animals was registered in the Federation of BiH (1, 3, 5, 14). The studies in the surrounding of BiH suggest that 1543 human blood samples were serologically examined in the period 1990 – 2007 at the Croatian Veterinary Institute in Zagreb, Croatia, as well as 452212 blood samples of cattle, 91948 blood samples of goats, 218949 blood samples of sheep, and 483036 blood samples of pigs from the area of the Republic of Croatia. In the sera of examined persons, specific antibodies were confirmed in 73 individuals, in cattle in seven cases, in goats in 478 cases, in sheep in 687 cases, and in pigs in 2040 cases (12).

In the period 2000 – 2005, 245 persons infected with brucellosis were registered in Bosnia and Herzegovina. In the period from 2000 to mid-2006, 152 infected persons were registered in the area of Zenica-Doboj Canton, with the morbidity rate of 37.2‰. Brucellosis was also registered in 1.57% of examined animals in the cantons of the Federation of Bosnia and Herzegovina (1, 14). These indications suggest that animals with the infection and disease are present in all areas of

Bosnia and Herzegovina, disseminating the causal agent of the disease in their nearby environment, which is transmitted to humans via the contact route, aerosol, i.e. via animal excretions, meat, milk, and dairy products. By presenting the results of examination of serum samples of 43 individuals, we wanted to highlight the significance of this disease in wider context. We also wanted to highlight the importance of serological methods applied in laboratory confirmation of the suspicion of this disease, i.e. significance of findings of specific antibodies in serum samples, or observation of dynamics of specific antibody titer in even-numbered serum samples, as a relevant laboratory finding.

In our examination of the age and gender structure of individuals, it is evident that the infections were more frequently registered in males, which matches the finding obtained in the study during the period 2000 – 2006, from a close or the same geographic area, where there were 62.50% of infected males. Although brucellosis is present in all age groups, due to general susceptibility of the population to this infectious agent, our study confirmed that it was present in persons older than 30, which matches the finding of other researches in the mentioned period and in the same geographic area (4, 14).

## 5. Conclusions

Brucellosis occurs in Bosnia and Herzegovina in sporadic and epidemic forms; during the past several years, it has acquired endemic nature of occurrence in certain regions of BiH and become a significant public-health problem.

Occurrence of the infection in humans is narrowly related to the occurrence of infection in domestic animals, cows, goats and sheep.

Positive result was obtained in 16 or 37.2% of a total of 43 tested individuals, using the adequate serological methods, and significant finding of specific antibody titer was obtained in 10 or 23.2% of individuals, using the CBR method.

Concerning the age and gender of infected individuals, our study confirmed that this disease was present both in males and persons older than 30.

In order to prevent the occurrence of brucellosis or to put it under medical control, close coope-

ration between veterinarian and human medicine is necessary, i.e. it is responsibility of veterinary institutions to introduce comprehensive measures, which will be implemented in certain areas, in co-operation with human medical institutions.

## 6. References

1. Velić Ramiz, Bajrović Tarik. *Brucellosis: Certain epizootic features in the Federation of Bosnia and Herzegovina. Brucellosis – public-health problem in Bosnia and Herzegovina and the region. The 20<sup>th</sup> Infectious Diseases Symposium with international participation. Sarajevo, 2006. Book of Abstracts, pg 50.*
2. Zvizdić Šukrija, Sadeta Hamzić, Dževad Čengić, et al. *Human Coxiella burnetii Infections in Region of Bosnia and Herzegovina 2002. Annals New York Academy of Sciences 2006: 1078(124-128).*
3. *Report on the occurrence of infectious diseases in Bosnia and Herzegovina. Ministry of Foreign Trade and Economic Relations of Bosnia and Herzegovina. Veterinary Office of Bosnia and Herzegovina, 2002, 2003, 2004, 2005, 2006.*
4. Dautović-Krkić Sajma. *Human brucellosis in Bosnia and Herzegovina - epidemiological and clinical aspects. Brucellosis – public-health problem in Bosnia and Herzegovina and the region. The 20<sup>th</sup> Infectious Diseases Symposium with international participation. Sarajevo, 2006. Book of Abstracts, pg 16-17.*
5. Dautović-Krkić Sajma, Mehanić S, Ahmetagić S, et al. *Brucellosis in Bosnia and Herzegovina 2000-2009. The 23<sup>rd</sup> Symposium of Infectologists of BiH with international participation. Book of Abstracts, pg 37.*
6. Murray PR, et al. *Manual of Clinical Microbiology. 8<sup>th</sup> eds, Washington, D.C.: ASM 2003.*
7. Young EI, Mandal GL, Benett JE, Dolin R. *Brucella species. Principles and Practice of Infectious Diseases, 2000; 2:2386-93.*
8. Mehanić S, Pinjo F, Hadžiosmanović V, et al. *Brucella endocarditis. 23. The 23<sup>rd</sup> Symposium of Infectologists of BiH with international participation. Book of Abstracts, pg 46.*
9. *Brucellosis in Humans and Animals. WHO guidance. Geneva, WHO, 2005.*
10. Heymann DL. *Control of communicable diseases manual: an official report of the American Public Health Association. 18<sup>th</sup> ed. Washington DC, World Health Organization/American Public Health Association, 2004.*
11. *World Health Organization: Brucellosis. Fact sheet N173. www.who.int/inffs/en/fact\_173.html (online) World Health Organization. Geneva, Switzerland, 1997.*
12. Cvetnić Ž, Velić R, Špirić S, et al. *Distribution of brucellosis in the Republic of Croatia, with an insight into the condition in Bosnia and Herzegovina. Journal of Infectology 2008; 28 (3):117-123.*
13. Gaon J, et al. *Special epidemiology of acute infectious diseases, Svjetlost, Sarajevo, 1989.*
14. Aličković I. *Epidemiological and clinical variations of infection with brucellosis and Q fever. Dissertation thesis, Sarajevo, 2007.*

### Corresponding author:

Zvizdic Sukrija,  
Medical Faculty,  
Bosnia and Herzegovina,  
e-mail: sukrijazvizdic@yahoo.com



# Modern Information Communication Technologies and educational technologies applied to education of medicine

## PRIMJENA SAVREMENIH INFORMACIONO KOMUNIKACIJSKIH TEHNOLOGIJA I OBRAZOVNIH TEHNOLOGIJA U MEDICINSKOJ EDUKACIJI

*Mensura Kudumovic<sup>1</sup>, Dzafer Kudumovic<sup>2</sup>, Nevres Mesanovic<sup>2</sup>, Eldin Huremovic<sup>3</sup>*

<sup>1</sup> Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina,

<sup>2</sup> University of Tuzla, Bosnia and Herzegovina,

<sup>3</sup> Tehnical school of Brcko District, Bosnia and Hercegovina

### Abstract

The introduction of ICT in medical education as well as additional qualification and training in medicine, contributing directly to improvement in the efficiency of this segment of the educational system, especially through reducing the cost, especially of visiting professors travel costs (video-conferencing).

This article describes the application of modern information and communication technologies and the introduction of combined methods of teaching at the Medical Faculty in Sarajevo.

This research aims to identify the information infrastructure and the possibility of introduction and application of modern ICT and education combined approach of medical education at the Medical Faculty in Sarajevo.

For the purposes of the application of *blended* methods of education is planned to develop electronic educational content, based on multimedia. This content can be developed and delivered through the platform for the development and content delivery, and monitoring activities of students, e e-Learning.

unsa, or e-learning e-net. For the purpose of electronic communication with students used will be forums, chat and e-mail on the same system.

Previous activities in this field are quite modest and done partially within the eContent program of WUS Austria program.

Focus of this research and the methods used in research at the Medical Faculty of the University in Sarajevo, as the central institution for medical education. Identification and testing of educational content is determined in advance, which require this type of research.

Applied is the methodology of system approach and system analysis and the analysis as a method for identifying opportunities and threats from the environment, or to analyze strengths and weaknesses.

In cooperation with Indiana University - USA and professor Dey Sukhen MD. PhD prepared is the frame (application) for online learning in the Moodle (LMS), aimed primarily to post graduate students and other target groups of students and health professionals

**Key words:** education, combined education, medicine, information technology

## Sažetak

Uvođenje ICT u medicinsku edukaciju i dodatnu kvalifikaciju i trening u medicini, doprinosi direktno poboljšanju efikasnosti ovog segmenta obrazovnog sistema kroz smanjenje troškova naročito gostujućih profesora troškova putovanja (videokonferencija).

U radu je prikazana primjena modernih informacijsko-komunikacijskih tehnologija i uvođenje kombinovane metode učenja na Medicinskom fakultetu u Sarajevu.

Ovo istraživanje ima za cilj identifikaciju informacione infrastrukture i mogućnosti uvođenja i primjena modernih ICT i kombinovanog obrazovnog pristupa medicinske edukacije na Medicinskom fakultetu u Sarajevu.

Za potrebe primjene *blended* metode obrazovanja na planirano je razviti elektronske obrazovne sadržaje, bazirane na multimediji. Navedeni sadržaji se mogu razviti i isporučivati na platformi za razvoj i isporuku sadržaja, te praćenje aktivnosti studenata, e-Learning.unsa, ili e-learning e-net .

U cilju elektronskog komuniciranja sa studentima koristiće se mogućnosti foruma, chat-a i e-maila na istom sistemu.

Dosadašnje aktivnosti na ovom polju su dosta skromne i odvijane su samu djelimično u okviru eContent WUS Austria programa.

Fokus ovih istraživanja kao i metode korištene u istraživanju je na Medicinskom fakultetu Univerziteta u Sarajevu, kao centralnoj instituciji medicinskog obrazovanja.

Identifikacija ispitivanih i obrazovnih sadržaja unaprijed je određena, što i zahtjeva ovaj tip istraživanja.

Primijenjena je metodologija sistemskog pristupa i sistemske analize i analiza kao metoda za identifikaciju šansi i prijetnji iz okruženja, odnosno za analizu snaga i slabosti.

U suradnji sa Indiana univerzitetom – USA i prof. Dr Sukhen Dey –om pripremljen je i okvir (aplikacija) za online learning u Moodle-u (LMS), naimjenjen prije svega, postdiplomcima i drugim ciljnim grupama studenata i zdravstvenih profesionalaca.

**Ključne riječi:** obrazovanje, kombinovano obrazovanje, medicina, informaciona, tehnologije

## Introduction

The introduction of ICT in medical education and additional qualifications and training in medicine, contributing directly to improving the efficiency of this segment of the educational system, especially through reducing the cost especially of visiting professors travel costs (videoconferencing).

Educational institutions that respond to these opportunities will stand out from other in the coming years by improving the quality of their educational program, which are enabled by the innovative integration of various information and communication technologies.

Time of development of global information and telecommunications technologies, with simultaneous development of web based educational material imposes a change in the philosophy of educational content delivery.

Not long ago, the teachers use only chalk and the board as a tool for delivering educational content. Implementation of projector and content in the form of prepared slides introduced novelty in this kind of education process. Today, more and more teachers use a computer and projector for the presentation of educational materials.

The first modern alternative to the delivery of educational material was computer-based system of learning (CBT - *Computer Based Training*) which used author systems, multimedia and CD. Web brings radical change and rapid transition from development technology like HTML (*Hyper-text Markup Language*) and scripting languages (*Java, ActiveX, XML*) on server systems that use databases (1). Due to the rapid development of electronic remote-supported education in the USA and Europe there was a need to develop web based tools for education in order to generate interactive educational content on the web.

At the same time, increased is the frequency of personal communication based on the use of computer technology, such as email, chat, forums and videoconferencing (2) The use of real time video content (streaming video) gets more and more importance in the delivery of courses on demand (3).

Previous activities in this field are quite modest and done partially within the eContent program of WUS Austria program.

The article describes the application of modern information and communication technologies and the introduction of combined methods of teaching at the Medical Faculty in Sarajevo.

The modern form of education offers a user-appropriate and adaptable education system. Unlike traditional education, here the student and teacher are generally physically separated. Educational materials are distributed through various media, in the classical and/or electronic form.

### Goals and research methods

This research aims to identify the information infrastructure and the possibility of introducing and application of modern ICT with combined education approach to medical education at the Medical Faculty in Sarajevo.

For the purposes of the application of *blended* methods of education is planned to develop electronic educational content, based on multimedia. The above mentioned content can be developed and delivered through platform for the development and content delivery, and monitoring activities of students, e e-Learning.unsa, or e-learning e-net.

For purpose of electronic communication with students should be used forums, chat and e-mail on the same system.

Focus of this research and the methods used in research are at the Medical Faculty in Sarajevo, as the central institution of medical education.

Identification of tested educational content is determined in advance, which require this type of research.

Applied is the methodology of system access, system analysis and analysis as a method for identifying opportunities and threats from the environment, or to analyze strengths and weaknesses.

### Previous activities

Previous activities in this field are quite modest and done partially within the eContent program of WUS Austria program and University Tele-information Centre.

In October 2003, University of Sarajevo began with e-learning education, the project conducted

by the University Tele-information Centre (UTIC) and four faculties from University of Sarajevo were involved: Electro-technical, Machine-engineering, Business and economy, and Medical faculty. On UTIC web site, seven students enrolled from Medical faculty.

Platform for the course of e-learning is achieved in collaboration with UTIC. University Tele-information Centre, established as part of University of Sarajevo and first ISP in Bosnia and Herzegovina in 1996 (www.utic.net.ba). It is scientific-organizational unit of the University of Sarajevo for improvement of scientific research work and through UTIC members of the University

can be gathered in the unique computer-communication structure. ). Objectives of UTIC are: to connect members of the University with similar institution in the country and abroad

due to more efficient use of scientific, research and educational resources, use of educational data bases and other information for the needs of the University and its members. Also, development

an integration of informatics computer technologies in education, creation of flexible infrastructure which will enable e-Learning to be accessible to all students and University

staff, improvement of general digital literacy of academic population, development of top quality educational content which could be integrated in the actual European processes of e-Learning revolution. With their help center for e-learning, "LUCUS CENTRUM", is created (Picture 1.). We hope that this is just a beginning step towards improvements of the

### Application of ICT in medical education

Project "Application of modern ICT and educational technology in medical education" which is endorsed by the Ministry of Education and Science of Sarajevo Canton provided the research activities for a period of 2 years on its implementation, so that during the 2008- 2009 the following activities are performed:

- Made is the identification and assessment of ICT infrastructure;
- Made are the necessary preparations of framework for educational content;

- Identification of ICT infrastructure is made at the macro and micro level and was evaluated as satisfactory for this project needs.

Also conducted is the selection of web platform for presentation of educational content, which should satisfy all the criteria and standards of e-learning, as well as the planned project activities.

Framework for educational content is the basis for presentation and publication of the educational content by selected topics/subject.

In cooperation with Indiana University - USA and professor Dey Sukhen MD. PhD prepared is the frame (application) for online learning in the Moodle (LMS), aimed primarily to post graduate students and other target groups of students and health professionals.

The appearance of web applications is given in the following figure:



Figure 1. Web applications for online medical courses

Participants in the project besides the teachers and associates are the students of postgraduate courses at the Faculty of Medicine in Sarajevo, a total of 12 of them, who directly participate in education, and development of the course "Information Technology in Healthcare."

Partner institutions in this project are the Maharaja Institute of Technology and Certification Institute PKCOE.

The Web application has the interface, or enables the communication from the student and the professor's environment.



Figure 2. Login to online medical courses

After login, student or teacher enters the desired course area.



Figure 3. The appearance of the home page from one medical course

Each course is developed according to the needs and demands of students.

### Information Technology in Healthcare

Healthcare is "Mission Critical". Caregivers, managers, physicians and any healthcare personnel are responsible for day-in-day-out welfare of patients. The ultimate goals of healthcare professionals including nurses are to preserve patient's welfare and "save lives". Every aspect of healthcare from patient admission, treatment, billing to post discharge care depend on Information Technology and that qualifies informatics as one of the most important knowledge base in making 'critical thinking' oriented decisions. This five week course discusses the various aspects of IT including foundation, application, infrastructure and future trends of Information Technology within healthcare settings.

## Conclusions

The rise of IT as an artefact of everyday life in the modern world has brought with it the dawn of a new era, often dubbed the “Age of Information”. These technologies are changing the way we perceive the world, how we think and communicate with another but especially in education human influence is irreplaceable

## References

1. Huremovic E, Mesanovic N *INTERNET AND TECHNICS: YESTERDAY – TOODAY – TOMORROW, TTEM* 2006, 1(2) 5-7
2. Masic I, Kudumovic M, Pasic E. *Education of Medical Informatics at Medical Faculty of Sarajevo. Med Arh*, 2004; 58(1, supl.2): 88-91.
3. <http://www.strategicwisdom.org/Courses>, 2010,
4. Kudumovic, M., Kudumovic, Dz., Kudumovic, L., Huremovic, E., Mesanovic, N. *Information communication technologies (ICT) and use of the internet related information at university of bosnia and herzegovina, TTEM*, 2009; 4(1): 33-37.
5. T. Mateljan, M. Kudumović, M. Tatlić *ICT Research Priorities for B&H2007-2013, TTEM*, 2007; 2(2): 50-58
6. Sukic C , Kudumovic M. *Informatičke tehnologije, Sejtarija*, 2009.

Corresponding author:

Mensura Kudumovic,  
Faculty of Medicine,  
University of Sarajevo,  
Bosnia and Herzegovina,  
e-mail: mensurak@yahoo.com



# Alopecia areata: New treatment modalities

*Emina Kasumagic-Halilovic<sup>1</sup>, Asja Prohic<sup>1</sup>, Semra Cavaljuga<sup>2</sup>*

<sup>1</sup> Department of Dermatovenerology, Sarajevo University Clinical Center Sarajevo, Bosnia and Herzegovina

<sup>2</sup> Institute of Epidemiology and Biostatistics, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina

## Abstract

Alopecia areata (AA) is a relatively common reversible hair loss disorder usually manifesting as patchy areas of complete hair loss on the scalp and other body parts that can progress to complete loss of all body hair. This disorder affects all age groups, with a higher prevalence in children and adolescents. The etiology of AA is unknown but is characterized by hair cycle dysfunction and the presence of peribulbar and perifollicular mononuclear cell infiltrates. Much evidence suggests that AA is tissue restricted autoimmune disease. Current traditional therapies are predominantly immunomodulating modalities, including corticosteroids, topical immunotherapy, anthralin, and photochemotherapy (PUVA). A nonspecific modality is topical minoxidil, which prolongs anagen and promotes growth of longer and wider hair. Improved future treatments may be immunosuppressive or immunomodulatory or they may otherwise protect hair follicles from the injurious effects of inflammation.

**Key words:** alopecia areata, immunosuppressive therapies

them than among the general population (1). The etiology and pathogenesis of AA is still uncertain, but many factors have been assumed concerning its pathogenesis, e. g. the patients genetic constitution, family history, the atopic state, non specific immune and organ-specific autoimmune reactions, possible emotional stress, infectious agents, and neurological factors (2, 3, 4). However, it is hypothesized that AA is an organ-specific autoimmune disease mediated by T lymphocytes directed to hair follicles. AA may be passively transferred by T cells and there is some evidence that serum IgG may also disturb hair cycling (5). Immunohistochemical studies have shown peri- and intra-follicular inflammatory infiltrate which damages hair follicles (6). Hair follicles are not destroyed in patients with AA, and the potential for regrowth always remains (1). The course of disease is not predictable and it is often associated with periods of hair loss and regrowth. The prognosis of AA is influenced by several factors, in particular by the type and extent of AA (7) with a worse prognosis for patients with AA totalis or universalis or with extensive patchy AA as compared to limited patchy AA.

## Introduction

Alopecia areata (AA) is a relatively common reversible hair loss disorder usually manifesting as patchy areas of complete hair loss on the scalp and other body parts that can progress to complete loss of all body hair (alopecia totalis, alopecia universalis). This disorder affects all age groups, with a higher prevalence in children and adolescents. The patients are usually otherwise healthy, but atopy, thyroid disease and vitiligo are more common

## Current treatments for alopecia areata

Because of their psychosocial stigmatization, the medical attendance and therapy of patients who suffer from distinct form of AA is difficult to challenge. Although spontaneous remission is possible, it occurs rarely. At present, all treatments are palliative, only controlling the problem, they certainly do not cure the condition.

Treatment of AA may be divided into four different categories of widely accepted therapeutic

modalities: immune inhibitors (steroids or psoralen and UVA light- PUVA therapy); topical sensitizers (squaric acid dibutylester and diphenylcyclopropanone); non specific irritants (anthralin) and the vasodilator minoxidil. These treatments stimulate hair growth but do not prevent hair loss and probably do not influence the course of the disease (1). Mild forms of AA are mostly treated by intralesional injection of a glucocorticoid, usually triamcinolone, every four to six weeks. Topical steroid application to areas of hair loss, usually applied twice daily, has also been found to be efficacious clinically, although combination treatment with minoxidil, anthralin or injected steroids is probably more therapeutic (1, 9). Systemic steroids are reserved for use in rapidly progressive or extensive AA. Topical sensitizers have proven efficacy in patients with long-standing AA involving more than 50 percent of the scalp (8). They have shown good tolerability and mild side effects. Photochemotherapy with psoralen and ultraviolet A light (PUVA) has also resulted in hair regrowth in some patients.

Current traditional therapies are predominantly immunomodulating modalities, these treatments have been used for many years, and new targeted therapies are particularly needed for children, for those with chronic, persistent patchy disease and for those with alopecia totalis and alopecia universalis.

### **New immunomodulatory therapies**

Topical immunomodulators are a new class of agent that acts locally on T cells by suppressing cytokine transcription (10). They are now emerging as the therapy of choice for several immune-mediated dermatoses, because of their comparable efficacy, ease of application and greater safety than their systemic counterparts (11). The two most studied topical immunomodulators are tacrolimus and pimecrolimus. A third new member of this group is topical cyclosporine A (CsA). All three drugs inhibit calcineurin, thereby inhibiting interleukin-2 production (12).

### **Topical tacrolimus**

It is macrolide, produced by *Streptomyces tsukubaensis*, a fungus found in the soil of Mount Tsukuba, the science city of Japan, where initial isolation and characterization of this drug was performed. The name of the drug is a neologism, composed of tsukuba, macrolide and immunosuppression.

Tacrolimus is an immunosuppressive agent that can be applied topically to the skin. It acts directly on T cells to inhibit interleukin-2 transcription, which results in decreased growth and proliferation of T lymphocytes in response to foreign antigens (13). It also inhibits other cytokines, including TNF- $\alpha$  and IFN- $\gamma$ , both important in T cell activation. Moreover, topical application of tacrolimus also has a hair growth stimulatory effect, independent of its T cell suppressive effect (14). Tacrolimus ointment does not cause skin atrophy, pigment changes, or teleangiectasia. It is only minimally absorbed, with 0,5 % of the locally applied drug detected in blood (11). Therefore, tacrolimus is promising candidate for the treatment of alopecia areata.

### **Topical pimecrolimus**

Pimecrolimus is a semi-synthetic product of ascomycin, which is a fermentation product of *Streptomyces hygroscopicus* var. *ascomycetes*. Similar to tacrolimus, it is a cell-selective cytokine inhibitor developed for the treatment of inflammatory skin diseases. It binds to macrophilin-12, inhibits calcineurin, inhibits synthesis of inflammatory cytokines, such as IL-2 and IFN- $\gamma$ , and inhibits T cell and mast cell activation (12). Pimecrolimus has high skin-specific-inflammatory activity with low potential for affecting the systemic immune response. The cream 1% formulation is safe and effective and does not cause skin atrophy or teleangiectasia. Adverse effect includes mild burning sensation. Unfortunately, the cream is not expected to be effective for hair regrowth because it permeates no lower than the superficial dermis, which is an insufficient depth for targeting T cells involved in AA (12).

## Topical cyclosporine A

Cyclosporine A (CsA), isolated from the fungus *Tolypocladium inflatum gams*, is a lipophilic cyclic polypeptide and calcineurin inhibitor. CsA is a potent immunomodulatory agent whose mechanism involves inhibition of T-4 lymphocyte activation (15). Although systemic CsA appears to be effective in AA, the adverse effect profile, recurrence rate after treatment discontinuation and inability to produce long-term remission make CsA unattractive for the treatment of AA.

In the past, topical formulations of CsA were ineffective because of poor skin penetration. To surmount this problem, a heptamer of arginine was conjugated to CsA through a pH-sensitive linker designed to release CsA at physiologic pH within the skin (16). The oligoarginine transporters enable full-skin-thickness penetration of CsA into cells throughout the epidermis and dermis of human skin, with functional inhibition of cutaneous inflammation (17).

## New biologic therapies

Biologic agents are proteins that possess pharmacologic activity and can be extracted from tissue. With the development of recombinant DNA technology, biologic agents can be synthesized in large quantities and designed to alter specific physiologic responses (18). Biologic therapies target cell surface receptors, and their theoretical advantage is that their greater specificity will provide better safety profiles (12). Biologics are larger than 'small-molecule' drugs and are most often administered by injection. They include etanercept, infliximab, efalizumab and alefacept.

## Liposomes

Another novel approach in treating AA is to create a vehicle that allows penetration to the subcutaneous fat where the bulbs of anagen hair follicles are located and where the pathomechanism takes place (19). Liposomal drug delivery may increase penetration of skin and allow slow release of active compound locally with diminished

toxicity. At present, liposomes seem to be the best candidate as a vehicle topical treatment. Topically applied liposomes have been shown to deliver melanin, proteins, genes and various small molecules selectively to hair follicles and hair shafts of mice in vivo (20). Liposome-targeting of molecules to human hair follicles has been demonstrated in human scalp in histoculture (21). However, future experiments have to show whether liposomes are able to deliver molecules to the hair bulb in human scalp in vivo.

## Miscellaneous agents

### Inhibition of the Fas-FasL system

Induction of hair follicle apoptosis by the Fas-FasL system seems to be involved in the pathogenesis of AA (6). Therefore, inhibition of the Fas-FasL system might protect hair follicles from injury caused by the inflammatory infiltrate. However, such treatment could only be applied topically and specifically limited to hair follicles, because systemic inhibition would disturb essential control mechanisms of lymphocyte homeostasis (19).

### Imiquimod

Imiquimod is the first member of a new class of immune response modifiers, it was first improved 1997 for the topical treatment of genital warts.

It is a synthetic molecule, which enhances both innate and acquired immune response, in particular, cell mediated pathways, by stimulating monocytes and macrophages via binding to cell surface receptors to produce several specific cytokines including IFN- $\alpha$ , IL-1, 6, 8, 10, 12 and tumor necrosis factor (11, 22), resulting in local immunoregulatory activity. Imiquimod also stimulates natural killer and B cells and enhances migration of Langerhans cells. In the future, imiquimod and newer generation of imidazoquinolines (resiquimod) require further investigation for potential clinical utility in treating AA.

## Conclusion

AA has an unpredictable course that is not easily altered by treatment. Different treatments will provoke regrowth of terminal hair to a variable extent but may not prevent further hair loss. At present, corticosteroids are the most popular form of treatment and can be given topically, intralesionally, or, in rare cases systemically. Minoxidil has had limited success in stimulating hair regrowth without altering the course of AA. Topical immunotherapy with diphenylcyclopropenone or PUVA therapy may be effective in longstanding and widespread disease. These treatments stimulate hair growth but do not prevent hair loss and probably do not influence the course of the disease (1).

As long as no causal treatment is available, future approaches should focus on a more specific targeting of the underlying pathomechanism with a topical action around the hair bulbs and without serious side-effects (19). New immunomodulators and biologic therapies target specific immunologic responses and offer new strategies for treating pathogenic T cells and the cytokines they produce (12). In the future, AA should receive first-line consideration for clinical studies with new specific therapies for T-cell-mediated inflammatory diseases.

## References

1. Price VH. Treatment of hair loss. *N Engl J Med* 1999; 341: 964-973
2. Madani S, Shapiro J. Alopecia areata update. *J Am Acad Dermatol* 2000; 42: 549-566.
3. McDonagh AJ, Tazi-Ahnini R. Epidemiology and genetics of alopecia areata. *Clin Exp Dermatol* 2002; 27: 405-409.
4. Gilhar A, Ullmann Y, Berkutzki T, Assay B, Kalish RS. Autoimmune hair loss (alopecia areata) transferred by T lymphocytes to human scalp explants on SCID mice. *J Clin Invest* 1998; 101: 62-67.
5. Tobin DJ, Gardner SH, Lindsey NJ, Hoffmann R, Happle R, Freyschmidt-Paul P. Diphenylcyclopropenone immunotherapy alters anti-hair follicles antibody status in patients with alopecia areata. *Eur J Dermatol* 2002; 12: 327-334.
6. Bodemer C, Peuchmaur M, Fraitaig S et al. Role of cytotoxic T cells in chronic alopecia areata. *J Invest Dermatol* 2000; 114: 112-116.
7. Wiseman MC, Shapiro J, MacDonald N, Lui H. Predictive model for immunotherapy of alopecia areata with diphenylcyclopropenone. *Arch Dermatol* 2001; 137: 1063-1068.
8. Shapiro J. Alopecia areata. Update on therapy. *Dermatol Clin* 1993; 11: 35-46.
9. Fiedler VC. Alopecia areata: a review of therapy, efficacy, safety, and mechanism. *Arch Dermatol* 1992; 128: 1519-1529.
10. Schneider LC. New treatment for atopic dermatitis. *Immunol Allergy Clinics North America* 2002; 22: 141-152.
11. Khandpur S, Sharma VK, Sumanth K. Topical immunomodulators in dermatology. *J Postgrad Med* 2004; 50: 131-139.
12. Price VH. Therapy of alopecia areata: on the cusp and in the future. *J Invest Dermatol Symp Proc* 2003; 8: 207-211.
13. Lawrence ID. Tacrolimus (FK 506): experience in dermatology. *Dermatologic Ther* 1998; 5: 74-84.
14. Yamamoto S, Jiang H, Kato R. Stimulation of hair growth by topical application of FK506, a potent immunosuppressive agent. *J Invest Dermatol* 1994; 102: 160-164.

15. Fernando J, Grimalt R. Partial response of several alopecia areata to cyclosporin A. *Dermatology* 1999; 199: 67-69.
16. Rothbard JB, Garlington S, Lin Q, et al. Conjugation of arginin oligomers to cyclosporine A facilitates topical delivery and inhibition of inflammation. *Nat Med* 2000; 6: 1253-1257.
17. Lin Q, Rothbard JB, Garlington S, McGrane P, Khavari PA. Addition of a poly arginine linker to cyclosporine A facilitates transcutaneous delivery and topical inhibition of cutaneous inflammation. *J Invest Dermatol* 2000; 114: 777.
18. Singri P, West DP, Gordon KB. Biologic therapy for psoriasis: the new therapeutic Frontier. *Arch Dermatol* 2002; 138: 657-663.
19. Freyschmidt-Paul P, Happle R, McElwee KJ, Hoffmann R. Alopecia areata: Treatment of today and tomorrow. *J Investig Dermatol Symp Proc* 2003; 8: 12-17.
20. Li L, Hoffman RM. Topical liposome delivery of molecules to hair follicles in mice. *J Dermatol Sci* 1997; 14: 101-108.
21. Li L, Hoffman RM. The feasibility of targeted selective gene therapy of the hair Follicle. *Nat Med* 1995; 1: 705-706.
22. Skinner RB. Imiquimod. *Dermatol Clin* 2003; 21: 291-300.

Corresponding author:

Emina Kasumagic-Halilovic  
Department of Dermatovenerology,  
University Clinical Center Sarajevo  
Bosnia and Herzegovina  
e-mail: kasumagicemina@yahoo.com



# Sustained attention in Bosnian war veterans diagnosed with PTSD

## ODGOĐENA PAŽNJA KOD BORACA ARMIJE BIH SA DIAGNOZOM PTSP–A

Aida Sarac – Hadzihalilovic<sup>1</sup>, Amela Kulenovic<sup>1</sup>, Abdulah Kucukalic<sup>2</sup>

<sup>1</sup> Institute of Anatomy, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina

<sup>2</sup> Psychiatric Clinics, University of Sarajevo Clinics Center, Bosnia and Herzegovina

### Abstract

The aim of our study was to examine sustained attention in Bosnian War Veterans diagnosed with PTSD.

The study was based on the testing of 79 Bosnian Army war veterans, who participated in the B&H war from 1992 to 1995. Of 79 subjects, 45 developed PTSD, while 34 remained PTSD free. The latter group constitutes the control. The subjects' age ranged from 30 to 50. Also, the groups were harmonized with respect to the education level (elementary and secondary education). The subjects were tested with Sustained Attention to Response Task – SART. The task includes frequent button pushing for untargeted stimuli or withholding from pushing as a response to the targeted stimulus. The duration of this computer based test is 4,3 minutes. During that time, 225 figures (1-9) randomly appear in the center of the screen. The results include three measures: the number of false positive responses, the number of false negative responses and average response time for all responses.

In the Sustained Attention to Response Task (SART) test, subjects with PTSD made more errors of both types (both false positive and negative responses). Statistically significant difference between PTSD and the control group was not found only in the case of average response time to task.

PTSD patients made more errors that were related to the difficulties with the maintenance of vigilance over time. PTSD patients reacted faster

which may be related to the anxiety symptoms that accompany PTSD. Excessive anxiety may be responsible for faster reaction as a response to these tasks, but for an increased error rate as well.

**Key words:** posttraumatic stress disorder, trauma, war veterans, sustained attention, SART

### Sažetak

**Cilj:** ovoga istraživanja je ispitati odgođenu pažnju kod boraca Armije BIH sa dijagnozom PTSP–a.

**Materijal:** za konstrukciju ove studije testirali smo 79 boraca Armije BIH, koji su učestvovali u predhodnom ratu u BIH od 1992–1995. Od 79 testiranih boraca Armije BIH 45 je bilo sa PTSP–om, a 34 bez PTSP–a (kontrolna grupa). Svi ispitanici su starosne dobi od 30–50 godina i istog obrazovnog nivoa (osnovna i srednja školska sprema). Svi participanti su pristupili testiranju sa testom održavane pažnje kako bi se odgovorilo na zadatak - sustained attentions to response task–SART. Zadatak uključuje frekventno pritiskanje dugmeta na svaki naciljani stimulus i zadržavanje, tj. nepritiskanje dugmeta kada se pojavi ciljani stimulus. Ovaj kompjuterizirani test traje 4,3 minute u toku kojih se 225 jednocifrenih brojeva (1-9) slučajnim redoslijedom pojavljuje u centru kompjuterskog ekrana. Kao rezultat dobivamo tri mjere: broj lažno pozitivnih odgovora, broj lažno negativnih odgovora i prosječno vrijeme reakcije svih odgovora.

**Rezultati:** Na testu održavane pažnje pri odgovoru na zadatak (SART), ispitanici sa PTSP–om pravili su više pogreški i jednog i drugog tipa (pozitivne i negativne greške). Statistički značajna razlika između PTSP grupe i kontrolne grupe jedino ne postoji kod mjerenja vremena reakcije na SART testu.

**Zaključci:** Pacijenti sa PTSP–om prave više pogreški, što je povezano sa poteškoćama održavanja vigijlnosti tokom vremena. Pacijenti sa PTSP–om imaju kraće vrijeme reakcije, što se može dovesti u vezu sa simptomima pobuđenosti kod osoba koje boluju od PTSP–a. Pretjerana pobuđenost može dovesti do toga da ove osobe reaguju brže u ovakvim zadacima, ali i prave više pogreški.

**Ključne riječi:** posttraumatski stresni sindrom, trauma, ratni veterani, odgođena pažnja, SART

## Introduction

The last two decades have witnessed worldwide growth of interest in the study of stress as a result of stressful events of the past century. The abundance of stress dedicated publications has increased, while stress became ever-present subject in everyday life, press and television. Thus the general opinion that we share particularly stressful era and that the consequences of stress for mental and somatic plane may be numerous and difficult to anticipate. War in a certain area fundamentally affects the course of life for large part of population and causes series of stressful events. We can establish with certainty that no person escapes the stress caused by war induced pressure, which requires new models of adjustment. The past war in our country provided us with an unfortunate opportunity to acquire better understanding of stress-related events and stress-induced psychotic disorders. Posttraumatic stress disorder (PTSD) certainly belongs into this group of disorders.

In consideration of the above, our study was constructed around patients – soldiers who were exposed to the atrocities of war and who sustained changes in the behaviour modalities along with psychotic disorders. Therefore, this study should represent a contribution to the research into association between PTSD and cognitive dysfunction.

The aim of our study was to examine damaged attention processes in patients with PTSD, which may underlie memory disorder as a cognitive dysfunction in these patients.

## Material and Methods

We have tested 79 B&H Army soldiers, who participated in the past B&H war between 1992 and 1995. Of the 79, 45 developed posttraumatic stress disorder (PTSD), while 34 remained PTSD-free and those constitute the control group. From the range of neuropsychological tests, we selected Sustained Attention to Response Task (SART) (1). It is a task of continuous performance. The attention sustenance deficit was assessed by SART (1). The task includes frequent button pushing for untargeted stimuli or withholding from pushing as a response to the targeted stimulus (1). The duration of this computer based test is 4,3 minutes. During that time, 225 figures (number from 1 to 9) randomly appear in the center of the screen (each figure appears 25 times). The appearing figures differ in size (12 to 29 mm in height, font sizes: 48, 72, 91, 100 and 120). Each figure remains on screen for 250 ms. It is followed by a 900 ms mask in the form of an “X” positioned in the center of the screen. The subjects’ task consists of pushing the response-box button each time a figure appears except in 25 cases when the screen displays figure 3 and the pushing response needs to be withheld.

The results include three measures: the number of false positive responses (pushing response to figure 3 – maximum number of errors is 25), the number of false negative responses (pushing response withheld to figures other than 3 – maximum number of errors is 200) and average response time for all responses. Prior to the formal test, the subjects had an opportunity to try and practice the response (with 16 untargeted figures and 2 figures 3). There is an association between the SART results and distraction and absentmindedness experienced on daily bases (2).

For the selection of PTSD patients, we relied on DSM IV classification system. PTSD evaluation was performed based on Mississippi scale (3). All the patients included into the study scored above 110 on the Mississippi scale. Thus, one of

the inclusion criteria was the duration of symptoms. For our group, we considered only chronic PTSD. Also, the subjects with head injury or surgical intervention during traumatic experience were excluded from the study. History of neurological disorders, psychotic disorders, and drug or alcohol addiction were also criteria for exclusion. The age of subjects ranged between 30 and 50 years. The groups were harmonized according to the age, education level and gender. Hence, it was a homogenous group of male subjects.

Testing Protocol and Informed Consent Forms were prepared specifically for this study. The study results were of confidential nature. Subjects' identity can never be revealed. The applied method is neither aggressive nor detrimental to the subjects. Ethical component of this study was rather high.

## Results

The difference between the PTSD group and the control is statistically highly significant. Value of  $t$ -test is:  $t = 5,942$ ; with significance threshold at  $p < 0,001$ .

The difference between the PTSD group and the control has high statistical significance. Value of  $t$ -test is:  $t = 4,485$ ; with significance threshold at  $p < 0,001$ .

The difference in values between PTSD group and the control is not statistically significant. Value of  $t$ -test is  $t = 0,429$ .

Table 1. Sustained attention to response task – SART /false negatives

| FN – FALSE NEGATIVE     | PTSD GROUP        |       | CONTROL GROUP     |       |
|-------------------------|-------------------|-------|-------------------|-------|
|                         | Nº                | %     | Nº                | %     |
| 0                       | -                 | -     | 2                 | 5,88  |
| 1 - 9                   | 5                 | 11,11 | 13                | 38,24 |
| 10 - 19                 | 3                 | 6,67  | 7                 | 20,59 |
| 20 - 39                 | 10                | 22,22 | 9                 | 26,47 |
| 40 - 59                 | 12                | 26,47 | 2                 | 5,88  |
| 60 and higher           | 13                | 28,89 | 1                 | 2,94  |
| Unable                  | 2                 | 4,54  | -                 | -     |
| TOTAL                   | 45                |       | 34                | 100   |
| Mean - $\bar{X}$        | $\bar{X} = 50,72$ |       | $\bar{X} = 16,21$ |       |
| Standard deviation - SD | SD = 31,90        |       | SD = 18,69        |       |

Table 2. Sustained attention to response task – SART /false positives

| FP – FALSE POSITIVE     | PTSD GROUP        |       | CONTROL GROUP   |       |
|-------------------------|-------------------|-------|-----------------|-------|
|                         | Nº                | %     | Nº              | %     |
| 0                       | -                 | -     | 1               | 2,94  |
| < 10                    | 9                 | 20,00 | 19              | 55,88 |
| 10 - 14                 | 18                | 40,00 | 11              | 32,35 |
| 15 - 19                 | 12                | 26,67 | 3               | 8,82  |
| 20 - 29                 | 4                 | 8,89  | -               | -     |
| Unable                  | 2                 | 4,44  | -               | -     |
| TOTAL                   | 45                | 100   | 34              | 100   |
| Mean - $\bar{X}$        | $\bar{X} = 13,40$ |       | $\bar{X} = 8,5$ |       |
| Standard deviation - SD | SD = 3,8409       |       | SD = 5,37       |       |

Table 3. Sustained attention to response task – SART / response time

| RESPONSE TIME           | PTSD GROUP              |       | CONTROL GROUP           |       |
|-------------------------|-------------------------|-------|-------------------------|-------|
|                         | N°                      | %     | N°                      | %     |
| < 300 msec              | 1                       | 2,22  | -                       | -     |
| 300 - 399 msec          | 11                      | 24,44 | 13                      | 38,23 |
| 400 - 499 msec          | 14                      | 31,11 | 6                       | 17,65 |
| 500 - 599 msec          | 12                      | 26,67 | 8                       | 23,53 |
| 600 msec and higher     | 5                       | 11,11 | 7                       | 20,59 |
| Unable                  | 2                       | 4,44  | -                       | -     |
| TOTAL                   | 45                      | 100   | 34                      | 100   |
| Mean - $\bar{X}$        | $\bar{X} = 451,07$ msec |       | $\bar{X} = 464,51$ msec |       |
| Standard deviation - SD | SD = 143,37             |       | SD = 131,15             |       |

## Discussion

Our study was aimed at examination of cognitive function in individuals with diagnosed PTSD. The results of earlier studies and theoretical background clearly indicate the existence of deficit in cognitive functions such as memory, attention, initiation and inhibition.

Sonia et al. (4) report activation within or in the proximity of hippocampal region during the encoding phase of memorizing process. Memory encoding is the process that may be divided into two different sub-processes, which may also be related to the hippocampal activation. One of the first roles (functions) attributed to hippocampus is the encoding process. Actually, PET and fMRI studies demonstrate that hippocampus and neighboring (bordering) cortex participate in the encoding of facial information but not in their retrieval. Hippocampal region also encodes (records) meaningful response (5) with respect to the meaningless one. Other studies report activation of hippocampal region during recording – encoding of visual objects (6,7), visual images and in depth word processing (8). Certain fMRI memory studies suggest hippocampal region role during novel encoding (recording). Tulving et al. (9) demonstrated intense hippocampal activity during recognition task where subjects had to decide whether the displayed object was “new” (never displayed before) or “old” (displayed earlier). This potential involvement of hippocampus in the detection of “new” is a powerful inducer of stress reaction in people

as well as animals (10). Golier et al. (11) explain the influence of trauma-dependent information on the memory in holocaust survivors.

PTSD group has significantly lower memory association compared to the PTSD-free group and unexposed group. This trauma-related connection with the explicit memory, joint with generally weaker explicit memory, may be explained by bi-directional nature of memory failure in PTSD.

Vermetten et al. (12) emphasize that animal studies demonstrate that stress associated with hippocampus injury inhibits neurogenesis and creates deficit and dysfunction in hippocampus-based memory. Studies on PTSD patients revealed deficit in hippocampus-based declarative verbal memory and reduced hippocampus volumes measured by MRI. Liberzon et al. (13) report different blood supply in amygdale, insula and medial prefrontal cortex in PTSD patients during emotional reactions. Preliminary results suggest that these neural substrates may be involved in the deficit of emotional process in PTSD on one side and trauma release on the other side. Zoellner et al. (14) indicate that recent research into posttraumatic sequelae suggests that intrusive rather than avoiding-dissociative models precisely represent encoding processes in the traumatized. Shin et al. (15) point out irregular regional cerebral blood supply in hippocampus during explicit remembrance of non-emotional material in individuals with PTSD.

Woike et al. (16) examine how personal motives influence the perception of traumatic event in the memory, and how such a perception may

relate to psychological unrest. In both studies, general motives influence the shaping of perception and integrate stress-induced growth. The findings indicate that motives play significant role in the perception of traumatic and difficult experience, and that the perception may be related to psychological influences in different manner. Yovell et al. (17) suggest that survivors of trauma share short and irreversible blanks, while longer, progressive and potentially reversible amnesia is characteristic for survivors who develop PTSD. Failure in acquiring episode memory may coincide with stress-dependent reduction in the function of hippocampal memory system. Nixon et al. (18) underline that earlier exposure to intense stress (i.e. child abuse, abduction) significantly correlates with poor performance in memory tasks. Regressive analysis, dedicated to the assessment of IQ and psychopathological edge demonstrate the importance of earlier trauma exposure and anticipate poor performance in memory tasks, thus suggesting that in current sample, deficit in verbal memory may be related (partly) to the level of stressful experiences accumulated (deposited) over lifetime. Danckwerts et al. (19) suggest that PTSD may be related to the deterioration in cognitive function. However, anomalies may appear and develop from various levels, thus masking the true nature of the relationship. At one level, there is vagueness (fogginess) of emotional and physical case of cognitive dysfunction, in particular with memory tasks, which is similar to those in neurological diseases. At another level, the problem apparently develops directly from both specific and general population. Then, there is an issue of application of narrowly focused neuropsychological instruments which cannot make distinction among summarized components of memory that are not related to everyday situation. Yehuda et al. (20) estimated the most profoundly affected memory component. The most profound deterioration in PTSD was observed in verbal learning, which may be either a risk factor or a consequence of chronic PTSD. Nutt et al. (21) underline that PTSD represents highly weakened condition which is associated with intrusive remembrance of traumatic event, hyper-stimulated, characterized by the avoidance of trauma associated issues and psychological dullness. Authors emphasize that PTSD symptoms

include exaggerated initial response to flashbacks which may be dependant on the weakening of higher neural regions (hippocampus, central frontal cortex). Silenced exaggerated excitation and anxiety symptoms mediate the response to memory of traumatic event through amygdale. Candell et al. (22) indicate that psychiatric references consider dissociative reactions at the moment of traumatic event (i.e. peri-traumatic dissociations) a risk factor for PTSD development. Also, it is established that the description of earlier emotional stress is hard to define. Restrictive factors play a role in the context of forgetting, functions and simulation.

Based on the analysis of our results related to cognitive functions in the group of PTSD subjects and the control group we may establish the following:

PTSD patients made more errors of both types (false positive and negative) at Sustained attention at response task (SART) test ( Table 1 and 2 ).

Statistically significant difference between PTSD group and the control group was not found only in the case of average response time in SART test (Table 3). Subjects with PTSD have shorter response time. This result may be associated with the excitation symptoms in PTSD subjects. Exaggerated excitation may cause these individuals to react faster but also to make more errors in such tasks.

Considering the information on cognitive functions obtained in neuropsychological tests and analyzing the results obtained in this study, we may establish that the deficits in our PTSD subjects are highly significant when compared with the control group. Such outcome may be explained by the type of trauma experienced by our participants. The issue is prolonged, constant stress induced by four-year long bestial war devastation. Also, along with fighting for their own life, our subjects protected bare lives of their loved ones.



## Conclusions

In comparison with the control group, subjects with diagnosed PTSD achieve significantly poorer results in the tests that evaluate focused attention, sustained attention and cognitive flexibility.

Significantly poorer achievement in SART test in PTSD subjects is related to the difficulties in the maintenance of vigilance over time.

Reduced response time in SART test is associated with the increased intensity of PTSD symptoms.

## Acknowledgments

The authors would like to thank Mis. Stefica Sergetlija for help with statistical analyses.

## List of Abbreviations

PTSD - Posttraumatic stress disorder  
 SART - Sustained Attention to Response Task  
 fMRI - Functional Magnet resonance Image  
 PET - Positron emission tomography

## References

1. Robertson I.H., Manly T., Andrade J., Baddeley B.T., Yied J. Performance correlates of everyday attentional failures in traumatic brain injured and normal subjects. *Neuropsychologia* 1997; 6:747 – 758.
2. Manly T, Robertson I.H., Galloway M., Hawkins K.: *The absent mind: further investigations of sustained attention to response*, *Neuropsychologia* 1999; 37: 661 – 670.
3. Keane T.M., Caddell J.M., Taylor K.L. *Mississippi Scala for Combat – Related Posttraumatic Stress Disorder: three studies in reliability and validity*, *J. Consult. Clin. Psychol.* 1988;56 (1): 85 – 90.
4. Lupin S.J., Lepage M. *Stress, memory, and the hippocampus: can t live with it, can t live without it*, *Behavioral Brain Research* 2001; 127 : 137 – 158.
5. Decety J., Grazes J., Costes N., Perani D., Jeannerod M., Procyk E., Grassi F., Fazio F. *Brain activity during observation of actions. Influence of action content and subjects strategy*. *Brain* 1997;120:1763-1777.
6. Roland P.E., Guly B. *Visual memory, visual imagery, and visual recognition of large field patterns by the human brain: functional anatomy by positron emission tomography*. *Cereb. Cortex* 1995;5:79 – 93.
7. Wiggs C.L., Weisberg J., Martin A. *Neural correlates of semantic and episodic memory retrieval*. *Neuropsychologia* 1999;37:103-118.
8. Vadenberghe R., Price C.J., Wise R., Josephs O., Frackowiak R.S.J. *Functional anatomy of a common semantic system for word and pictures*. *Nature* 1996;383:254-256.
9. Tulving E., Markowitsh H.J., Kapur S., Habib R., Houle S. *Novelty encoding networks in the human brain: positron emission tomography data*. *Neuroreport* 1994; 5:2525 – 2528.
10. Mason J.W. *A review of psychoendocrine research on the pituitary – adrenal cortical system*. *Psychosom. Med.* 1968;30:576 – 607.
11. Golier J.A., Yehuda R., Lupien S.J., Harvey P.D. *Memory for trauma – related information in Holocaust survivors with PTSD*. *Psychiatry Res.* 2003 121(2):133-143.

12. Vermetten E., Bremner J.D. Olfaction as a traumatic reminder in posttraumatic stress disorder: case reports and review. *J. Clin. Psychiatry.* 2003;64(2):202–207.
13. Liberzon I., Britton J.C., Phan K.L. Neural correlates of traumatic recall in posttraumatic stress disorder; *Stress.* 2003 Sep;6( 3 ): 151-156
14. Zoellner L.A., Sacks M.B., Foa E.B. Directed forgetting following mood induction in chronic posttraumatic stress disorder patients. *J. Abnorm. Psychol.* 2003;112(3):508 – 514.
15. Shin L.M., Shin P.S., Heckers S., Krangler T.S., Macklin M.L., Orr S.P., Lasko N., Segal E., Markis N., Richert K. Hippocampal function in posttraumatic stress disorder. *Hippocampus* 2004;14( 3 ): 292 – 300.
16. Woike B., Matic D. Cognitive complexity in response to traumatic experiences. *J. Pres.* 2004;72(3):633 – 657.
17. Yovell Y., Bannett Y., Shalev A.Y. Amnesia for traumatic events among recent survivors: a pilot study. *CNS Spectr.* 2003;8(9): 638 – 635; 676 – 680
18. Nixon RD, Nishith P, Resick PA.: The accumulative effect of trauma exposure on short – term and delayed verbal memory in a treatment – seeking sample of female rape victims, *J Trauma Stress.* 2004; 17 (1): 31 – 35.
19. Danckwerts A., Leathem J. Questioning the link between PTSD and cognitive dysfunction, *Neuropsychol. Rev.* 2003; 13(4): 221-235.
20. Yehuda R., Golier J.A., Halligan S.L., Harvey P.D. Learning and memory in Holocaust survivors with posttraumatic stress disorder. *Biol. Psychiatry.* 2004; 55(3):291 – 295.
21. Nutt D.J., Malizia A.L. Structural and functional brain change in posttraumatic stress disorder; *J. Clin. Psychiatry.* 2004; 65 (Suppl 1): 11 – 17.
22. Candel I., Merckelbach H. Peritraumatic dissociation as a predictor of posttraumatic stress disorder: a critical review. *Compr. Psychiatry* 2004; 45(1): 44 – 55.

Corresponding author:

Aida Sarac – Hadzihalilovic,  
Institute of Anatomy,  
Faculty of Medicine,  
University of Sarajevo,  
Bosnia and Herzegovina  
e-mail: aida024@bih.net

# Re-affirmation of traditional values of space for the sake of healthier manner of life – analysis of traditional and modern materials, their influence to the human health and giving directions for future actions in the materialization of contemporary facilities

*Chabbouh-Aksamija Lemja*

Cathedra for Theory and History of Architecture, Faculty of Architecture in Sarajevo, University in Sarajevo, Bosnia and Herzegovina

## Abstract

**Background and goal:** Neglecting of traditional values in the architectural heritage, has brought not only to the loss of the consciousness on the identity but also to the loss of the quality in using housing space. Negative aspects of globalization, especially at “small peoples” who haven’t affirmed themselves yet, out of unknown reason, brought into question their traditional specific characteristics under which we certainly place the traditional architecture. Development of urban structure of all traditional city compounds led towards the changes produced by different way of life including the utilization of space. Conditional-consequential relation of the living culture, as the expression of modern life trends and the formation of space which is the result of new technological knowledge, contemporary modeled ideas and innovation approaches, resulted with general change of urbanism of all levels of settlements but also with the change of living space and general change of ambience. The goal of

this work is to point out the values of traditional materials and forms, recognized as the Bosnia and Herzegovina traditional values, and by comparing it with modern materials and forms being classified into universal one, to point out the advantages of these traditional values in relation to the health of their users.

**Methodology:** By using the DeAngelis D Osat method it is common to analyze the overall facility, in detail and in relation to the ambience, but for this analysis the most important is to express the attitude on:

1. Cultural and historic aspects;
2. Artistic-esthetic aspect;
3. Constructive aspect and materials.

In this way, characteristics of traditional facilities, traditional ambience and traditional materials that were used for their construction will be defined. By comparing it to modern facilities, modern ambience and modern materials, the advantage and disadvantages of the both will be defined.

**Target Group:** On one side, those are traditional facilities out of which we chose the urban housing heritage of the Ottoman period in Sarajevo and rural heritage of the mountainous regions of the Sarajevo surrounding, and on the other side, it is modern housing construction from the year 1975 by today, whose characteristic are the block constructions from the prefabricated elements with cheap construction materials intended for the collective residence.

**Results:** The results of the research showed that from the urbane spaces almost disappeared the group of facilities that we call vernacular architecture which was the reflection of traditional values of the local national constructors, that we consider as the bearer of the elementary message of the identity of the space and of historic stratify. This architecture almost disappeared from rural ambiances thus only rear remains witness about their existence. The aspect of mental and physical health was analyzed through possible advantages and disadvantages which characterize the use of modern spaces, so as the advantages and disadvantages while using traditional facilities. The form of the space, materials the facility was constructed from and the ambience which was created by mutual relation of the facilities were taken into consideration. The concept of the contemporary use of traditional facilities is given through the overview of the characteristics of the rural and urban heritage and the values which additionally influence to one and another group of facilities were defined.

**Conclusion:** \*The advantages of modern facilities are the designed and planned space, adjusted to modern needs – infrastructural network in the function of the facility, isolation characteristics of the facilities; and disadvantages are in the use of materials and construction elements harmful to health, alienation of the users, the form subject to the function and construction elements, non identification with the residence place and lack of genius loci.

\*The advantages of traditional facilities are the standards adjusted to a man, the form providing the open communication with the surrounding, the use of healthy and ecologically acceptable materials, constructive elements subject to the form, function and form in harmonized relation, the feeling of the user to belong to the space, offering the sen-

se of the identity to the users, mutual communication of the users and... Disadvantages are in insufficient infrastructural network, difficulties at maintenance of the facilities, isolation characteristics of the facilities, obsolete of mounted materials and insufficient capacity of traditional ambiances.

By subliming the both, only at look confronted poles, we come to the conclusion that the defining of traditional values, their affirmation through modern life trends and determination of solid frameworks of actions in forming and conception of facilities, may contribute that modern construction with the reminiscence to traditional values would be a form of the expressing all modern needs through modeling and materialization directed towards mental and physical health of the user.

**Key words:** Traditional materials and forms, traditional values, restoration of traditional facilities. Healthy materials, ecology, vernacular architecture, physical and mental health.

## Introduction

Relation towards heritage reflects also the relation towards own identity but also the level consciousness on the characteristics of traditional values and level of civilization. Actual situation in architecture opens confuse and non-articulated situation in society, thus it is not unusual that people for very long time have stopped to take care of healthy way of life and universal values of residing in architectural facilities. Construction is in greatest extent directly towards the economic aspect of sustainable development while very often social and ecological aspects are only formally present. Contemporary architecture, especially one of the residing character, is modeled in way that the form follow the function, so according to all known theoretic postulates, architecture does not exist at all. It is not our goal for this research to have the character of the critic of contemporary residing architecture, but to point out its disadvantages and to give propositions for its improvement. The aspect of traditional construction is unfortunately confronted to contemporary construction, mostly from the fact that once more care was directed to the final ser of the space, that is to a man. Actual situation is such that architectural approach to the

housing space is reduced to simple satisfaction of basic human needs which I certainly caused by numerous socio-economic factors which are not subject to this research. These same factors influence not only the modeling but the choice of materials, so it is not rare that the house becomes the source of mental and physical diseases for their tenants. Reasons for these problems are numerous and may be caused by wrong disposition, use of unhealthy or even toxic materials, bad bio-climate conditions and not adequate position of the facility on the ground. Contrary to the actual construction, traditional facilities were carefully located on the ground with special care to isolation ‘‘rose of winds’’, the position of facility in relation to neighboring facilities, selection of materials and their combination and mutual interaction, disposition of anterior and-people loved the space they lived in, used very often gardens or yards and communicated with nature, traditional city blocks of the Ottoman period ‘‘Mahala’’ same as actual new city blocks were exclusively zones of residence but the people there did not feel alienated. The reason is in the fact that once the public place in those zones was reduced only to the street or small narrow street, and private space, separated by high wall, was divided to opened and closed, but those private units mutually communicated by some internal communicational ways. Enclosed porches on the upper floors of the houses were surpassed over the street and on three sides opened with windows, thus the visual communication was established with outer ‘‘world’’ which existed outside this private oasis. All this was dimensioned according to human standards so that all views to those facilities, but also those internal towards outside, were subject to the feeling of closeness to the space. The use of material was reduced of stone, unbacked brick and wood. Construction form coming out from the characteristics of materials from one side and technological knowledge from the other side resulted in a solution which produced specific traditional form.

Second example talking about harmonized interaction of architectural form, its materialization and surrounding ambience, is the rural traditional architecture. Its bio-climate characteristics is expressed much stronger than at urban architecture, but here also, it is possible to talk about the mo-

deling of space that is subordinated to its final user and the use of the material which is ecological, but also in construction sense, a clear answer of a local builder to all stimulus of natural environment. The observer has a feeling that all facilities are surfacing from the landscape joining with it. Dominant accent is a monolith four leaned roof, covered with shingle, which we consider as the bearer of the traditional picture of the space but also the characteristic location of the facilities on the inclined terrain with the stone supportive wall on the front house representing the storage dig into the terrain. The only floor is in the proportion 1:2,5 to 1:3 in relation to the roof and is constructed from stones or woods depending on the disposed materials in the environment. These characteristics of the traditional rural construction stand opposite to the contemporary rural construction which has no characteristics, it is no more formed by bio-climate and geo-morphological conditions of the region but is confronting to them by technological and technical achievements, providing to their users bigger comfort, which price is, in this case, extremely high. It is paid in healthy life in new projected space, under the losing of identity and the spirits of the space atmosphere they live in.

### **Elaboration**

#### **Cultural-historic aspect**

For this research which I referred to the man’s health, cultural-historical aspect of the space he lives in has a strong spiritual value. The feeling of alienation or the feeling of belonging, are the characteristic emotions of the users towards the space they live in. Globalization is, with strong and aggressive urbanization, affected the accelerated development and uncontrolled construction. Consequences of these influences are seen at constructed spaces but also from the relation of the users towards that space. City blocks without identity, which remind one to another, and which remind to any other in any other city in the world, are the picture of today’s architectural urbane reality. Reasons for these relations towards the space are found in insensible society but also the authors, architects, for traditional values. Their inspirations



are widely accepted clichés, universalized on the level of the whole planet. Unification of values in the formed sense, is a result of the unification within mental scope, and transitional countries, we belong to, are mostly fond to these influences. Lack of self-awareness and non articulated globalization, jeopardize the harmony of the relation between a man and his surrounding space. Existential space, has outgrown the constructed architectural space, which has jeopardized his own existence, and the question of modern construction brought to an absurd. This aspect, is far more important when we speak about the protection of the construction heritage, where the research and documentation is the starting point for the final conservation and restoration practice. When talking about the utilization of this traditional space, we will only say that it is created as a result of the traditional living culture and as such, even though old, is still more humane and better solution, for its many characteristics than it is today's concept of living.

### **Artistic-esthetic aspect**

According to Kant, esthetic is a science which treats the conditions of the emotional perception. May we the thing considered as non-esthetic pronounce unemotional, or is non-esthetic suppression of emotions. Just such definition is corresponding to the spirit of the users of contemporary living space in new city blocks. Here it is important to mention that all of our analytic critics towards contemporary housing construction is directed to non care of its final users, so the conclusion is that it is about self purpose, and that it reduced the housing standards to the minimum. In neither case, it is not appropriate to talk about artistic character of the facility because in the first case it is related to the housing vernacular architecture, so, about the architecture without the architect, whose task is not to express itself with its exceptionality, but to, collecting previous knowledge and experience, present construction skills, as well as to improvise with empiric knowledge and produce a form which will provide satisfaction of the needs for shelter, and create the pleasant residence. This position that we have described, is related to rural heri-

tage, and urban vernacular architecture will differ by the fact that its goal is to create a beautiful and pleasant home for its family. Contemporary architecture itself, which we have defined within target group, can also be treated as vernacular, neglecting the fact that it has its own authors and because it is a result of planned architectural project. It evaluated very much in its form, which can be seen on these facilities facades, with notable interventions of the users, and which has transformed uniformed facilities into colorful and non-attractive spaces with differently closed balconies, inadequate annexes, non unified windows openings, inadequate attics, and...All those subsequent interventions, significantly devastated original look of the facility, but these examples clearly witness to the contribution of our thesis that the tenants of these facilities are non sensible towards them in emotional sense as it is completely not important what external impression their house gives. They have introvert perception of that space and they are solely interested in their own micro space. It is clear that then we can talk about the alienation from the space in which people live and about lack of the interest for the wider space in which they reside.

Both those aspect certainly have strong influence to the level of human consciousness, which then provoke changes in behavior, depressive states, expressed aggressiveness...

### **Materials and constructive aspect**

All traditional architecture of this region is based upon three elementary materials of natural origin:

Stone, woods and brick (which in the Ottoman architecture appeared in the form of blocks made of unbaked dirt, so called *čerpić*).

All these three basic materials belong to the group of ecologically acceptable materials even those considered as extremely recommendable for the extern and intern use, because they are not harmful to health. Construction forms are characteristic for the time of the construction, as well as in traditional architecture so as today, they are the reflection of the characteristic level of technical and technological knowledge.

Talking about healthy and unhealthy materials, or more precisely about constructive forms which make living space, we will see that it is about physical and psychological causes which produce disturbances or have negative effect to the space users. Physical causes may be divided into small particles of the smallest structures and into radiation produced by the materials. These causes result in negative effects of the construction materials. Psychological causes are provoked by structuring of inner and outer space and mutual relation of the facilities in the space, surroundings and spirit of the space (*genius loci*).

Physical causes are divided into live and non live, where the live ones are divided into: microbes, eggs, cocoons and..., and non live to: atoms, molecules or basic forms of some substances in solid, liquid and gas conditions.

Radiation understands emission of harmful waves and the activity of energetic radiation on to the people being in the constructed space.

Harmful influences produced by physical causes are predictable and known, but the very radiation represents huge danger for the users of the space. Some authors classify radiation to one which can be registered by the apparatus and the one which is difficult to be registered, even though it is known that they exist. In the first group we place: electro magnetic, ionization, radio active and cosmic radiation, while in the second one we place: radiation from earth, houses and appliances – radio cesian radiation.

Modern materials made in chemical manner, which characteristics are closely directed to salvation of some constructive task, mostly those materials which composition is of the mineral origin, contain radon, radio active gas causes lung cancer.

Up to day researches of the harmful influences of the construction materials separated six most dangerous space polluters: asbestos, toxic materials, dissolvent, radon, biological polluters and the means for the protection against them.

Radiation of radio cesian origin, were known even in the traditional construction, and the successor constructors based upon some forgotten and neglected knowledge, such as oarlock, plumb line and similar methods, which in this moment look in ephemeral way. However, we consider these

influences extremely harmful and we divide them into:

- natural waves originating from composition and physical features of the soil;
- telluric stream – influences being the consequence of all soil movements;
- underground constructions;
- technological facilities;
- buildings, depending on the materials they are build from and the type and the amount of installations;
- geo-biological (Hartman) net;
- own radiation from individual objects and living beings;

Beside these usual or some more concrete divisions we made, definition of the harmfulness of some materials in architecture is still hardly realistic, but according to the results from the Engineer R. Kovačević, generally we can conclude the following:

- metals are principally not suitable, especially if used on the surface (tins, nets);
- concrete is not suitable specially when reinforced or when the aggregate originates from not suitable source;
- artificial materials are principally non suitable;
- stone is suitable depending on the finding place;
- wood is suitable;
- brick is suitable.

Given this analysis provides only partial insight of harmful influences of the construction materials to the humans, using the table from R. Kovačević, we will give the preview of the individual materials and their features.

After one such review of harmful influences, it is clear by itself, that for each individual material we try to determine its features from the point of view of the healthy house, being the subject of the following text.

### Features of the construction materials being the most common in contemporary construction

- **Concrete**—We consider it as the material of all choices for all construction and construction forms. It becomes definition sine qua non in civil engineering, and at complicated static constructions. Given the concrete non consistent behavior at low temperatures and at temperature changes, so as at humidity presence, it is not recommended in hosing spaces. Its harmfulness is increased while introducing reinforced concrete in its construction and while using aggregates of the volcano sand or in combination with pucoaine cement it becomes extremely harmful for health. Modest use is recommended and the avoidance of this construction material. In traditional housing architecture this material has never been used, although in its original form it has been known for centuries.
- **Steel and other metals** – Characteristics of these materials are very applicable in construction and with time they became one of the basic constructive elements. However, their conductivity features and a possibility of producing electro-magnetic fields and waves on this basis including negative emission in space, declares this material as highly not suitable for its use in housing construction. Only the usage of copper foils and led connectors, which is normally the part of traditional construction, has no harmful influence to the space.
- **Brick** – This is one of the three traditional materials which do not have any harmful influence to the humans. One of its derivates, *čerpić* - non baked brick mixed with straw, is considered as the safest material for life, not taking into consideration its bad physical characteristic, because it's very sensitive to humidity and it is not slid enough to pressure and movement. To the contrary, brick has outstanding physical characteristics, it is solid, resistant to the temperature changes and it is only one having the feature of thermo isolation and accumulation at the same time. For this material we may say that it can be harmful if the clay the brick is made from is extracted from radio active finding place, even thou in such situation radiation is decreased during baking time.
- **Stone**—We consider stone as historic material, which is, because of its persistence, preserved artifacts of many historical facilities up today. Architecture is, mostly because of the stone and its persistence, become an element of historic remembrances. However, stone does not have even closely the features of the brick, so it is bad isolator, and it is hard to be manipulated during the construction. Its basic characteristic, besides esthetic, is solidness. It is not principally harmful for the health but depending on its volcano origin, it may radiate. Even its granulation used in mortar, in such way may represent potential danger to health. In housing construction, sediment and metamorphic stones are recommended, that is, lime stone and marble. Lime stone is mostly used in urban and rural traditional architecture and granulate for mortar is made of it.
- **Wood** – It appears through history as the faithful partner of brick and stone in the unity of the construction form, and represents a unique connection of healthy and useful construction material. Depending on the sort, we have different wood characteristics, so that we have soft and less resistant sorts and those solid and in static sense extremely resistant sorts. Some of them are resistant to humidity so they are used in exterior, although the key shortage of this material is non resistance to living organisms, pests, which may be removed by certain protections, but at this procedure the wood becomes harmful for the environment. Given these protective coats are chemically based and intended to the elimination of living pests attacking the wood, their use is not recommended in interior of living facilities.
- **Artificial coats for wood** – As it was already said, coats for the wood act against the insects and fungus who habitat in and on the wood surface. These insecticide and fungicide may cause severe damages to humans, especially during their application, so we should prefer

organic coats or adequate techniques of the wood processing and avoid the others. In traditional architecture, the wood was exposed to smoke, so that soot may the protective layer which was impenetrable for all the pests, while the external surfaces were coated by different sorts of oils and waxes thus being protected from humidity.

- **Light concrete** – Its application in contemporary construction is of extreme importance considering that it is about the material which weight has decisive importance in construction forms, and is eagerly used because they do not burden bearing construction making the construction cheaper. However, these materials do not have physical stability, so they radiate small particles in physical sense, but even in chemical sense, they radiate unstable general structures. Development of these materials is ongoing thus it may be expected the improvement of their characteristics, but in this moment the caution is necessary while using them.
- **Artificial wall coats** – It's completely illusory to expect these materials to be excluded from the usage because in relation to the natural one, they are much more economic and have good characteristics. Objective, it is about material which completely prevents the circulation through the walls thus preventing micro circulation between inside and outside space. Circulation process enables eventual exit of negative particles from the space, but thanks to this coat, it is prevented. Among others, these materials contain toxic metals and their dissolvent by its toxic activity directly jeopardize human health and causes many diseases.
- **Materials made from mineral fibers** – Thermo isolation materials are made out of this material. Their exceptional isolation features have become inevitable, because of the fact that the isolation aspect, in construction sense, is one of the main advantages of the contemporary in relation to traditional construction. There are different types of the mineral fibers processing and their origin. People mostly know about glass and stone wool, both may be found in various forms.

There are pressed fibers, which are mounted in compact blocks as isolation, but there are also densities structures, which are the most harmful for their small particles which never stop to radiate into the space. Still, there are those which are ecologically certified and which are completely acceptable or at least less harmful. In any case, this material should be mounted with protection.

- **Plastics** – This material produces static electricity by which, on one side, it attracts small particles from the air, but it releases them during movements in space, thus it is not recommended for the emplacement on the floor surfaces. It has indirectly harmful effect, because it is constantly emitting micro particles into space.

Table review shows the behavior of the quoted materials and their harmful features

Still, we may not neglect the usage of the materials for the construction of the architectural details, openings, windows and doors. This paper will treat characteristics of plastic and wood carpentry.

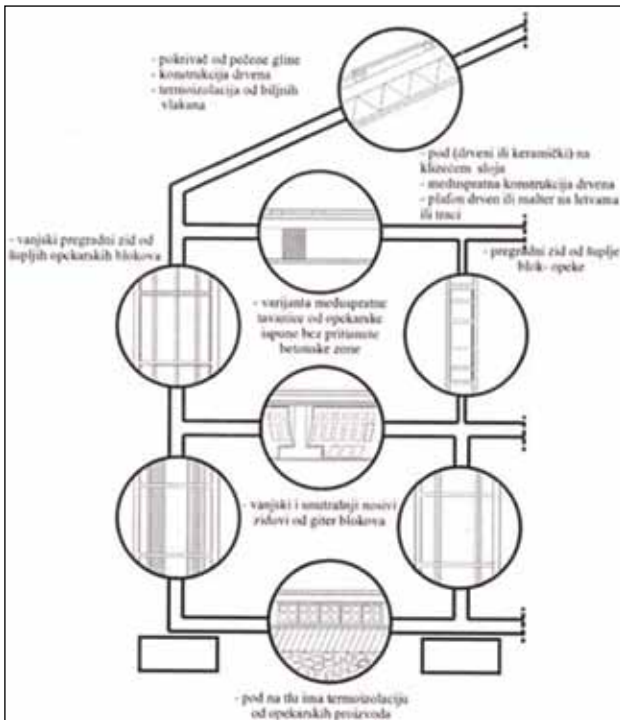
Common opinion that the plastic carpentry may have harmful influence to the human health, mostly have no basis except in two cases. Because of good isolation provided by plastic carpentry, humidity may appear in houses and apartments, which at some persons may cause respiratory problems, and damages on the facilities related surface works on the walls because of condensates being the place for fungus and molds. Other problem appears in the case of fire because during melting the gases are released being very harmful for human health.

Wood as material has been already elaborated, and its usage in carpentry is traditionally present in this region for centuries. As the material suitable for processing, with good technical characteristics, this carpentry, varying in its technical characteristics was changed during the existing period of the facility, but always with the same functional value. It is, expressing the characteristics of the wood as a material and responding to temperature changes and changes of the air humidity, creating the final barrier between inner and outer space, micro movements characteristic to wood, always have had a small dosage of semi-conductivity, so it enables the breathing of space thus ventilating



and airing the living space. In judging over the advantages and disadvantages between the two types, wooden carpentry is recommended.

Upon reviewing the characteristics of quoted materials, it is clear how difficult is to choose those which combination can reach suitable construction of the living house. Architect R. Kovačević offers a schema of the construction, which is mostly acceptable and which we present here.



Picture 1. Review of harmfulness of certain materials

| MATERIJALI                          | KRITERIJUMI |          |         |          |            |          |             | NAPOMENA                                                  |
|-------------------------------------|-------------|----------|---------|----------|------------|----------|-------------|-----------------------------------------------------------|
|                                     | FIZIČKI     |          |         |          |            |          |             |                                                           |
|                                     | HEMIBI      | BIOLOŠKI | TOPLINA | VLAŽNOST | ELEKTRICNA | RAZLIČNI | RAZLIČNOSTI |                                                           |
| 1. BETON                            | ○           | ○        | ●       | ○        | ○          | ○        | ○           | opaziti: volanski sprag i pregradni zidovi                |
| 2. ČELIK                            | ○           | ○        | ○       | ○        | ○          | ○        | ○           |                                                           |
| 3. OPEKA                            | ○           | ○        | ○       | ○        | ○          | ○        | ○           | opaziti: ako je gips u sastavu zidova ili pregradnog zida |
| 4. KAMEN                            | ○           | ○        | ○       | ○        | ○          | ○        | ○           | opaziti: volanski, substrukcijski i pregradni zidovi      |
| 5. DRVO                             | ○           | ○        | ○       | ○        | ○          | ○        | ○           |                                                           |
| 6. LAKI BETONI                      | ○           | ○        | ○       | ○        | ○          | ○        | ○           |                                                           |
| 7. VJEŠTAČKI PREMAZI ZA ZIDOVE      | ○           | ○        | ○       | ○        | ○          | ○        | ○           |                                                           |
| 8. VJEŠTAČKI PREMAZI ZA DRVO        | ○           | ○        | ○       | ○        | ○          | ○        | ○           |                                                           |
| 9. MATERIJALI OD MINERALNIH VLAKANA | ○           | ○        | ○       | ○        | ○          | ○        | ○           |                                                           |
| 10. PLASTIKA                        | ○           | ○        | ○       | ○        | ○          | ○        | ○           |                                                           |

○ POZITIVAN ● NEGATIVAN ◐ USLOVNO NEGATIVAN ○ BEZ UČINKA

Picture 2. Review of the possible application of existing materials and traditional principles

Analysis of the construction form of the traditional living house differed in different periods of construction and such form element is a true reflection of the technological conscious of the local builders. Getting knowledge on the materials and their characteristics will not be difficult to choose adequate construction for design. These are concrete quoted examples which speak about harmfulness of construction materials to human health, but also the designing of the space influences human organism, thus making the responsibility of the creator bigger, and in such way we will point the possible mistakes, while we have already determined which are the advantages of traditional spaces in relation to those newly designed. Nowadays, from the construction of the facility it is expected: bearing, thermo isolation, sound isolation, steam conductivity (breathing), accumulation, water proof, non hygroscopic, ecological fitness, acceptable price and rapid construction. It seems that construction profession puts before itself high demands, but not fulfilling them not in the smallest percentage. Not taking care of final users and their health, demands are finally reduced to acceptable price and rapid construction.



a)



b)





c)

Picture 3. Review of urban traditional house, The Svrzo family house.

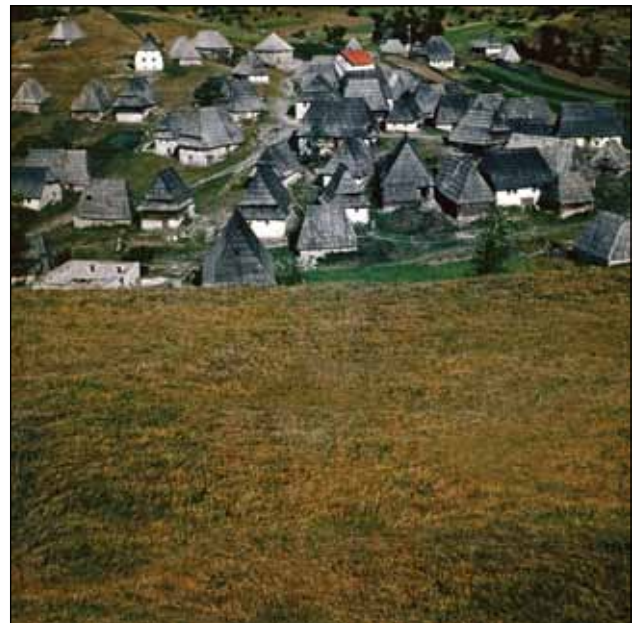
Except physical damage to human health, ambience, atmosphere that ambience carries, memory of the place, socio-economic aspect of the space, they can all influence the psycho-physical condition of people in one specific environment.



b)



a)



c)

Picture 4. Review of rural traditional village in Bjelasnica, Umoljani Village, 1968

Aggression, conflict and anxiety can often be the consequence of small or confuse space. These conditions are caused by different reasons, but the space in which such persons reside very often has the role of a trigger, which provokes such behavior. On the other hand, the ambience being pleasant for living, which by its form, presence of the green or water, for example, is calming and soothing. Just to recall the rooms of the dervish tekia, which were among the first to cure mentally ill with



poetry, music, silence, murmuring of water and birds singing inside their gardens, inside the walls of tekia or zavija. Some of the most known such dervish hospitals were placed in Sarajevo (Hadži Sinanova Tekia in Sagrdžije and Mevlevijska Zavija on Bentbaša).



a)



b)



c)



d)



e)



f)

Picture 5. Review of typical urban block in the city

There are certainly many different situations coming from the influence from construction phenomenon to the behavior and experiences of people and such phenomenon analysis will demand much wider research. We may put the consideration that in today's moment many factors represent more important dominants of human behavior from the surrounding ambience we move inside, but it is non-doubtful that just architectural space, as the part of the general existence space, represents the framework of the individual and social life. It would be an interesting example the space ruined by war, which in the same moment, for that place inhabitants, represents the memory of the place, remembering of some horrible moment from the past, and then, when the place is reconstructed, rehabilitated and returned to the previous condition, a man start to forget horrible images the place reminded him of. Historic memory is always connected to constructed space, although natural landscape may represent a part of the place memory, if it is about important, accented points in space. We have already talked about the need of the space user for recognizing the own identity through the space identity in which he permanently resides and the alienation from the own surrounding on the other side. Presumption is that even such conditions affect mental health of people moving in their own defined space. Such detailed analysis would be useful for architectural and medical practices, because by this mutual interaction, the results would be followed up and compared.

## Conclusion

We may conclude that forming and materialization directly participate to the process of the protection and preservation of human health, mental and physical. Application of traditional materials and their careful selection for the sake of structuring new facility with the reminiscence of traditional construction values, will produce good results. Contemporary construction today is based on cheap and non quality materials, which represent danger to human health, thus the caution is recommended while selecting them. Traditional materials are not binding today's constructors to traditional constructions, if there is creative desire to create new vales, but traditional constructions represent tried out and always balanced concept of building, where for the centuries almost nothing has been changed. Forming of architectural space represents the form in which we move and live. That form is a parameter by which all its inhabitants are recognized, by which they are identified and with whom they sympathize. Emotional bounds between inhabitants and the location at certain way are mutual, and equally as a harmony which is seen and measurable, so as disharmony between the tenants and space is promptly noticed. Negative manifestations are aggression, depression, anxiety and ...while ambience is neglected, devastated, confused and...

Research and adoption of traditional value of space, its authenticity in form, materials, content and ambience, would contribute to better physical and mental health of people, the users of the space.

## References

1. *De Angelis d'Ossat, Guglielmo., Guide to the methodical study of monuments and causes of their deterioration = Guida allo studio metodico dei monumenti e delle loro cause di deterioramento.; International Centre for the Study of Preservation and Restoration of Cultural Property., Rome., 1972, 1907- 237;*
2. *Norberg- Shulz, Christian, Existence, space and architecture ( Original title: Existance, Space & Architecture. Translation: M. Maksimović). Construction book , Belgrade, 1976*
3. *Norberg- Shulz, Christian.; Intancies in architecture.,; Library Culture of surrounding ;press Jesenski and Turk.; 2009. 189;*
4. *Rosi, A.: Architecture of city , Construction book - Premis, Belgrade, 1996*
5. *Vitruvijevih ten books on architecture, Svjetlost, Sarajevo, 1951*
6. *Rosi, A.: Architecture of city, Contruction book- Premis, Belgrade, 1996*
7. *Giedion,S.: Space, time and architecture, Construction book, Belgrade, 1969*
8. *Frampton, K.: Modern architecture: Critical history, Globus publishing house, Zagreb, 1992*
9. *Kadić, Rešad.; Last bašeskija notes, El kalem, Sarajevo,1997, 248;*
10. *Kovačević, Ranko.;Principles of materialization of ecologic house; Traditional architecture of Montenegro, University Montenegro, Faculty of Civil Engineering, Monography ,Podgorica, 2005*
11. *Bruks Fajfer, Brus.; Rajt.; Taschen, 2008, 96;*
12. *Charleson,W., Andrew.; Structure as Architecture, A source Book for Architects and structural Engineers.;Elsevier Book Aid International.; Oxford, UK.; 2005; 241*

## Corresponding author:

*Chabbouh-Akšamija Lemja,  
Cathedra for Theory and History of Architecture,  
Faculty of Architecture in Sarajevo,  
University in Sarajevo,  
Bosnia and Herzegovina,  
e-mail: lemjaaksamija@yahoo.com*

# New information about Von Willebrand's factor?

## ŠTA JE NOVO O VON WILLEBRANDOVOM FAKTORU?

Nermina Babic<sup>1</sup>, Jasminko Huskic<sup>1</sup>, Emina Nakas-Icindic<sup>1</sup>, Suzana Tihic-Kapidzic<sup>2</sup>, Miralem Music<sup>3</sup>

<sup>1</sup> Institute of Physiology and Biochemistry, School of Medicine, University of Sarajevo, Bosnia and Herzegovina

<sup>2</sup> Institute of Clinical Chemistry and Biochemistry, Clinical Centre University of Sarajevo, Bosnia and Herzegovina

<sup>3</sup> Department of Patophysiology, School of Medicine, University of Sarajevo, Bosnia and Herzegovina

### Abstract

The Von Willebrand factor (vWf) is a multi-metric plasma glycoprotein that participates in the adhesion and aggregation of the platelets at the blood vessel injury sites. The vWf serves as a coagulation factor VIII carrier. The vWf is synthesized in endothelial cells and megakaryocytes, in the form of large polymers, composed of identical subunits. The size of a circulating vWf multimer is determinant of its function in the adhesion of platelets, and is controlled by proteolytic actions of a specific protease. Reduced synthesis or reduced functional activity of the vWf causes a hemorrhagic diathesis. Elevated vWf levels indicate an increased risk of arterial and venous thrombosis. The vWf is an acute phase reactant. Its level may increase due to inflammation, stress, physical exercise, and following a surgery. The vWf is also a marker of endothelial dysfunction.

This article intends to illustrate the current methodical findings about the structure, the biosynthesis and the role of the vWf at various physiological and pathophysiological states.

**Key words:** von Willebrand factor (vWf), multimers, platelets, endothelial dysfunction

### Sažetak

Von Willebrandov faktor (vWf) je multimetrički glikoprotein plazme koji sudjeluje u adheziji i agregaciji trombocita na mjestu oštećenja zida krvnog suda. Nosač je VIII faktora koagulacije krvi. Sintetiziraju ga endotelne stanice i megakariociti u

formi polimera građenih od različitog broja identičnih podjedinica. Funkciju vWf u adheziji trombocita određuje veličina cirkulirajućeg multimera koja je regulirana proteolitičkim djelovanjem specifične proteaze. Smanjena sinteza ili smanjena funkcionala aktivnost vWf je uzrok hemoragične dijateze. Povećan nivo vWf u plazmi udružen je sa povećanim rizikom od arterijske i venske tromboze. Nivo vWf u plazmi, kao reaktanta akutne faze, može se povećati u toku upalnog procesa, stresa, fizičke aktivnosti, operativnog zahvata. vWf je i marker disfunkcije endotela.

Cilj ovog revijalnog članka je prezentirati nova saznanja o strukturi, biosintezi i ulozi vWf u različitim fiziološkim i patofiziološkim stanjima.

**Ključne riječi:** von Willebrand faktor (vWf), multimeri, trombociti, disfunkcija endotela

### Introduction

Von Willebrand factor (vWf) was named after dr. Erik von Willebrand, a Finnish doctor. Dr. Willebrand first described the hereditary bleeding disorder with cutaneous and mucosal bleeding, including menorrhagia in 1926. The vWf was isolated in 1970.

The vWf, as a large adhesive glycoprotein was confirmed in the Weibel-Palade bodies of endothelial cells, the  $\alpha$ -granules of platelets and megakaryocytes, in plasma and in the subendothelial connective tissue (1).

The vWf plays an important role in primary hemostasis, supporting adhesion and aggregation of the platelets at the site of blood vessel injury under



a high shear stress. The vWf also participates in a secondary hemostasis, as a protein carrier and stabilizer of blood coagulation factor VIII. The vWf is considered an indicator of endothelial dysfunction and in determining of a level of activity of the atherosclerotic process.

### A Structure of vWf

The circulating vWf is one of the largest soluble proteins with a molecular mass of 520 to 20,000 kDa. The mature vWf protein is a multimetric glycoprotein molecule, composed of numerous identical subunits (M 260,000), monomers, which are connected by disulfide bonds. Their number in the vWf molecule may range from only two to a very large number, where it forms the so-called ultra-large multimer vWf (UL vWf).

Each vWf monomer contains many specific domains with specific functions: the D ' / D3 domain (which binds to factor VIII), the A1 domain (which binds to platelet gp 1 b $\alpha$  receptor, heparin, heparin-like molecules, proteoglycans, sulfatides, and possibly collagen), the A3 domain (which binds to collagen), the C1 domain (containing Arg-Gly-Asp (RGD) sequence which binds to platelet integrin  $\alpha_{IIb}\beta_3$  when this is activated), the "cysteine knot" domain at C-terminal end of protein, which vWf shares with platelet growth factor (PDGF), transformed growth factor  $\beta$  (TGF $\beta$ ) and  $\beta$ -human chorionic gonadotropin ( $\beta$ HCG) (2).

Small, medium and very large multimers of vWf exist in the plasma.

### Synthesis of vWf

The Von Willebrand factor is synthesized primarily by vascular endothelial cells and also in small quantities, by megakaryocytes (3). Only 10-20% of the vWf in humans have a platelet origin.

The synthesis of the vWf is a complex process, involving multiple steps. The vWf is synthesized as a precursor, polypeptide of 2813 amino acids (pre-pro-vWf), including the 22- residue signal peptide, the 741- residue propeptide, also known as von Willebrand antigen II, and the 2050 residue mature vWf protein. After cleavage of a

signal peptide in the endoplasmic reticulum, the pro-vWf molecules forms C-terminal dimers, then undergoes intense posttranslational modifications. This includes glycosylation, sulfation, then polymerization, multimerization, and finally splitting the pro-vWf in vWf multimer (mature vWf) and propeptide. Both aminoterminal multimerization and propeptide cleavage are thought to occur in the trans-Golgi network, where the furin was detected, as the vWf proteolytic processing enzyme (4). After the proteolytic action of furin, the propeptide remains noncovalently associated with vWf, and is transmitted along the secretory granules: The Weibel-Palade bodies of endothelial cells, and the platelet  $\alpha$  granules.

The propeptide plays an active role in targeting, sorting and storage of the vWf in secretory granules (5). The connection between the vWf and the propeptide is pH-dependent. At a low pH and at the presence of calcium ions, as in the trans-Golgi network, these two proteins are noncovalently associated. However, at pH 7.4, this interaction is not sustained (6). The secretory granules have a lower pH than the Golgi apparatus, so the vWf and propeptide are constantly connected. Both proteins are found in an equimolar ratio in the Weibel-Palade bodies. The vWf propeptide facilitates multimerization of vWf, as one of the most characteristic post-translational event, which takes place in the secretory granules. It remains unknown what is the influence on the final length and on the number of dimers in the UL vWf multimer. The Weibel-Palade bodies contain only the high-molecular vWf multimers, whereas vWf released constitutive composed of dimers and small multimers. A degree of polymerization of the vWf directly correlated with its prothrombotic activity. High-molecular weight vWf multimers are most effective in hemostasis.

Michaux and al. (7) have shown that vWf is stored in the secretory granules in the form of tubules, giving a characteristic elongated shape of Weibel-Palade bodies. The tubular conformation of vWf is essential for a rapid unfurling of 100 micrometers long vWf filaments, when exposed to neutral pH after exocytosis in cell culture or in living blood vessels. The increase in pH (from pH 5.5 in Weibel-Palade bodies to pH 7.4 in the blood) causes release of vWf propeptide, after which the chain of vWf multimer unfold (7).

Stored vWf plays an important role in the secretion of other proteins from Weibel-Palade bodies (P-selectin, interleukin-8), and may modulate inflammatory processes (8).

### The vWf Secretion

Quantitative analysis of secretion, both in resting and in stimulated cultured endothelial cells, found that the vWf is released together with propeptide at 1:1 ratio. The propeptide has a circulating half-life of only 2-3 hours compared to the vWf, whose half-life is approximately 18 hours (9).

The vWf is secreted by two pathways: constitutive, requiring no cellular stimulation and regulated pathway, responsive to secretagogues. The most of vWf from unstimulated, resting cells are secreted constitutively, continuously in small amounts (9). This type of secretion is found in endothelial cells and platelets during thrombogenesis. Megakaryocytes do not constitutively secrete vWf. Endothelial cells constitutively secrete the vWf both into the circulating blood and the subendothelial matrix. This secreted low molecular weight of the vWf was detected on the basal membranes and is free in plasma (10). Functionally, this vWf is less competent. It shows little affinity for binding to subendothelial structures. In regulated secretion, the vWf is released only through luminal membranes in the lumen of blood vessels. Secretion of vWf from specialized storage granules of the endothelial cell is triggered by several substances, some which are important mediators of thrombosis and inflammation (11, 12). In vivo, a release of the vWf from secreting granules and increase its concentration in the blood, may induce adrenaline, vasopressin or its analogue, the nicotinic acid, interleukin-1, a tumor necrosis factor  $\alpha$  (TNF $\alpha$ ). Also, as a rapid response to vascular injury and endothelial damage leads to releasing of large vWf multimers from the secreting granules.

Vischer et al. (12) investigated the pattern of release and the cytoskeletal requirements for secretion in response to  $Ca^{2+}$  raising agents or to cAMP-raising agents. Thrombin and histamine induced rapid vWf release (less than 5 minutes), which is dependent on a rise in concentration of ions  $Ca^{2+}$  in the cytosol. It involves released from central and peripheral secretory granules and is accompa-

nied by cell retraction. On the other hand, adenosine, adrenaline and prostacyclin induced a slower response (more than 10 minutes), dependent on the rise c-AMP with the release of only peripheral secretory granules, without cell retraction (12).

Giblin et al. (13), in experiments on human endothelial cells pointed to a third pathway of the vWf secretion, which the authors call basal secretion (also called constitutive-like secretion). They demonstrated that most of the vWf secreted by the unstimulated cells was not a product of constitutive secretion but a basal release of post-Golgi storage organelle, presumably the Weibel-Palade bodies. Their findings sparked considerable public interest in science in this area (14) and generated a huge interest.

### The Physiological Functions of the vWf

The primary function of the vWf is binding with different proteins. Its function in thrombogenesis is to promote a thrombus formation by creating adhesion of platelets to the injured vessel wall and to one another, under flow conditions, especially under high shear rate as arterioles in the normal circulation and in stenosed arteries. In plasma, vWf forms noncovalent complex with coagulation factor VIII, whose is essential for normal survival of factor VIII. It protects factor VIII from proteolytic inactivation and appears to prolong this half-life about 5 times. Factor VIII is separated from the vWf by a thrombin activity.

Dong et al. (11) showed that UL vWf multimers secreted from the stimulated endothelial cells remained anchored onto the endothelial surface, in form several millimeters long chains. Shear stress enhances binding of UL vWf with platelets, potentially accelerating a platelet aggregation and thrombus formation. Platelets had no measurable interaction with the soluble vWf in the circulation but adhered promptly to the exposed immobilized vWf. The platelets adhered to the long strings attached to stimulated endothelial cells as "beads on a string". These structures moved together back and forth, in the blood stream (11).

The first contact between platelets and immobilized vWf, under high flow conditions, is mediated by the binding of platelet GP Iba receptor to

the A1 domain of vWf (15). The connection is achieved fast, by the multimetric nature of the vWf and a high density of the active A1 domain sites. However, this relationship has a limited half-life and cannot provide bonds supporting irreversible platelet adhesion. The platelets, tethered to the vessel wall, move constantly in the direction of flow. During the slow translocation platelets become activated. Activated platelet integrin  $\alpha\text{IIb}\beta\text{3}$  binds to the RGD sequence in the vWf C1 domain, and create an irreversible platelet adhesion. This interaction plays a role in platelet accumulation during the thrombus growth (16).

### The vWf in Pathophysiological Conditions

When the endothelium is damaged, the vWf binds to the collagen in subendothelial tissue. In areas with small and slow blood flow, the platelets bind to the walls of blood vessels, as an independent entity from the vWf. The fibrinogen stimulates platelet aggregation at low shear levels. The vWf is essential for platelet adhesion in the areas with high shear force caused by changing the size of a blood flow.

There is evidence that vWf in different anatomical locations participates in hemostatic processes (16). Although the vWf originating from subendothelial tissue directly supports an adhesion of platelets. Its distribution is inhomogeneous and is lacking a protein in many vessels where it is necessary for platelet function in hemostasis, as the subendothelium of arterioles and arterial capillaries. The plasma vWf is well suited to mediate early adhesion because it is adapted to quickly binds to collagen, particularly the proteoglycans, at the site of vascular damage. For optimum adhesion and aggregation of platelets, the additional vWf, released from endothelial cells or platelets, is required (17).

The vWf secreted from the endothelial cells also rapidly binds to the collagen fibers at the site of a vascular injury. It is exposed to high levels of shear stress, deformation of endothelial cells in the direction of blood flow, which unfold the conformation of vWf from globular to an elongated form, providing the substrate to support platelet adhesion and aggregation. Under normal flowing conditions, the vWf multimers circulate in a glo-

bular form. However, when the vWf is exposed to increased shear forces, these molecules unravel into a "stringlike" conformation. This increases the number of exposed platelet/matrix binding sites and thus enhances the platelet tethering potential of the vWf molecule. The vWf is also released by the activated platelets and is active in platelet aggregation under conditions of high shear stress and has a contribution at a later stage of creation of thrombus (18).

Fibrin is actively involved in platelet reactions essential for thrombus growth. Keuren et al. (21) have shown that the C domain of vWf is critical determinant of platelet adhesion to fibrin under conditions of high shear stress. This authors suggest that fibrin-bound vWf present at an injured vessel wall or disrupted atherosclerotic plaque may critically contribute to thrombus growth by tethering (nonactivated) platelets from fast flowing blood.

Deficit of vWf, especially in large multimers and also reduced activities of the vWf causes the von Willebrand's disease, a bleeding diathesis of skin and mucous membranes, including menorrhagia. Increase in the vWf levels observed in several clinical conditions such as diabetes mellitus, hypertension, renal failure, liver diseases and malignant tumors poses a significant risk factor for development of thrombosis (19,20). Persons with increased cardiovascular risk: smokers, people with hypertension, hypercholesterolemia, diabetes mellitus have higher values of vWf.

### Degradation of the vWf

The proteolytic cleavage of the endothelial cells derived from the UL vWF multimers is a rapid (one second to 2 minutes) physiological process that occurs on endothelial cell surfaces. The metalloprotease ADAMTS-13 (*a disintegrin and metalloprotease with a thrombospondin type 1 Motif, member 13*) enzymatically converts the prothrombotic UL vWF multimers to smaller and much less adhesive, but hemostatically active forms (22).

Thus, ADAMS-13 appears to be a key physiologic regulator of the vWf platelet functions. Shear stress facilitates UL vWF proteolysis by stretching the UL vWF multimers to an open, elongated conformation. ADAMS-13 cleaves vWf whenever

one or more of its cleavage sites are exposed by shear stress (11). Cleavage of the UL vWf strings under shear stress is up to a thousand fold faster than under static condition (22). Smaller vWf fragments were degraded by the other peptidases after separation from the endothelial cells.

An ADAMS-13 deficiency or has been inhibited by antibodies directed at the enzyme, the uncleaved, or only partially cleaved, the UI vWF will accumulate on the endothelial surface and in plasma. This results in systemic thrombotic microangiopathies, as seen in the thrombotic thrombocytopenic purpura. The patients with ADAMS-13 deficiency have an increased risk of thrombosis.

### **Factors and Conditions Affecting Concentration and Activity of the vWf In Plasma**

Concentration and activity of the vWf in plasma are influenced by many factors. In addition to age, estrogen levels, blood type, medication effects and the impact they have; it can cause some physiological (pregnancy, physical exertion) and pathological conditions (inflammation, proteolysis with ADAMS13, genetic variations, stress and malignant diseases).

Previous studies have shown that there is no difference in the concentration of the vWf in relation to gender. The vWf concentration increases with age in both sexes (23). The vWf concentration increases 2-3 times in the second and third trimester of pregnancy. There is a connection between a blood group type and a vWf concentration. Persons with blood type 0 have the lowest concentration and activity of the vWf, while the persons with blood type AB have the highest values of the vWf (24). There are disagreements about the effect of statins in reduction of the vWf concentration. Some studies show that the statins do not reduce level of the vWF. However, level of the vWf is reduced when using statins, as demonstrated in patients with hyperlipidemia, coronary artery disease (25), cerebrovascular disease and diabetic dyslipidaemia. Recent studies have shown that the fluvastatin inhibits a regulated secretion of the vWf in endothelial cells, in response to diverse secretagogues (26). In patients with the acute coronary

syndrome, an early rise in the vWf levels can be reduced by a low molecular weight heparin and by thienopyridines, such as the clopidogrel. In patients with the coronary syndrome, who do not have diabetes, the level of vWf is reduced by applying of the oral antihyperglycaemic agent. Damin and al. (27) have found the increased concentrations of the vWf in patients with colorectal cancer, and these values tend to increase, in accordance with progression of a disease.

### **The vWf as a Marker of Endothelial Damage**

Boneu et al. (28) were the first to propose measuring of plasma vWF as an indicator of endothelial damage in vascular diseases. The circulating vWf originates mainly from endothelial cells. The increased levels of this factor in plasma, probably indicates endothelial dysfunction and damage. However, the vWf is an inflammatory plasma reactant in acute phase, so that the increase of its concentration may indicate activation and endothelial stimulation, without primary damage.

Increased concentrations of the vWF were detected in the peripheral, cerebral and atherothrombotic coronary arterial disease. Increased shear stress on the stenosis arteries accelerates vWf binding to platelets and making thrombus formation. Whincup et al. (19) showed that increased concentrations of circulating vWf may be associated with an incident coronary heart disease. Constans et al. (29) while examining several biological markers, found that the vWf was found as the best indicator of endothelial biomarkers and indicator of risk of ischemic heart disease and stroke. Kiki et al. (30) determined the vWf activity in patients with acute myocardial infarction. They compared these values with the efficiency of a thrombolytic therapy. They found that the activity of the vWf was significantly lower in patients with a successful recanalization of thrombolytic therapy, applied within 6 hours of the myocardial infarction, compared to patients in whom the recanalization was unsuccessful. The patients with acute myocardial infarction and a high vWf activity had significantly less successful fibrinolytic therapy. According to these findings, the vWf can be used as an indicator of the performance impact of thrombolytic therapy. Hor-

vath et al. (31) point out that activity of the vWf may be an indicator of the progression of vascular disease. The vWf activity is higher in patients with acute than chronic vascular disease (31). The vWf is increased in patients with diabetes, especially in those with microalbuminuria and macroalbuminuria (32,33) and positive history of coronary artery disease (34). Significantly higher level of vWf has been proven in patients with diabetic than in patients with nondiabetic claudication (35). Dejanov et al. (36) have found a significant increase in biological activity of vWf in patients with venous thrombosis, diabetes mellitus, chronic renal disease, ischemic coronary disease and, according to the type of hemodialyses membrane, in hemodialysis patients. Also, these authors have highlighted the importance of vWf as markers of endothelial damage, possible markers of biocompatibility hemodialyses membrane, and markers of the risk of thrombosis, AV fistula.

Lu Guo-yuan et al. (37) found that the vWf concentration increased significantly and the activity ADAMS13 significantly reduced in patients with chronic renal disease (lupus nephritis, diabetic nephropathy, nephrotic syndrome, and chronic glomerulonephritis). Other authors had similar findings (increase of the vWf and decrease of ADAMS13) in patients with myocardial infarction, thrombotic thrombocytopenic purpura and stroke (38,39,40).

## Conclusion

The Von Willebrand factor (vWf) plays a critical role in adhesion and aggregation of platelets in the blood vessels, occurring in places of a large shear stress. The vWf participates in the secondary hemostasis, extending a half-life of a coagulation factor VIII in plasma. The increased levels of the vWf in plasma possibly indicate damage to the endothelial dysfunction, because the circulating vWf comes mainly from the endothelial cells. Increase in the vWf concentration may indicate activation and endothelial stimulation, without his primary damage, because the vWf is an inflammatory acute phase reactant. The future clinical research should focus on examining importance and role of the vWf, as an indicator of the endothelial damage, and the potential markers of atherosclerotic development and thrombotic process.



## References

1. Wagner DD. Cell biology of von Willebrand factor. *Annu Rev Cell Biol* 1990;6:217-46.
2. Andrews RK, Berndt MC. Platelet adhesion: A game of catch and release. *J Clin Invest* 2008; 118(9): 3009-11.
3. Girma JP. Biology of von Willebrand factor. *Nephrol Ther.* 2006 Jan;2 Suppl 2:143-8.
4. Shapiro J, Sclaky N, Lee J, Bosshart H Angeletti RH, Bonifacino JS. Localization of endogenous furin in cultured cell lines. *J Histochem Cytochem* 1997;45:3-12.
5. Haberichter SL, Scot AF, Montgomery RR. Von Willebrand factor storage and multimerization: 2 independent intracellular processes. *Blood* 2000;96:1808-15.
6. Vischer UM, Wagner DD. Von Willebrand factor proteolytic processing and multimerization precede the formation of Weibel-Palade bodies. *Blood* 1994;83:3536-44.
7. Michaux G, Abbitt KB, Collinson LM, Haberichter SL, Norman KE, Cutler DF. The physiological function of von Willebrand's factor depends on its tubular storage in endothelial Weibel-Palade bodies. *Dev Cell* 2006;10(2):223-32.
8. Mourik JA van, Romani de Wit T, Voorberg J. Biogenesis and exocytosis of Weibel-Palade bodies. *Histochem Cell Biol* 2002;117:113-22.
9. Borchiellini A, Fijnvandraat K, JW ten Cate, Pajkrt D, SJ van Deventer, Pasterkamp G, Meijer-Huizinga F, Zwart-Huinink L, Voorberg J, JA van Mourik. Quantitative analysis of von Willebrand factor propeptide release in vivo: Effect of experimental endotoxemia and administration of 1-deamino-8-D-arginine vasopressin in humans. *Blood* 1996;88(8):2951-8.
10. Lip GYH, Blann A. Von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? *Cardiovascular research* 1997;34:255-65.
11. Dong JF. Cleavage of ultra-large von Willebrand factor by ADAMTS-13 under flow conditions. *J Thromb Haemost* 2005;3:1710-6.
12. Vischer UM, Barth H, Wollheim CB. Regulated von Willebrand factor secretion is associated with agonist-specific patterns of cytoskeletal remodeling in cultured endothelial cells. *Arterioscler Thromb Vasc Biol.* 2000;20:883-91.
13. Giblin JP, Hewlett LJ, Hannah MJ. Basal secretion of von Willebrand factor from human endothelial cells. *Blood* 2008;112(4):957-64.
14. Johnsen J, López JA. vWf secretion: what's in a name?. *Blood* 2008;112(4):926-7.
15. Andrews RK, Berndt MC. Platelet adhesion: a game of catch and release. *J Clin Invest* 2008;118(9):3009-11.
16. Ruggeri ZM. Von Willebrand factor, platelets and endothelial cell interactions. *J Thromb Haemost.* 2003;1(7):1335-42.
17. Ruggeri ZM. Von Willebrand factor: Looking back and looking forward. *Thromb Haemost* 2007;98:55-62.
18. Nichols T, Samama C, Bellinger D, Roussi J, Reddick R, , Bonneau M. Function of von Willebrand factor after crossed bone marrow transplantation between normal and von Willebrand disease pigs: effect on arterial thrombosis in chimeras. *Proc Natl Acad Sci USA* 1995;92:2455-9.
19. Whincup PH, Danesh J, Walker M, Lennon L, Thomson A, Appleby P, Rumley A, Lowe GD: von Willebrand factor and coronary heart disease: prospective study and meta-analysis. *Eur Heart J* 2002;23:1764-70.
20. Franchini M, Lippi G: von Willebrand factor and thrombosis. *Ann Hematol* 2006;85:415-23.
21. Keuren JFW , Baruch D, Legendre P, Denis CV, Lenting PJ, Girma JP, Lindhout T. Von Willebrand factor C1C2 domain is involved in platelet adhesion to polymerized fibrin at high shear rate. *Blood* 2004;103:1741-6.
22. Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, Schade AJ, McIntire LV, Fujikawa K, Lopez JA. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood* 2002;100:4033-9.
23. Kumari M, Marmot M, Brunner E. Social determinants of von Willebrand factor The Whitehall II study. *Arterioscler Thromb Vasc Biol.* 2000;20:1842-7.
24. Franchini M, Capra F, Targher G, Montagnana M, Lippi G. Relationship between ABO blood group and von Willebrand factor levels: from biology to clinical implications. *Thrombosis Journal* 2007;5:14-8.

25. Ordulu E, Erdogan O. Early effects of low versus high dose atorvastatin treatment on coagulation and inflammation parameters in patients with acute coronary syndromes. *Int J Card* 2008;128(2):282-4.
26. Fish RJ, Yang H, Viglino CH, Schorer R, Dunoyer-Geindre S, Kruithof EKO. Fluvastatin inhibits regulated secretion of endothelial cell von Willebrand factor in response to diverse secretagogues. *Biochem J*. 2007 August 1; 405(Pt 3): 597-604.
27. Damin DC, Rosito MA, Gus P, Roisemberg I, Bandinelli E, Schwartzmann G. Von Willebrand factor in colorectal cancer. *Int J Colorectal Dis*. 2002 Jan;17(1):42-5.
28. Boneu B, Abbal M, Plante J, Bierme R. Factor VIII complex and endothelial damage. *Lancet* 1975;1:1430
29. Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. *Clin Chim Acta* 2006;368:33-47.
30. Kiki I, Köse N, Gündoğdu M, Kaya H, Çetinkaya R. Relationship between von Willebrand factor activity and therapeutic success of thrombolytic therapy in patients with myocardial infarction. *Eur J Gen Med* 2007;4(2):57-61.
31. Horvath B, Hegedus D, Szapary L, Marton Z, Alexy T, Koltai K, Gyevnar Z, Juricskay I, Toth K, Kesmarky G. Investigation of von Willebrand factor as a marker of endothelial dysfunction in atherosclerotic patients. *Orvosi hetilap* 2003;144(50):2471-6.
32. Hirano T, Ookubo K, Kashiwazaki K, Tajima H, Yoshino G, Adachi M. Vascular endothelial markers, von Willebrand factor and thrombomodulin index, are specifically elevated in type 2 diabetic patients with nephropathy: comparison of primary renal disease. *Clinica chimica acta* 2000;299(1-2):65-75.
33. Chan NN, Fuller JH, Rubens M, Colhoun HM. Von Willebrand factor in type 1 diabetes: its relationship with endothelial nitric oxide production and coronary artery calcification. *Med Sci Monit* 2003;9(7):297-303.
34. Ibrahim HA, El-Meligi AA, Abdel-Hamid M, Elhendy A. Relations between von Willebrand factor, markers of oxidative stress and microalbuminuria in patients with type 2 diabetes mellitus. *Med Sci Monit* 2004;10(3):85-9.
35. Gosk-Bierska I, Adamiec R, Alexewicz P, Wysokin-ski WE. Coagulation in diabetic and non-diabetic claudicants. *Int Angiol* 2002 Jun;21(2):128-33.
36. Dejanov P, Polenaković M, Oncevski A, Sikole A, Dejanova B, Panov S, Kostovska S. The role of the von Willebrand factor in renal diseases and haemodialysis patients. *Prilozi*. 2004;25(1-2):5-15.
37. Lu GY, Shen L, Wang ZY, Guo XF, Bai X, Su J, Ruan CG. Significance of plasma von Willebrand factor level and von Willebrand factor-cleaving protease activity in patients with chronic renal diseases. *Chin Med J (Engl)*. 2008 Jan 20;121(2):133-6.
38. Kaikita K, Soejima K, Matsukawa M, Nakagaki T, Ogawa H. Reduced von Willebrand factor-cleaving protease (ADAMTS13) activity in acute myocardial infarction. *J Thromb Haemost*. 2006; 4: 2490-3.
39. Vischer UM. von Willebrand factor, endothelial dysfunction, and cardiovascular disease. *J Thromb Haemost* 2006;4:1186-93.
40. Yoo G, Blombäck M, Schenck-Gustafsson K, He S. Decreased levels of von Willebrand factor-cleaving protease in coronary heart disease and thrombotic thrombocytopenic purpura: study of a simplified method for assaying the enzyme activity based on ristocetin-induced platelet aggregation. *Br J Haematol* 2003;121:123-9.

Corresponding author:

Nermina Babic

Institute of Physiology and Biochemistry

University of Sarajevo,

School of Medicine

Bosnia and Herzegovina

e-mail: nerminab@yahoo.com

# INSTRUCTIONS FOR THE AUTHORS

*All papers need to be sent to e-mail: healthmed\_bih@yahoo.com*

Every sent magazine gets its number, and author(s) will be notified if their paper is accepted and what is the number of paper. Every correspondence will use that number. The paper has to be typed on a standard size paper (format A4), leaving left margins to be at least 3 cm. All materials, including tables and references, have to be typed double-spaced, so one page has no more than 2000 alphanumeric characters (30 lines). Sent paper needs to be in the form of triplicate, considering that original one enclosure of the material can be photocopied. Presenting paper depends on its content, but usually it consists of a page title, summary, text references, legends for pictures and pictures. Type your paper in MS Word and send it on a diskette or a CD-ROM.

## TITLE PAGE

Every article has to have a title page with a title of no more than 10 words: name (s), last and first of the author (s), name of the institution the authors (s) belongs to, abstract with maximum of 45 letters (including space), footnote with acknowledgments, name of the first author or another person with whom correspondence will be maintained.

## SUMMARY

Second page needs to contain paper summary, 200 words at the most. Summary needs to hold all essential facts of the work-purpose of work, used methods (with specific data, if possible) and basic facts. Summaries must have review of underlined data, ideas and conclusions from text. Summary has no quoted references. For key words, at the most, need to be placed below the text.

## CENTRAL PART OF THE ARTICLE

Authentic papers contain these parts: introduction, goal, methods, results, discussion and conclusion. Introduction is brief and clear review of a problem. Methods are shown so that interested reader is able to repeat described research. Known methods don't need to be identified, it is cited (referenced). Results need to be shown clearly and logically, and their significance proven by statistical analysis. In discussion, results are interpreted and compared to existing, previously published findings in the same field. Conclusions have to give an answer to author's goal.

## REFERENCES

Quoting references must be in a scale in which they are really used. Quoting most recent literature is recommended. Only published articles (or articles accepted for publishing) can be used as references. Not-published observations and personal notifications need to be in text in brackets. Showing references is as how they appear in text. References cited in tables or pictures are also numbered according to quoting order. Citing paper with six or less authors must have cited names of all authors; if seven or more authors' wrote the paper, the name of the first three authors are cited with a note "et al". If the author is unknown, at the beginning of papers reference, the article is named as "unknown". Titles of the publications are abbreviated in accordance to Index Medicus, but if not listed in the index, whole title of the journal has to be written.

Footnote-comments, explanations, etc., cannot be used in the paper.

## STATISTICAL ANALYSIS

Tests used for statistical analysis need to be shown in text and in tables or pictures containing statistical analysis.

## TABLES AND PICTURES

Tables have to be numbered and shown by their order, so they can be understood without having to read the paper. Every column needs to have title, every measuring unit (SI) has to be clearly marked, preferably in footnotes below the table, in Arabian numbers or symbols. Pictures also have to be numbered as they appear in text. Drawings need to be enclosed on a white paper or tracing paper, while black and white photo have to be printed on a radiant paper. Legends next to pictures and photos have to be written on a separate A4 format paper. All illustrations (pictures, drawings, diagrams) have to be original and on their backs contain illustration number, first author last name, abbreviated title of the paper and picture top. It is appreciated if author marks the place for table or picture. Preferable the pictures format is TIF, quality 300 DPI.

## USE OF ABBREVIATIONS

Use of abbreviations has to be reduced to minimum. Conventional units can be used without their definitions.

# UPUTSTVO ZA AUTORE

*Sve rukopise treba slati na e-mail adresu healthmed\_bih@yahoo.com*

Svaki upućeni časopis dobija svoj broj i autor(i) se obavještavaju o prijemu rada i njegovom broju. Taj broj koristeće se u svakoj korespondenciji. Rukopis treba otipkati na standardnoj veličini papira (format A4), ostavljajući s lijeve strane marginu od najmanje 3 cm. Sav materijal, uključujući tabele i reference, mora biti otipkan dvostrukim proredom, tako da na jednoj strani nema više od 2.000 alfanumeričkih karaktera (30 linija). Rad treba slati u triplicatu, s tim da original jedan prilog materijala može biti i fotokopija. Način prezentacije rada ovisi o prirodi materijala, a (uobičajeno) treba da se sastoji od naslovne stranice, sažetka, teksta, referenci, tabela, legendi za slike i slika. Svoj rad otipkajte u MS Wordu i dostavite na disketi ili kompaktnom disku Redakcijskom odboru, čime će te olakšati redakciju vašeg rada.

## NASLOVNA STRANA

Svaki rukopis mora imati naslovnu stranicu s naslovom rada ne više od 10 riječi: imena autora; naziv ustanove ili ustanova kojima autori pripadaju; skraćeni naslov rada s najviše 45 slovnih mjesta i praznina; fusnotu u kojoj se izražavaju zahvale i/ili finansijska potpora i pomoć u realizaciji rada, te ime i adresa prvog autora ili osobe koja će s Redakcijskim odborom održavati i korespondenciju.

## SAŽETAK

Sažetak treba da sadrži sve bitne činjenice rada-svrhu rada, korištene metode, bitne rezultate (sa specifičnim podacima, ako je to moguće) i osnovne zaključke. Sažeci trebaju da imaju prikaz istaknutih podataka, ideja i zaključaka iz teksta. U sažetku se ne citiraju reference. Ispod teksta treba dodati najviše četiri ključne riječi.

## SAŽETAK NA BOSANSKOM JEZIKU

Prilog radu je i prošireni struktuirani sažetak (cilj), metode, rezultati, rasprava, zaključak) na bosanskom jeziku od 500 do 600 riječi, uz naslov rada, inicijale imena i prezimena autora te naziv ustanova na engleskom jeziku. Ispod sažetka navode se ključne riječi koje su bitne za brzu identifikaciju i klasifikaciju sadržaja rada.

## CENTRALNI DIO RUKOPISA

Izvorni radovi sadrže ove dijelove: uvod, cilj rada, metode rada, rezultati, rasprava i zaključci. Uvod je kratak i jasan prikaz problema, cilj sadrži kratak opis svrhe istraživanja. Metode se prikazuju tako da čitaoci omoguću ponavljanje opisanog istraživanja. Poznate metode se ne navode nego se navode izvorni literaturni podaci.

Rezultate treba prikazati jasno i logički, a njihovu značajnost dokazati odgovarajućim statističkim metodama. U raspravi se tumače dobiveni rezultati i uspoređuju s postojećim spoznajama na tom području. Zaključci moraju odgovoriti postavljenom cilju rada.

## REFERENCE

Reference treba navoditi u onom obimu koliko su stvarno korištene. Preporučuje se navođenje novije literature. Samo publicirani radovi (ili radovi koji su prihvaćeni za objavljivanje) mogu se smatrati referencama. Neobjavljena zapažanja i lična saopćenja treba navoditi u tekstu u zagradama. Reference se označavaju onim redom kako s pojavljuju u tekstu. One koje se citiraju u tabelama ili uz slike također se numeriraju u skladu s redoslijedom citiranja. Ako se navodi rad sa šest ili manje autora, sva imena autora treba citirati; ako je u citirani članak uključeno sedam ili više autora, navode se samo prva tri imena autora s dodatkom "et al". Kada je autor nepoznat, treba na početku citiranog članka označiti "Anon". Naslovi časopisa skraćuju se prema Index Medicusu, a ako se u njemu ne navode, naslov časopisa treba pisati u cjelini. Fusnote-komentare, objašnjenja, itd. Ne treba koristiti u radu.

## STATISTIČKA ANALIZA

Testove koji se koriste u statističkim analizama treba prikazivati i u tekstu i na tabelama ili slikama koje sadrže statistička poređenja.

## TABELE I SLIKE

Tabele treba numerirati prema redoslijedu i tako ih prikazati da se mogu razumjeti i bez čitanja teksta. Svaki stubac mora imati svoje zaglavlje, a mjerne jedinice (SI) moraju biti jasno označene, najbolje u fusnotama ispod tabela, arapskim brojevima ili simbolima. Slike također, treba numerisati po redoslijedu kojim se javljaju u tekstu. Crteže treba priložiti na bijelom papiru ili paus papiru, a crno-bijele fotografije na sjajnom papiru. Legende uz crteže i slike treba napisati na posebnom papiru formata A4. Sve ilustracije (slike, crteži, dijagrami) moraju biti originalne i na poleđini sadržavati broj ilustracije, prezime prvog autora, skraćeni naslov rada i vrh slike. Poželjno je da u tekstu autor označi mjesto za tabelu ili sliku. Slike je potrebno dostavljati u TIFF formatu rezolucije 300 DPI.

## KORIŠTENJE KRATICA

Upotrebu kratica treba svesti na minimum. Konvencionalne SI jedinice mogu se koristiti i bez njihovih definicija.