ISSN 1840-2291

ſ

Journal of Society for development in new net environment in B&H



# Volume 8 / Number 2 / 2014



#### **EDITORIAL BOARD**

Technical Editor Eldin Huremovic

Editor-in-chief Mensura Kudumovic

Members

Paul Andrew Bourne (Jamaica) Xiuxiang Liu (China) Nicolas Zdanowicz (Belgique) Farah Mustafa (Pakistan) Yann Meunier (USA) Suresh Vatsyayann (New Zealand) Maizirwan Mel (Malaysia) Budimka Novakovic (Serbia) Diaa Eldin Abdel Hameed Mohamad (Egypt) Omar G. Baker (Kingdom of Saudi Arabia) Amit Shankar Singh (India) Chao Chen (Canada) Zmago Turk (Slovenia) Edvin Dervisevic (Slovenia) Aleksandar Dzakula (Croatia) Farid Ljuca (Bosnia & Herzegovina) Sukrija Zvizdic (Bosnia & Herzegovina) Bozo Banjanin (Bosnia & Herzegovina) Gordana Manic (Bosnia & Herzegovina)

Address Hamdije Kresevljakovica 7A, 71 000 Sarajevo, Bosnia and Herzegovina. **Editorial Board** e-mail: healthmedjournal@gmail.com web page: http://www.healthmed.ba Published by DRUNPP, Sarajevo Volume 8 Number 2, 2014 1840-2291 e-ISSN 1986-8103 ISSN

#### HealthMED journal is indexed in:

- EBSCO Academic Search Complete
- EBSCO Academic Search Premier,
- EMBASE,
- SJR Scopus,
- Index Copernicus,
- Universal Impact Factor: Impact Factor is 1.0312 (UIF 2012)
- Electronic Social and Science Citation Index (ESSCI),
- Direct Science,
- ISI institute of science index,
- SCImago Journal and Country Rank,
- ISC Master Journal List,
- Genamics Journal Seek,
- World Cat,
- Research Gate,
- CIRRIE,
- getCITED and etc.

# Sadržaj / Table of Contents

Acute Organophosphate Poisoning in Adults: A 10-Year Analysis	Factors related to the frequency of citationof the Journal of Orthopaedic & SportsPhysical Therapy253Bayram Unver; Fatma U. Kocak, Mehmet Erduran
Founder effect analysis of disease haplotypes in DFNB23/ USH1F linked Pakistani families	<b>The correlation of a postural status and isometric</b> <b>endurance with rowers of school age264</b> <i>Sasa Milenkovic, Mladen Zivkovic, Dobrica Zivkovic,</i> <i>Sasa Bubanj, Zoran Bogdanovic, Sladjan Karaleic</i>
Nakhshab Chaudhary, Fridoon Jawad Ahmad Role of metacognitive beliefs and thought control strategies in mental health	Species distribution and Caspofungin susceptibility of Candida spp. isolated from blood cultures269Yesim Cekin, Nevgun Sepin Ozen, Nilgun Gur, Hamit Yasar Ellidag
Total hip artroplasty with long oblique subtrochanteric shortening osteotomy in high developmental dysplasia of hip	Diabetic kidney disease and its associated complications
Hicabi Sezgin   Trends in epidemiology of tuberculosis in   HIV-infected patients   Iosif Marincu, Simona Claudia Cambrea, Adelina Mavrea,	White matter hyperintensities and relatedrisk factors in Chinese normal aging :A community based study
Mirela Cleopatra Tomescu   ABO/Rh blood groups distribution and serum   lipid profile: Is there any association?	Radiological classification of newly formed alveolar bone: A Cone Beam CT study
Nania Menrzad, Shiva Khalegnparast A computer vision based system for a rehabilitation of a human hand	Relevance of evaluation for quality assurance in the field of health education work - evaluation of midwifery studies
Radiologic assessment of apical periodontitis and its relation with root canal filling quality	Imported visceral Leishmaniasis in   western Romania: Report of four cases   Iosif Marincu, Nicoleta Bertici, Livius Tirnea   Instructions for the authors   298
Effects of berberine on the expression of AQP-2, IL-1 and IL-6 in a rat model of streptozotocin-induced diabetes mellitus	Instructions for the authors
The prevalence and risk factors associated with otorhinolaryngologic diseases among the students of Hakkari City center	
The prevalence and the nature of violence directed at the medical staff in psychiatric health care in Slovenia	
<b>The dependence of explosive strength and</b> <b>speed on feet posture</b>	
Effect of WIN51,708, a NK <sub>1</sub> receptor antagonist, on the signal transmission between two endings of primary afferent nerves from adjacent spinal segments*	

# Acute Organophosphate Poisoning in Adults: A 10-Year Analysis

Ahmet Baydin<sup>1</sup>, Ali Kemal Erenler<sup>2</sup>, Turker Yardan<sup>1</sup>, Celal Kati<sup>2</sup>, Latif Duran<sup>2</sup>, Ahmet Dilek<sup>3</sup>

<sup>1</sup> Ondokuz Mayis University, Faculty of Medicine, Department of Emergency Medicine, Samsun, Turkey,

<sup>2</sup> Corum State Hospital, Corum, Turkey,

<sup>3</sup> Ondokuz Mayis University, Faculty of Medicine, Department of Intensive Care, Samsun, Turkey.

#### Abstract

Organophosphate poisoning (OP) is an important medical emergency that may have serious clinical outcomes. The aim of this study was to report our 10-year experience in patients with acute OP. This cross-sectional study was designed retrospectively by examining the files of patients with OP who were admitted to the emergency department (ED). Diagnosis was based on a clinical assessment and serum acetylcholinesterase (AChE) level at the time of hospital admission. A total of 225 patients poisoned by organophosphates (mean age, 41.6±16.9 years; 53.3% male) were studied. Most of the poisonings were with suicidal intent (58.7%). The common symptoms and complaints on admission were nausea and vomiting (54.7%). The most frequent route of exposure to organophosphates was oral (71.1%). Most of the poisonings occurred in summer (40.4%). The mean serum AChE level on admission was 3804.4±3300.2 (normal range: 5400-13200 U/L). The patients were treated in the emergency medicine ward (78.2%) and intensive care unit (21.8%). Hemoperfusion was performed in 8.4% of all patients. Intermediate Syndrome developed in 16% of all patients. The duration of hospitalization was 4.3±3.6 days. Twenty-eight patients (12.4%) died in the hospital due to complications. The majority of OP in this study was a result of attempted suicide. Physicians in the ED must be more alert to the possibility of organophosphates in summer, when these chemicals are commonly used for pest control. Prohibition of use of these chemicals by uneducated and unequipped people will help to decrease the incidence of OP.

Key words: organophosphate poisoning, emergency department, acetylcholinesterase, intoxication

#### Introduction

Organophosphates are toxic substances that are factors in suicidal, accidental or occupational poisoning, and they are responsible for the death of hundreds of thousands of people annually [1,2]. Poisonings with these toxic substances are frequent among those who are unemployed, uneducated or with lower socioeconomic status and among farmers [3-6].

Most organophosphates are highly lipid-soluble agents that are well absorbed by all routes - gastrointestinal, respiratory and skin. Organophosphates show their toxic effects by inhibiting acetylcholinesterase (AChE) enzyme after entering the body. As a result of AChE enzyme inhibition, the substrate acetylcholine accumulates at the cholinergic synapses of the central nervous system, neuromuscular junction, parasympathetic nerve endings and some sympathetic nerve endings such as sweat glands (muscarinic effects), and somatic nerves and ganglionic synapses of autonomic ganglia (nicotinic synapses). The continued stimulation and eventual paralysis of the acetylcholine receptors account for the clinical signs and symptoms of organophosphate poisoning (OP) [2,7]. The onset of symptoms and signs occur within the first 8 hours and nearly all within the first 24 hours [8].

Organophosphate poisoning is generally a serious condition for patients in the emergency medicine ward or intensive care unit (ICU). Therefore, early diagnosis and appropriate treatment are often life saving. It is known that physicians should be on alert for the diagnosis and treatment of OP. The purpose of this study was to report our 10-year experience in patients with acute OP.

## **Materials and Methods**

This cross-sectional study was designed retrospectively by examining the files of patients with OP who were admitted to Ondokuz Mayis University Emergency Department (ED) between January 1, 2000 and January 1, 2010. During this period, we determined that 260 adult patients were admitted to the ED due to OP. Patients whose medical records could not be obtained, those with carbamate poisonings, those admitted after 24 hours, and 35 others with inadequate medical records were excluded from the study. The patients either admitted primarily to the ED or were referred from other regional hospitals. The study protocol was approved by the local ethics committee.

A form was prepared on which the following information was recorded: demographic data (age, sex), complaint, pulse rate, respiratory rate, blood pressure, time elapsed between exposure and admission to the ED, mental status, route of intake (ingestion, inhalation, skin contact, injection), intent of ingestion, duration of hospital stay, month of OP occurrence, laboratory findings, therapeutic options, and clinical outcomes. Patients were subdivided into four age groups as: <25, 25-34, 35-44, and >44 years. The Glasgow Coma Scale (GCS) was used to assess the patient's level of consciousness.

The diagnosis of acute OP was based on the presence of the following criteria: (I) history of exposure to or contact with organophosphates within the last 24 hours (h); (II) characteristic clinical signs and symptoms of OP; (III) improvement in signs and symptoms after treatment with atropine and oximes; (IV) definition of responsible organophosphate agents; and (V) decreased serum cholinesterase activity [9,10].

The patients were divided into two groups according to the intent of the ingestion (Group 1, suicidal poisoning; Group 2, accidental poisoning). Additionally, the patients were allocated to two groups according to the severity of their clinical status (Group 1, severe poisoning; Group 2, mild or moderate poisoning).

The severity of the clinical status was assessed on admission according to: (I) increased severity and number of findings and symptoms; (II) decreased blood pressure; and (III) presence of specific clinical respiratory system and central nervous system findings [9-15].

Serum pseudocholinesterase activity and other laboratory parameters such as electrolytes, amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, glucose, and white blood cell (WBC) count were measured on admission.

After the diagnosis, all patients received standard treatment for OP, including gastric lavage, fluid resuscitation, and activated charcoal via nasogastric tubes. Patients received atropine to counteract muscarinic effects, such as hypersecretion, lacrimation and bradycardia, and also Pralidoxime to reactivate AChE enzyme inhibited by the organophosphates. Atropine was administered as intravenous infusion (0.02-0.08 mg/kg/h) or intermittent bolus infusions (1-3 mg per 20 minutes). Pralidoxime was administered as a starting dose of 2 g daily (divided into four doses) up to 100-200 mg/h (continuous infusion) according to the clinical severity of the condition. Most of the patients were observed in the emergency medicine ward. Patients who required mechanical ventilation (MV) were observed in the ICU. The indications for endotracheal intubation and MV were as follows: a loss of consciousness; excessive secretions, which cause an inability to protect the airway; poor gas exchanges unresponsive to oxygen treatment; cardiorespiratory arrest; and severe metabolic acidosis with hemodynamic instability (systolic blood pressure <90 mmHg).

All statistical calculations were made using the SPSS<sup>®</sup> for Windows 13.0 (SPSS Inc. Headquarters, Chicago, IL, USA) software program. Data were presented as mean  $\pm$  SD and frequencies. Mann-Whitney U test was used to compare the groups according to continuous variables for the data that were not distributed normally. The discrete variables were evaluated by Chi-square test. The outcomes with p values <0.05 were considered significant.

# Results

The study group consisted of 120 (53.3%) male and 105 (46.7%) female patients, with a mean age of 41.6 $\pm$ 16.9 years (45.2 $\pm$ 17.2 in males and 37.5 $\pm$ 15.8 in females p<0.05). There were two causes of OP: suicidal attempt (n=132, 58.7%) or accidental exposure (n=93, 41.3%). Of the patients who attempted suicide, the route of exposure was oral in 129 and intramuscular in 3. The routes of accidental exposure were inhalation, oral and via the skin in 55 (59.1%), 31 (33.3%) and 7 (7.6%) patients, respectively. The mean ages of the patients according to groups were as follows: suicidal attempt group:  $38.8\pm15.1$  years, accidental exposure group:  $45.8\pm18.7$  years, clinically severe group:  $42.3\pm17.7$  years, and clinically mild or moderate group:  $41.7\pm16.7$  years. While there

was no statistically significant difference between the clinically severe and mild groups, the mean age of the suicidal attempt group was significantly lower than that of the accidental exposure group (p<0.01) (Tables 1, 2). The distribution of gender according to age groups is shown in Table 3.

The most common responsible agents for OP were Methamidophos and Dichlorvos. The distribution of cases according to responsible organophosphate agents is shown in Table 4.

	Suicidal attempt (n=134)	Accidental (n=91)	<i>p</i> Value
Age (year)	38.8±15.1	45.8±18.7	< 0.01
Pseudocholinesterase (U/L)	3035.9±3213.3	4936.1±3112.1	< 0.001
White Blood Cell (Thousand/uL)	13413.9±7235.9	10764.4±4706.9	< 0.01
Glucose (mg/dL)	153.3±98.4	119.5±41.5	< 0.01
Amylase (U/L)	209.0±243.9	110.9±76.7	< 0.001
AST (U/L)	55.8±284.2	27.8±58.2	< 0.01
ALT (U/L)	35.8±148.3	26.1±53.1	>0.05
Creatinine (mg/dL)	0.8±0.6	0.7±0.3	>0.05
Sodium (mEq/L)	137.4±4.3	137.5±3.4	>0.05
Potasium (mEq/L)	3.7±0.6	3.9±0.4	< 0.01
Calcium (mg/dL)	8.8±0.8	8.9±0.7	>0.05
Arrival time (hour)	3.3±1.0	3.6±1.1	>0.05
Pulse rate (beats/min)	93.3±23.6	83.3±17.3	< 0.001
Respiratory rate (breath/min)	23.1±3.9	21.9±2.5	< 0.01
Systolic blood pressure (mmHg)	117.1±24.5	124.1±27.7	>0.05
Duration of hospital stay (day)	5.1±4.3	2.9±1.8	< 0.001

Table 1. The findings of the study group according to aim of ingestion (mean±SD)

*Table 2. The findings of the study group according to clinical severity (mean±SD)* 

	Severe (n=61)	Mild (n=164)	<i>p</i> Value
Age (year)	42.3±17.7	41.7±16.7	>0.05
Pseudocholinesterase (U/L)	2277.2±2984.1	4372.5±3239.2	< 0.001
White Blood Cell (Thousand/uL)	15057.4±8650.8	11332.5±5094.8	< 0.01
Glucose (mg/dL)	201.5±132.4	119.5±41.5	< 0.001
Amylase (U/L)	272.5±264.1	130.9±154.4	< 0.001
AST (U/L)	91.3±419.9	27.1±45.2	< 0.001
ALT (U/L)	53.4±219.1	23.9±40.4	>0.05
Creatinine (mg/dL)	0.9±0.7	0.7±0.4	>0.05
Sodium (mEq/L)	136.4±5.1	137.8±3.4	< 0.05
Potasium (mEq/L)	3.7±0.7	3.8±0.5	>0.05
Calcium (mg/dL)	8.6±0.9	8.9±0.7	< 0.001
Arrival time (hour)	3.6±0.9	3.3±1.1	>0.05
Pulse rate (beats/min)	95.2±29.5	87.0±17.8	>0.05
Respiratory rate (breath/min)	22.9±4.4	22.5±3.1	>0.05
Systolic blood pressure (mmHg)	110.5±30.4	123.5±23.3	< 0.01
Duration of hospital stay (day)	6.5±4.9	3.4±2.6	< 0.001

Age groups (years)	Female*	Male*	Total**	р			
<25	27 (60.0%)	18 (40.0%)	45 (20.0%)				
25–34	30 (56.6%)	23 (43.4%)	53 (23.6%)	X <sup>2</sup> =12.8			
35–44	20 (50.0%)	20 (50%)	40 (17.8%)	df=3			
>44	28 (32.2%)	59 (67.8%)	87 (38.7%)	p <0.01			
Total	105 (46.7%)	120 (53.3%)	225 (100%)				

*Table 3. The distribution of gender according to age groups* 

\* Line percent

\*\* Column percent

*Table 4. General characteristics of the study group (n=225)* 

Characteristics	Number of patients (%)	p value
Gender		
Male	120 (53.3%)	
Female	105 (46.7%)	
Age (years)		
25<	45 (20.0%)	X <sup>2</sup> =23.9
25–34	53 (23.6%)	df=3
35–44	40 (17.8%)	p < 0.001
> 44	87 (38.7%)	
Season		
Spring	50 (28.0%)	X <sup>2</sup> =43.1
Summer	55 (40.4%)	df=3
Autumn	37 (23.4%)	p < 0.001
Winter	22 (10.3%)	
Mental status		X72 56 2
Fully awake	128 (56.9%)	$X^2=56.2$
Drowsy or stupor	49 (21.8%)	df=2
Coma	48 (21.3%)	p <0.001
Aim of ingestion		
Suicidal attempt	132 (58,7%)	
Accidental	93 (41.3%)	
Route of poisoning		
Oral ingestion	160 (71.1%)	X <sup>2</sup> =284.9
Inhalation	55 (24.4%)	df=3
Skin contact	7 (3.1%)	p < 0.001
Intramuscular	3(1.4%)	1
Responsible Agents		
Methamidophos	65 (28.9%)	
Dichlorvos	51 (22.7%)	X <sup>2</sup> =37.7
Parathion	31 (13.8%)	df=5
Dikloro difenol trikloroethan	29 (12.9%)	p < 0.001
Malathion	28(12.4%)	P
Fenthion	21 (9.3%)	
Clinical severity		
Severe	61 (27.1%)	
Mild	164 (72.9%)	
Care unit		
Emergency Medicine Ward	176 (78.2%)	
Intensive Care Unit	49 (21.8%)	
Intermediate syndrome		
Developed	36 (16%)	
Not developed	189 (84%)	
Clinical outcomes		
Survived	197 (87.6%)	
Death	28 (12.4%)	

The most common symptoms and complaints on admission were nausea and vomiting (54.7%), altered mental status (26.2%), respiratory distress (9.8%), weakness (4.9%), and headache (4.4%).

The mental status of patients on admission was generally good. The distribution of patients according to mental status is shown in Table 4.

The mean time lapse from exposure to arrival at the hospital according to groups was as follows: suicidal attempt group:  $3.3\pm1.0$  hours, accidental exposure group:  $3.6\pm1.1$  hours, clinically severe group:  $3.6\pm0.9$  hours, and clinically mild group:  $3.3\pm1.1$  hours. There was no statistically significant difference between groups [suicidal vs. accidental (p>0.05), severe vs. mild (p>0.05)] according to arrival time (Tables 1, 2).

The majority of intoxication cases occurred in the summer. The seasonal distribution of patients is shown in Table 4.

One hundred and seventy-six (78.2%) of the patients were admitted to the emergency medicine ward, and 49 (21.8%) to the ICU. The mean hospitalization period of the study group was  $4.3\pm3.6$  days (range: 1-21 days). The hospitalization periods of the patients according to groups were as follows: Suicidal attempt group:  $5.1\pm4.3$  days, accidental exposure group:  $2.9\pm1.8$  days, clinically severe group:  $6.5\pm4.9$  days and clinically mild group:  $3.4\pm2.6$  days. There were statistically significant differences between groups [suicidal vs. accidental (p<0.001), severe vs. mild (p<0.001)].

The means of systolic pressure, pulse and respiratory rate on admission were  $119.9\pm26.0$  mmHg,  $89.2\pm21.8$ /minute and  $22.6\pm3.5$ /minute, respectively.

Serum pseudocholinesterase activity was determined in all patients on admission. The mean serum pseudocholinesterase level on admission was  $3804.4.2\pm3300.2$  (normal range: 5400-13200U/L). The values in 150 (66.7%) cases were lower than the normal range. The lowest serum pseudocholinesterase level was 79 U/L. There was a statistically significant difference between the suicidal attempt and accidental exposure groups (p<0.001), and also between the clinically severe and mild groups (p<0.001). Findings of the patients according to the intent of ingestion and clinical severity are presented in Tables 1 and 2.

Initial treatment was given to all patients in the ED. While 49 (21.8%) of the patients needing endo-

tracheal intubation and MV were treated in the ICU, 176 (78.2%) patients were treated in the emergency medicine ward. The treatment methods were as follows: 176 (78.2%) patients underwent gastrointestinal decontamination, 177 (78.7%) were administered pralidoxime, 197 (87.6%) were administered atropine, and 19 (8.4%) were administered hemoperfusion. While death occurred in 4(21.1%) of the patients who had undergone hemoperfusion therapy, 24 (11.7%) of the patients who had not undergone hemoperfusion therapy died. There was no statistically significant difference between patients who underwent hemoperfusion or not with respect to mortality. Intermediate syndrome (IS) was observed in 36 (16%) patients. Although frequency of IMS was found higher in patients with Folidon (29.0%) and Tamaron (21.5%) poisonings there was no statistically significant difference between the groups those were poisoned with different organophosphate compounds (X<sup>2</sup>=9.2, df=5 p>0.05). The mortality rate of the study group was 12.4% (28 patients) (Table 4).

# Discussion

This retrospective study describes the demographic and clinical features and laboratory findings of patients with OP in Samsun province. Samsun is located in the northern part of Turkey, with a population of approximately 1.2 million people who are primarily involved in agriculture. As a result, organophosphates are widely used to increase production and raise crop quality, and there are many cases of intoxication, either accidental or with suicidal intent. Our results showed that more poisoning cases were with suicidal intent rather than due to accidental exposure. Moreover, the majority of cases in the suicidal attempt group were young females. Previous studies conducted in Turkey also reported that females were admitted more frequently because of suicidal poisoning [4,5,16,17].

Poisonings due to organophosphate compounds that are used as pesticide occur frequently in other developing countries as well. The frequency of poisonings due to this toxic compound varies between countries and by gender. It was reported that while males are mostly affected by organophosphate compounds in Australia, Portugal and Korea [18-20], females are more often affected in Singapore, Tunisia and Jordan [3,13,21]. Various studies from our country have reported that females are more commonly affected by organophosphate compounds than males [4-6,17]. The overall male to female ratio was 1.1:1 in our study.

Every year, patients of various age groups are poisoned due to organophosphate exposure in both developed and developing countries and some outcomes are fatal. Therefore, OP is a serious public health problem that must be solved. In a small study in Crete, Greece, patients' ages ranged from 13 to 74 years, with the highest number of cases being over 44 years of age [22]. In their study, Kang et al. [20] reported that patients' ages varied between 16-91 years, and the mean age was 54.5 years. Lee and Tai [21] determined the clinical features of 23 patients with OP who required intensive care and reported that the patients' ages ranged between 19-87 years, with a mean of 40.0±18.5 years. In another study with 75 patients in South Korea, it was reported that patients' ages varied between 6-79 years (mean: 45.0±17.2 years). It was also reported that in serious OP cases, this average age tends to increase [23]. In our study, the mean age was  $41.6\pm16.9$ years, and the majority of the patients (38.7%) were older than 44 years. This result is concordant with the other reports.

In developing third-world countries, it has been reported that young adults commit suicide using organophosphate compounds for various reasons, such as unemployment, low income, depression, and single status, and for this intent, the oral route is frequently preferred [3,13,23]. In developed countries, accidental exposures are seen more frequently [5,18,24]. While suicidal OPs are observed at a ratio of 90% in third-world countries, this ratio is 13-36% in developed countries [13,18,19,23]. Different studies from Turkey have reported that suicidal attempts with organophosphate compounds were observed at ratios of 68-84% [4,6,17]. In our study, we determined that 59.5% of the patients had suicidal intent, and the majority preferred the oral route. The ratio of suicidal attempts in our study was lower than seen in developing countries but higher than in developed countries. We think that the high rate of organophosphate compound poisoning in our country can be attributed to the uncontrolled trade of this material, its inappropriate conservation, and its widespread use by the public and consequent availability.

The duration of hospitalization after poisoning differs according to the severity of the poisoning, presence of complications such as cardiovascular collapse, respiratory failure, aspiration pneumonia, septic shock, and IS, presence of accompanying diseases, and developmental levels of the country. While duration of hospital stay ranges between 2-7 days in developed countries, it can range between 3-12 days in developing countries [18,21,25]. In our country, this period was reported as 4.1-6.7 days in previous studies. In our study, the average stay in the hospital was 4.3±3.6 days (range: 1-21 days) [4,6,26]. While the duration of hospital stay in our study was similar to other results from our country and developed countries, it was shorter than that observed in the developing countries. The longer hospital stay is likely due to the facts that most cases of suicidal attempt ingest large amounts of organophosphate compounds and a high percentage of them require MV, and because of the possible complications that can develop in severely poisoned patients managed in the ICU. While the percentage of suicidal attempts was high in our study (59.5%), the percentage of those who required intensive care was lower than in developing countries (21.8%).

In studies conducted in Turkey, it was reported that accidental and suicidal OPs were mostly seen in July because of the increase in agricultural activities in this month [4,27,28]. Similarly, Soysal et al. [29] reported that OP was frequently seen in the summer months. Dippenaar et al. [30] reported that OPs were frequently seen in January in South Africa. We determined that poisonings were mostly seen in summer, especially in June and July. In our region, organophosphate compounds are widely used as pesticides both in agriculture and at home.

After exposure to organophosphates, the occurrence of toxic effects is dependent on the amount of the compound, and this delay until the onset of effects ranges from 30 minutes to 2 hours [31]. The period between exposure and treatment affects morbidity and mortality. An increase in this period negatively affects morbidity and mortality rates. Thus, the early admission of poisoned patients is of critical importance. In the literature, it was reported that the time of admission of poisoned patients varied between 2.4 - 9.4 hours [4-6,17,25,26,32]. The variety in admission times may be due to the distance between hospitals and city centers; the need to refer the poisoned patients to an advanced center with intensive care unit; delayed referral of patients under follow-up with worsening findings to an advanced center; and suicidal versus accidental etiology. In our study, the mean arrival time to the emergency unit was  $3.4\pm1.0$  h (range: 1–24 h) after the exposure. This finding is similar to that of other studies in the literature.

In the literature, both in case reports and clinical studies, abnormalities in laboratory findings of patients with OP have been reported. In many studies, increases in glucose level and WBC count were defined as the most common laboratory finding abnormalities after OP. Electrolyte abnormalities such as hyponatremia and hypokalemia, and increase in AST and ALT enzymes and hyperamylasemia are reported less commonly [5,6,16]. Amanvermez et al. [16] reported that there was a correlation between the severity of poisoning and glucose and WBC count levels in their study investigating suicidal poisoning with organophosphates. In another study that compared the initial laboratory findings of patients who died versus survived after OP, it was found that mean AST and ALT levels were higher in the deceased patients than in those who survived, but there was no statistically significant difference between the groups [20]. In a study involving 47 patients in the ICU, Sungur and Guven [17] reported increases in WBC count in 72.3%, in serum glucose level in 31.9%, in lactate dehydrogenase (LDH) level in 31.9%, and in AST and ALT levels in 23.4% of the patients. Ozer et al. [6] reported hyperglycemia in 74.6%, hyperamylasemia in 73.0%, hyponatremia in 42.8%, and hypokalemia in 41.2%, and increases in LDH in 58.7%, and in ALT levels in 19% of the patients. In another study, leukocytosis in 68.7%, hyperamylasemia in 36.1%, increase in AST in 33%, and increase in LDH in 29.8% of the patients were reported. [32] Naggar et al. [25] reported that liver function tests were normal in 89.4% of the patients after poisoning but in 8.5%, both AST and ALT levels were elevated. They also reported that the most common electrolyte abnormalities were hyponatremia (61.7%) and hypokalemia (61.7%). In our study, we evaluated laboratory findings on admission to the emergency room as mean values according to both clinical severity and intent of intake. While there were no statistically significant differences in ALT and creatine levels between groups according to clinical severity and intent of intake, there were statistically significant differences in AST, calcium, WBC count, glucose, amylase, and sodium between the clinically mild and severe groups, and in WBC count, glucose, amylase, AST, and potassium levels between the suicidal attempt and accidental exposure groups (Tables 1, 2).

Intermediate syndrome (IS), first defined by Senanayake and Karaliedde, is a clinical situation resulting from the neurotoxic effects of organophosphates. The frequency of IS varies between 7.7-42.1% [33,34]. It is characterized by respiratory paralysis, proximal muscle weakness and motor cranial nerve palsies [13,33,35,36]. There are numerous theories about the etiology of IS, such as the severity of poisoning, some varieties of organophosphates (methamidophos, dimethoate), inadequate or delayed initiation of oxime therapy, and the persistence of organophosphate in the body [33,35]. In our study, we determined IS development in 16% of the patients in spite of general supportive therapy and early Pralidoxime treatment on admission to the ED. Although there wasn't statistically significant difference between the groups those were poisoned with different organophosphate compounds, it's thought that IMS was more likely to develop in patients with highly toxigenic organophosphate compound poisoning. The majority of the patients (86.1%) who developed IS were admitted due to suicidal attempt. It was thought that great amounts of organophosphates ingested to terminate life and the consequent prolonged inhibition of AChE can explain this result in the patients who attempted suicide.

Lee and Tsai [21], in their study investigating the clinical features of patients with acute OP who required ICU follow-up, reported that the serum cholinesterase level was found to be less than 500 U/L in severely poisoned patients. They divided patients into three groups according to the degree of cholinesterase activity repression on admission as mild, moderate and severe. In another retrospective study of patients with suicidal attempt, patients were divided into three groups as mild, moderate and severe according to their clinical and laboratory findings. It was reported that serum cholinesterase levels in the mild, moderate

and severe poisoned groups were 2892.4±2458.8 U/L, 1329.0±1250.2 U/L, 593.4±422.5 U/L, respectively, and that serum cholinesterase levels in the severe poisoned group were significantly more repressed in comparison with the other groups [16]. Nouira et al. [13] reported that mean serum cholinesterase level was 448±409 U/L in patients with life-threatening poisoning and 611±575 U/L in the mild and severe groups without life-threatening poisoning. In another study, the mean serum cholinesterase level was found to be 110.7±167.9 IU/L in patients who died, and it was stated that the cholinesterase level was an important marker in estimating prognosis and closely related with mortality.<sup>20</sup> In contrast to the reports of Nouira [13] and Kang [20], Aygun et al. [9] reported that decreased levels of serum cholinesterase do not indicate the severity in acute OP but support the diagnosis. In another study, it was reported that the mean serum cholinesterase level in patients with OP was 1782.5±1965.7 U/L and that serum cholinesterase levels tended to decrease inversely proportional to the severity of poisoning [26]. Yurumez et al. [32] declared that serum cholinesterase levels on admission were more repressed in females than males (mean cholinesterase levels were 1592.6±1735 U/L and 1918±2155.4 U/L in females and males, respectively). In our study, a significant difference was found in the serum cholinesterase levels between groups according to both severity and intent of intake (severe group 2277.2±2984.1 U/L vs. mild group 4372.5±3239.2 U/L; suicidal group 3035.9±3213.3 U/L vs. accidental group 4936.1±3112.1 U/L).

Current therapy for OP consists of airway control, intensive respiratory support, general supportive measures, decontamination, prevention of absorption, and the administration of antidotes [7,8]. In addition, hemoperfusion would be considered in the treatment of severe OP; however, its effectiveness is controversial [10,20]. In our study, nasogastric irrigation was administered to 78.2%, pralidoxime to 78.7% and atropine to 88.0% of the patients, and 19 of them (8.4%) had undergone hemoperfusion therapy. No statistically significant difference was found between the patients who underwent hemoperfusion and those who did not with respect to mortality, demonstrating that hemoperfusion is ineffective in severe OP patients. In conclusion, OP is a public health problem that threatens many lives especially in developing countries. Best way of prevention from OP is to make concerned people conscious of this product and make the necessary legal arrangements. Early and appropriate treatment in ED may prevent development of IS and so reduce mortality and morbidity. The role of cholinesterase level in estimating prognosis is controversial. However results of our study support the idea that it may be a good marker in determining the clinical severity. We believe that, as in all poisonings, economical and cultural development of countries may reduce the number of OP cases.

## Limitations of the study

Although plasma cholinesterase is more available and easier to assay than red blood cell cholinesterase (ChE), it is less specific than red blood cell ChE and often impacted by many factors causing falsely low levels that could confound the magnitude and severity of the poisoning including liver dysfunction, malnutrition, alcoholism, pregnancy, chronic therapy with other plasma ChE metabolized drugs, and chronic exposures to organophosphates in farmers and people living in agricultural communities with frequent organophosphate applications. For we cannot assay the level of red blood cell ChE at our laboratory we used the level of plasma cholinesterase as a diagnostic criterion and that is one of the conditions limited our study.

# Acknowledgements

We would like to thank Associate Professor Ahmet Tevfik Sunter for providing the statistical advice.

#### References

- 1. Sivangnanam S. Potential therapeutic agents in the management of organophosphorus poisoning. Critical Care 2002; 6: 260–261.
- Eddleston M, Buckley NA, Eyer P, Dawson AH. Management of acute organophosphorus pesticide poisoning. Lancet 2008; 371: 597–607.
- Saadeh AM, Al-Ali MK, Farsakh NA, Ghani MA. Clinical and Sociodemographic Features of Acute Carbamate and Organophosphate Poisoning: A Study of 70 Adult Patients in North Jordan. Clinical Toxicology 1996; 34: 45–51.
- Sahin HA, Sahin I, Arabaci F. Sociodemographic factors in organophosphate poisonings: a prospective study. Human & Experimental Toxicology 2003; 22: 349–353.
- Kara IH, Güloğlu C, Karbulut A, Orak M. Sociodemographic, clinical, and laboratory features of cases of organic phosphorus intoxication who attended the Emergency Department in the Southeast Anatolian Region of Turkey. Environ Res 2002; 88: 82–88.
- 6. Ozer C, Kuvandik G, Gokel Y, Duru M, Helvaci MR. Clinical Presentation and Laboratory Findings of Organic Phosphorus Poisoning. Adv Ther 2007; 24: 1321–1329.
- Eddleston M, Clark RF. Insecticides: Organic phosphorus compounds and carbamates. In: Nelson LS, Lewin NA, Howland MA, Hoffman RS, Goldfrank LR, Flomenbaum NE, eds. Goldfrank's Toxicologic Emergencies. Ninth edition. New York, McGraw-Hill Co., 2011: 1450–1466.
- 8. Robey III WC, Meggs WJ. Pesticides. In: Tintinalli JE, Kelen GD, Stapczynski JS, eds. Emergency Medicine a comprehensive study guide. Seventh edition. New York, McGraw-Hill Co., 2011: 1297–1301.
- 9. Aygun D, Doganay Z, Altintop L, Guven H, Onar M, Deniz T, et al. Serum Acetylcholinesterase and Prognosis of Acute Organophosphate Poioning. Clinical Toxicology 2002; 40: 903–910.
- Altintop L, Aygun D, Sahin H, Doganay Z, Guven H, Bek Y, et al. In Acute Organophosphate Poisoning, the Efficacy of Hemoperfusion on Clinical Status and Mortality. J Intensive Care Med 2005; 20: 298–302.
- Chuang FR, Jang SW, Lin JL, Chern MS, Chen JB, Hsu KT. QT, Prolongation Indicates a Poor Prognosis in Patients With Organophosphate Poisoning. Am J Emerg Med 1996; 14: 451–453.

- 12. Yılmazlar A, Özyurt G. Brain Involvement in Organophosphate Poisoning. Environmental research 1997; 74: 104–109.
- 13. Nouira S, Abroug F, Elatrous S, Boujdaria R, Bouchoucha S. Prognostic Value of Serum Cholinesterase in Organophosphate Poisoning. Chest 1994; 106: 1811–1814.
- 14. Yen DHT, Yien HW, Wang LM, Lee CH, Chan SHH. Spectral analysis of systemic arterial pressure and heart rate signals of patients with acute respiratory failure induced by severe organophosphate poisoning. Crit Care Med 2000; 28: 2805–2811.
- 15. Yelamos F, Diez F, Martin C, Blanco JL, Garcia MJ, Lardelli A, Pena JF. Acute organophosphate insecticide poisonings in the province of Almería. A study of 187 cases. Med Clin 1992; 98: 681–684.
- 16. Amanvermez R, Baydin A, Yardan T, Başol N, Günay M. Emergency Laboratory Abnormalities in Suicidal Patients with Acute Organophosphate Poisoning. Turk J Biochem 2010; 35: 29–34.
- 17. Sungur M, Güven M. Intensive care management of organophosphate insecticide poisoning. Critical Care 2001; 5: 211–215.
- Emerson GM, Gray NM, Jelinek GA, Mountain D, Mead HJ. Organophosphate Poisoning in Perth, Western Australia, 1987–1996. J Emerg Med 1999; 17: 273–277.
- 19. Teixeira H, Proença P, Alvarenga M, Oliveira M, Marques EP, Vieira DN. Pesticide intoxications in the centre of Portugal: three years analysis. Forensic Sci Int 2004; 143: 199–204.
- 20. Kang EJ, Seok SJ, Lee KH, Gil HW, Yang JO, Lee EY, et al. Factors for Determining Survival in Acute Organophosphate Poisoning. Korean J Intern Med 2009; 24: 362–367.
- 21. Lee P, Tai TYH. Clinical Features of Patients with Acute Organophosphate Poisoning Requiring Intensive Care. Intensive Care Med 2001; 27: 694–699.
- 22. Bertsias GK, Katonis P, Tzanakakis G, Tsatsakis AM. Review of clinical and toxicological features of acute pesticide poisonings in Crete (Greece) during the period 1991–2001. Med Sci Monit 2004; 10: 622–627.
- 23. Tsai JR, Sheu CC, Cheng MH, Hung JY, Wang CS, ChongIW, et al. Organophosphate Poisoning: 10 Years of Experience in Southhern Taiwan. Kaohsiung J Med Sci 2007; 23: 112–118.

- 24. Levy-Khademi F, Tenenbaum AN, Wexler ID, Amitai Y. Unintentional organophosphate intoxication in children. Pediatric Emergency Care 2007; 23: 716–718.
- 25. El-Naggar AR, Abdalla MS, El-Sebaey AS, Badawy SM. Clinical Findings and cholinesterase Levels in Children of Organophosphates and Carbamate Poisoning. Eur J Pediatr 2009; 168: 951–956.
- 26. Akdur O, Durukan P, Ozkan S, Avsarogullari L, Vardar A, Kavalci C, et al. Poisoning Severity Score, Glasgow Coma Scale, Corrected QT Interval in Acute Organophosphate Poisoning. Human & Experimental Toxicology 2010; 29: 419–425.
- 27. Guloglu C, Kara IH. Acute poisoning cases admitted to a university hospital emergency department in Diyarbakir, Turkey. Human&Experimental Toxicology 2005; 24: 49–54.
- 28. Mert E, Bilgin NG. Demographical, aetiological and clinbical characteristics of poisonings in Mersin, Turkey. Human&Experimental Toxicology 2006; 25: 217–223.
- 29. Soysal D, Karakuş V, Soysal A, Tatar E, Yıldız B, Şimşek H. Evaluation of Cases with Acute Organophosphate Pesticide Poisoning Presenting at a Tertiary Training Hospital Emergency Department: Intoxication or Suicide? JAEM 2011; 10: 156–160.
- 30. Dippenaar R, Diedericks RJ. Paediatric organophosphate poisoning–a rural hospital experience. S Afr Med J 2005; 95: 678–681.
- Linden CH, Lovejoy FH. Poisoning and drug overdose. In: Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo GL, eds. Harrison's Principles of internal medicine. 14th edition. New York, McGraw-Hill Co., 1998: 2523–2544.
- 32. Yurumez Y, Durukan P, Yavuz Y, Ikizceli I, Avsarogullari L, Ozkan S, et al. Acute Organophosphate Poisoning in University Hospital Emergency Room Patients. Intern Med 2007; 46: 965–969.
- *33.* He F, Xu H, Qin F, Xu L, Huang J, He X. Intermediate myasthenia syndrome following acute organophophates poisoning–an analysis of 21 cases. Human & Experimental Toxicology 1998; 17: 40–45.
- 34. De Bleecker J, Van Den Neucker K, Colardayn F. Intermediate syndrome in organophosphorus poisoning: A prospective study. Crit Care Med 1993; 21: 1706–1711.
- Senanayake N, Karalliedde L. Neurotoxic Effects of Organohosphorus Insecticides. N Engl J Med 1987; 316: 761–763.

36. Karalliedde L. Organophosphorus poisoning and anaesthesia. Anaesthesia 1999; 54: 1073–1088.

Corresponding Author Ahmet Baydin, Ondokuz Mayis University, Faculty of Medicine, Emergency Medicine, Samsun, Turkey, E-mail: abaydin@omu.edu.tr

# Founder effect analysis of disease haplotypes in DFNB23/ USH1F linked Pakistani families

Riffat Mehboob<sup>1</sup>, Adnan Shahzad Syed<sup>2</sup>, Nakhshab Chaudhary<sup>1</sup>, Fridoon Jawad Ahmad<sup>1</sup>

<sup>1</sup> Department of Biomedical Sciences, King Edward Medical University, Lahore, Pakistan,

<sup>2</sup> Institute of Genetics, University of Cologne, Cologne, Germany.

#### Abstract

Usher syndromes are a group of autosomal recessive disorders characterized by moderate to profound sensorineural hearing loss and progressive visual loss from retinitis pigmentosa. Clinically they are classified into three types on the basis of phenotypes. Within each clinical group molecular heterogeneity exists and people with indistinguishable phenotypes have mutations in different genes. Protocadherin-15 (PCDH15) is one of the five genes identified as being mutated in Usher 1 syndrome and defines Usher syndrome type 1F (USH1F). Mutation in this gene also causenonsyndromic deafness DFNB23. A total of 25 families were collected in which pattern of inheritance was autosomal recessive and were screened for locus DFNB23 by using fluorescently labeled markers D10S2529, D10S546, and D10S2522. Three families were found to be linked with DFNB23. Haplotypes of these families were compared with 12 previously linked families obtained from CEMB repository. Seven families divided into two groups shared same haplotypes while in other eight families, no correlation was found between the haplotypes. Variability of haplotypes among families indicate presence of different type of mutations and families with same haplotypes may have same founder. These results will lead to better understanding of hearing impairment caused by mutations in PCDH15 and will help in identification of carriers and genetic counselling.

**Key words:** Usher syndrome, USH1F, Deafness, Linkage analysis, haplotype analysis, Founder effect analysis.

#### Introduction

Deafness, partial or complete hearing impairment, is most prevalent sensory deficit in humans with both genetic and environmental etiologies [1]. It is estimated that 1 in 1,000 births are affected with serious permanent hearing impairment [2], of which about 60% are attributed to genetic factors which in most cases are due to single gene mutations and the rest are due to environmental causes. The major pattern of inheritance in deafness is autosomal recessive (over 75%) while autosomal dominant (12-24%), X linked (1-3%) and mitochondrial is also involved[3]. Autosomal recessive forms of hearing loss are the most severe and account for almost all-congenital profound deafness. They are almost exclusively due to cochlear defects and are fully penetrant and bilateral. The autosomal dominant form of deafness seems to be progressive, post-lingual and is often unilateral or mild bilateral associated with conductive and sensorineural impairments [4].

These monogenic forms of deafness may be syndromic or non-syndromic[5]. Approximately 70% of prelingual hereditary hearing loss (HL) is non-syndromic; the remaining 30% is syndromic[6]. Syndromic HL may be conductive, sensorineural, or mixed [7]. Syndromic deafness can be either Dominant (Wardenburg syndrome, Branchial-oto-renal syndrome, Stickler syndrome), Recessive (Ushers syndrome, Pendred Syndrome) or X linked (Alport syndrome, Nance syndrome, Hunter syndrome). Presently, there are more than 120 non-syndromic deafness loci and 21 of the corresponding genes have been reported [8].

Usher syndrome (USH) is the most frequent cause of deafness accompanied by blindness due to retinitis pigmentosa. Usher syndrome accounts for more than 50% of individuals who are both deaf and blind [9], about 18% of retinitis pigmentosa cases, and 3-6% of congenital deafness cases [10]. Usher syndrome can be classified into three different clinical subtypes type I (USH I), type II (USH 2) and type III (USH3) and involves 12 loci

(7 for USH1, 3 for USH2 and 2 for USH3). Seven genes have been identified, namely *MYO7A*, *USH1C*, *CDH 23*, *PCDH15*, *SANS*, *USH2A* and *USH3A* (Supplementary table)[11].

Usher Syndrome Type I is further subdivided into 5 types. Mutations in the MYO7A, USH1C, CDH23, PCDH15, and USH1G (SANS) genes cause Usher Syndrome Type 1B, Type 1C, Type 1D, Type 1F and Type 1G respectively. Mutations in these genes account for most cases of Usher syndrome Type I. Mutations in MYO7A are the most common accounting for 39-55% of cases (Keats and Lentz, 2006).

The last protein known to be involved in USH1 is related to Otocadherin, and like all the other USH1 genes, the encoding gene is expressed in the retina and hair cells. This gene is named Protocadherin 15 (PCDH15) and is underlying USH1F (Figure 1 and supplementary Table). In addition to its localization in retina and cochlea, PCDH15 (supplementary Table) is expressed in brain, kidney, lung, and spleen, but mutations in PCDH15 do not cause additional dysfunctions despite RP and deafness. Protocadherins are required for neural development and synapse formation. The function of PCDH15 in the mammalian inner ear is yet unsolved, but a role in the formation of stereocilia from microvilli has been suggested [12].

The mouse model of USH1F is called the Ames *Waltzer* mouse (av). Like sh-1 mice, Ames Waltzer (Table 1) mice do not exhibit an ocular phenotype, indicating a different retinal function of PCDH15 between men and mice [13]. In conclusion, USH1 has to be considered as a disease entity caused by impaired development of structural components of cilia in the receptors of the visual, the auditory, and the balance system. This impairs the production of the nerve impulse and causes early deafness and progressing retinal degeneration. USH 1F was mapped to chromosome 10q21-22 (Figure 1; supplementary Table), in a 15cM interval centromeric to DFNB12/ USH1D [14]. DFNB23, an autosomal non-syndromic recessive deafness locus, was also mapped to an interval that overlapped the location of USH1F. Mutation of novel PCDH15 gene that encodes Protocadherin 15 was found to be the cause of USH1F (Supplementary Table) [13]. DFNB23 an autosomal non-syndromic recessive deafness locus was mapped to an interval that overlapped the location of USH1F [15]. The mouse Ames waltzer (av) phenotype is due to a recessive mutation and is used as the mouse model for USH1F [12]. Homozygous mutant mice show degeneration of the inner ear neuroepithelia and vestibular dysfunction but not retinal abnormality.

Although hearing loss is a worldwide problem [7] but mapping of gene responsible for isolated deafness is hindered due to combination of the extreme genetic heterogeneity of this disorder, the absence of clinically distinctive sign and symptoms for the various gene defects, the high frequency of unions between deaf people in developed countries. Mutation in different genes even in the same family can cause the same clinical phenotype in hearing impaired individuals. On the other hand, extreme phenotypic variation between different families can be due to mutations in the same gene. Different mutations in PCDH15 are responsible for non-syndromic recessive deafness of DFNB23 and USH1F. Therefore, recessive splice site and non-sense mutation of PCDH15, encoding Protocadherin15, is known to cause deafness and retinitis pigmentosa in usher syndrome type 1F [11]. Non-syndromic recessive hearing loss is caused by missense mutations of PCDH15 (supplementary Table).

PCDH15 is one of the five genes identified as being mutated in Usher 1 syndrome and defines Usher syndrome type 1F (USH1F). PCDH15 spans approximately 1.6 MB of genomic DNA on 10q21. 1. The gene contains 33 exons (Figure 1) of which only the first 2 are situated in the Usher syndrome type IF (USH1F; 602803) critical region defined by one of the Pakistani families studied. The start codon is at 396 bp in exon 2 and the stop codon is at 6,263 bp in exon 33 (Figure 1). PCDH15 encodes a predicted protein of 1,955 amino acids with a molecular weight of approximately 216 KD [13] reported 2 mutations of the PCDH15 gene in 2 families segregating Usher syndrome type IF. PCDH15 is expressed in the retina, and causes retinitis pigmentosa associated with USH1F.

There are six major subclasses of the cadherin superfamily, including protocadherin, almost all of which are expressed in the neuronal tissues. A typical protocadherin have up to seven extracellular calcium binding domains, one transmembranedomian and a unique intracellular domain [16, 17]. Some procadherin genes occur in clusters, which



Figure 1. Schematic representation of PCDH15 gene and protein. PCDH15 has 33 exons spaced by several introns and it encodes PCDH15 protein with 11 extracellular domains

may allow the generation of many isoforms. *Pro-tocadherin 15* is expressed in the sensory epithelia of the eye and ear (Supplementary Table).

In higher vertebrates, protocadherins are thought to be involved in a variety of functions including neural circuit formation and synapse formation. It is important inmorphogenesis and cohesion of sterocilia bundles and retinal photoreceptor cell maintenance or functions. Abnormal development of sterocilia results in hair cell and it progresses to nearly total degeneration of the cochlear neuroepithelium dysfunction. Sensory and supporting cell pathology is also observed during later stages.

Recessive splice site and nonsense mutations of *PCDH15* (Table 3), encoding *Protocadherin15*, are known to cause deafness and retinitis pigmentosa in usher syndrome type 1F [18]. Non-syndromic recessive hearing loss (DFNB23/USH1F) is caused by missense mutations of *PCDH15*. This suggests a genotype-phenotype correlation in which hypomorphicalleles cause non-syndromic hearing loss, while more severe mutations of this gene results in USH1F (Supplementary Table).

Linkage analysis is a powerful method not only for identification of new gene loci but also for refining intervals where deafness causing loci have been previously mapped. This strategy has helped in gene identification studies of deafness loci [8]. When a linkage has been obtained, then other families can be analyzed to check if they link to these locations. If they have recombination different from the family used to map locus, they can reduce the candidate interval. By linkage analysis, we can define the prevalence of particular Usher Type 1F locus in Pakistani population.

**Founder effect** is the phenomenon that occurs within microevolution. Microevolution refers to small scale changes in gene frequencies in a population over the course of a few generations. Evolution generally refers to any process of change over time. However, in the context of the life sciences, evolution is change in the genetic makeup of a group. Such a population shares a common gene pool and members exhibit a degree of genetic relatedness. Since 1940s, evolution was defined more specifically as a change in the frequency of alleles from one generation to the next. Founder effects are common in island ecology, but the isolation need not to be geographical. For example, the Amish populations in the United States, which have grown from a very few founders but have not recruited newcomers, and tend to marry within the community, exhibit founder effects: phenomena such as polydactyly (extra fingers and toes, a symptom of Ellis-van Creveld syndrome), though still rare absolutely, are more common in Amish communities than in the US population at large. In extreme cases, founder effects may lead to the evolution of new species.

One good aspect of doing this research in Pakistani population is because of traditional consanguineous marriages within the same ethnic groups [19], which increases the risk of homozygosity, by descent. Therefore, for this reason, studying deaf families living in geographic, cultural or religious isolates or large consanguineous families with more affected individuals has proved helpful [7]. As a consequence, families with multiple affected individuals showing clear segregation will be effective for the linkage studies[20]. In such studies Pakistan can play a vital role. These families provide excellent resource materials for conventional linkage analysis and refining candidate interval for positional cloning of gene causing deafness.

Studies of comparison of haplotypes among different families give us an insight about the type of mutations in deafness causing gene and help in founder effect study. The benefits from this research will be applicable to the hearing loss caused by the mutations in *PCDH15*. For recessive cases of deafness it is possible to reduce the incidence by increasing awareness about the effects of cous-in marriages and identification of carriers.

#### Materials and methods

#### **Enrollment of families**

Families with three or more deafness-affected individuals were contacted through the schools for deaf and from different hospitals. If a family had other relatives affected with deafness then they were also included in the study depending on their willingness and availability. Informed consents were obtained from all family members who participated in the study. Detailed history was taken from each family to minimize the presence of other abnormalities and environmental causes for deafness. Families were questioned about skin pigmentation, hair pigmentation, and problems relating to balance, vision, night blindness, thyroid, kidneys, heart, and infectious diseases like meningitis, antibiotic usage, injury, and typhoid. The pedigree structures were based upon interviews with multiple family members and pedigrees of the enrolled families were drawn, using Cyrillic program (Cyrillic 2. 1). The families provided convincing evidence for an autosomal recessive mode of inheritance. Family members rarely marry outside the kindred, and consequently consanguineous reunions were common. All affected individuals regardless of age displayed profound hearing loss affecting all frequencies, implying that hearing impairment in the families was not progressive. Audiometric testing was performed for all deaf individuals, where possible. Pure tone Audiometry with air conduction at 250, 500, 1000, 2000, 4000, 8000 Hz was performed with Siemens, SD-25 or Beltone 112 audiometer.

#### Blood collection and dna extraction

Blood samples from twenty-five families willing to participate in the study were collected. 5-10 ml of venous blood was collected in 50 ml falcon tubes having 100-200 µl of 0. 5 M EDTA from all participating individuals. Genomic DNA was extracted by a standard non-organic protocol (Grimberget al., 1989). For 10 ml of blood 30 ml of lysis buffer TE (10mM TrisHCl pH8 and 2mM EDTA) was used. The pellets were washed with NaCl and EDTA buffer (0. 075M and 0. 025M respectively). 0. 5mg Proteinase K was added along with 200 µl of 10% SDS (Grimberget al., 1989) for protein digestion. These were left in incubator shaker for overnight at 37°C. After overnight incubation 1ml saturated NaCl was used to precipitate the excess proteins. DNA was precipitated from the supernatant with equal volume of isopropanol (Miller et al., 1988). After washing with 70% ethanol, DNA was dissolved in TE and heated at 70°C for one hour to inactivate any remaining nucleases. DNA concentrations were determined by spectrophotometric reading at optical density 260nm & 280nm. Working DNA concentration was kept at 25 ng/µl and 2µl was used for10µl PCR reaction.

#### Swabs collection and dna extraction

Buccal swabs were collected from small children and individuals unwilling to give blood samples. Master Amp Buccal Swab Brush, (Epicentre Technologies WI, Medical Package Corporation, CA, USA. www. epicenter. com), was used to take cheek cells. Each brush was swirled inside the cheek for 20 times and then stored back in the container at 4°C. Two swabs were taken from each individual. DNA was extracted from these cells by using Master Amp Buccal swab DNA extraction solution from the same company. 500ul of the MasterAmpBuccal Swab DNA Extraction solution was aliquoted into an appropriate number of 1.5 ml micro centrifuge tubes, placed in ice. Buccal brush was placed in a tube containing DNA extraction solution and it was rotated for a minimum of 20 minutes. Brush was pressed against the side of tube and rotated while removing it from the tube to ensure most of the liquid remains in the tube. Cap was screwed on the tube tightly and vortex mixed for 10 seconds. Tube was incubated at 60°C for 30 minutes. Vortex mixed for 15 seconds. Tube was transferred to 98°C and incubated for 8 minutes. Again vortex mixed for 15 seconds and tube was returned to 98°C and incubated for an additional 8 minutes. Vortex mixed for 15 seconds and chilled on ice briefly to reduce the temperature. Cellular debris was pelleted by centrifugation at 14,000 rpm at 4°C for 5 minutes. transfered the supernatant containing the DNA carefully to a clean tube without including any of the beads. DNA was stored at -20°C, or at -70°C for long-term storage.

#### PCR for microsatellites

For PCR amplification of microsatellites, 10µl reaction volume was used. 3 fluorescently labeled primers (with forward primer labeled with fluorescent dye FAM was used for linkage analysis of the locus DFNB23). PCR reaction mixture contained 1X Taq Buffer (50mM KCl, 0. 1X Triton, 1. 5mM or 2. 0mM MgCl2), 200µl dNTPs (Pharmacia), 0. 5 units of Taq DNA polymerase and 0. 3mMof each primer. The samples were amplified using following PCR cycles, first denatured at 96°C for 4 minutes, then 35 cycles were repeated as denaturation at 95°C for 30 seconds annealing at 54°C for 30 seconds then extension at 60°C for 2 minutes. A post PCR step for 10 minutes at 72°C was added to extend all unfinished products. GeneAmp PCR system 9700 and 2700 (Perkin Elmer) were used for PCR. The markers used for each linkage encompassed the chromosomal location reported for deafness locus DFNB23 (http://dnalab-www. uia. ac. be/dnalab/ hhh). The markers were mostly dinucleotide repeats and were chosen from the Marshfield Comprehensive Human Genetic Maps (http://www.marshmed. org/genetics/) or Genthon Human Genetic Map for chromosome 10q. The primer sequences for amplification of each marker were listed in the genome database (http://gdwww.gdb.org).

# Preparation of samples for abi 3100 genetic analyzer

Fluorescent-labeled markers were used for screening DFNB23; PCR products of different

sizes labeled with different dyes were pooled by adding 1µl of each PCR product in 11. 8µl of deionized formamide and 0. 2µl of LIZ size standard (Perkin Elmer). The samples were denatured at 95°C for 5 minutes before running in the ABI Prism 3100 genetic analyzer.

#### Genotyping

Alleles in base pairs for each marker were recorded. For initial screening few members from each family were genotyped for three markers for this locus. Additional markers from the corresponding regions (http://www. marshmed. org/genetics/) were also typed if some of these markers were uninformative. Markers were run to define the region of homozygosity and all family members were genotyped and haplotypes generated to either include or exclude the linkage region.

#### Lod score calculations

Lod score calculations were calculated using different utility program of the FASTLINK computer package (Schaffer, 1996). MLINK was used for two point lod scores. Multipoint lod scores were calculated using LINKMAP. A fully penetrant recessive model with no phenocopies and disease allele frequency of 0. 001 was assumed. Marker order and map distances were chosen from the Marshfield genetic map (http://research.marshfieldclinic.org/). Meiotic recombination frequencies were considered to be equal for males and females. Allele frequencies for microsatellite markers were calculated by genotyping 90 randomly collected unaffected individuals from the same population.

#### Results

Twenty-five families with autosomal recessive deafness were collected from different areas of Pakistan. All affected members of these families had prelingual, severe to profound sensorineural deafness. Detailed medical history was taken to

Table 1. Sts markers used for linkage analysis of DFNB23 locus

Locus DFNB23	Marker	cM	Dye	PCR Program	Conditions	Product Size
1	D10S2522	75.57	FAM	64-54	2. 5mM	243bp
2	D10S2529	75.57	FAM	64-54	1. 5mM	200bp
3	D10S546	75.57	FAM	64-54	1. 5mM	148bp

Journal of Society for development in new net environment in B&H

exclude environmental causes. All families were non-syndromic and had no other medical related problems except PKDF875, which had history of night blindness and balance problem.

The caste and ethnicity of all families was recorded to observe any association between particular haplotypes/mutations with ethnic group. Initially all families were screened through linkage analysis to known deafness loci. Deafness phenotype in three families showed linkage with DFNB23/USH1F.

#### Dfnb23 linked families

#### Family PKDF875

PKDF875 was enrolled from Joharabad (Punjab) and belongs to Punjabi ethnic group. PKDF875 had five affected individuals (Figure 2). Ages of all affected individuals are between three to eighteen years. All affected individuals displayed bilateral hearing loss. Tandem gait and Romberg test revealed balance dysfunction in affected individuals. Affected individuals of pedigree have problem of night vision loss co-segregating with hearing loss. Five affected and two normal individuals were genotyped. All the deaf individuals were homozygous for the marker D10S2522, D10S546 and D10S2529 and the normals were heterozygous. Father (II: 3), a normal individual (II: 1) were carrier of the mutated alleles of DFNB23/USH1F. Haplotype analysis of DFNB23/USH1F linked markers of PKDF875, showed no resemblance with other DFNB23/US-H1F haplotypes. This indicates that a new mutated allele is segregating in PKDF875.



Figure 2. Pedigree Drawing of Family PKDF875

#### FamilyPKDF756

The family was enrolled from Faisalabad (Punjab) and belongs to cast 'Malik'. PKDF756, a small family with three affected individuals in a single consanguineous loop (Figure 3). The family had three affected individuals with severe to profound deafness. The family also had no history of night blindness or any other medical related problem such as goiter, heart disease and blood pressure. Three affected (V: 1, V: 2 and V: 3) and three normal individuals (IV: 2, V: 4 and V: 5) were genotyped (Fig. 12). All deaf were homozygous for markers D10S2522 (75. 57cM), D10S546 (75. 57cM) and D10S2529 (75. 57cM) located on chromosome 10. The normal individuals were carriers. This family was therefore linked to DFNB23. The haplotype of this family was unique and matched with none of previously linked families and probably harbors a novel mutant allele of DFNB23/USH1F.



Figure 3. Pedigree Drawing of Family PKDF756

#### Family PKDF801

This family was enrolled from Chiniot (Punjab). A highly consanguineous pedigree having six affected members with inherited deafness in eight sibships (Figure 4). The patients were profound deaf with no history of night blindness or other medical related problems such as goiter, balance problem, heart disease or blood pressure. On genotyping all the deaf individuals (V: 2, V: 3, V: 10, V: 15, V: 16 and V: 19) were genotyped and were found homozygous for the marker D10S2522 (75. 57cM), D10S546 (75. 57cM) and D10S2529 (75. 57cM) used for DFNB23/USH1F screening. Few normal individuals (IV: 1, V: 17, V: 6, V: 11, V: 12 and V: 13) inherited normal alleles from both parents while other normal individuals (IV: 2, IV: 3, IV: 4, IV: 5, IV: 8, IV: 6, IV: 7, IV: 9, IV: 11, IV: 12, V: 18, V: 20, V: 1, V: 4, V: 5, V: 7, V: 8 and V: 21) are carriers and inherited one normal and other mutated allele from their parents (Figure 4).

# Comparison of haplotypes

Twelve families were provided by CEMB DNA bank, which were previously linked with DFNB23 to compare haplotypes. Necessary information about the families regarding the city from where they were enrolled, their ethnic groups and caste were taken from CEMB Data bank. Comparison of haplotypes revealed that PKSR54a, PKDF875, PKDF809 and PKDF231 segregate the same haplotype. Mutation for only PKSR54a 785G>A (G262D, Exon 8) is known, and there is a high possibility that other three families with same haplotype might also have same mutation, which can be confirmed by sequencing. So, it can be anticipated that these families may have common founder (Table, 2).



Figure 4. Pedigree Drawing of Family PKDF801

Journal of Society for development in new net environment in B&H

Families PKDF70 and PKDF756 also have same haplotype. A missense mutation i. e. 400C>G (R134G) is found in PKDF70. These two families (PKDF70 and PKDF756) have same caste and ethnic group and same haplotype in all three markers and there are high chances of founder effect and same mutations in both families.

Eight families (PKDF801, PKDF181, PKDF338, PKSR17, PKDF248, PKDF627, PKSR8b and PKDF409) harbor unique haplotypes across the linked interval. This finding shows that eight different mutant alleles of DFNB23/USH1F are segregating in these families.

#### Discussion

Large consanguineous families are a powerful resource for mapping and identifying deafness loci and genes that modify deafness phenotype [21, 22]. Pakistan has diverse ethnic groups and marriages are usually done within families, castes and ethnic groups, hence, these families have been instrumental in mapping recessive deafness loci and genes.

Twenty-five families belonging to different ethnic groups were screened for a locus DFNB23 and out of these; three families were linked to *PCDH15/ DFNB23*. The remaining families were unlinked to this locus and these families could hopefully be linked to some other known deafness loci or there

*Table 2. Comparison of Haplotype with previously linked families having reported mutations and correlation of haplotypes and mutations* 

Padigraa				Screening markers for DFNB23			
No	City	Ethnic group	Caste	D10S2529	D10S546	D1092522	Mutation
190.				(Intronic)	(Intronic)	D1052522	Wintation
		Families h	naving simi	ilar Haplotype	es asPKSR54	a	
DVSD540	Powolnindi	Dunichi		100 100	145 145	255 255	785G>A(G262D,
T KSKJ4a	Kawaipinui	r unjaon	-	199 199	145 145	233 233	Exon8)
PKDF875	Joharabad	Punjabi	Mohal	199 199	145 145	261 261	
PKDF809	Chaniot	Punjabi	Rajput	199 199	145 145	265 265	
DVDE221	Nohshero-	Sindhi	Jakhia	100 100	145 145	262 262	
FKDF251	Feroz	Silidili	JOKIIIO	199 199	143 143	203 203	
		<b>Families</b>	having sim	ilar Haplotype	es asPKDF7(	9	
PKDF70	Lahore	Punjabi	Malik	197 197	155 155	267 267	400C>G (R134G)
PKDF756	Faisalabad	Punjabi	Malik	197 197	155 155	267 267	
DVDE120	Multon	Urdu speaking	Mughal	107 107	155 155	250 250	1927C>T
1 KDF139	Withtan	Oldu speaking	Iviugilai	19/19/	155 155	239 239	(R643X)
		Families	for which	no correlatio	n was found		
PKDF801	Chiniot	Punjabi	Bhatti	199 199	155 155	261 261	
PKDF181	Kasur	Punjabi	Kamboh	199 199	155 155	255 255	
PKDF338	Sialkot	Punjabi	Arain	203 203	143 143	261 261	
PKSR17	Lahore	Punjabi		203 203	143 143	261 261	
PKDF248	Thattha	Sindhi	Palijo	199 199	151 151	257 257	
PKDF627	Pashin	Balochi	Kakar	201 201	155 155	259 259	
PKSR8b	Faisalabad	Punjabi	_	199 199	157 157	255 255	
PKDF409	Rawalpindi	Pathan	Pashto	197 197	145 145	255 255	

Table 3. Haplotypes of Recently linked pedigrees with PCDH15/DFNB23

Pedigree	City	Ethnic	Casta	Screening mar	kers for PCDH15/DF	NB23
No.	City	group	Caste	D10S2459 (Intronic)	D10S546 (Intronic)	D10S2522
PKDF875	Joharabad	Punjabi	Mohal	199 199	145 145	261 261
PKDF756	Faisalabad	Punjabi	Malik	197 197	155 155	267 267
PKDF801	Chiniot	Punjabi	Bhatti	199 199	155 155	261 261

is also a high probability of finding new loci/genes due to molecular heterogeneity in our population. Three polymorphic markers D10S2529, D10S546 and D10S2522 were used for screening (Table 1). By keeping in view the above data in mind it is clear that, those individuals who are phenotypically normal are genotypically carriers. DFNB23 locus present on chromosome 10 is an allelic form of USH1F. In the same way some other non-syndromic recessive deafness and Usher syndrome loci are also allelic variant. These are DFNB2/USH1B, DFNB12/US-H1C, DFNB15/USH1B and DFNB18/USH1C. Families linked to these non-syndromic deafness loci may help in narrowing down the region of the particular locus and identification of genes. Mutation in the PCDH15 gene cause primary defects in sensory cells in the inner ear, and probably also in retina of the eye. In the inner ear PCDH15 gene is involved in the signaling pathway, which control development and maintenance of hair bundles of the sensory cells. In the present study as mentioned earlier, three families were found linked to DFNB23 and these families are PKDF875, PKDF756 and PKDF801. In addition to these, PKSR54a, PKDF809, PKDF231, PKDF70, PKDF139, PKDF181, PKDF338, PKSR17, PKDF248, PKDF627, PKSR8b and PKDF409 were provided by CEMB repository.

Linkage analysis and haplotype comparison revealed that PKDF875, PKDF809 and PKDF231 segregate the same haplotypes as PKSR54a, that has 785G>A (G262D) mutation in exon 8. All of these families may have the same mutation. Same haplotype in these families support the idea of the common founder (Yan *et al.*, 2003 and Yolanda *et al.*, 2002). Three out of four families belong to Punjabi ethnic group and fourth belong to Sindhi ethnic group. Therefore, it is more likely that these families share the same founder haplotype and mutation. PKDF231 may also have the same founder as that of other three families and may have migrated from Punjab to Sindh.

The haplotypes of PKDF756 and PKDF70 are similar. Both families are Punjabi Malik and have same haplotypes in all the three markers, while PKDF139 is urdu speaking Mughal and the haplotype is similar to PKDF756 and PKDF70 in the first two intronic markers while it is different in 3<sup>rd</sup> distal marker. PKDF756 is more similar to PKDF70 for which mutation is already known i.

e. 400C>G (R134G). It is anticipated that they might have same mutation and a common founder. PKDF139 has mutation 1927C>T (R643X) which is different from mutation of PKDF70. So, we can infer from the above study that PKDF139 also had the same founder as that of PKDF756 and PKDF70. PKDF139 might have migrated and segregated independently from the other two families before the mutational event could occur. That's why PKDF139 has different mutation, which has been acquired independently after separation.

On the other hand, no correlation was found among the families PKDF801, PKDF181, PKDF338, PKSR17, PKDF248, PKDF627, PKSR8b and PKDF409. PKDF801 and PKDF181 have the same haplotype and ethnic group (Punjabi), so, they might have same type of mutation and same founder. As, this haplotype is different from all other DFNB23 linked families with known mutations; it is possible that these families might have a novel mutation, which is responsible for deafness. PKDF338 and PKSR17 have the same haplotypes and ethnic group i. e. Punjabi, they also might have same mutation and same founder. There are more chances that this mutation may also be novel mutation because haplotypes of these families is very different from all other families having known mutations.

PKDF248, PKDF627, PKSR8b and PKDF409 have different haplotypes, castes, locations and ethnic groups. All these families may have different founders and have no correlation at all with each other or with any other family linked with DFNB23 for which mutations are already known. These families may also have some novel mutations.

It may be inferred from the above results that there is a correlation of haplotypes and mutations. Families having similar haplotypes have same founder because the investigated markers are highly polymorphic and the existence of common haplotypes in the general population is very unlikely. In the present study haplotype sharing extends to D10S2529 and D10S546 in all families. D7S2522 is not an informative marker in case of establishing correlation of haplotypes and mutations. To correlate haplotypes and mutations there should be other markers close to the gene.

Founder mutations are more commonly found in isolated populations such as Ashkenazi-Jews.

One particular mutation of GJB2-35delG is an ancient mutation and the most common cause of deafness in Caucasian. Recent studies demonstrated that 35delG is probably a founder mutation rather than a mutational hot spot in GJB2, which was the prevailing hypothesis for Caucasians [23]. The conserved haplotype flanking 167delT suggest that this mutant allele of GJB2 is a founder mutation segregating in Ashkenazi Jews [24]. Mutations of PCDH15 causing USH1 seem to be rare in most populations, as only four families (Pakistani, Indian and Hutterite) carrying mutations of PCDH15 have been reported [12, 13]. However R245X, a nonsense mutation of PCDH15, appears to be a founder mutation segregating exclusively among Ashkenazi Jews [24].

It may also be inferred from the results of the present study that same sets of haplotypes in different families may have different mutations in different exons. The same set of haplotypes in these families might have common founder allele that has mutated differently in different populations through the process of evolution. It may also be inferred that same mutations on two different sets of haplotypes can also appear.

# Conclusions

These studies have demonstrated that correlation of haplotypes and mutations is a useful method for the identification of population specific mutations. This strategy can save time and available resources in terms of mutational studies. Possible mutation in family under study can be predicted from haplotype analysis. Until now three mutations of PCDH15 have been identified. A planned study is therefore needed for identification of new mutations in Pakistani families, which will add to the repository of mutations and help in understanding the nature and function of the PCDH15 gene. Linkage analysis is done for screening of carrier's status, as consanguineous marriages are common in Pakistan, therefore, it is important to identify and offer genetic counseling to the families to reduce the incidence of deafness and socioeconomic burden on the affected families.

# Funding

Financial support for this project was provided by Center for Excellence in Molecular Biology, Lahore, Pakistan

## References

- 1. McKusick V, et al. Mendelian Inheritance in Man. 1992.
- 2. Morton NE. Genetic epidemiology of hearing impairment. Ann NY Acad Sci. 1991; 630: 16-31.
- 3. Marazita ML, et al. Genetic epidemiological studies of early-onset deafness in the U. S. school-age population. Am J Med Genet. 1993; 46(5): 486-91.
- 4. Petit C. Genes responsible for human hereditary deafness: symphony of a thousand. Nat Genet. 1996; 14(4): 385-91.
- 5. Kalatzis V, Petit C. The fundamental and medical impacts of recent progress in research on hereditary hearing loss. Hum Mol Genet. 1998; 7(10): 1589-97.
- 6. Friedman TB, Griffith AJ. Human nonsyndromic sensorineural deafness. Annu Rev Genomics Hum Genet,. 2003; 4: 341-402.
- 7. Petit C. Usher syndrome: from genetics to pathogenesis. Annu Rev Genomics Hum Genet. 2001; 2: 271-97.
- 8. Van Camp GaS, RJH. Hereditary hearing loss homepage [http:dnalab-www.uia.ac.be/dnalab/hhh]. 2000.
- 9. Vernon M. Usher's syndrome--deafness and progressive blindness. Clinical cases, prevention, theory and literature survey. J Chronic Dis. 1969; 22(3): 133-51.
- 10. Boughman JA, Vernon M, Shaver KA. Usher syndrome: definition and estimate of prevalence from two high-risk populations. J Chronic Dis. 1983; 36(8): 595-603.
- 11. Ahmed ZM, et al. Mutations of MYO6 are associated with recessive deafness, DFNB37. Am J Hum Genet. 2003; 72(5): 1315-22.
- 12. Alagramam KN, et al. Mutations in the novel protocadherin PCDH15 cause Usher syndrome type 1F. Hum Mol Genet. 2001; 10(16): 1709-18.
- 13. Ahmed, Z. M., et al., Mutations of the protocadherin gene PCDH15 cause Usher syndrome type 1F. Am J Hum Genet. 2001. 69(1): 25-34.
- 14. Bork JM, et al. Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are cau-

sed by allelic mutations of the novel cadherin-like gene CDH23. Am J Hum Genet. 2001; 68(1): 26-37.

- 15. Jaijo T, et al. Mutation screening of the PCDH15 gene in Spanish patients with Usher syndrome type I. Mol Vis. 2012; 18: 1719-26.
- 16. Angst BD, Marcozzi C, Magee AI. The cadherin superfamily: diversity in form and function. J Cell Sci. 2001; 114(Pt 4): 629-41.
- 17. Angst BD, Marcozzi C, Magee AI. The cadherin superfamily. J Cell Sci. 2001; 114(Pt 4): 625-6.
- 18. Ahmed ZM, et al. PCDH15 is expressed in the neurosensory epithelium of the eye and ear and mutant alleles are responsible for both USH1F and DFNB23. Hum Mol Genet. 2003; 12(24): 3215-3223.
- 19. Shami SA, Schmitt LH, Bittles AH. Consanguinity related prenatal and postnatal mortality of the populations of seven Pakistani Punjab cities. J Med Genet. 1989; 26(4): 267-71.
- 20. Guilford P, et al. A human gene responsible for neurosensory, non-syndromic recessive deafness is a candidate homologue of the mouse sh-1 gene. Hum Mol Genet. 1994; 3(6): 989-93.
- 21. Guilford P, et al. A non-syndrome form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. Nat Genet. 1994; 6(1): 24-8.
- 22. Baldwin CT, et al. Linkage of congenital, recessive deafness (DFNB4) to chromosome 7q31 and evidence for genetic heterogeneity in the Middle Eastern Druze population. Hum Mol Genet. 1995; 4(9): 1637-42.
- 23. Rothrock CR, et al. Connexin 26 35delG does not represent a mutational hotspot. Hum Genet. 2003. 113(1): 18-23.
- 24. Ben-Yosef T, et al. A mutation of PCDH15 among Ashkenazi Jews with the type 1 Usher syndrome. N Engl J Med. 2003; 348(17): 1664-70.

Corresponding Author Riffat Mehboob, Department of Biomedical Sciences, King Edward Medical University, Lahore, Pakistan, E-mail: mehboob.riffat@gmail.com

# Role of metacognitive beliefs and thought control strategies in mental health

Hossein Dadashzadeh<sup>1</sup>, Mahdi Vazifeh Zinabad<sup>2</sup>, Akbar Mohammadi<sup>3</sup>

- <sup>1</sup> Department of Psychiatry, Tabriz University of Medical Sciences, Clinical Psychiatry Research Center, Tabriz, Iran,
- <sup>2</sup> Department of Psychology, Ardabil Branch, Islamic Azad University, Ardabil, Iran,
- <sup>3</sup> Young Researchers and Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

## Abstract

**Background and Objectives**: Nowadays, the ever-increasing development of societies towards industrialization, the increase in population, and intense economic fluctuations are intensifying the stress and pressure endured by individuals. The studies conducted into this area are indicative of the importance and necessity of mental health and the impact of metacognitions and thought control strategies on students' physical and mental health. This study aims to investigate the role of thought control strategies and meta-cognitive beliefs on mental health of students studying at Ardebil Azad University.

**Materials and Method:** To this aim, 160 students were selected using cluster-random sampling from those studying at Bachelor's and Master's levels. They were then assessed using Kumar Mental Health Checklist (MHC), Metacognitions Questionnaire (MCQ-30), and Though Control Questionnaire (TCQ) and were then analyzed with multiple stepwise regressions.

**Findings:** There is a negative correlation between metacognitive beliefs (uncontrollability and danger, poor cognitive self-consciousness, need to control thoughts) and thought control strategies (punishment, worry, and reappraisal) and mental health. In addition, mental health could be predicted by metacognitive beliefs and thought control strategies in 86% of men and 77% of women.

**Conclusion:** It could be stated that the students studied in this research demonstrated poor mental health and a considerable percentage of the students were using inefficient thought control strategies and metacognitive beliefs.

**Key words:** Metacognition, Metacognitive Beliefs, Though Control Strategies, Mental Health.

# Introduction

The negative effects of industrialization of societies on physical and mental health of people are known issues. Many cases of cardiovascular diseases, hypertension and digestive diseases, which are known as *psychosomatic disorders* in today's studies, show the effects of mental problems on body (Weiten, 2003). It is taken for granted that an effective coping with mental stresses in everyday life is one the important strategies in preventing psychosomatic disorders.

Protecting mental health of people to enhance individual's competence and capability in everyday life and ensuring people's welfare are among the important objectives and activities of healthcare specialists in human societies.

It is said that an individual is enjoying mental health if he/she gets rid of anxiety and symptoms of helplessness, enables to establish a mutual and effective relationship with others, and cope with the pressures of life (Kamav, 1992).

Student population is an important part of the young and active group of a society which play a determinant role in conducting future of their own society. Needless to say that the efficiency, competence, innovation, management and welfare of these people is dependent on their physical and psychological health and it is necessary for the country's healthcare authorities to pay more attention to the health of this group of people.

As metacognition field and its two important scopes including *Metacognitive Beliefs* and *Thought Control Strategies* are of almost new fields of psychology and almost a fair amount of studies have been carried out on it. As this new field in psychology has shown its abilities in controlling factors such as stress, anxiety, depression, and their treatments, it is necessary to help to improve mental health of students by conducting an appropriate research on mental health, metacognition, and increasing knowledge in this concern.

In a research carried out by Abdi et al. (2012) there was a positive and significant correlation between the overall score of metacognitions questionnaire and score of general health questionnaire, and also there was positive and significant correlation between metacognitive factors such as uncontrollability and danger, positive beliefs about anxiety, poor cognitive confidence, and need to control thoughts. In other words, by increasing an individual's score in general factor of metacognitive beliefs and each of the relevant factors, his/her score in general health questionnaire increased as well, which indicated worsening of his/her general condition. As we know, increasing the score of an individual in a general health questionnaire indicates low degrees of his/her health.

The results of the research carried out by Roussis and Wells (2006) indicate that there is a positive and significant relationship between metacognitive negative beliefs about uncontrollability and danger and continuation of stress. Spada et al. (2008) believe that activation of metacognitive beliefs, uncontrollability, and danger expose people to emotional stress. It seems that emotional stress makes those with high score in uncontrollability and danger aspect engage maladaptive coping strategies. The use of such strategies causes these threat concepts to be available in processing and increasing stress and negative emotions. In fact, these processes make people overestimate environmental threats and underestimate their coping ability, which leads to continuation of mental disorder. The Results obtained from the research of Abdi et al. (2012) indicated that among 5 metacognitive factors, metacognitive factor of uncontrollability and danger is the predictor of a worse situation of general health; in other words, by increasing the score of this factor, the individual's mental health decreases. The theoretical studies carried out in this concern show that coping strategies of *punishment* and *worry* accompany pathological symptoms of psychological state and there are relationships between metacognitive strategies and vulnerability, and emotional turmoil (Wells, 2000, 2011).

In their studies, Dupuy and Ladouceur (2008) realized that there is a positive and significant relationship between the scores of people in the aspects of uncontrollability and danger, positive beliefs about worry, poor cognitive confidence, need to control thoughts and the total score of an individual in general health questionnaire in which the high score indicates many problems and the low score shows the health degrees of an individual.

In addition, the results obtained from studies show that there is a positive relationship between metacognitive beliefs and an individual's predisposition to pathological worry (Cartwright-Hatton and **Wells**, 1997; Wells and Matthews, 1995)

Indeed, the use of maladaptive coping mechanisms leads to formation and continuation of psychological disorders. In this concern, Spada et al. (2008) believe that metacognitive beliefs play the role of a mediator between stress perception and incidence of negative emotions. In addition, the increase of the score of an individual in the factor of uncontrollability of thought leads to the feeling of "lack of control". In this situation, an individual concludes that he/she has no control over his/her environment. The positive beliefs about worry cause formation and increase of second-order worry, which predispose the individual for anxiety. In addition, metacognitive factor of need to control thoughts and negative beliefs about anxiety may lead to formation of anxiety disorders and depression. In conclusion, these metacognitive beliefs have negative effect on general health and increase mental disorders.

In addition, Matthews et al. (1999) that metacognition, emotion-oriented problem solving, and worry are the indicators by which stress, worry, irrelevant thought about exam, and physical symptoms associated with test anxiety can be predicted.

Regarding the studies, the importance and necessity of the studies, which are capable of clarifying the relationships between thought and information processing in brain and condition of mental health for behavioral sciences specialists, seem not only useful but also necessary. The present study aims to discuss the role of thought control strategies and metacognitive beliefs in mental health of the students of Ardabil Azad University.

#### **Materials and Method**

Due to describing the variables under study and analyzing relationships between them, the present research is considered as a cross-sectional study.

By one dependent variable and two independent variables (metacognitive beliefs and thought control strategies) and two moderator variables (gender and marital status) and a few intervening variables, including family problems, economic problems, etc and each unpredicted and uncontrollable factor, which affect dependent variable (mental health), the present research was conducted in four groups. The sample of the present research was selected among the students of Islamic Azad University of Ardabil through stratified cluster sampling. There are 160 samples comprising two groups of undergraduate students and postgraduate students; each group is divided into two groups of females and males. Ninety-eight students (46 female students and 52 male students) and 63 students (23 male students and 40 female students) were selected from the undergraduate group and the postgraduate group, respectively.

In this research, data collection was performed by questionnaires. The questionnaires include (11item) Kumar Mental Health Checklist (MHC) (Kumar, 1992), which studies health at two levels of mental and physical. The overall score of mental and physical health is obtained by adding the scores of both levels, which is between 11 and 44. The more the score of an individual, the less his/ her mental health is. The (30-item) Metacognitions Questionnaire (MCQ-30) that measures metacognitive beliefs within 5 aspects are positive beliefs about worry, negative beliefs about worry associated with uncontrollability and danger, need to control thoughts, poor cognitive confidence, and cognitive self-consciousness (Wells and Cartwright-Hatton, 2004). Gaining more score indicates further belief of an individual to that aspect of the metacognitive beliefs. The (30-item) Thought Control Strategies Questionnaire (TCQ-30) that measures strategies within 5 levels, are punishment, reappraisal, worry, social control, and distraction. Gaining more score in each of the strategies indicates further application of thought strategy. There was also a (10-item) Personal Information Questionnaire (PIQ).

Due to the high education of the participants and the capability of the questionnaire to be applied in groups, collection of data in groups was performed in one stage in each class simultaneously with the presence of an examiner and his/her initial explanation on the plan. After data collection, all the obtained data were used using descriptive statistics methods, inferential statistics methods, including correlation and stepwise multiple regression to analyze the research hypotheses. Statistical analysis of data was performed using SPSS statistical software.

# Results

According to the results obtained from the metacognitive beliefs questionnaire, in males, the mean score for the questions related to the positive beliefs factors about worry was 13.16, uncontrollability and danger was 15.14, poor cognitive confidence was 12.55, need to control thoughts was 16.72, and cognitive self-consciousness was 18.16. In females, the mean score for the questions related to the positive beliefs factors about worry was 14.99, controllability and danger was 14.19, poor cognitive confidence was 13.00, need to control thoughts was 16.04, and cognitive self-consciousness was 18.15.

In the thought control strategies questionnaire, in males, the mean scores for social control, worry, reappraisal, punishment, distraction were 14.38, 11.76, 14.85, 12.34 and 15.28, respectively. In females, the average scores of social control, worry, reappraisal, punishment, and distraction were 14.9, 12.69, 15.66, 13.15, and 15.71, respectively.

In males, the mean scores for mental health, physical health, and overall score of males were 13.41, 9.43, and 22.84, respectively. In females, the mean scores for mental health, physical health, and total score of females were 13.59, 9.94, and 23.52, respectively.

Based on the obtained results, values of multiple regressions between mental health and metacognitive beliefs in males and females were 0.68 and 0.69, respectively. Value of F is significant at the level of 0.01 and it indicates that almost 45 percent of the variance of mental health in males and 47 percent in females are explained by the metacognition factors.

According to the results (Tables 1 & 2), uncontrollability and danger factors, cognitive selfconsciousness and poor cognitive confidence in males and uncontrollability and danger in females respectively can predict mental health, whereas positive beliefs about worry and need to control thoughts in females and males and cognitive self-consciousness and poor cognitive confidence in females cannot predict mental health significantly.

Test of multiple regression analysis was performed to study the relationship between the factors of thought control strategies and mental health in males and females (Tables 3 & 4). Based on the results, multiple regression values for males and females are 0.64 and 0.54, respectively. Value of F is significant at the level of 0.01, which shows that almost 41 percent of variance of mental health in males and 30 percent in females are explained by the factors of thought control strategy.

*Table 1. Coefficients of test of multiple regression analysis to study the relationship between metacognitive and mental health factors in males* 

Madal	Non-standard Coefficient		Standard Coefficient	t Valua	Significance
Model	В	<b>Standard Error</b>	Beta	t value	level
Constant	10.174	4.219	-	2.412	0.018
Positive Beliefs	0.268	0.162	0.152	1.652	0.102
Uncontrollability and Danger	0.521	0.165	0.328	3.161	0.002
Cognitive Confidence	0.47	0.189	0.246	2.486	0.015
Need to Control Thought	0.353	0.203	0.183	1.745	0.085
Cognitive confidence	-0.581	0.227	-0.226	-2.561	0.012

*Table 2. Coefficients of test of multiple regression analysis to study the relationship between metacognitive and mental health factors in females* 

Madal	Non-standard Coefficient		Standard Coefficient	4 Valua	Significance
Model	В	Standard Error	Beta	tvalue	level
Constant	1.518	4.097	-	0.371	0.712
Positive Beliefs	0.269	0.165	0.16	1.635	0.107
Uncontrollability and Danger	0.847	0.226	0.453	3.743	0.01
Cognitive Confidence	0.201	0.136	1.151	1.478	0.144
Need to Control Thought	0.283	0.227	0.142	1.246	0.218
Cognitive Self-consciousness	-0.066	0.124	-0.049	-0.529	0.599

Table 3. Coefficients of test of multiple regression analysis to study the relationship between thought control strategy and mental health factors in males

Madal	Non-sta	ndard Coefficient	Standard Coefficient	4 Value	Sim:Goon oo lovol	
Niouei	В	Standard Error	Beta	t value	Significance level	
Constant	18.482	5.435	-	3.4	0.001	
Social Control	-0.065	0.207	-0.027	-0.314	0.755	
Worry	7650	0.199	0.4	3.852	0.001	
Reappraisal	-0.11	0.225	-0.044	-0.506	0.614	
Punishment	0.492	0.243	0.213	2.027	0.046	
Distraction	-0.528	1700	-0.267	-3.107	0.003	

Table 4. Coefficients of test of multiple regression analysis to study the relationship between thought control strategy and mental health factors in females

Madal	Non-stan	dard Coefficient	Standard Coefficient	4 Value	Significance level	
widdei	В	Standard Error	Beta	tvalue		
Constant	15.032	5.262	-	2.857	0.006	
Social Control	-0.165	0.254	-0.078	-0.649	0.519	
Worry	0.425	0.278	0.231	1.53	0.131	
Reappraisal	0.108	0.281	0.052	0.386	0.701	
Punishment	0.64	0.269	0.352	2.376	0.021	
Distraction	-0.289	0.24	-0.138	-1.204	0.233	

Coefficients of test of multiple regression analysis were calculated to study the relationship between the factors of thought control strategies and mental health in males and females. According to the stipulated results, worry, distraction and punishment in males and punishment strategy in females can predict mental health significantly, while both social control and reappraisal in females and males and distraction and worry in females cannot predict mental health significantly.

#### Discussion

Regarding the results obtained and the studies carried out in data analysis section and with respect to the moderator variables examined by the personal information questionnaire, an almost significant difference was observed only in gender and the rest of moderator variables had either less frequency or they had not an appropriate effect to change test result. In this study, the scores obtained from Kumar Mental Health Checklist (MHC) indicate poor quality of health in university students. MHC is divided into two scales of physical health and mental health and the maximum scores at two scales are 20 and 24, respectively. The mean score for males' health at physical health scale was 9.43 out of the maximum score of 20. The same condition is true for females. This means that both male population and female population have approximately 50% of the symptoms of lack of physical health caused by mental and psychosomatic symptoms.

Such a condition at the scale of mental health is worse than the scale of physical health. Maximum score for mental health scale for both genders is 24. Males and females gained 13.41 and 13.59, respectively out of 24, which exceeds 50% of the maximum score of this scale.

The mean score of the participants in the overall score of mental health follows this procedure. Out of the maximum score of this test, that is 24, the overall score of males and females are 22.85 and 23.53, respectively. In the percentage norm of Kumar Mental Health Checklist, the overall score of males is close to 90 percent and the overall score of females is above 90 percent. That is, in general, the sample used in this research, are under high psychological stress and they are in pressing need for more accurate assessment. In a simple statistical review of the application of metacognitive beliefs between females and males, it can be figured out that there is no considerable difference between these two genders. In the application of metacognitive beliefs, female and male population believe more in cognitive self-consciousness and need to control thoughts; the overall score of these two factors in both genders exceeds the rest of factors and the rest of factors are in the following categories.

High scores at the scale of cognitive self-consciousness and need to control thoughts and consistency of these results with the earlier studies indicate that the participants used more appropriate metacognitive factors.

There is no significant difference between the strategic factors of thought control in the two groups of females and males. In order of priority, both groups use strategies of distraction, reappraisal, social control, punishment, and worry.

In the studies carried out by Wells and Davies (1994) on TCQ-30 test, worry and punishment strategies were of the most inappropriate strategies of thought control, which had further significant relationship with symptoms of anxiety, as compared with the rest of factors (Wells and Matthews, 1995). Meanwhile, other strategies such as social control, reappraisal, and distraction, respectively, were of the most appropriate methods of thought control strategy; they were considered as the positive factors to provide psychological health. However, in the present study, priority of application of safe strategies was changed considerably, that is, distraction strategy has the highest score. According to the predictions of self regulatory executive function (S-REF) model, under certain conditions, some of the thought control strategies (distraction) may be more harmful for cognitive and emotional self-regulation (Wells, 2000). In other words, those who use distraction instead of processing disturbing thoughts and adapting that to the social controls do not solve the postulate of disturbing thought and merely focus on the other issue; whereas, social control strategy and reappraisal are considered as the most appropriate methods to solve a disturbing thought and its mental control.

The results of the present study show that there is a significant relationship between mental health and metacognitive beliefs in males and females. This significant relationship has more size is between four metacognitive factors (uncontrollability and danger, poor cognitive confidence, need to control thought, positive beliefs about worry).

There were no positive relationship between cognitive self-consciousness and the score of an individual in the mental health checklist. In other words, with the increase of the score of an individual in cognitive self-consciousness, his/her score in mental health checklist did not increase and it did not lead to his/her lack of mental health. The results of this research are consistent with the ones of the earlier studies in this concern (Abdi et al. (2012); Roussis and Wells (2006), Dupuy and Ladouceur, 2008; Cartwright-Hatton and Wells, 1997; Wells and Matthews, 1996; Bidi, et al., 2012; Wells and Papageorgiou, 2001).

Likewise according to the results of the present study, there is a significant relationship between thought control strategies and mental health in males and females. This significant relationship is among 3 factors of thought control strategies in males (including worry, punishment, reappraisal) and 2 factors of thought control strategies in females (including worry, punishment). There is no significant relationship between distraction and social control and mental health of males and females.

According to Wells' theory, worry and punishment strategies are of the most inappropriate strategies of though control, which have a strong and significant relationship with anxiety and lack of emotional control (Wells, 2000). It can be concluded that in male and female group, with respect to the use of worry and punishment strategies, the sample under study does not have an appropriate mental health, which is due to the excessive use of these two inappropriate strategies.

Less application of social control and reappraisal strategies, which are more suitable strategies than the rest of strategies, caused individuals not to be successful in dealing with intrusive thoughts and to suffer stress. This research is consistent with the studies of Wells and Matthews (1995). It means that *worry* and *punishment* strategies are the predictors of lack of mental health; since with the increase of the score of an individual and more application of this strategy, his/her score in mental health checklist increases; this indicates the decrease of mental health. In this research, social control and distraction have a negative and significant correlation with the mental health questionnaire; in other words, with the increase of the score of an individual in these factors, his/her score in mental health does not increase. Therefore, they are known as the best strategy of thought control. Since the increase of the score in mental health questionnaire indicates worse situation of mental health. This part of the finings is consistent with the studies conducted on TCQ-30 test (Wells, 2000).

The overall objective of the plan was to discuss the role of thought control strategies and metacognitive beliefs in mental health of students. Concerning the studies and results, there is a significant relationship between thought control strategies and metacognitive beliefs. In other words, 77 to 86 percents of mental health in female and male students of Ardabil Azad University are explained by thought control strategies and metacognitive beliefs; this indicates the role of these strategies and beliefs in mental health of individuals.

With the increase of the scores of students in metacognitive beliefs factors and thought control strategies, the score of their mental health checklist increases as well. As scoring of Kumar Mental Health Checklist is reversed, having a high mark in this checklist indicates the low level of mental health.

The general results of the present research is consistent with the earlier studies carried out on metacognitive beliefs (Abdi et al. (2012); Roussis and Wells, 2006; Dupuy and Ladouceur, 2008; Cartwright-Hatton and Wells, 1997; Wells and Matthews, 1996; Bidi et al.,2012; Wells and Papageorgiou, 1998) and the studies conducted by Wells and Matthews (1995) and the research carried out on the test of Wells' thought control strategies and thought control strategies (Wells, 2000).

#### Conclusion

As a general conclusion obtained from the findings of the present research, it can be stated that among the metacognitive beliefs, "cognitive selfconsciousness" and among thought control strategies, "social control", "reappraisal" and "distraction" are considered as the appropriate beliefs and strategies. On the other hand, "negative beliefs about worry" and "poor cognitive confidence" are considered as the inappropriate metacognitive beliefs. In addition, thought control strategies, "worry" and "punishment", are of the inappropriate strategies in both female and male groups.

With respect to the multiple regression analysis in males' mental health on metacognitive beliefs and thought control strategies, up to 45% and 41% of males' mental health in this research can be explained by metacognitive beliefs and thought control strategies, respectively. In other words, 86% of mental health in male group of students of Ardabil Azad University is predicted by metacognitive beliefs and thought control strategies.

With respect to the multiple regression analysis in females' mental health on metacognitive beliefs and thought control strategies, 47% and 30% of females' mental health in this research can be explained by metacognitive beliefs and thought control strategies, respectively. In other words, 77% of mental health in female group of students of Ardabil Azad University is predicted by metacognitive beliefs and thought control strategies.

As 75 percent of the participants were single in this research, we were unable to study beliefs and strategies in two groups of single and married. The present research was conducted on the students of Ardabil Azad University and cautions should be exercised to generalize the findings about the other social classes and the rest of universities (restriction of generalization).

Finally, it is better to consider that the present research used self-expression inventories. Such inventories may be influenced by mentality of individuals.

#### References

- 1. Weiten W. Psychology: Themes and Variations (with Concept Charts and InfoTrac). 6<sup>th</sup> Edition, Wadsworth Press. 2003.
- 2. Kamav CW. Locus of control and Mental Health of Teachers in Eastern Province of Kenya. Burnout, Unpublished Doctoral Thesis. Punjab University, Chandigarh. 1992.
- 3. Abdi HM, Bageri S, Shoghi S, Goodarzi S, Hosseinzadeh A. The Role of Metacognitive and Self-Efficacy Beliefs in Students' Test Anxiety and Academic Achievement. Australian Journal of Basic and Applied Sciences, 2012; 6(12): 418-422.
- 4. Roussis P, Wells A. Post-traumatic stress symptoms: Tests of relationship with thought control strategies and beliefs as predicted by the Meta cognitive model. Personality and Individual Differences, 2006; 40(1): 111-220.
- 5. Wells A, Carter K. Further tests of a cognitive model of GAD: meta-cognitive and worry in GAD, panic disorder, social phobia, depression and non- patients. Behavior Therapy, 2002; 32(1): 85-102.
- 6. Spada MM, Nikčevič AV, Moneta GB, Wells A. Metacognitive, perceived stress and negative emotion. Personality and Individual Differences, 2008; 44(5): 1172-1181.
- 7. Wells A. Emotional disorders and metacognition. Innovative cognitive Therapy. 1th Edition, Wiley Press. 2000.
- 8. Wells A. Metacognitive Therapy for Anxiety and Depression. Reprint Edition, New York: Guilford Press. 2011.
- 9. Dupuy JB, Ladouceur R. Cognitive processes of generalized anxiety disorder in comorbid generalized anxiety disorder and major depressive disorder. Journal of Anxiety Disorders, 2008; 22(3): 505-514.
- 10. Cartwright-Hatton S, Wells A. Beliefs about worry and intrusions: The Meta-Cognitions Questionnaire and its correlates. Journal of Anxiety Disorders, 1997; 11(3): 279-296.
- 11. Wells A, Matthews G. Attention and Emotion: A clinical perspective. New Ed Edition, Psychology Press. 1995.
- 12. Matthews G, Hilliard EJ, Campbell SE. Meta-cognitive and maladaptive coping as components of test anxiety. Clinical Psychology and Psychotherapy, 1999; 6(2): 111-125.
- 13. Wells A, Matthews G. Modelling cognition in emotional disorder: The S-REF model. Behaviour Research and Therapy, 1996; 34(11-12): 881-888.

- 14. Bidi F, Namdari M, Kareshki H, Ahmadnia H. The Mediating Role of Metacognition in the Relationship between Internet Addiction and General Health. Addict and Health, 2012; 4(1-2): 49-56.
- 15. Wells A, Papageorgiou C. Social phobic interoception: effects of bodily information on anxiety, beliefs and self-processing. Behaviour Research and Therapy, 2001; 39(1): 1-11.
- 16. Wells A, Papageorgiou C. Relation between worry, obsessive - compulsive symptoms and Meta- cognitive beliefs. Behaviour Research and Therapy: 1998; 36(9): 899-913.
- 17. Wells A, Davies MI. The Thought Control Questionnaire: A measure of individual differences in the control of unwanted thoughts. Behaviour Research and Therapy, 1994; 32(8): 871-878.
- 18. Kumar P. Mental Health Checklist. National Psychological Corporation, Agra. 1992.
- Wells A, Cartwright-Hatton S. A short form of the meta-cognitions questionnaire: Properties of the MCQ-30. Behavior Therapy, 2004; 42(4): 385–396.

Corresponding Author Hossein Dadashzadeh, Department of Psychiatry, Tabriz University of Medical Sciences, Clinical Psychiatry Research Center, Tabriz, Iran, E-mail: hossein.dadashzade@gmail.com

# Total hip artroplasty with long oblique subtrochanteric shortening osteotomy in high developmental dysplasia of hip

Bulent Koksal, Ali Terkuran, N. Turgut Karaismailoglu, Hicabi Sezgin

Department of Orthopaedics and Traumatology, Medical Faculty, Ondokuz Mayis University, Samsun, Turkey.

## Abstract

**Objectives**: We have conducted a research on the complications and difficulties in the patients with developmental dysplasia of hip and undergoing subtrochanteric long oblique shortening osteotomy procedure on femur site as well as total hip arthroplasty. The success ratios of this treatment method have been analyzed.

**Patients and Methods:** 68 hips of 52 patients with high developmental dysplasia of hip were exposed to subtrochanteric long oblique femoral osteotomy and cementless total hip arthroplasty between May 2000 and May 2008. Of these patients 49 (94.2%) were female and 3 (5.8%) were male and mean age was determined to be 42 (range 32-68). All these patients presented with crowe type IV dislocation and all of them were operated under general anesthesia by using posterolateral approach.

**Results:** These results were clinically and radiologically evaluated. The patient was followed for 54 months in average (range 36-96 months). Nonunion was not observed in the osteotomy line of the patients. In three patients postoperative peroneal nerve disorder developed. 2 patients (3.8%) experienced dislocation. These patients were clinically assessed before and after operation according to Harris hip scoring system. Mean preoperative Harris hip score was detected to be 41.2 (range 29-60) while the mean postoperative value was increased up to 86.8 (range 65-97).

**Conclusion:** In subtrocantheric osteotomy total hip prosthesis procedure, hip reduction is facilitated and complication ratios such as sciatic nerve disorder are reduced. In the subtrocantheric osteotomy performed in the metaphyseal region, nonunion probability is minimized.

**Key words:** Developmental dysplasia of hip, arthroplasty, osteoarthritis, long oblique femoral shortening osteotomy.

# Introduction

The patients presenting with untreated developmental dysplasia of hip frequently experience osteoarthritis in the 4th and 5th decades of their lives. It is characterized by pain and walking difficulty. Total hip prosthesis in the patients with high dysplasia of hip is an opened surgical intervention complex and including complication risk. Such patients have numerous anatomic disorders. In such cases, acetabulum bone stock is reduced, anterversion is increased and anterior wall is thinned and becomes hystoplastic. In the soft tissues of joint periphery chronic dislocation related contractions have been detected. As in acetabulum, some modifications have been detected in femur as well. Femur neck anterversion increases, femur neck object angle increases and femoral duct size is reduced and large trochanter has modified to posterior site (1,2). While applying hip prosthesis, it has been reported that acetabulum should be placed in its actual place and these results are better (3). However, placing the acetabulum in its anatomic place is difficult and particularly if the secondary modifications are not intervened, some complications may occur. In addition, subtrochanteric femoral osteotomies facilitate the prosthesis reduction and minimize the neurological disorders that may develop following the athroplasties.

In this retrospective study, the complications, union ratios and durations detected in the patients exposed to total hip prosthesis and subthrocantheric long oblique shortening osteotomy due to high hip dislocation have been examined. Harris hip scores of the operated patients have been evaluated and success ratios have been researched.

#### Patients and methods

68 hips of 52 patients with high developmental dysplasia of hip were exposed to subtrochanteric

long oblique femoral osteotomy and total hip arthroplasty without cement between May 2000 and May 2008. In sixteen (30.7%) patients bilateral high dysplasia of hip, in thirty six (69.3%) lateral high dysplasia of hip was detected. Of these patients 49 (94.2%) were female and 3 (5.8%) were male and mean age was determined to be 42 (range 32-68). Mean follow up period was 54 months (range 36-96 months). Of these affected 68 hips 29 (42.6%) were right sided and 39 (47.4%) were left sided. In 11 hips 38 mm, in 51 hips 40 mm and in 6 hips 42 mm acetabular cups were used (Graph 1). Due to the failure of acetabulum superior wall, in 7 hips (10.2%) exposed to acetabular graft, autograft taken from femur head was inserted in the superolateral of acetabulum and fixed with screw. In four patients, femur valgus osteotomy was performed in another center in advance. All these patients presented with crowe type IV dislocation and all of them were operated under general anesthesia.

While determining the indication of total hip prosthesis in the patient, difficulty in daily motions, difficulty in walking, secondary osteoarthritic modifications and most importantly severe pain were evaluated. In all the patients, in lateral decubit position, posterolateral incision was performed. Anatomic hip center was restored and acetabular cup was inserted in its current place. For this, femur head was dragged to the inferior site. Inferior extension ratio of hip rotation center was measured. Femoral osteotomy was performed under trochanter as an oblique pattern. In order for avoiding the traction injury of sciatic nerve, not more than 4 cm extension in the lower extremity was performed. Preoperative and postoperative lower extremity differences were measured. In all the patients, square sectional femoral sterns exactly filling the femoral duct and with distal stability were applied. On the PO 3<sup>rd</sup> day, partial load was delivered and the patients were mobilized. In the subsequent weeks, load delivery was increased step by step and exact load was delivered in the end of 2<sup>nd</sup> month. Following the hospitalization of the patients for 5 (3-10) days in average, they were invited to the control in the months 1st,3rd,6th and 12<sup>th</sup> in the subsequent year.

#### Surgical Technique

While the patient was bedding in the supine position, hip adductors were examined in the hip

abduction. In case of adductor contracture determination, with fine tip scalpel, some points were intervened and adductor tenotomy was performed. With posterolateral incision, while the patient was in the lateral position and as positioning the operation site in the upper direction, the hip site was incised.

Soft tissues in the femur proximal were loosened. Inferior adhesion site of the capsule was observed thereby determining the acetabulum site. Anterior and posterior site of the capsule were separated from the junction point and it was all excised. Femur head was accordingly incised (Figure 1). With a long and narrowed curette, medulla was intervened. Medullar duct was caved with a scrapper. From the lower site of throcanther minor, femur was exposed to long oblique osteotomy. Prior to preparing the acetabulum, as femur was exposed to osteotomy, it was easily intervened.

In order for preparing the acetabulum, ecators were inserted in appropriate positions. Afterwards, osteophyts and capsule wastes were removed. Carving of acetabulum was performed with a 38 mm sized apparatus. Medial cortex of acetabulum was tried to be preserved. Following the carving procedure, appropriate sized acetabular shell was inserted in the acetabulum as to be appropriate with anterversion. It was fixed with two or three screws.

With preoperative radiological planning and by aligning the intraoperative femur to the lower section of acetabulum, subtrocantheric femoral shortening was determined (Figure 1). Following the femoral shortening, it was scrapped until it was complying with the size of medullar duct. Appropriate scrapper was inserted in the femur. Trial femur head was inserted and hip was reduced. Movement size and stability of the hip were controlled. Femoral stem was inserted in the femur, appropriate head was inserted and hip was reduced (Figure 2, 3).

#### **Clinical evaluation**

Preoperative and postoperative patients were clinically evaluated with Harris hip scoring (4). All the patients in the control were evaluated by considering the criteria of Harris and scored accordingly. Those total scores were compared with the preoperative scores and thereby determining the success of the operation. While performing the postoperative scoring, last control values were considered. If the total score acquired according to Harris scale were 86-100, results were considered to be excellent, 71-85, very good, 61-70 good, 41-60 medium, and less than 40 poor. In addition, it was considered whether the patient could raise his/her extremity or not and resistant against force. If it was resistant, muscle strength was evaluated according to standard Medical Research Council scale (MRC grade between 0-5) (5). Length differences in the lower extremity were measured. Trendelenburg sign was considered. Abductor muscle strength was evaluated. Early complications were considered.



Figure 1. Oblique femoral subtrochanteric shortening and osteotomy demonstration on a model

#### Radiological evaluation

Anterior – posterior and lateral femur hip graphs of the patients were taken during the control. These graphs were compared with those graphs taken immediately after the operation and the stability of femoral stem was evaluated by considering the criteria determined by Gruen et al (6) and divided into 7 zones and these zones were assessed by Engh et al (7). The evaluation was based upon the zones disclosed by Asetabum Dee Lee and Charnley (8). Femur is divided into 7 anterior – posterior and 7 lateral sites in total 14 zones in the graph described by Gruen. That the radiolucent lines in the femur in these radiological sites were 2 mm and over was considered to be substantial for the favor of loosening if they were complying with the clinical results in the end of 1<sup>st</sup> year.





Figure 2. (a) Female patient, 14 years old and experienced valgus osteotomy, 45 years old, hip graph taken before operation of type 4 hip dislocation. (b) 36 month graph of the patient experiencing subtrochanteric oblique femoral shortening and fracture line fixed with cable.





Figure 3. (a) 52 years old patient, preoperative graph of bilateral type 4 hip dislocation. (b) Right sided postoperative 45<sup>th</sup> month and left sided postoperative 48<sup>th</sup> month graphs of the patient experiencing subtrochanteric oblique femoral shortening.

Similarly, on these graphs, the distance between trochanter minor and superomedial margin of femoral stem was measured. It was kept for use in the determination of possible stem collapse (vertical migration). Vertical collapses more than 5 mm were considered to be associated with instability of femoral component. Modification of acetabular cup and eye tear figure lower margins, vertical replacement, and Kohler line and modification of cup outer wall distance were considered to be horizontal replacement. In the control graph, by considering the operation period, vertical and/or horizontal replacement over 2 mm was considered to be instability of acetabular component. Additionally, fracture, dislocation, infection and osteotomy line union results were evaluated.

# Results

68 hips exposed to subtrochanteric long oblique osteotomy were followed for 54 months (range 36-96 months) in average. No nonunion was detected in the osteotomy lines of the patients and union was detected to be exact and mean union duration was detected to be 3.8 months (range 3-6 months) (Figure 2, 3). In two (2.9%) patients, dislocation was detected. One patient fell down from bed on the PO 7<sup>th</sup> day, therefore it developed. The other case developed in the 2<sup>nd</sup> month.

Both cases were reduced under general anesthesia with closed reduction, as well. During the follow up no nonunion was detected in the dislocation and osteotomy line of the patient. Three (4.4%)patients presented with postoperative peronoal nerve disorder. Two of these patients recovered exactly within six months. Only one patient did not exactly recover from peronoal nerve disorder exactly and in the end of one year tendon transfer was applied to the patient. Postoperative length differences were detected to be 3.6 cm (range 2-6 cm) in the patients. Length difference measurement taken in the postoperative period was detected to be 1.15 cm (range 0-4 cm). Out of sixty eight hips, 59 (86.7%) were positive, 9(13.3%) were detected to be negative in the trendelenburg test performed in preoperative period. In the postoperative trendelenburg test, of the patients 6 (8.8%) were detected to be positive while 62 (91.2%) were detected to be negative. Abductor muscle strength examination was measured to be 4 (range 3-5) in average.

During the surgical intervention, hip rotation center was extended to the inferior site 6 cm in average (range 2.5-11 cm) and placed in the current acetabulum. During the placement in the current acetabulum, it was medialized 2.4 cm (range 1-3.5 cm) in average and arm raising strength was increased. 4.3 cm (range 3.5-7 cm) shortening
from femur site was performed in average. In addition, 3 cm (range 2-4 cm) extension in average was performed in the lower extremity. In the sciatic nerve, in order for not causing traction injury, other extension was avoided. The patients were clinically evaluated according to postoperative and preoperative Harris hip scoring. With Harris Hip Score preoperative average was detected to be 41.2 (range 29-60), while this value was postoperatively increased up to 86.8 (range 65-97). In 1 (1.9%) patient medium, and in 10 (19.2%) patients very good and in 41 (78.8%) patients, excellent results ware taken (graph 2).



Graph 1. Acetabular cup distribution according



*Graph 2. Postop. Haris Hip score results to patients* 

In the radiographic controls during the postoperative period, in the acetabular component, radiolucent site and migration results were not acquired. In the femoral site, however, in 3 patients in zones I-II, asymptomatic radiolucent sites and in one patient in zone I focal osteolysis site was detected. In one patient, in femoral component, 3 mm sized, and in another patient 4 mm sized vertical migration was detected. Revision was not performed due to the fact that all these patients were clinically stabile. Due to the failure of superior wall of acetabulum 7 (10.2%) patients were exposed to roof graft and of these 9% (range 5-20%) in average resorption was detected, as well. With 20% even in the hip with highest graft resorption ratio, acetabulum coating was detected to be more than 90%. None of these patients presented with loosening.

# Discussion

Those adult patients presenting with hip pathologies but who did not undergo sufficient and effective treatment might refer with hip osteoarthritis and pains. Most widespread underlying reason of such pathologies is developmental dysplasia of hip (9, 10).

Patient population in the hip osteoarthritis secondary to developmental dysplasia of hip varies compared to patient population with primary osteoarthritis. This pathology causes joint degeneration in early ages, and this leads to pain in hip and functional disorder, as well. In addition, mostly in young and active patients, more instant revision need is seen compared to older and more inactive patients. As a result of the study conducted by Dorr et al in the 49 hips of the patients under 45 years old, revision ratios have been detected to be 12% for 5 years, 33% for 9 years, 67% for 16 years (11). In other studies, it has been reported that revision ratios are 18% and 39% for 5 and 12 years respectively in the patients under 50 years old (12, 13).

Total hip arthroplasty indication demonstrates similarity with primary osteoarthritis cases in the developmental dysplasia of hip. Pain is one of the most substantial criteria in arthroplasty indication.

Table 1. THA and complication numbers in the patients with high hip dislocation and Harris Hip Scores

Study by	Numbers of hips	Age	Nerve injury	Nonunion	Dislocation	Fracture	Mean Harris Score
Eskelinen et al. (26)	75	Not statated	5	5	2	5	84
Kim et al. (34)	62	22-66	2	3	3	3	89
Current study	68	32-68	3	0	2	0	87

Extremity length inequality, hobbling and lumbar scoliosis accompanying low back pain do not establish exact arthroplasty indication (14, 15). In these cases abductor muscles, iliopsoas, rectus femoris, gluteus maximus, fascia lata and other muscle lengths have been shortened and thightness has been increased. Priformis muscle has been shortened and other external rotators have been extended. Following the arthroplasty, femur should be regulated to distal site in order for enabling the sufficient functioning of particularly abductor muscles, for regulating the trendelenburg hobbling and for enabling the equal extremity neck in the lateral dislocations or for reducing the difference (16). Therefore as in total knee prosthesis, establishing soft tissue balance is of great importance.

In such cases, total hip arthroplasty is a surgical intervention technically complex and opened to complications. If soft tissue contractures, insufficient bone stock and hip rotation center are detected in the proximal site, abnormal neurovascular structures make it difficult for the hip rotation center to be placed in its original location (17). Subtrochanteric osteotomy facilitates the regulation of hip rotation center in the inferior site and prevents the traction injury during the complex reduction of neurovascular structures. However, nonunion in the osteotomy line may cause some problems such as insufficient restoring of length difference and shortening of revision duration of femoral stem. Various femoral shortening osteotomy techniques have been determined. These techniques include femoral shortening osteotomy and trochanteric developmental osteotomy, step cut, double chevron, oblique and transverse osteotomies (18, 19). Step cut is more stable than double osteotomy and oblique osteotomy transverse osteotomy for rotational angle. We have used oblique osteotomy. However, we have extended the duration of oblique osteotomy. During the operation, other than plate and screws and cable in three patients, we have not used any fixation materials. Due to extra fixation need other than prosthesis and better rotational stability as well as union of bone within a short time, we have preferred this osteotomy. In addition, on the postoperative 3rd day, by delivering partial load, we have mobilized our patients. Most frequently detected complications of subtrochanteric osteotomy include fracture development in femur, and postoperative nonunion problem in the osteotomy line. During the insertion of intraoperative femoral stem, fracture development risk has been reported to be 5-22% (20). In our patients, no femur fracture has been detected.

Chareancholvanich et al (21) have not reported nonunion and loosening in 15 cases exposed to subtrocantheric double chevron osteotomy. Paavilainen et al (22) have not determined any nonunion in 55 cases exposed to throcantheric developmental osteotomy and femoral shortening from metaphysal region. However, Anwar et al (23) have solely performed trocantheric osteotomy in 34 patients and in 10 patients nonunion has been detected. In general sense, in subtrocantheric osteotomy nonunion frequency varies from 8% to 29% (24, 25). We have supported the osteotomy line by inserting the spongious grafts prepared from femur head if any space is detected between the proximal and distal parts. In solely three patients, we have used wire cable. We have not used another fixation material for the stabilization of fracture line. In none of our patients exposed to such osteotomy has not presented with nonunion.

With subtrocantheric shortening osteotomy, the narrowest section of femoral duct may be resected. This enables us to insert appropriate femoral stem. It is substantial for ensuring the stability. We generally have used square sectional femoral stems with more distal stability and which fill the femoral duct as much as possible in the anatomic structure. If the femoral duct is so narrowed, fine stem containing prosthesis has been used. The most substantial disadvantage of oblique osteotomy is that it does not permit the rotation required for correction of femoral anterversion. We have inserted the acetabulum as to compensate the femur anterversion. We have tried to insert the femoral anterversion to reduce the femoral anterversion anterversion. In none of the patients, no additional procedure is required to correct the femoral anterversion.

Compared to standard total hip prosthesis, loosening period of total hip prosthesis is shorter in the patients presenting with high hip dislocation. Because, in such cases, due to hypoplastic acetabulum and poor bone quality, relatively smaller acetabular cup insertion is required (if the hip rotation center is inserted in its original location). In order for preparing the dysplastic acetabulum, various techniques such as insertion of small cup together with medialization, perforation in the controlled medial wall and structural graft use have been determined. We have used small cups (38, 40 and 42 cm sized cups) in our patients. Small sized acetabular component use leads to more percentage cup coverage in most of the patients and less superolateral structural graft need. In our study, we have inserted the graft acquired from femur head with the help of 2 screws on the acetabulum in which superolateral region is not supported in 7 hips. We have experienced 9% graft resorption in average. Nevertheless, we have not detected loosening in the acetabular component. On the other hand, use of small cup has some disadvantages, as well. It requires for the use of fine linear and 22 mm sized head. This may cause linear failure and proliferation of disclocation risk. Dislocation has been detected in two patients. One patient fell down from bed on the PO 7<sup>th</sup> day, therefore it developed. The other case developed in the second month.Both cases were reduced under general anesthesia with closed reduction, as well. During the follow up, no dislocation was detected in the patients.

In the hip osteoarthritis developing secondary to high hip dislocation, modifications in the soft tissue accompany the pathological bone modifications in the femur proximal (1,10,26). Numerous complications may occur if the acetabulum is inserted in the original location thereby requiring the extension of femur in the distal section and the secondary soft tissue modifications are not intervened. Of these complications the most important one is the neurological damage developing during the complex reduction. Edward et al (27) have concluded that for peroneal palsy in the lower extremity mean 2.7 cm and for nerve palsy in the lower extremity more than 4.4 cm extension are required. Eggli et al (28) have reported that neurological damage is not directly associated with extension in the lower extremity but rather related to more complex reduction and complex surgical procedure. In our study, we have endeavored to extend 2 cm in the lower extremity in the patients with bilateral hip dislocation but not more than 4 cm in the lower extremity. Preoperative shortening has been considered following the radiological determination while shortening the tensile of soft tissues in the periphery of intraoperative joint. By keeping the knee in flexion and hip in extension postoperatively, we have reduced the sciatic tensile. Nevertheless, in 3 patients, postoperative peroneal nerve disorder has developed. Of these patients two have completely recovered. In one patient peroneal nerve disorders has not fully recovered and in the end of one year the patient has been exposed to tendon transfer.

In the clinical evaluation, Harris hip score was used. Eskeilen et al (20) have reported and published postoperative harris hip score as 84 following a 12 year of follow up of 75 hips, Kim et al (29) as 89 following a 13 and 17 year of follow up of 62 hips. In another study, in 103 hips Harris score was reported to be increased from 39.3 to 89.5 (30). In our study, we have detected that preoperative Harris hip score was 41.2 in average (ranfe 29-60) and this value was detected to be 86.8 (range 65-97) in average.

In conclusion, total joint arthroplasty is a complex and high complication risk containing procedure in the developmental hip dislocation compared to primary total joint arthroplasty. Subtrocantheric osteotomy facilitates the hip reduction in the total hip prosthesis application and reduces the complications such as sciatic nerve disorder. In some patients with very fine femur medulla, it enables the resection of that piece thereby closing the femur to more normal. In case of subtrocantheric osteotomy performed in the metaphysal region, nonunion possibility is less. In addition, oblique osteotomy is of great importance for enabling the rotational stability.

#### References

- Sponseller PD, McBeath A. Subtrochanteric osteotomy for arthroplasty of the dysplastic hip. J. Arthroplasty 1998; 3: 151.
- 2. Ermiş MN, Dilaveroğlu B, Erçeltik O, Tuhanioğlu U, Karakaş ES, Durakbaşa MO. Intermediate-term results after uncemented total hip arthroplasty for the treatment Dysplastic and dislocated hip arthroplasty 15 of developmental dysplasia of the hip. Eklem Hastalikları ve Cerrahisi 2010; 21: 15-22.
- 3. Linde F, Jensen J, Pilgaart S. Charnley arthroplasty in osteoarthritis secondary to congenital dislocation or subluxation of the hip. Clin Orthop, 1988; 227: 164-171.
- 4. Harris WH. Traumatic arthritis of the hip after dislocation and acetabular fractures: Treatment by mold arthroplasty. An end result study using a new method of result evaluation. J Bone Surg, 1969; 51-A: 737-75.

- 5. Seddon HJ, ed. Peripheral Nerve Injuries. Medical Research Council Special Report Series Number 282. London, UK: Her Majesty's Stationery Office; 1954
- 6. Gruen TA, McNeice GM, Amstutz HC. "Modes of failure" of cemented stem-type femoral components: a radiographic analysis of loosening. Clin Orthop, 1979; 141: 17-27.
- 7. Engh CA, Bobyn JD, Glassman AH. Poros-coated hip replacement: The factors governing bone. İngrowth, stress shielding, and clinical results. JBJS. 1987; 69-B: 45.
- 8. De Lee JG, Charnley J. Radiological demercation of cemented sockets in total hip replacement. Clin Orthop, 1976; 121: 20-32.
- 9. Mc Queary FG, Johnston RC. Coxarthrosis after congenital dysplasia. Treatment by total hip arthroplasty without acetabuler bone grafting. J Bone Joint Surg, 1988; 70-A: 1140-1144.
- 10. Haddad FS, Masri BA, Garbuz DS, et al. Primary total replacement of the dysplastic hip. AAOS Instruct Course LECT. J Bone Joint Surg, 1999; 81-A: 1462-1482.
- 11. Dorr LD, Kane TJ III, Conaty JP. Long-term results of cemented total hip arthroplasty in patients 45 years old or younger: a 16-year follow-up study. J Arthroplasty. 1994; 9: 453–456.
- 12. Callaghan JJ, Forest EE, Sporer SM, Goetz DD, Johnston RC. Total hip arthroplasty in the young adult. Clin Orthop Relat Res.1997; 344: 257–262.
- 13. Emery DF, Clarke HJ, Grover ML. Stanmore total hip replacement in younger patients: review of a group of patients under 50 years of age at operation. J Bone Joint Surg Br. 1997; 79: 240–246.
- 14. Harris WH. Total hip arthroplasty in the management of the congenital hip dislocation. In Callaghan JJ, Rosenberg AG, Rubash HE (eds): The Adult Hip. Lippincott-Rawen, Philadelphia: 1988; 1651-1682.
- 15. Hess WH, Umber JS. Total hip arthroplasty in chronically dislocated hips. J Bone Joint Surg, 1978; 60-A: 948-951.
- 16. Yasgur DJ, Stuchin SA, Adler EM, DiCesare PE. Subtrochanterik femoral shortening osteotomy in total hip arthroplasty for hi-riding developmental dislocation of the hip. J Arthroplasty, 1997; 12: 880-888.
- 17. Reikeras O, Haaland JE, Lereim P. Femoral Shortening in Total Hip Arthroplasty for High Developmental Dysplasia of the Hip Clin Orthop Relat Res: 2010; 468: 1949–1955
- 18. Paavilainen T, Hoikka V, Solonen KA. Cementless total hip replacement for severely dysplastic or dislocated hips. JBone Joint Surg Br 1990; 72: 205-11.

- 19. Sener N, Tozun I, Asik M. Femoral shortening and cementless arthroplasty in high congenital dislocation of the hip. J Arthroplasty 2002; 17: 41-8.
- 20. Eskelinen A, Helenius I, Remes V, Ylinen P, Tallroth K, Paavilainen T. Cementless total hip arthroplasty in patients with high congenital hip dislocation. J Bone Joint Surg Am. 2006; 88: 80–91.
- 21. Chareancholvanich K, Becker DA, Gustilo RB. Treatment of congenital dislocated hip by arthroplasty with femoral shortening. Clin Orthop 1999; 360: 127–135
- 22. Paavilainen T. Total hip replacement for developmental dysplasia of the hip. Acta Orthop Scand. 1997; 68: 77–84
- 23. Anwar MM, Sugano N, Masuhara K, Kadowaki T, Takaoka K, Ono K. Total hip arthroplasty in the neglected congenital dislocation of the hip. A five- to 14-year follow-up study.Clin Orthop: 1993295: 127–134
- 24. Symeonides PP, Pournaras J, Petsatodes G, Christoforides J, Hatzokos I, Pantazis E. Total hip arthroplasty in neglected congenital dislocation of the hip. Clin Orthop Relat Res.1997; 341: 55–61.
- 25. Hartofilakidis G, Stamos K, Ioannidis TT. Low friction arthroplasty for old untreated congenital dislocation of the hip. J Bone Joint Surg Br. 1988; 70: 182–186.
- 26. Crowe JF, Mani J, Ranawat C. Total hip replacement in congenital dislocation and dysplsia of the hip. J Bone Joint Surg: 1979; 61-A: 15-23,.
- 27. Edwards BN, Tullos HS, Noble PC. Contributory factors and etiology of sciatic nerve palsy in total hip arthroplasty. Clin Orthop: 1987; 218: 136–141
- 28. Eggli S, Hankemayer S, Muller ME. Nerve palsy after leg lengthening in total replacement arthroplasty for developmental dysplasia of the hip. J Bone Joint Surg Br: 1999; 81: 843–845
- 29. Kim YH, Seo HS, Kim JS. Outcomes after THA in patients with high hip dislocation after childhood sepsis. Clin Orthop Relat Res. 2009; 467: 2371–2378.
- 30. Kılıçarslan K, Yalçın N, Karataş F, Çatma F, Yıldırım H. Cementless total hip arthroplasty for dysplastic and dislocated hips. Eklem hastalıkları ve cerrahisi. 2011; 22(1): 8-15

Corresponding Author Bulent Koksal, Department of Orthopaedics and Traumatology, Medical Faculty, Ondokuz Mayis University, Samsun, Turkey, E-mail: bulentkoksal19@hotmail.com

# Trends in epidemiology of tuberculosis in HIVinfected patients

Iosif Marincu<sup>1</sup>, Simona Claudia Cambrea<sup>2</sup>, Adelina Mavrea<sup>3</sup>, Mirela Cleopatra Tomescu<sup>3</sup>

<sup>1</sup> Department of Infectious Diseases, "Victor Babes" University of Medicine and Pharmacy Timisoara, Romania,

<sup>2</sup> Department of Infectious Diseases, "Ovidius" Faculty of Medicine, University Constanta, Romania,

<sup>3</sup> Department of Internal Medicine, "Victor Babes" University of Medicine and Pharmacy Timisoara, Romania.

## Abstract

**Background:** Tuberculosis (TB) is one of the most common opportunistic infections in patients with HIV/AIDS. The aim of the study was to assess the epidemiological and clinical features of tuberculosis in HIV-infected patients using two algorithms.

**Materials and Methods:** HIV infection was confirmed by Western blot. CD4 counts were determined by flow cytometry. First algorithm identified TB patients using symptoms, chest X-ray and tuberculosis skin test. Second algorithm comprised sputum cultures associated with direct microscopy.

**Results:** A total of 102 HIV-infected patients were identified. Cough was present in 18 while fever, 20 patients were having pulmonary infiltrates, 6 adenopathy and 3 infiltrates and adenopathy. After applying both algorithms, 5 HIV-infected patients (4.90%) with definite TB were found.

**Conclusion:** A diagnostic management strategy using two simple clinical decision rules is effective in the evaluation and management of HIV-infected patients with clinically suspected tuberculosis.

**Key words:** Tuberculosis, HIV-infected patients, tuberculin skin test, CD4.

#### Introduction

The Human Immunodeficiency Virus (HIV) is causing the most destructive epidemic of recent times, having been responsible for the deaths of more than 25 million people since it was first recognized in 1981 (1). HIV infection remains of major public health importance in Europe, with an estimated 802,000 people living with HIV in EU/ EEA countries and more than 30 percent of HIVinfected people are unaware of their infection (2).

In Romania, latest data reported that 16,000 people were living with HIV/AIDS in 2009. Romania is the only country in Central and Eastern

Europe providing universal access to treatment and care for this disease (3).

Dual infection with *Mycobacterium tuberculosis* and HIV affects nearly 11 million people worldwide (4). Co-infection of HIV-infected patients with tuberculosis together with problems in health care systems and infrastructure, have an important impact on treatment success. In the absence of anti-retroviral therapy, HIV-infected patients with latent tuberculosis infection have 5–10% annual risk of TB in contrast to 10% during the life-time in HIV negative patients (5, 6).

Pulmonary tuberculosis (TB) is conventionally diagnosed by a combination of symptoms, chest X-ray (CXR), direct staining of *Mycobacterium tuberculosis* in sputum, sputum culture or by nucleic acid amplification techniques, where these are available (7).

Furthermore, the World Health Organization (WHO) recommends cough as the trigger for tuberculosis screening in HIV-infected patients, with acid-fast bacillus (AFB) smear as the initial diagnostic test (8). The most commonly used and reliable specimen for bacteriological examination is sputum for the diagnosis of pulmonary TB, but when the patient cannot expectorate sputum, several methods, such as laryngeal swab, sputum induction, gastric aspiration, and bronchoalveolar lavage, can be used to obtain specimens for smear or culture examination for acid-fast bacilli (9).

A great challenge in the diagnostic work-up of HIV-infected patients with clinically suspected TB is to accurately and quickly diagnose the presence of TB, for an early treatment. Because of the very frequent association of tuberculosis and HIV, it has become necessary to look for tuberculosis in HIV-infected patients and vice versa (10).

The aim of this study was to assess the epidemiological and clinical features of tuberculosis in HIV-infected patients in a 6 months period in 2 infectious diseases centres from Romania and to propose two simple algorithms for detection of TB in HIV-infected patients.

#### Material and methods

The study included all HIV-infected patients, aged at least 15 years, which received antiretroviral therapy, from the evidence of Infectious Disease Clinics of Timisoara and Constanta. We excluded from the study HIV-infected patients with serious opportunistic infections, sepsis, meningoencephalitis, and coma, cardiac, respiratory, hepatic, renal or circulatory failure, neoplasm or hematologic malignancies. Data concerning demographic (age, sex, location) and clinical characteristics (symptoms) were recorded.

#### Diagnosis of HIV status

In all patients, HIV infection was confirmed by Western blot and HIV-1 env DNA gene detection by PCR (11). The immune status was evaluated, and staging of HIV/AIDS was done according to international criteria, developed by CDC Atlanta, in 1993. CD4 cell counts were also evaluated by flow cytometry in all subjects.

#### **Diagnosis of TB**

All patients were subjected to chest radiograph and sputum smear AFB examination of three samples. Mantoux test using 5 TU of purified protein derivative (PPD) was done and transverse diameter of induration was noted in mm in all patients.

Than, first screening algorithm was applied to identify TB suspects using a short, structured screening questionnaire (symptoms), chest X-ray (CXR) and IDR to tuberculin (TST) (Figure 1). Xray images were scored as showing no abnormalities or abnormalities not suggestive of TB (negative) or abnormalities suggestive of TB (positive).

Then, second screening algorithm that comprised bacteriological investigation was applied to all HIV-infected patients with positive symptoms and positive CXR. The bacteriological examination was based on the detection of BK by sputum cultures (spontaneous or induced sputum and bronchoalveolar lavage), associated with direct microscopy of AFB stained sputum smear (Figure 2).



*Figure 1. First screening algorithm to detect TB in HIV-infected patients* 



*Figure 2. Second screening algorithm to detect TB in HIV-infected patients* 

#### Statistical analysis

Data were analysed using statistical computer software, MedCalc version 12.1.4.0. The descriptive data were given as means  $\pm$  standard deviation (SD). The differences were considered to be statistically significant when the *p* value obtained is less than 0.05.

#### Ethical approval

The study was approved by the research ethics committees of the "Victor Babes" University of Medicine and Pharmacy Timisoara, Romania and "Ovidius" Faculty of Medicine and University Constanta, Romania and written informed consent was obtained from all participants.

#### Results

The study included 102 HIV-infected patients (M = 52, W = 50, mean age =  $27.74 \pm 12.45$  years with a range of 19-65 years), retrospectively recruited from two centers for infectious diseases, Timisoara and Constanta from Romania.

The age distribution revealed that the highest incidence of TB suspects in HIVinfected patients was found in the groups of age under 20 years (45%). Most patients (n=65) included in the study were from urban areas, and the rest (n=37) were from rural areas (Table 1).

Table I	!. Age, 1	sex and	location	distrib	ution	of HIV-
infecte	d patier	nts				

Age	Number	Percentage (%)			
<20	46	45			
20-34	31	30.4			
35-44	5	5			
45-54	14	13.8			
>55	6	5.8			
Total	102	100			
	Sex	ζ			
Male	52	51			
Female	50	49			
Total	102	100			
	Location				
Rural	37	36.2			
Urban	65	63.8			
Total	102	100			

Cough was the most common symptom present in 18 patients (17%), followed by fever, weight loss and sweating present in 5 (5%), 5 (5%) and 3 (3%) patients, respectively.

Following the number of symptoms per patient, we noted that most patients (16) had just a symptom, 2 patients were registered with two symptoms, 1 patient experienced three symptoms, and 2 patients reported four symptoms. Radiological examination is known as a routine procedure used in identifying patients with TB. In this study, 20 patients (20%) were having pulmonary infiltrates, 6 (6%) adenopathy, and 3 (3%) infiltrates and adenopathy. There is no patient with caseous or ulcerative lesions (cavitations).

Using the algorithm proposed, 51 patients were categorised as "non TB", 12 patients with "TB probably likely" were monitored at the Prevention Centre for TB, and 39 patients with "TB likely" continued bacteriological investigation. Bacteriological confirmation was indicated in all potential positive TB patients. Considering the results of bacteriological investigation, patients with positive BK results on cultures or microscopy were diagnosed as "definite TB cases" and referred to anti-TB treatment. "Non-TB cases" were considered those with negative BK results on cultures of secretions obtained by bronchoalveolar lavage, associated with negative BK microscopy.

By all HIV-infected patients studied, 2 patients were found with a positive sputum culture of spontaneous BK and 3 patients had positive BK culture from bronchoalveolar lavage. All 5 HIV-infected patients (4.90%) were considered definite cases with TB.

An association between symptoms and the presence of radiological changes was observed in 5 (5%) patients with severe immunosuppression, in 5 (5%) with moderate immunosuppression, and in 1 (1%) patient with balanced immune status. Although all 15 patients with a history of TB were under antiretroviral therapy, 2 had severe immunosuppression (Li CD4 < 200/mm<sup>3</sup>), 7 had moderate immunosuppression (CD4 between 200-499/ mm<sup>3</sup> Li), and 6 were in balanced immune status (Li CD4 > 500/mm<sup>3</sup>) (Table 2).

Table 2. Association between symptoms, chest X-ray and values of Li CD4

Clinical Categories	Li CD4 levels	Pacient number n (%)	Patients with symptoms	Patients with positive CXR	Patients with symptoms and positive CXR	<i>p</i> value
C3	$< 200/mm^{3}$	34 (33%)	11 (11%)	5 (5%)	5 (5%)	NS
C2	200-499/mm <sup>3</sup>	15 (15%)	8 (8%)	9 (9%)	5 (5%)	NS
C1	$> 500/mm^{3}$	30 (29%)	2 (2%)	10 (10%)	1 (1%)	NS

CXR: Chest X-ray NS: Nonsignificant (p>0.05)

# Discussion

In 1989, Romania experienced a unique, major nosocomial HIV epidemic in which several thousand institutionalised children contracted HIV through blood transfusions. Since then, the rates of diagnosed HIV cases doubled between 2000 and 2009 in countries like Bulgaria, Hungary, Lithuania, Slovakia and Slovenia, but in Romania, new HIV cases decreased by more than 20%. The proportion of eligible patients that received antiretroviral therapy in Romania at the end of 2010 was about 60-79 % (12, 13).

HIV promotes the progression of latent TB infection to disease, and TB accelerates the progression of the HIV disease. Delayed diagnosis and improper management of people with TB and HIV co-infection may lead to spread of TB within the community, contribute to the development of drug resistant TB, and lead to poorer health outcomes for HIV-infected patients. Tuberculosis co-infection is associated with a doubling of the mortality rate in HIV-infected people. The most significant predictor of survival for HIV-infected patients with coexistent TB is the degree of immunodeficiency (14).

In our study, distribution by age showed the predominance of people less than 25 years (45%), confirming that TB coinfection in HIV-infected patients is more common in sexually active age group. Given the risk of TB and the unknown risk of opportunistic infections in HIV-infected patients, monitorisation should include preliminary detection of TB in this population.

Tuberculosis in HIV-infected patients may have unusual clinical features and can cause diagnostic difficulties. Signs and symptoms (fever, weight loss, and fatigue) can be caused by tuberculosis and other atypical mycobacterium infections (lymphoma, AIDS Wasting syndrome, CMV infection, etc.). This was confirmed by the results we obtained in the study group where only 21 patients had symptoms of TB diagnosis oriented. Therefore, the authors included in the algorithm for detecting TB in these patients, in addition to symptoms and radiological changes, the IDR to tuberculin. Similar with other studies, cough was the most common symptom, followed by fever, weight loss and sweating (15, 16). In people with HIV infection, the diagnosis of TB may be problematic due to confusion with other opportunistic infections and other HIV related diseases. Tuberculosis may present as a disseminated disease or with atypical clinical (or radiological) presentations, including enlarged hilar and/or mediastinal lymph nodes, pleural effusion, and lower lobe infiltrates; this is particularly the case in those with severe immunodeficiency (17-19).

Typical and cavitary lesions are usually observed in patients with higher CD4 counts, and more atypical patterns are observed in patients with lower CD4 counts. In patients with symptoms and signs of TB, a negative chest radiograph result does not exclude TB (20).

Inour study, 20 patients (20%) were having pulmonary infiltrates, 6 (6%) adenopathy, and 3 (3%) infiltrates and adenopathy. There is no patient with caseous or ulcerative lesions (cavitations). In The Terry Beirn Community Programs for Clinical Research on AIDS, pulmonary infiltrates were seen among 67%, adenopathy in 7%, pulmonary nodule in 20%, cavity in 20% and pleural effusion in 10% (21).

CD4 count measure the degree of immunosuppression in HIV-infected patients (22). Tuberculosis, unlike other HIV-associated opportunistic infections may occur at relatively high levels of CD4, although its frequency markedly increases in patients with more severe immunosuppression (23). Clinical symptoms indicative of tuberculosis were more frequent among patients with CD4 <200/mm3, while radiological changes occurred at a lower frequency among persons with low CD4 Li, but the differences were not statistically significant. In our study, stage C of HIV/AIDS was found in 81(79%) of HIV-infected patients. This requires the establishment of rigorous monitoring measures associated with early detection as TB in these patients. The number of patients with marked immunosuppression (35%) was close to the number of those with controlled immune status (29%) due to antiretroviral therapy administered. Moreover, this feature could explain the selected group and number of patients (n=5) identified as definite pulmonary TB. These HIVinfected patients could be clinically and biologically supervised in two traditional university clinics. The clinical experience in this consistent pathology is

relatively new and difficult for some hospitals in other geographic areas, where the application of this algorithm could lead to other results.

Monitored cases (n=12), respectively negative symptoms and negative CXR, but positive TST will be strictly controlled by both TB Prevention Centres from Timisoara and Constanta, and the Infectious Diseases Clinic where they are in record time to capture the appearance of clinical or radiological changes. Possible TB cases (n=49) resulting from the combination of three basic elements - positive symptoms, or positive chest X-ray or positive TST, will also be closely monitored in parallel clinics by specialists in infectious diseases and TB for detection of subclinical forms, latent bacteriological confirmed TB, but will be treated according to known rules.

Using proposed algorithms, 49 of the 102 HIV/ AIDS patients were identified with suspected TB, which would not have been possible through a routine examination. It shows that sometimes a single factor represents the main element of suspicion of TB. A special attention should be given to HIV-infected patients with a history of pulmonary or extrapulmonary TB (15 patients in the study). In these patients, due to immunosuppression, the risk of reinfection or relapse is high and has to be early identified, properly monitored, and effectively treated.

In order to avoid false-negative results that may occur through direct bacterioscopy or sputum cultures or need for cooperation and compliance from patients, but also seriously and conscientiously from health professionals involved in the diagnostic laborious process. We suggest, where possible, to advise and prepare the patient by health professionals who work with the office of psychological counseling in the hospital.

#### Conclusions

A diagnostic management strategy using two simple clinical decision rules is effective in the evaluation and management of HIV-infected patients with clinically suspected tuberculosis.

#### **Study limitation**

Although sputum cultures remain the gold standard in diagnosing TB, we met difficulties

with patients compliance and collaboration. Some patients refused bronchoscopy and preferred to give spontaneous sputum that was saliva leading to erroneous results and requiring a fresh sample from induced sputum or secretions obtained by laryngeal-tracheal lavage. Although they had agreed to participate in the study, the patients had difficulties in accepting all investigations, which indicates the need for their psychologically counselling during the study.

#### Acknowledgments/Funding

We thank leadership, specialists, and staff from the Centre for Health Policy and Services Bucharest (CPSS) that contributed to this Grant with funding from the Global Fund against HIV/AIDS, Tuberculosis, and Malaria through Romanian Angel Appeal. We thank all laboratory staff and Infectious Diseases and TB clinics which contributed to complete this project conducted in partnership between specialist clinics in Timisoara and Constanta from Romania.

#### References

- French N, Kaleebu P, Pisani E, Whitworth JA. Human immunodeficiency virus (HIV) in developing countries. Annals of tropical medicine and parasitology. 2006; 100(5-6): 433-54. Epub 2006/08/11.
- Deblonde J, Meulemans H, Callens S, Luchters S, Temmerman M, Hamers FF. HIV testing in Europe: mapping policies. Health Policy. 2011; 103(2-3): 101-10. Epub 2011/07/29.
- 3. Ruta S, Cernescu C. Influence of social changes on the evolution of HIV infection in Romania. The International journal of environmental studies. 2008; 65(4): 501-13. Epub 2008/01/01.
- Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Archives of internal medicine. 2003; 163(9): 1009-21. Epub 2003/05/14.
- Rook GA, Dheda K, Zumla A. Immune responses to tuberculosis in developing countries: implications for new vaccines. Nature reviews Immunology. 2005; 5(8): 661-7. Epub 2005/08/02.
- 6. Bordon J, Plankey MW, Young M, Greenblatt RM, Villacres MC, French AL, et al. Lower levels of interleukin-12 precede the development of tuberculosis

among HIV-infected women. Cytokine. 2011; 56(2): 325-31. Epub 2011/09/02.

- 7. Cho SN, Brennan PJ. Tuberculosis: diagnostics. Tuberculosis (Edinb). 2007; 87 Suppl 1: S14-7. Epub 2007/06/23.
- Bassett IV, Wang B, Chetty S, Giddy J, Losina E, Mazibuko M, et al. Intensive tuberculosis screening for HIV-infected patients starting antiretroviral therapy in Durban, South Africa. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2010; 51(7): 823-9. Epub 2010/08/26.
- 9. Uskul BT, Turker H, Kant A, Partal M. Comparison of bronchoscopic washing and gastric lavage in the diagnosis of smear-negative pulmonary tuberculosis. Southern medical journal. 2009; 102(2): 154-8. Epub 2009/01/14.
- Patel AK, Thakrar SJ, Ghanchi FD. Clinical and laboratory profile of patients with TB/HIV coinfection: A case series of 50 patients. Lung India: official organ of Indian Chest Society. 2011; 28(2): 93-6. Epub 2011/06/30.
- 11. Delwart EL, Shpaer EG, Louwagie J, McCutchan FE, Grez M, Rubsamen-Waigmann H, et al. Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 env genes. Science. 1993; 262(5137): 1257-61. Epub 1993/11/19.
- 12. European Centre for Disease Prevention and Control. Summary of key publications 2010: ECDC corporate. Stockholm: European Centre for Disease Prevention and Control; 2011. iii, 35 p. p.
- 13. Fraser G, Spiteri G, European Centre for Disease Prevention and Control. Annual epidemiological report reporting on 2009 surveillance data and 2010 epidemic intelligence data. Stockholm: European Centre for Disease Prevention and Control; 2011. xvi, 227 p. p.
- Ackah AN, Coulibaly D, Digbeu H, Diallo K, Vetter KM, Coulibaly IM, et al. Response to treatment, mortality, and CD4 lymphocyte counts in HIV-infected persons with tuberculosis in Abidjan, Cote d'Ivoire. Lancet. 1995; 345(8950): 607-10. Epub 1995/03/11.
- 15. Kim L, Heilig CM, McCarthy KD, Phanuphak N, Chheng P, Kanara N, et al. Symptom screen for identification of highly infectious tuberculosis in people living with HIV in Southeast Asia. J Acquir Immune Defic Syndr. 2012; 60(5): 519-24. Epub 2012/04/11.
- 16. Ayles H, Schaap A, Nota A, Sismanidis C, Tembwe R, De Haas P, et al. Prevalence of tuberculosis, HIV and respiratory symptoms in two Zambian communities: implications for tuberculosis control in the era of HIV. PloS one. 2009; 4(5): e5602. Epub 2009/05/15.

- 17. Brassard P, Hottes TS, Lalonde RG, Klein MB. Tuberculosis screening and active tuberculosis among HIVinfected persons in a Canadian tertiary care centre. The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale / AMMI Canada. 2009; 20(2): 51-7. Epub 2010/06/02.
- Komati S, Shaw PA, Stubbs N, Mathibedi MJ, Malan L, Sangweni P, et al. Tuberculosis risk factors and mortality for HIV-infected persons receiving antiretroviral therapy in South Africa. AIDS. 2010; 24(12): 1849-55. Epub 2010/07/14.
- 19. Verma SC, Dhungana GP, Joshi HS, Kunwar HB, Pokhrel AK. Prevalence of pulmonary tuberculosis among HIV infected persons in Pokhara, Nepal. Journal of Nepal Health Research Council. 2012; 10(1): 32-6. Epub 2012/08/30.
- Jensen PA, Lambert LA, Iademarco MF, Ridzon R. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings, 2005. MMWR Recommendations and reports: Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control. 2005; 54(RR-17): 1-141. Epub 2005/12/31.
- 21. Perlman DC, el-Sadr WM, Nelson ET, Matts JP, Telzak EE, Salomon N, et al. Variation of chest radiographic patterns in pulmonary tuberculosis by degree of human immunodeficiency virus-related immunosuppression. The Terry Beirn Community Programs for Clinical Research on AIDS (CPCRA). The AIDS Clinical Trials Group (ACTG). Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1997; 25(2): 242-6. Epub 1997/08/01.
- 22. Akinbami A, Dosunmu A, Adediran A, Ajibola S, Oshinaike O, Wright K, et al. CD4 Count Pattern and Demographic Distribution of Treatment-Naive HIV Patients in Lagos, Nigeria. AIDS research and treatment. 2012; 2012: 352753. Epub 2012/10/12.
- 23. Padyana M, Bhat RV, Dinesha M, Nawaz A. HIV-Tuberculosis: A Study of Chest XRay Patterns in Relation to CD4 Count. North American journal of medical sciences 2012; 4(5): 221-5. Epub 2012/06/02.

Corresponding Author Iosif Marincu, Department of Infectious Diseases, Pneumology and Parasitology, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania, E-mail: imarincu@umft.ro

# ABO/Rh blood groups distribution and serum lipid profile: Is there any association?

Sepideh Parchami Ghazaee<sup>1</sup>, Hooman Bakhshandeh<sup>1</sup>, Nahid Mehrzad<sup>2</sup>, Shiva Khaleghparast<sup>3</sup>

<sup>2</sup> Medical Laboratory Science, Clinical Biochemistry Department, Massoud Clinical Laboratory, Tehran, Iran,

<sup>3</sup> Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.

#### Abstract

**Background:** Dyslipidemia acts synergistically with other major risk factors of coronary artery disease (CAD). Variety in the distribution, type, and multiplicity of each of the blood groups in the human societies has prompted different types of investigations into the relationship between ABO blood groups and different diseases. Reducing risk factors constitutes the primary clinical approach to decreasing the mortality and morbidity of CAD; consequently, we sought to probe into the relationship between ABO blood groups and the serum lipid profile in the Iranian population with a view to designing a population strategy for the primary prevention of CAD.

Method and patients: This cross-sectional study was conducted on 2815 individuals who referred to Massoud Medical Laboratory and the Medical Laboratory of Rajaie Cardiovascular, Medical and Research Center, Tehran. ABO blood groups/ Rhesus and the serum lipid profile were determined after 12-14 fasting hours, and the subjects were classified according to ABO/Rhesus blood typing.

**Results**: There was no marked correlation between the serum lipid values and the different ABO/Rhesus blood groups. Family history of CAD and family history of hyperlipidemia were most prevalent in blood group A (p value =0.003), blood group O<sup>+</sup>(p value <0.001), and negative Rhesus factor (p value <0.001).

**Conclusion**: We suggest that individuals with phenotype A or O may be more predisposed to cardiovascular diseases and blood group screening may be an important way to prevent heart troubles in society.

**Key words:** Coronary artery disease, hyperlipidemia, ABO blood group system, Rh factor, Iran.

#### Introduction

Coronary artery disease (CAD) is the most common form of cardiovascular diseases and the leading cause of mortality, morbidity, and disability in the Iranian population. The incidence of CAD is strongly associated with the pattern of distribution and the number of CAD risk factors (1). It has been established that dyslipidemia is one of the major risk factors of CAD and that it acts synergistically with other major risk factors of this disease (1-2).

The discovery of ABO blood groups in 1900 is one of the most important events in the medical history. Variety in the distribution, type, and multiplicity of each of the blood groups in the human societies has been previously established (3), and it has prompted various types of investigations into the relationship between ABO blood groups and different diseases. Several studies have revealed a correlation between ABO blood groups and the risk of cardiovascular diseases (4-5). There are inconsistent results in different studies with respect to the distribution of ABO blood groups and major cardiovascular risk factors in different regions (5-6). Nevertheless, in the majority of these investigations, the understudy subjects have been identified cases of CAD, awaiting coronary artery angiography or bypass graft surgery. Moreover, in some of these studies there is no concrete information on the association between ABO blood groups and serum lipids as a major CAD risk factor and various lipid parameters have not been indicated objectively and comprehensively.

Since reducing risk factors is the primary clinical approach to lessening the mortality and morbidity caused by CAD (2), we sought to clarify the relationship between ABO blood groups and the serum lipid profile in a sample of the Iranian population in Tehran with a view to designing a population strategy for the primary prevention of CAD.

<sup>&</sup>lt;sup>1</sup> Cardiovascular Intervention Research Center, Rajaie Cardiovascular, Medical and Research Center, Iran University Of Medical Sciences, Tehran, Iran,

#### **Subjects and Methods**

This cross-sectional study was conducted on 2815 consecutive individuals who referred (for regular occupational or general medical checkup or according to the physician's order) to Massoud Medical Laboratory and the Medical Laboratory of Rajaie Cardiovascular, Medical and Research Center, Tehran, Iran, between March and September 2012.

The exclusion criteria were consumption of lipid-lowering agents or existence of liver obstructive diseases. ABO blood groups were determined via cell typing and back typing methods. Blood Rhesus was determined using Lorne kits (Germany), and direct Coomb's test was used to determine D antigen in Rhesus cases.

Samples for serum lipid profile parameters [total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein (VLDL), and triglyceride (TG)]were collected after 12-14 fasting hours and measured via the enzymatic colorimetric method with a biochemistry auto analyzer (Hitachi 917). The subjects were classified according to ABO/Rhesus blood typing. The normal ranges for serum lipids were considered as follows: TC <200 mg/dl; LDL-C <130 mg/dl; HDL-C>50 mg/dl; TG<200 mg/dl; and VLDLs<38mg/dl (7).

All the participants provided written, informed consent for participation in the study, and the study protocol was approved by the institutional Review Board and the Ethics Committee of Tehran University of Medical Sciences.

Statistical analysis was performed with SPSS software (SPSS 18.0 for Windows, SPSS Inc., Chicago, Illinois). The data are expressed as mean  $\pm$ SD for the continuous and as percentages for the discrete variables. The chi-square test was used to compare the nominal variables, and the Fisher exact test was employed if necessary. The Kruskal-Wallis test was utilized to compare the nominal variables with the ranked variables. Additionally, the *t*-test or one-way ANOVA was used to compare the nominal variables and the Mann Whitney U test was used if necessary. A p value<0.05 was considered statistically significant.

# Results

A total of 3058 participants from the general population referring to Massoud Medical Laboratory and the Medical Laboratory of Rajaie, Cardiovascular Medical and Research Center, Tehran, Iran, were enrolled in our study. Of this total, 243 subjects were excluded. Consequently, 2815 participants (1251 men and 1564 women) at an average age of  $44.5\pm15.9$  years (range =20-84 years) were considered for analysis.

The prevalence of cardiovascular risk factors for the participants was: diabetes mellitus 17%; hypertension 17.6%; smoking 16.3%; obesity (body mass index [BMI]> 25) 49.7%; and family history of CAD 43.3%. According to Rhesus factor status, 86.7% of the subjects were Rh<sup>+</sup> and 13.3% were Rh<sup>-</sup>. As is illustrated in Figure 1, blood group O was predominant.



*Figure 1. Distribution of blood groups in the participants* 

Table 1 depicts the demographic characteristics of the participants according to ABO blood groups classification. A family history of CAD and a family history of hyperlipidemia were more likely to be found in the participants with blood group A (p value =0.003) and blood group O (p value <0.001). Also, those in blood group O were more likely to be older (p value =0.035) and smokers (p value =0.049). The other characteristics were insignificantly associated with the different types of blood groups.

There were no reliable statistical changes in the prevalence of Rh positivity and negativity concerning sex and different ABO blood groups (Table 2). The statistical analyses revealed that as regards Rhesus factor, there were significant differences in the prevalence of diabetes (18% in Rh<sup>+</sup> vs. 10.7%

Characteristics	O <sup>a or b</sup> 949(33.7)	A 935 (33.2)	B 683(24.3)	AB 248(8.8)	P value
Age	45.1±16.1†	$44.5 \pm 16.1$	44.6±15.7	41.8±14.5†	0.035
Sex (%)M/F	46.4/ 53.6	42/58	43/57	42.3/ 57.7	0.257
Diabetes	168(35.1)	159(33.2)	115(24)	37(7.7)	0.694
Hypertension	161(32.9)	174(35.6)	116(23.7)	38(7.8)	0.695
Family history of CAD	365(29.8)	416(34)	323(26.4)	120(9.8)	0.003
Family history of hyperlipidemia	159(43.8)	118 (32.5)	61(16.8)	25(6.9)	< 0.001
Current smoking	170(37.1)	140(30.6)	118(25.8)	30(6.6)	0.049
BMI>25	462(33.3)	460(33.2)	345(24.9)	120(8.7)	0.493

Table 1. Demographic characteristics of the participants according to blood groups

*Values are expressed as Mean* $\pm$ *SD or n(%); CAD: Coronary artery disease; BMI: Body mass index;* †: *Significantly different(p value* =0.023),

in Rh<sup>-</sup>; p value <0.001), family history of CAD (41% in Rh<sup>+</sup>vs.59.7% in Rh<sup>-</sup>; p value <0.001), and family history of hyperlipidemia (13.8% in Rh<sup>+</sup>vs. 7.2% in Rh<sup>-</sup>; p value <0.001).

Table 2. Prevalence of Rhesus positivity and ne-<br/>gativity according to sex and blood group

Characteristics	Rh <sup>+</sup>	Rh⁻	P value
Sex			
Male	87.3	12.7	0.266
Female	85.9	14.1	
Blood group			
Α	86.9	13.1	
В	84.5	15.5	0.086
AB	84.7	15.3	
0	88.5	11.5	

Values are expressed as %, Rh: Rhesus factor

The average values of the lipid profile in the participants were as follows: TC (174.6±42.4 mg/dl); LDL\_C (102.3±32.9 mg/dl); TG (124±72.9 mg/dl); VLDL (21.9± 13.6 mg/dl); and HDL\_C (45.8±13.4 mg/dl).

There were no reliable differences in the lipid profile parameters between the different blood groups. Similar differences were obtained in the lipid indices according to Rhesus factor (Table 3). These results were supported by multivariate analysis after adjustment for different independent variables, which are mentioned in Table 1.

## Discussion

In our study, the most frequent blood group was O (33.7%), followed by blood groups A (33.2), B (24.3%),and AB(8.8%): this chimes in with the data on the Iranian population reported by the Iranian Blood Transfusion Organization, according to which, blood group O (33.6%) is the most prevalent, followed by blood group A(30.2%) and blood group B (24.4%),whereas blood group AB (11.8%) is the least prevalent (5-8).

The main finding of the present study was that there were no significant differences in terms of the lipid profile distribution between the different types

Table 3. Serum lipid profile levels according to different blood groups and Rh

Blood group/Rh	Number of patients (%)	TC (mg/dl)	LDL_C(mg/dl)	HDL_C(mg/dl)	TG(mg/dl)	VLDL(mg/dl)
0	949 (33.7)	173.5±43.2	102.1±34.4	45.6±13.6	125.5±75.1	22.7±15.1
А	935 (33.2)	176.1±41.4	103.5±32.1	45.7±12.9	122.7±74	21.6±13.6
В	683(24.3)	174.4±43	102.1±33.1	46.2±14	123.8±68.4	21.5±11.9
AB	248(8.8)	173.7±40.1	99.7±29.3	46.2±12.5	122.6±71.2	21.3±12.2
P value		0.222	0.524	0.500	0.242	0.063
$Rh^+$	2440(86.7)	174.3±42.4	102.2±33.1	45.9±13.7	124.8±74.2	22.2±13.9
Rh <sup>-</sup>	375(13.3)	177.3±41.5	103.1±31.6	45.7±11.4	118±62.9	20.2±11.2
P value		0.222	0.524	0.500	0.242	0.063

*Values are expressed as mean*±SD; *Rh: Rhesus factor; TC: Total cholesterol; LDL\_C: Low-density lipoprotein cholesterol; HDL\_C: High-density lipoprotein cholesterol; TG: Triglyceride; VLDL: Very low-density lipoprotein cholesterol* 

of ABO/Rhesus groups. The literature contains some evidence on a positive association between the different blood groups and cardiovascular diseases (9-10). Nonetheless, Amirzadegan and colleagues (8) posited that there was no correlation between the different blood groups and the development of CAD and the prevalence of major cardiovascular risk factors in patients who underwent coronary artery bypass graft. In contrast, the results of another study on the Iranian population revealed that hyperlipidemia was less frequent in participants with blood group B than in the other groups (6).

Data analysis of the Japanese population demonstrated that the average TC levels were significantly elevated in phenotype A compared to non-A (11). Kanbay et al. (12) reported that in patients who underwent coronary artery bypass surgery, no significant differences in the lipid levels between the different blood types were obtained, although the Rh<sup>+</sup> group had a significantly lower mean HDL-C level than the Rh<sup>-</sup> group. The latter finding is not consistent with our finding regarding HDL-C levels, which were equal in the different ABO/ Rhesus groups. Another study on patients who underwent coronary angiography in Budapest reported that although the mean serum cholesterol level was almost identical in the ABO blood groups, according to Rhesus it was significantly higher in the Rh<sup>-</sup> patients (13).

It is necessary to note that we found reliable differences in the prevalence of some other cardiovascular risk factors, including a family history of CAD, which was more prevalent in the participants with blood group A, and smoking, which was more frequent in the subjects with blood group O. Abdollahi and co-authors (5) demonstrated a significant relationship only between a family history of CAD and ABO blood groups among all the cardiovascular risk factors. The authors, however, reported no data on the association between hyperlipidemia (as a major risk factor for CAD) and ABO or Rhesus groups.

Our biochemical analysis showed that according to Rhesus factor, although the Rh subjects were more likely to have a family history of CAD, the prevalence of diabetes was more likely to be in the Rh<sup>+</sup> group. The results of various studies on the prevalence of diabetes mellitus regarding Rhesus groups in different regions are controversial. Our finding agrees with the results of Kumar et al. (14), who demonstrated a strong association between the prevalence of diabetes mellitus and Rh<sup>+</sup> group in the Indian population. However, *Dali Sahiand and colleagues (15) did not observe any significant association* between ABO/Rh blood groups and diabetes mellitus in the Algerian population. Given these conflicting reported data on the possible association between the different blood groups and the important risk factors of CAD, it is advisable that sufficient heed be paid to environmental conditions and hereditary factors involved in the prevalence of cardiovascular risk factors.

Since the lipid profile is a genetic component, one of the key risk factors for hyperlipidemia is family history (16). As much as we found no reliable association between the lipid values andthe different ABO/Rhesus groups, a family history of hyperlipidemia was strikingly more likely to be observed in blood group O<sup>+</sup>. It seems important to screen people at increased risk of lipid disturbances. Phenotype O individuals may be more predisposed to CAD through one of its major risk factors. There is strong evidence to suggest that those with a family history of hyperlipidemia require much more aggressive management through dietary recommendations, change in life style, and consideration of referral for pharmacological management for the prevention of CAD (15).

## Conclusion

There was no marked correlation between the serum lipid values and the different ABO/Rhesus blood groups in our sample of the Iranian general population. A family history of CAD and a family history of hyperlipidemia were more prevalent in our blood group A and blood group O<sup>+</sup> subjects. We would posit that individuals with phenotype A or O may be more predisposed to cardiovascular diseases and blood group screening may be an important way to prevent heart troubles in society.

The data presented herein were collected from a sample of the Iranian general population by two clinical laboratory centers. Be that as it may, further research with particular focus on blood group AB is required because it had the lowest prevalence of all the different blood groups in our study population.

#### Acknowledgment

We wish to thank Massoud Medical Laboratory managers and staff.

#### References

- 1. Hatmi ZN, Tahvildari S, Gafarzadeh Motlag A, Sabouri Kashani A. Prevalence of coronary artery disease risk factors in Iran: a population based survey. BMC Cardiovascular Disorders. 2007; 7: 32.
- Klop B, Elte JW, Cabezas MC. Dyslipidemia in obesity: mechanisms and potential targets. Nutrients. 2013; 5(4): 1218-1240. Doi: 10.3390/nu5041218.
- 3. Anstee DJ. The relationship between blood groups and disease. Blood. 2010; 115(23): 4635-4643. Doi: 10.1182/blood-2010-01-261859.
- 4. Carpeggiani C, Coceani M, Landi P, Michelassi C, L'abbate A. ABO blood group alleles: A risk factor for coronary artery disease. An angiographic study. Atherosclerosis. 2010; 211(2): 461-466. Doi: 10.1016/j. atherosclerosis.
- Abdollahi AA, Qorbani M, Salehi A, Mansourian M. ABO Blood Groups Distribution and Cardiovascular Major Risk Factors in Healthy Population. Iranian J Publ Health. 2009; 38(3): 123-126.
- Sotoudeh Anvari M, Boroumand MA, Emami B, Karimi A, SoleymanzadehM, Abbasi SH, et al. ABO Blood Group and Coronary Artery Diseases in Iranian Patients Awaiting Coronary Artery Bypass Graft Surgery: A Review of 10,641 Cases. Lab Medicine. 2009; 40(10): 528-530. Doi: 10.1309/LM0XULJ-3JAYARH9K.
- 7. Young DS. Effects of disease on Clinical Lab. Tests. 4rd ed. Washington, DC: AACC; 2001.
- 8. Amirzadegan A, Salarifar M, Sadeghian S, Davoodi G, Darabian C, Goodarzynejad H. Correlation between ABO blood groups, major risk factors, and coronary artery disease. Int J Cardiol. 2006; 110(2): 256-258.
- 9. Lee HF, Lin YC, Lin CP, Wang CL, Chang CJ, Hsu LA. Association of blood group A with coronary artery disease in young adults in Taiwan. Intern Med. 2012; 51(14): 1815-1820.
- Biswas J, Islam MA, Rudra S, Haque MA, Bhuiyan ZR, Husain M, et al. Relationship between blood groups and coronary artery disease. Mymensingh Med J. 2008; 17(2suppl): S22-S27.

- 11. Wong FL, Kodama K, Sasaki H, Yamada M, Hamilton HB. Longitudinal study of the association between ABO phenotype and total serum cholesterol level in a Japanese cohort. Genet Epidemiol. 1992; 9(6): 405-418.
- 12. Kanbay M, Yildirir A, Ulus T, Bilgi M, Kucuk A, Muderrisoglu H. Rhesus Positivity and Low High-Density Lipoprotein Cholesterol: A New Link? Asian Cardiovasc Thorac Ann. 2006; 14(2): 119-122.
- 13. Tarján Z, Tonelli M, Duba J, Zorándi A. Correlation between ABO and Rh blood groups, serum cholesterol and ischemic heart disease in patients undergoing coronarography. OrvHetil. 1995; 136(15): 767-769.
- 14. Kumar MV, Sambaia K, Ramesh BN, Manohar B, Lokesh BR. A comprehensive study on the serum lipid profile and risk factor analysis for cardiovascular diseases in a cross-sectional Indian population. J Indian Med Assoc. 2010; 108(3): 156, 158-160.
- 15. Dali Sahi M, Metria A, Belmokhtar F, Belmokhtar R, Bouazza F. The relationship between ABO/rhesus blood groups and type 2 diabetes mellitus in Maghnia, western Algeria. S Afr FAM Pract. 2011; 53(6): 568-572.
- 16. Daniels SR, Greer FR. Committee on Nutrition. Lipid screening and cardiovascular health in childhood. Pediatrics. 2008; 122(1): 198-208. Doi: 10.1542/peds.

Corresponding Author Shiva Khaleghparast, Rajaie Cardiovascular, Medical and Research Center, Tehran, Iran, E mail: sh khaleghparast@yahoo.com

# A computer vision based system for a rehabilitation of a human hand

# Peter Peer, Ales Jaklic, Luka Sajn

University of Ljubljana, Computer Vision Laboratory, Faculty of Computer and Information Science, Ljubljana, Slovenia.

# Abstract

Paper presents a rehabilitation system for patients who suffer from arm or wrist injury or similar. The idea of the rehabilitation using computer and additional hardware is not new, but our solution differs significantly. We tried to make it easily accessible and thus started with a limitation that only a personal computer and one standard web camera is required. Patient holds a simple object, cuboid, and moves it around. Camera records his movement while the software in real-time calculates position of the object in 3D space on the basis of color information and cuboid model. Object is then placed in the virtual 3D space, where another similar object is already present. The patient's task is to move the real object in the position, which matches the position of the virtual object. Doing so the patient trains specific movements that speed up the recovery. Evaluation of the system shows that presented solution is suitable in cases where accuracy is not very critical and smaller 3D reconstruction deviations do not thwart the process of rehabilitation.

**Key words:** computer vision, 3D reconstruction, cuboid model, real time execution, web camera, arm rehabilitation, injury, stroke

# Introduction

According to the World Health Organization, 15 million people have a stroke worldwide each year. Of all people who have a stroke, about a third are likely to die within the first ten days, about a third are likely to make a recovery within one month, and about a third are likely to be left with disabilities and needing rehabilitation. The types and degrees of disability that follow a stroke depend upon which area of the brain is affected. Generally, stroke can cause five types of disabilities: paralysis or problems controlling movement; sensory disturbances including pain; problems using or understanding language; problems with thinking and memory; and emotional disturbances. The paralysis is one of the most common disabilities resulting from stroke. The paralysis is usually on the side of the body that is opposite to the side of the brain affected by stroke: it may affect the face, an arm, a leg, or the entire side of the body. Movement impairments after stroke are typically treated with intensive, hands-on physical and occupational therapy for several weeks. Unfortunately, due to economic pressures on health care providers, stroke patients are receiving less therapy and going home sooner [1]-[3]. Additionally, there are various types of physical injuries, for instance due to car or sport accidents, where rehabilitation is required. Therefore we developed a system, called FRI Rehab 3D, which would help the patients to continue the rehabilitation and practice of intensive movement training also at home without the expense of an always-present therapist and special expensive equipment.

We explored existing solutions in Section 2, which are based on computers, robots, sensors and cameras. Since most of these systems require specialized hardware they can be very expensive, not always easy to use, and difficult to carry or set up at home. Thus, the biggest differences between existing systems and our solution are the price and the availability of required hardware. Whereas some solutions can cost from few thousand USD (not counting in a personal computer) up to over one hundred thousand USD, the cost of our solution is merely a personal computer and a single web camera, which people usually already have. Our goal was therefore to develop a solution that would allow rehabilitation for as many people as possible.

We developed and tested our system on a personal computer with Dual Core 1.86 GHz processor and a Logitech Quickcam Pro 5000 color web camera. The system was running Windows XP and the software was developed in Microsoft Visual Studio 2005 in C++ programming language. We decided for Windows family operating system because it is by far the most widely used operating system and thus probably preferred choice for most users [4]. We extensively used an open source package OpenCV, which offers many useful algorithms to solve computer vision and graphics related problems [5]-[8].

In Section 3 we describe how to set the working space environment to achieve best results. Discussion of segmentation methods can be found in Section 4. Next, the step of moving into 3D space based on the information from segmentation process is described in Section 5. In Section 6 the process of matching the real and virtual object is presented. Results of the quantitative and qualitative experiments are presented in Section 7 to demonstrate effectiveness of the proposed solution. Finally, Section 8 gives an overall conclusion and presents possible directions for improving the functionality of our system.

#### **Existing solutions**

The first examined solution, Rehab 2D, was also developed at the University of Ljubljana in Slovenia [9]. This method requires only a personal computer and a monochromatic camera, which tracks and detects the object that is moved by the patient. In the environment with sufficient lightning and under the condition that the object is not being moved too fast, the system successfully detects the object even if it is partly occluded. Unfortunately this method works only in two dimensions, so we expanded the basic idea of this solution and moved into three dimensions by using color information and cuboid model.

The MTi is a miniature size and low weight 3DOF Attitude and Heading Reference System (AHRS). The MTi contains accelerometers, gyroscopes, and magnetometers in 3D. Its internal low-power signal processor provides real-time and drift-free 3D orientation as well as calibrated 3D acceleration, 3D rate of a turn and 3D earth-magnetic field data. As such it could also be used for rehabilitation purposes. The price of the system is about 3,400 USD. According to the specifications the error of a moving sensor is less than two degrees [10]. This means that for the cuboid that we used for testing our system (length 14 cm, width 8 cm, height 6 cm), the error would be less than 5.35 mm.

The system Optotrak Certus provides accuracy of up to 0.1 mm and resolution of 0.01 mm. It can track up to 512 markers, with a maximum marker frequency of 4,600 Hz. Their motion path is tracked in 3D by the three infrared cameras. This system costs about 150,000 USD. It is not limited though only to rehabilitation, but is used in different industries, biomechanics, universities, and research institutions around the world [11], [12].

Y. Tao and H. Hu developed a real-time hybrid solution to 3D arm motion tracking for home-based rehabilitation by combining visual and inertial sensors. The Extended Kalman Filter (EKF) [13] Blackand Particle Filters (PF) [14]Black were used to fuse the different data modalities from two sensors and exploit complementary sensor characteristics. Due to the non-linear property of the arm motion tracking, upper limb geometry information and the pin-hole camera model are used to improve the tracking performance. With additional optimization algorithms Blackand introduction of additional constraintsBlack they achieved the accuracy of  $\pm 5$  cm.

ARMin is a system for robot-aided arm therapy [15]. A pilot study with ten healthy subjects and six patients was carried out to analyze comfort, functionality and acceptance of the ARMin training. Using a scale from 1 to 10 an average mark of 8.5 was assigned by the patients. In a second pilot study, the effects of the ARMin training were analyzed with three chronic stroke patients. After eight weeks of training there was a reduction in motor impairment assessed by the upper limb portion of the Fugl-Meyer Assessment. The improvements in this assessment are in a similar range as in other studies on arm therapy in chronic stroke patients. Unfortunately, quantitative results are not presented.

Here we can also mention the Stroke Rehabilitation Exerciser from Philips Research, which uses wireless motion sensors to fully capture the patient's upper-body movements. The idea is to develop a motion sensor exerciser to allow patients to perform vital rehabilitation exercises at home. This system is currently in the prototype state and is being tested and refined in clinical settings [16].

#### Preparations

One of the major factors that affects the efficiency of our solution, FRI Rehab 3D, is the work space arrangement (Figure 1). First, we have to understand that the only thing that is important on the picture that the camera captures is the cuboid. Therefore we have to make sure that it is easy to segment it out from the whole picture. We use the technique called blue or green screen, which is commonly used in weather forecast broadcasts, but with black color [17], [18]. Therefore we use a black curtain for the background and wear a black glove on the hand with which we hold the object. This makes it easy to segment out the cuboid.

Since we are limited to web cameras, which are quite sensitive to lightning conditions, we have to pay special attention to the light sources in the room. Ideally the room should be well lit and the cuboid should be illuminated evenly from all sides. We noticed that the combination of daylight and one or two artificial lights work very well. The lights should not be pointing directly towards the cuboid but should produce more diffuse light.

The material, that the sides of the cuboid are made of, plays a big role. The material should be bright enough, but not too reflective. We used a wooden cuboid, which sides we precisely covered with colored matt photograph paper. BlackOur cuboid reconstruction method is based on detection of cuboid corners, thus appropriate uniform colors of the sides are the easiest and fastest way to obtain the edges and corners, i.e. the cuboid framework. The three needed colors are specified in the HSV color space. In this color space the definition of three as different as possible colors is quite straightforward: the interval in which the parameter H is defined has to be split into the three equal parts. Knowing that, the colors of the sides were defined as: (H=60, S=100, V=100), (H=180, S=100, V=100), (H=300, S=100, V=100). Black

Another important aspect of the cuboid is its size which has to be input before the start of the rehabilitation process. On the basis of cuboid dimensions and its colors the algorithm can construct a correct cuboid model and use it for 3D reconstruction.

In the preparation stage the camera's parameters, such as brightness, contrast, white balance, exposure, and gain, should also be configured properly. Usually the camera's driver sets these parameters automatically, but in our experience it was always better to set and adjust them manually. We also need to know the camera's field of view and its resolution to compute 3D coordinates from the information obtained from the original, captured image [19], [20].



Figure 1. Work-space arrangement

# Segmentation

Since our approach does not use specialized external hardware, the computer processor has to do much more work and therefore our method can be computationally very demanding. Consequently it is necessary to implement time-efficient algorithms. Thus, in the first step we crop the image to get only the region of interest (using cvBo-undingRect [5]).

As will be described in more detail in the following sections, we represent the cuboid in the virtual 3D space with eight corners that are computed from detected corners in the input image. Therefore, it is important to develop a good method for identifying the corners in the captured image. Black Figure 2 gives basic steps of the whole system in a flow-chart.Black

The whole problem of identifying the corners is split into three subproblems: if one side of the cuboid is visible, if two sides are visible, and if three sides are visible. The first step is to determine how many sides are visible – one, two or three. This is done using the histogram of H values on the image converted to HSV color space [21]. Then the H interval is split into bins according to the colors of the cuboid sides, which were input in the preparation stage. Every pixel of the picture is then put in proper bin on the basis of its H value. In our case this method gave very good results. To reduce the calculation time the picture is resized to typically one eighth of its original size.



Figure 2. Basic steps of the whole system

This speeds up the process but leaves the ratios between bins practically the same. Ratios between bins determine which sides are present on the image and consequently which of the three subproblems needs to be solved. BlackThe histogram of H values in the case where three sides are visible is shown in Figure 3. Black



Figure 3. Histogram of H values of an image, where three cuboid sides are visible

# One side

This subproblem is solved in two steps. In the first step we detect approximate corners, which are then optimized in the second step (Figure 4). The approximate corners are detected by finding the smallest bounding rectangle around the visible cuboids' side. The bounding rectangle touches the cuboid side at four points, which are approximate corners. These corners are further refined with local optimization in area close to the corner. For every white pixel in this area the number of white surrounding pixels is calculated. The pixel with the least white surrounding pixels is most likely the corner.



Figure 4. Upper two figures show approximate corners, which are optimized with local optimization visible in two lower figures



Figure 5. Obtaining the edge information by intersecting two cuboid sides after isolating and processing them

# Two sides

In the case when two sides are visible the intersection method is used. Here the edges of the cuboid are detected first. The original image is split on the basis of color information in two images with only one cuboid side per image. Each image is further processed to remove any non-belonging surface and dilated to make it a bit bigger and more smooth. Then the intersection of both images is performed, which leaves only a narrow line of pixels that represents the edge. This set of pixels is further narrowed, eroded. On this basis the line defining the edge is calculated with linear regression. Figure 5 shows detection of one line defining the edge on the example where three sides are visible.



Figure 6. Detecting the outer four corners by analyzing perpendiculars along the edge

From the line defining the edge start and end points of the edge can be obtained. The line is traced until non-black pixel is found. This is the start of the edge. Then the line is traced further until a black pixel is detected. This is the end point of the edge. Thus, the first two corners are detected.

We continue by detecting the remaining four corners. In the vicinity of the previously detected corner (beginning of the edge) n perpendiculars on the edge are defined. BlackEach perpendicular starts at the edge and is tracked till the outer edge of the cuboid.Black If all pixels of a perpendicular are black, its length is set to zero. If the perpendicular runs across the cuboid, its length is calculated. BlackThe new corner is found when the algorithm detects two consecutive perpendiculars with big length difference (while both perpendiculars in the next pair are of the maximal length found in the vicinity of the already found corner).Black This new corner is further refined by the already mentioned local optimization. This method is repeated also for the other three remaining corners. Figure 6 illustrates this process.

# Three sides

The first step of this subproblem is similar to the subproblem of two cuboid sides. Here though sides intersection method is performed three times for the three pairs of sides and so three edges are detected. Intersection of all three edges defines one of the corners of the cuboid, let us call it the central corner.



Figure 7. Left figure shows the first four corners and three approximately detected corners. The figure on the right shows the result after local optimization

Next three corners are detected by following the three edges. For each of these three corners we start at the central corner and trace the edge until a black pixel is found. Additional check based on color information along the edge is performed, which confirms that the resulting corners are the right ones; note that the line defining the edge can be traced in two directions. Now, there are only three unknown remaining corners. These can be obtained by mirroring the central corner across the middle of the lines defined by the pairs of in the previous step detected three corners. After local optimization is applied, these three corners are usually well detected (Figure 7).

#### Moving into 3D space

The 3D coordinates are calculated when all the characteristics, namely sides colors, number of sides and corners are known. To calculate all eight corners of the cuboid it is theoretically enough if we detect only four corners of one side. But due to the fact that the detection is not always very precise, we get better result by including other corners as well. This is possible of course only when two or three sides are present. The method for obtaining 3D coordinates is therefore not completely the same for the three subproblems, though they are all based on the same idea. To obtain 3D coordinates the following data is required:

- the 2D coordinates of corners,
- the cuboid dimensions,
- the camera resolution,
- the camera field of view or focal length.

The detection of the corners and their 2D coordinates was already described in the previous section. In addition, it is necessary to know which side of the cuboid each detected corner belongs to, so the distances between the corners can be identified. The resolution of the camera can be set directly in the software, though we have to be careful that the camera supports the resolution that we set. We could not find the specification of camera's field of view or focal length so we had to measure it ourselves.

Figure 8 shows how an object is projected on the image plane. In this case the object is just one side of the cuboid. The camera is put in the center of the 3D coordinate system and is marked with label **o**. Points on the image are labeled **a**, **b**, **c** and **d**. These represent the corners that are detected on the captured image. Labels **a'**, **b'**, **c'** and **d'** refer to the actual corners of the cuboids' side that we need. Since focal length *f* is the distance between the camera and the image plane, we can extend the 2D corners **a**, **b**, **c** and **d** in the following way:

Thus, the new values of **a**, **b**, **c** and **d** are:

We introduce four new scalars  $k_1, k_2, k_3$  and  $k_4$ :

$$\overrightarrow{oa'} = k_1 \cdot \overrightarrow{oa}$$
  

$$\overrightarrow{ob'} = k_2 \cdot \overrightarrow{ob}$$
  

$$\overrightarrow{oc'} = k_3 \cdot \overrightarrow{oc}$$
  

$$\overrightarrow{od'} = k_4 \cdot \overrightarrow{odod'} = k_4 \cdot \overrightarrow{od}$$
.....(3)



*Figure 8. Detailed illustration of how an object is projected on the image plane* 

We can see that the scalars  $k_1$ ,  $k_2$ ,  $k_3$  and  $k_4$  are uniquely defined if two limitations, which are based on the cuboid model, are introduced. First limitation takes into consideration the intersection of both diagonals of the cuboid side. The first diagonal is defined by **a'** and **c'**, the second diagonal is defined by **b'** and **d'**:

The second limitation is the length  $l(\mathbf{a',b'})$ . Note that the lengths are given in the preparation step (Section 3), where we input the dimensions of the cuboid.

Both limitations together form four equations, which uniquely define needed four scalars  $k_1$ ,  $k_2$ ,  $k_3$  and  $k_4$ :

Thus, the scalars  $k_1$ ,  $k_2$ ,  $k_3$  and  $k_4$  are calculated and real cuboid corners **a'**, **b'**, **c'** and **d'** are obtained from Eq. (3).:

$$a'=(x'_{a}, y'_{a}, z'_{a})$$
  

$$b'=(x'_{b}, y'_{b}, z'_{b})$$
  

$$c'=(x'_{c}, y'_{c}, z'_{c})$$
  

$$d'=(x'_{d}, y'_{d}, z'_{d})$$
.....(6)

This concludes the calculation of the 3D coordinates of the four corners of the first side. The remaining four corners are calculated using normalized vector product. With altogether eight corners we can fully reconstruct the cuboid and visualize it in the virtual 3D space.

#### Matching

After obtaining 3D coordinates the cuboid is placed in the virtual 3D space in which another cuboid with the same characteristics as the real one is placed at for therapy appropriate position and orientation. The goal of the user is to move the real cuboid and to match it to the virtual cuboid as well as possible. In this way the user trains specific movements of his hand that assist in the recovery after the injury.



Figure 9. Successful matching of the virtual and real cuboid displays a success message. (Live image is on the left

Detection of the matching of the real and virtual cuboid is a two step process. In the first step the centers of both cuboids are calculated. If the two centers are close enough, we move on to the next step, where distances between corresponding corners of real and virtual cuboid are calculated. When these distances are under a predefined limit (defined by the therapist), matching is detected and a success message is displayed (Figure 9). The parameters that define minimal distance can be set in the initialization file of the system. Setting these two parameters to higher or lower value the difficulty can be adjusted to fit the user's needs.

#### Results

The system was developed and tested on a personal computer with the Intel Core2 Duo 1.86 GHz processor. Logitech Quickcam Pro 5000 web camera was used to capture images at resolution of  $640 \times 480$  pixels. On such equipment the achieved frame rate of our system was usually somewhere between four and nine frames per second. This is not a lot, but we have to keep in mind that the used equipment was not very fast and that our system at this point has not been optimized with regards to the computing time.

Black Furthermore, since lens distortion is not corrected on images, the reconstructions inherit its error. Processing undistorted images in general brings better reconstruction results, but in our solution processing distorted images does not give the impression of any worse performance to the end user, which is of main importance in our solution. Having in mind that undistorting image sequence means that much more processing time is needed, we should be satisfied with the results gained using the distorted sequence if we want to run in real-time. Another drawback of undistorted images is that they are more blurred in comparison to distorted originals, which can influence the subsequent processing steps. [22] Black

Two types of tests were performed: a quantitative and a qualitative analysis. But before we describe both of them, let us take a look at commonly occurring problems.

#### **Common problems**

In some cases the corners were very inaccurately detected due to the environment conditions. One of the common reasons was the bad lightning in the room Black (Figure 10) Black. When there is not enough light the sides are not completely visible and the result can be quite unpredictable. Fortunately, it is easy to add more light sources and avoid this problem. But when adding more light, we have to be careful not to cause another possible problem. If the light source is too strong and directed towards the object, a very strong reflection can occur on the object, making the side appear to be of different color (usually white or very bright) than it actually is Black (Figure 11) Black.



*Figure 10. Inaccurate reconstruction due to bad lightning* 



Figure 11. Strong light source is directed towards the object, causing a very strong reflection on the object, and thus inaccurate reconstruction

Again, the result is incorrect. This can be avoided by careful setting of the light and by using non reflective material for the cuboid sides. The third problem occurs when the object is moved too far away from the camera. Here not much can be done, except using higher resolution. This though is not a very good solution since it requires much more computational time. However, if the maximal distance is not more than 85-90 cm (which proved to be more than acceptable in practice), the accuracy and stability is good enough.

#### Quantitative analysis

Black Proper evaluation normally demands that we test the proposed solution on synthetic and real scenes, to perform quantitative and qualitative evaluation. As it is very hard (if not even near to impossible, especially without a big budget to buy a device like Optotrak [11, 12] or at least MTi [10]) to get ground truth information for real scenes of appropriate quality, but we can assure perfect ground truth information for synthetic scenes, we choose synthetic scenes for quantitative evaluation.

In the case of Tao and Hu system [14], which is the closest to our system in comparison to the cost and Blackemployed sensors, the tracking results from the marker-based system Qualisys (not their system) are regarded as a ground truth. But the error of Qualisys system itself is not reported and taken into account in their evaluation. Furthermore, they perform the evaluation on simple circular motion on the desk, which actually is a 2D example.

Some of the real world conditions aren't taken into account in the synthetic scenes, but the most obvious are. All the real world conditions are taken into account in our qualitative evaluation, where the user is the one making the judgment, similar as in the ARMin system [15] evaluation. Black

The quantitative test was performed mainly to identify the ideal capabilities of the system and to evaluate the main idea of the 3D reconstruction using single camera, color information and cuboid model. To get the perfect conditions, i.e. the ground truth information, we used 3ds Max application and in it we defined a space very similar to our own workspace arrangement. We placed a camera, light sources and a proper cuboid in this virtual space. We rendered the scenes at the resolution of 640×480 pixels and used rendered images as an input to our own system. In the end we compared the coordinates of real corners (of 3ds Max scene) and calculated corners obtained by our solution. Two types of errors were defined in this experiment. A quantitative error was defined

as a distance between coordinates of corner in 3ds Max and a calculated corner. Since this type of error does not always give a correct feedback about how the error appears to the user, second error estimator was defined, which we call approximate qualitative error. This type of error neglects the error on z axis, because the error on this coordinate is much less noticeable to the user than the errors on x and y axes. For both types of errors several metrices were calculated: average error with standard deviation, geometric mean and median [23], [24].

Altogether 45 cases with 226 corners (15 cases with one visible side, 15 cases with two visible sides and 15 cases with three visible sides) were examined. The results are shown in Tables 1 and 2.

Table 1. Calculated errors on the basis of 45 cases and 226 corners

	geometric mean	median	average
quantitative	11.73 mm	11.58 mm	19.38 mm
approx. qual.	2.51 mm	2.34 mm	4.42 mm

Table 2. Average quantitative error expressed inthe percentages of the actual distances

	average error ± standard deviation
distance from origin	3.02 % ± 3.84 %



Figure 12. Graph shows the correlation between the distance from the origin and the error size

Figure 12 shows the correlation between the distance of the object from the origin of the coordinate system (camera) and error size for both types of errors. Both graphs are also modelled with linear functions which, as we would expect, show that the error increases with the distance.



*Figure 13. Distribution of the quantitative and the approximately qualitative errors* 

Figure 13 shows the distribution of the error sizes, where we can notice the difference between the quantitative and the approximate qualitative errors functions. The approximate qualitative errors function has much narrower area of the expected errors than the quantitative errors function, which means that the stability from the user point of view is better than what one would expect looking only at the quantitative errors function.

Table 3. Comparison of different solutions (seeSection 2 for more details)

	reported error	cost
FRI Rehab 3D	11.58 mm	price of the camera
Optotrak [11, 12]	0.1 mm	approx. 150,000 USD
Xsens MTi [10]	max. 5.35 mm	approx. 3,400 USD
Tao, Hu [13, 14]	50 mm	price of the camera and the sensor

Table 3 compares the quantitative errors of other methods with our solution. We can see that the more expensive methods with specialized hardware are more accurate, but their cost/error ratio is not so good.

# Qualitative analysis

The second test was the qualitative analysis of our system which was performed on real input from the camera. Before we initiated the test, we calibrated the colors again and re-adjusted the light sources to get the best conditions and therefore best results. The test comprised of making three videos and evaluating each frame. In the first video (2160 frames, 72 seconds) we moved and rotated the cuboid so that most of the time only one side was visible at once. In the second video (2677 frames, 89 seconds) we moved and rotated the cuboid so that mostly two sides were visible, and in the third video (3477 frames, 116 seconds) we moved the cuboid so that three sides were visible at once. Then, we introduced three grades: good (no deformation), acceptable (Blackthe cuboid is in the right position, but one of its sides is only a bit deformedBlack), and bad (very deformed cuboid). According to them we graded each frame of all three videos. Table 4 summarizes the results. *Table 4. Qualitative analysis results* 

video	good	acceptable	bad
#1 [frames]	2107	0	53
#1 [percentage]	97.5 %	0 %	2.5 %
#2 [frames]	2553	93	31
#2 [percentage]	94.5 %	4.3 %	1.2 %
#3 [frames]	3307	154	16
#3 [percentage]	95.1 %	4.4 %	0.5 %
all [frames]	8314	247	100
all [percentage]	95.8 %	3.0 %	1.2 %

The results show that of all frames only 4.2 % were incorrect, i.e. graded acceptable or bad. However, only 1.2 % are bad and other 3 % are acceptable. The results are very satisfactory, especially if we take into consideration that the algorithms used can be further optimized, as it will be described in the next section. These results are possible though only with proper lightning adjustment, correct color calibration, good camera settings and can vary from video to video due to different factors (the distance of the cuboid from the camera, the speed of movement, the quality of the cuboid etc.).

# Conclusion

Our system, FRI Rehab 3D, uses very different approach than other, more expensive systems. Whereas they use advanced and specific hardware, we limited the requirements to only a single web camera. This brings both advantages and disadvantages:

Main advantages:

- low price and affordable hardware,
- can be used even at home,
- accuracy will be better with optimized algorithms and better cameras.

Main disadvantages:

- accuracy is lower than in more expensive systems,
- sensitivity to lightning conditions,
- computationally demanding.

Basically, we can separate our method in two steps. In the first step as many characteristics as possible are found in the captured image. The results suggest that this step works quite good, though it would be possible to improve it by additional analysis of the most commonly occurring errors. The second step calculates 3D coordinates from previously detected characteristics, by using color information and the cuboid model. This step is theoretically easily and uniquely solvable, but as it turns out in practice, it is not always very accurate. One of the main reasons is low camera resolution. This problem could be avoided by using better camera with higher resolution and with more time efficient algorithms. And, indeed, the errors of tests at resolution of 1,600×1,200 pixels were typically two or three times smaller (but so was the frame rate). Fortunately, using higher resolution is not the only possibility to improve the accuracy and the stability of detection and usability of the whole system. Here we list a few more ideas:

- The algorithms could be optimized to reduce computational demands.
- The accuracy of the 3D reconstruction could be significantly improved by implementing an algorithm that would use the cuboid model in a more efficient way. BlackFor instance, if a correction step of all corners together is added after detecting eight corners according to the constraints on the whole cuboid, the precision could be improved.Black
- One of the possible improvements comes from the observation that even a small change in 2D coordinates of a corner can cause big changes in 3D reconstruction. This is especially noticeable at bigger distances. If, for example, at one moment the corner is well detected, but at the next moment it is off by just a pixel, the error can increase significantly. Therefore, we need subpixel accuracy [25], [26]. This could be done in the following way: first, the 2D coordinates

would be detected in the same way as they already are. Then two neighboring sides would be calculated. Two corners of these two sides that touch would be compared and distances between them calculated. Ideally, the distances would be as low as possible. Then the 2D coordinates would be slightly changed and two sides calculated again and distances between the corners re-evaluated. This process could be repeated until the error is small enough, although the number of iterations would have to be limited to retain real-time execution.

- The system could compare new detection with previous one and check if the change is logical. If it is not, it could re-evaluate or skip the frame.
- BlackThe reconstruction results could be improved by eliminating the lens distortion problem, i.e. by undistorting the images in the preprocessing step [22].Black
- The stereo reconstruction approach could be implemented [27], [28].
- The system could also include patients database and their statistics, which could be analyzed by physiotherapist to evaluate the improvement of the patient.

Based on presented results and discussion, we conclude that under the condition that the user is willing to put in some effort on his own, the presented system serves its purpose good enough despite some faults. The presented system is suitable mostly in cases, where accuracy is not critical and smaller deviations of the 3D reconstruction do not thwart the process of the rehabilitation. Although the accuracy is already quite good under the right conditions, if previously suggested improvements would be implemented the overall performance would be significantly higher.

#### References

- 1. National Institute of Neurological Disorders and Stroke (NINDS), "Post-Stroke Rehabilitation Fact Sheet," in NIH Publication No. 08-4846, 2008.
- 2. American Heart Association, "Heart Disease and Stroke Statistics – 2009 Update," in American Heart Association, 2009.

- 3. Sucar LE, Azcárate G, Leder RS, Reinkensmeyer D, Hernández J, Sanchez I, Saucedo P. "Gesture Therapy: A Vision–Based System for Arm Rehabilitation after Stroke, "in Proceedings of the First International Conference on Health Informatics, 2008; 107–111.
- 4. OS Platform Statistics [Online]. Available: http:// www.w3schools.com/browsers/browsers\_os.asp
- 5. CXCORE Reference Manual [Online]. Available: http://opencv.willowgarage.com/wiki/CxCore
- 6. *CV* Reference Manual [Online]. Available: http:// opencv.willowgarage.com/wiki/CvReference
- 7. Experimental and Obsolete Functionality Reference [Online]. Available: http://opencv.willowgarage.com/ wiki/CvAux
- 8. *HighGUI Reference Manual [Online]. Available: http://opencv.willowgarage.com/wiki/HighGui*
- Katrašnik J, Veber M, Peer P. "Using computer vision in a rehabilitation method of a human hand," in Mediterranean Conference on Medical and Biological Engineering and Computing MEDICON 2007; 947–949.
- 10. Xsens MTi description and specifications [Online]. Available: http://www.xsens.com/en/general/mti
- 11. Hinesly D. "Technology in Motion Assessing the latest motion capture technologies," in Physical Therapy Products, 2008; vol. 6.
- 12. Technology in Motion description and specifications [Online]. Available: http://www.ndigital.com/ lifesciences/certus.php
- 13. Tao Y, Hu H. "3D Arm Motion Tracking for Homebased Rehabilitation," in Proceedings of the 3rd Cambridge Workshop on universal access and assistive technology, Cambridge, U.K., 2006; 105–111.
- 14. Tao Y, Hu H. "A hybrid approach to 3D arm motion tracking," in Transactions of the Institute of Measurement and Control, 2008; vol. 30, no. 3/4: 259–273.
- 15. ETH Zurich, Department of Mechanical and Process Engineering (D-MAVT), ARMin – description and specifications [Online]. Available: http://www.sms. mavt.ethz.ch/research/projects/armin/therapy
- 16. Philips Research, Stroke Rehabilitation Exerciser [Online]. Available: http://www.research.philips. com/technologies/projects/strokerehab/
- 17. Yamashita H, Agata T, Kaneko. "Every Color Chromakey," Department of Mechanical Engineering, Shizuoka University, 2008.

- 18. Smith R, Blinn JF. "Blue Screen Matting," Proc. SI-GGRAPH2004, 1996; 259–268.
- Ryberg A, Christiansson K, Lennartson B, Eriksson K. "Camera Modelling and Calibration – with Applications," in Computer vision In-Tech, 2008; 303–333.
- 20. Alter TD. "3D Pose from 3 Corresponding Points under Weak-Perspective Projection," MIT, Artificial Intelligence Laboratory, 1992; 1–9.
- 21. Forsyth DA, Ponce J, Computer Vision: A Modern Approach, Prentice Hall, 2003, 53–94.
- 22. Black P. Peer, Solina F. Multiperspective panoramic depth imaging, In: J. X. Liu (Ed.), Computer vision and robotics, Nova Science, 2006, 135–188. Black
- 23. Wolfram Research Inc. and Dr. Eric Weisstein, Encyclopedia Of Mathematics, 2008; 124: 1881.
- 24. Montgomery DC, Runger GC. Applied Statistics and Probability for Engineers, John Wiley & Sons, 2003; 59–89.
- 25. Mohr R, Boufama B, Brand P. "Accurate projective reconstruction," Applications of Invariance in Computer Vision, 2006; 257–276.
- 26. Mohr R, Brand P. "Accuracy in image measure," Spie, Videometrics III, 1994; 218–228.
- 27. Poggio GF, Poggio T. "The Analysis of Stereopsis," Annual Review of Neuroscience, 1984; vol. 7: 379–412.
- 28. Yu-Hui Z, Guo-Qiang L, Yue-Hui H, Wen-Wen L. "Algorithm and implementation of binocular stereopsis models," Computer Engineering and Applications, 2006; vol. 42, no. 35: 65–67.

Corresponding Author Luka Sajn, Faculty of Computer and Information Science, Ljubljana, Slovenia, E-mail: luka.sajn@fri.uni-lj.si

# Radiologic assessment of apical periodontitis and its relation with root canal filling quality

Ehsani Maryam<sup>1</sup>, Abesi Farida<sup>2</sup>, Khafri Soraya<sup>3</sup>, Mirkarimpour Seyed Sobhan<sup>4</sup>

- <sup>1</sup> Dental Materials Research Center, Department of Endodontic, Faculty of Dentistry, Babol University of Medical Sciences, Babol, Iran,
- <sup>2</sup> Dental Materials Research Center, Department of Oral & Maxillofacial Radiology, Faculty of Dentistry, Babol University of Medical Sciences, Babol, Iran,
- <sup>3</sup> Social Medicine Department, Babol University of Medical Sciences, Babol, Iran,
- <sup>4</sup> Student Research Center, Faculty of Dentistry, Babol University of Medical Sciences, Babol, Iran.

# Abstract

**Introduction:** Apical periodontitis (AP) is a local inflammatory process in the apical periodontal ligament and surrounding bone as a reaction to pulp necrosis or may be caused by severe periodontal diseases. The aim of the present study was to radiologic assessment of the rate of AP and its relation with the quality of root canal filling (RCF).

**Materials and methods:** In this cross-sectional study, 374 radiographs were selected based on the following inclusion criteria: 1) presence of > 10 natural teeth; 2) age > 18 years old; 3) root canal treatment performed during the last 2 years; 4) absence of medical complications; 5) good quality of radiographs; 6) teeth without periapical surgery.

The quality of RCF was evaluated by two observers who recorded the following parameters: length and density of RCF, presence or absence of AP and its size. The type kind and quality of coronal restoration was assessed during clinical and radiographic examination. Data were statistically analyzed using the SPSS software version 18 and Chi-Square test. P-values < 0.05 were considered significant.

**Results:** The rate of apical periodontitis in different groups demonstrated that its presence was increased in improper situations: length, density and coronal restoration quality (39.8%, 35.9% & 39.8 respectively).

In the situation that all the three parameters were appropriate, apical periodontitis was present in 6.4% of canals. If only one of these parameters: filling density, length or coronal restoration, was inappropriate, apical periodontitis was present in 30.3%, 24.7% and 22.7% of the cases, respectively. When all of them were inappropriate, apical periodontitis was present in 57% of canals.

Sensitivity and specificity of presence of three parameters simultaneously: Length, Density and Coronal restoration quality for predicting apical periodontitis were 20.3% and 94.3%, respectively.

**Conclusion**: The results of this study showed that there was a significant relationship between the radiographic quality of RCF and presence of AP. The higher rate of specificity and lower rate of sensitivity showed that the absence of three improper parameters simultaneously can predict AP absence strongly, but in presence of these parameters together; it is not qualified for predicting of AP presence.

**Key words:** Apical periodontitis, Panoramic radiography, Root canal filling quality.

# Introduction

The outcome of RCT is determined through the evaluation of tooth function, presence of signs and symptoms, radiographic changes or histopathology examination of the surrounding tissue of the tooth<sup>1</sup>. The most important goal of RCF is to prevent re infection of the root canal system and healing of the periapical tissues. To determine root canal treatment outcome, clinical and radiographic evaluation of RCF is necessary<sup>2</sup>. Panoramic radiography or dental panoramic has become a very popular technique in dentistry despite of its low resolution comparing to periapical radiography. And it is the first choice in initial visit of most of the patients. The radiologic features of inflammatory lesions are different and related to the time course of the lesion<sup>3</sup>. RCT is widely known as a highly complicated procedure, and epidemiologic studies have reported a higher rate of low quality RCF<sup>4, 5</sup>. Failure in RCF is associated with technical factors and uncorrectable anatomical problems in the root canal system<sup>6,7</sup>.

Apical periodontitis is a local inflammatory process in the apical periodontal ligament and surrounding bone as a reaction to pulp necrosis or may also be caused by severe periodontal diseases. Several studies have reported different frequency of AP in root treated teeth on panoramic or periapical radiographs like 48.57% by Kashan<sup>8</sup>, 46.31% by Kamberi<sup>9</sup>, 64.5% by Egea <sup>10</sup>, 52% by Kirkevang<sup>11</sup>, 61% by Weiqer<sup>12</sup>, 37.99% by Ozbas<sup>13</sup>, 73.9% by Gencoglu<sup>14</sup>, 52% by Asgari<sup>15</sup>, 40.4% by De Moor<sup>16</sup> and 25% by Loftus<sup>17</sup>. Therefore, the aim of the present study was to radiologic assessment of apical periodontitis and its relation with RCF quality.

# Materials and methods

Out of 1052 digital panoramic radiographs of patients who referred to a private Oral & Maxillofacial Radiographic Center, 437 radiographs were included in the study. The data were collected and assessed from December to May 2012. Inclusion criteria of studied radiographs were as follows: 1) presence of > 10 natural teeth; 2) age > 18 years old; 3) RCT done during the last 2 years; 4) absence of medical complications; 5) radiographs without artifact related to patient positioning and abnormal anatomical variation; 6) teeth without periapical surgery. Table 1 showed the parameters were considered in the study<sup>15</sup> (Table 1). Also, the quality of coronal restoration was evaluated through a clinical and radiographic examination. Two observers, an oral & maxillofacial radiologist and an endodontist evaluated the radiographs simultaneously in a semi-obscure and quiet room on a 1360x768 pixels resolution

Parameters	
Apical periodontitis	<ol> <li>Absence of apical normal structure or small changes in apical structure</li> <li>Change in bony structure and decrease in mineral part, radiolucent area with defined border or severe or diffuse periodontitis</li> </ol>
Size of apical periodontitis	1. <3 mm 2. <3 mm ->5 mm 3. >5 mm
Filling length	<ol> <li>Appropriate (&lt;2 mm from the radiographic apex)</li> <li>Inappropriate (&gt;2 mm from the radiographic apex)</li> </ol>
Filling density	<ol> <li>Appropriate (even density and adaptation to canal wall)</li> <li>Inappropriate (lateral space, void in filling and missed canal)</li> </ol>
Coronal restoration	<ol> <li>Appropriate (marginal adaptation, no sign of leakage)</li> <li>Inappropriate (overhang, recurrent caries, temporary or lack of restoration)</li> </ol>

Table 1. Parameters description

monitor. Density and contrast of the images were adjusted according to the conditions. The data were recorded after an agreement between the two specialists. In case of a disagreement between them, the opinion of a third observer was requested in order to have a majority coordinated decision. Data were statistically analyzed using the SPSS software version 18 (SPSS Inc., Chicago, IL, USA) and Chi-Square test. P values < 0.05 were considered significant.

# Results

Regarding the type of the teeth, apical periodontitis was present in 21.9% of premolars and 78.1% of the molar teeth. The data summarized in Chart 1 showed that the improper RCF density had the most frequency. Apical periodontitis was present in 26.73% of teeth. And size 1 apical periodontitis was more than other sizes (86.1% v.s 10% in size 2 and 4% in size 3).



*Chart 1. Rate of appropriate and inappropriate length, density and coronal restoration* 

The rate of apical periodontitis in different groups demonstrated that its presence was increased in improper situations (Table 2).

When all the three parameters were appropriate, apical periodontitis was present in 6.4% of canals. When only one of these parameters: filling density, length or coronal restoration, was inappropriate, apical periodontitis was present in 30.3%, 24.1% and 22.7% of the cases, respectively. When all of them were inappropriate, apical periodontitis was present in 57% of canals.

According to Table 3, sensitivity and specificity of presence of three parameters simultaneously: Length, Density and Coronal restoration quality for predicting apical periodontitis were 20.3% and 94.3%, respectively.

# Discussion

The general trend of the present study's findings revealed that the rate of AP (26.73%) was lower than other similar studies<sup>8-16</sup>. In contrast, a lower rate of AP was reported in one study.<sup>17</sup> The lower percentage of AP comparing to others might be a consequence of the sample size and the radiographs type (digital panoramic or periapical ones).

Also this study showed that in each of the improper RCF length, density and coronal restoration separately, the rate of AP was more than the appropriate ones. And some studies confirm this finding.<sup>8, 10, 11, 14, 15, 18</sup> In current study, 41% canals had appropriate filling length, density and coronal restoration that was significantly more than the 17.2% reported by Egea et al.<sup>10</sup>

Also, RCF density had the most significant effect on the presence of apical periodontitis in this study and it maybe due to the effect of filling density on the root canal seal and prevention of canal re infection.

Regarding to the teeth type, the highest rate of apical periodontitis was related to mandibular molar teeth (61.3%), and the lowest rate was observed in mandibular premolars (9.2%). This can be explained by the complexity of the molar canals. Also, it should be noted that there is more clarity associated with mandibular teeth canals compared

*Table 2. Comparison of treatment quality according to different parameters in teeth with apical periodontitis* 

	Apical periodontitis	P-value	RR(CI95%)		
Length					
Appropriate	271(18.4)	0.001>	2.91(2.26-3.76)		
Inappropriate	138(39.8)	0.001>			
Density					
Appropriate	128(12.4)	<0.001	3.96(3.13-5.01)		
Inappropriate	282(35.9)	<0.001			
Coronal restoration Quality					
Appropriate	270(18.4)	<0.001	2.93(2.28-3.67)		
Inappropriate	140(39.8)	<0.001			

- Values in table are n (%).

*Table 3. Sensitivity and specificity of length, density and coronal restoration in predicting apical periodontitis* 

*Predicting parameter Apical periodontitis	Absence	Presence
Absence	1331(94.3)	80(5.7)
Presence	326(79.7)	83(20.3)

\*Predicting parameter: represents the presence of three improper parameters simultaneously: Length, Density and Coronal restoration quality

- Values in table are n (%).

to maxillary ones in radiographs mainly because of canal morphology and the anatomical structures, such as the maxillary sinus and zygomatic process.

In addition, the data showed that if all of the parameters were appropriate, the absence of apical periodontitis could be predicted to up to 94.3%. On the other hand, if all of them were inappropriate, the presence of apical periodontitis could be predicted to up to 20.3%. This demonstrates that the other factors such as proper sterilization, cleaning & shaping, body defense system, etc., could be involved. As mentioned earlier, panoramic radiographs were used in this study because they represent the first radiographs prescribed by dentists in the initial visit. Ordering full mouth periapical radiographs is not economic and moral in first visit for most of the patients.

One of the limitations of this study was the presence of apical scars that radiologically resemble apical periodontitis and might have been misdiagnosed. However, because of their low prevalence, they were not considered in this study. Also, the radiographic interpretation might have varied among the observers due to the different radio-opacity of canal sealers or cements, different commercial gutta-percha, bone anatomy, radiographic angle, two-dimensional nature of radiographs and missed or additional canal and superimposed canals. The presence of voids or lack of filling adaptation to canal wall might be masked with radiopacity of sealers.<sup>19</sup>This means that the canals with radiographically appropriate filling length and density are not essentially clinically appropriate. The higher rate of specificity and lower rate of sensitivity showed that the absence of three improper parameters simultaneously can predict AP absence strongly, but in presence of these parameters together; it is not qualified for predicting of AP presence.

At the end of this study, it is suggested that to perform the study with more samples and different radiographic angle (periapical) and new techniques like Cone Beam Computed Tomography.

#### Conclusion

The results of this study showed that there was a significant relation between radiographic quality of RCF and the presence of AP. And more specialized training is needed to improve RCF standards and decrease the frequency of AP.

#### References

- 1. Salehrabi R, Rotstein I. Endodontic treatment outcomes in a large patient population in the USA: an epidemiological study. J Endod 2004; 30: 846-50.
- Moussa-Badran S, Roy B, Bessart du Parc AS, Bruyant M, Lefevere B, Maurin JC. Technical quality of root fillings performed by dental students at the dental teaching center in Reims, France. Int Endod J 2008; 41: 679-684.
- 3. White S, Pharoan M. Oral radiology, 6<sup>th</sup> ed, Mosbey, Philadelphia, 2006; 175: 326-337
- Kirkevang LL, Vaeth M, Horsted-Bindslev P, Bahrami G, Wenzel A. Risk factors for developing apical periodontitis in a general population. Int Endod J, 2007; 40: 290-299.
- 5. Kabak Y, Abbott PV. Prevalence of apical periodontitis and the quality of endodontic treatment in an adult Belarusian population. Int Endod J, 2005; 38(4): 238-245.
- 6. *Abbott PV. The periapical space–a dynamic interface. Aust Endod J, 2002; 28(3): 96-107.*
- 7. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med, 2004; 15(6): 348-381.
- 8. Kashan A, Neamat H, Yahia E. Pilot study on relation of the periapical status and quality of endodontic treatment in an adult Sudanese population. Archives of Orofacial Sciences. 2011; 6(1): 3-8.
- 9. Kamberi B, Hoxha W, Stavileci M, Dragusha E. Prevalence of apical periodontitis and endodontic treatment in a Kosovar adult population. BMC Oral Health 2011; 11: 32.
- Segura-Egea JJ, Jime' nez-Pinzo' n J, Poyato-Ferrera M, Velasco-Ortega E. Ri'os-Santos JV. Periapical status and quality of root fillings and coronal restorations in an adult Spanish population. Int Endod J, 2004; 37:; 525-530.
- 11. Kirkevang LL, Ørstavik D, Hörsted-Bindslev P, Wenzel A. Periapical status and quality of root fillings and coronal restorations in a Danish population. Int Endod J, 2000; 33: 509-515.
- 12. Weiger R, Hitzler S, Hermle G, Löst C. Periapical status, quality of root canal fillings and estimated endodontic treatment needs in an urban German population. Endod Dent Traumatol 1997; 13(2): 69-74.

- 13. Ozbas H, Aşçı S, Aydın Y. Examination of the prevalence of periapical lesions and technical quality of endodontic treatment in a Turkish subpopulation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112: 136-142
- 14. Gencoglu N, Pekiner FN, Gumru B, Helvacioglu D. Periapical status and quality of root fillings and coronal restorations in an adult Turkish subpopulation. Eur J Dent. 2010 Jan; 4(1): 17-22.
- 15. Asgary S, Shadman B, Ghalamkarpour Z, Shahravan A, Ghoddusi J, Bagherpour A, Akbarzadeh Baghban A, Hashemipour M, Ghasemian Pour M. Periapical Status and Quality of Root canal Fillings and Coronal Restorations in Iranian Population. Iran Endod J. 2010; 5(2): 74-82.
- 16. De Moor RJG, Hommez GMG, De Boever JG, Delme KIM, Martens GEI. Periapical health related to the quality of root canal treatment in a Belgian population. Int Endod J 2000; 33: 113-120.
- 17. Loftus JJ, Keating AP, McCartan BE. Periapical status and quality of endodontic treatment in an adult Irish population. Int Endod J. 2005 Feb; 38(2): 81-6.
- Georgopoulou MK, Spanaki-Voreadi AP, Pantazis N, Kontakiotis EG, MorfisAS.Periapical status and quality of root canal fillings and coronal restorations in a Greek population. Quintessence Int;2008; 39(2): e85-92
- Cohen S, Hargreaves KM. Pathway of Pulp.10<sup>th</sup> ed. Mosby; Elsevier, Philadelphia, 2011; 349-372.

Corresponding Author Abesi Farida, Dental Materials Research Center, Department of Oral & Maxillofacial Radiology, Faculty of Dentistry, Babol University of Medical Sciences, Babol, Iran, E-mail: f\_abesi@yahoo.com

# Effects of berberine on the expression of AQP-2, IL-1 and IL-6 in a rat model of streptozotocin-induced diabetes mellitus

Guo Zhentao, Zhang Huamin, Zhang Yanfeng, Sun Hongri

Department of Nephrology, Affiliated Hospital of Weifang Medical University, P.R. China.

## Abstract

**Objectives:** Berberine, an extract from traditional medicine herbs, has been used for the management of diabetes mellitus (DM) and its complications. The purpose of this study was to detect the effects berberine on the renal function and the expression of aquaporin-2 (AQP-2), interleukin 1 (IL-1) and interleukin 6 (IL-6) in kidney in streptozotocin-induced diabetic rats.

**Methods:** Sprange-Dawley rats received a single intraperitoneal injection of streptozotocin (50 mg/kg) 10 hours after the deprivation of food and water. Sixteen animals with blood glucose levels between 16 and 30 mmmol/L were taken as diabetic rats and assigned into DM group and berberine group with 8 animals in each group. Rats of the berberine group were given intragastric administration of berberine (100 mg/kg/day) for 21 days. Urine volume, urinary albumin excretion and creatinine clearance rate (Ccr) were measured or calculated. The expression of AQP-2 in kidney was examined with immunofluorescence staining, as well as the expression of IL-1 and IL-6 in kidney was respectively measured using ELISA.

**Results:** The diabetic rats in the DM group had more urine volume, more urinary albumin excretion, higher expression of AQP-2, IL-1, IL-6 and lower Ccr than the control rats. The treatment with berberine significantly decreased the urine volume, urinary albumin excretion, as well as the expression of IL-1 and IL-6 in kidney, and increased Ccr as compared to the animals in the DM group. However, berberine had no distinct influence on the expression of AQP-2 in collecting ducts.

**Conclusions:** Berberine improved the renal function of the diabetic rats induced by streptozotocin injection and the drug might exert its effects by inhibiting the local expression of IL-1 and IL-6 in kidney. **Key words:** Berberine, diabetes mellitus, AQP-2, IL-1, IL-6.

## Introduction

Diabetes mellitus is a group of metabolic disorders with hyperglycaemia that affect almost 300 million people worldwide (1). Diabetic patients, regardless of suffering from type 1 diabetes or type 2 diabetes, may have various complications developed as the consequence of the metabolic disorders, such as heart disease, nerve lesion, and renal dysfunction. Among the multiple complications, diabetic nephropathy (DN) characterized by basement membrane thickening, glomerular hypertrophy, disruption of the glomerular permeability barrier, is a serious condition that may lead to renal failure if not well treated (2).

The pathogenesis of kidney lesion in diabetic subjects is complex and involves many mechanisms leading to renal damage. Recently, the inflammatory factors are considered to contribute to the development and exacerbation of diabetesinduced renal damage (3, 4). Some inflammatory cytokines, such as IL-1 and IL-6 have been demonstrated to play important roles in the pathogenesis of DN (5, 6,7). Clinical and animal studies showed the subjects with DN usually had high levels of these cytokines, and the levels were reduced if DN was well treated (8). Therefore, inflammation might be a therapeutic target of DN. Besides, the aquaporins (AQPs), water channels involved in the regulation of urinary salt and water excretion, are believed to have a role in the pathophysiology of DN (9, 10,11). Of the AQPs expressed in kidney, AQP 1 mainly locates in the proximal tubule and thin descending limb of Henle epithelia, AQP2, AQP3 and AQP4 locates in the collecting ducts. There is an increasing body of evidence suggesting that AQP 2 expression sites (in apical

membrane or cytoplasm) in principal cells are controlled by vasopressin, and disrupted function or regulation of AQP2 may result in nephrogenic diabetes insipidus. Although the function of AQP2 in DN has been studied, the changes of AQP2 expression in diabetic subjects are still debated. Some studies showed increased AQP2 expression in the collecting ducts of the subjects with DN or diabetes and the AQP2 expression could be inhibited if the hyperglycemia was controlled (10, 12, 13, 14); while some other studies showed hyperglycemia might cause down-regulation of AQP2 expression in the STZ-induced diabetic animals and the protein expression of AQP2 was restored to the control level after insulin treatment (15).

Berberine, a Chinese patent medicine, has been used in the management of diabetes mellitus and some other inflammatory diseases (16, 17, 18). But to our knowledge, very limited studies detected the effects of berberine on DN and the mechanisms of its effects on DN remain unclear. This study intended to investigate whether treatment of diabetic rats with berberine could prevent the diabetes-induced renal damage and influence the expression of some substances that are involved in pathogenesis of DN.

# Methods

## Animals

The Animal Research Ethics Committee at the Affiliated Hospital of Weifang Medical University approved the animal experimental protocols of the study. All animal experimental protocols were conducted in strict in accordance with the Guidelines for Experimental Animal Welfare approved by the Ministry of Science and Technology of China. Male Sprague–Dawley rats (180-220 g) purchased from Shandong University were used for the experiments.

# **Diabetic Model**

In order to induce diabetes, a single intraperitoneal injection of streptozotocin (50 mg/kg) (dissolved in 0.1 M cold citrate buffer, pH 4.5 ) was given to the Sprague–Dawley rats. Blood glucose was examined using a glucometer 3 days after the streptozotocin injection, and rats with blood glucose less than 16 mmol/L or higher than 30 mmol/L were excluded from this study. The diabetic rats were further randomly divided into DM group and berberine group with 8 animals in each group, and another 8 normal Sprague–Dawley rats were taken as the control group.

# Drug treatment

Three days after the administration of streptozotocin, the animals in the berberine group received intragastric administration of berberine (100 mg/ kg, qd, dissolved in saline) for 21 consecutive days. The animals of the DM group and the control rats were treated with the same volume of saline only.

# **Renal function analysis**

After the 21 days' drug treatment, rats were placed in metabolic cages for 24 h to collect the urine (under oil to prevent from evaporation). 24 h urinary albumin excretion and Ccr were calculated using the following equations to assess the renal function, following the measurements of the concentrations of urinary albumin and creatinine in both urine and serum.

24 h urinary albumin excretion= Concentration of urinary albumin ×24 h urine volume Ccr = (Concentration of urinary creatinine × urine volume produced per minute)/ Concentration of Serum creatinine

# *Immunohistochemistry*

After the deep anesthesia, the rats were sacrificed. The right kidneys were removed, washed in PBS, fixed in 10% phosphate-buffered formalin and embedded in paraffin. Fixed kidneys were sectioned at 4 µm and attached to class slides. Following the rehydration protocol, the sections were incubated in the blocking buffer (1% donkey serum in 0.01 M PBS) for 10 min to prevent the non-specific binding to antibody, then incubated in the anti-AQP 2 antibody (1:100, diluted in PBS. Boster Company, China) overnight at 4 °C. After rinsed for 3min in PBS (repeated 3 times), sections were incubated with the secondary antibody (1:200, diluted in PBS. Boster Company, China) for 1 h at room temperature, followed by repeated washing in PBS and incubation with biotinylated FITC for 30 min in humid dark box. The expression of AQP 2 in kidney was observed immediately after rinsing for 30 minutes with 0.01 M PBS.

# ELISA assays of IL-1 and IL-6

To detect the influence of berberine on the expression of IL-1 and IL-6 in local kidney tissue which promote the pathogenesis of diabetic nephropathy, 1 gram of kidney tissue containing renal cortex and renal medulla was collected after the remove of the left kidney, stored at -70 °C and subsequently homogenized in 2 ml saline (4 °C). The supernatant was collected after the homogenization and centrifugation at 3000 rpm for 15 min. Specific ELISA kits (Boster Company, China) were used to analyze the concentrations of IL-1 and IL-6 in the supernatant according to the manufacturer's recommendations.

# Statistical analysis

The statistical analyses were performed using SPSS 13.0. Data were presented as mean  $\pm$  SD. The differences between the groups were compared using One-way analysis of variance (ANOVA) followed by Scheffe test. P values less than 0.05 were considered statistically significant.

# Results

# Effects on renal function

The 24 h urine volume was collected in metabolic cages and the mean values of the volumes were shown in table 1. A significant increase of the 24 h urine volume of the rats in the DM group was seen if compared with that of the controls (P<0.05). There was a marked reduction in 24 h urine volume of the berberine treated animals compared to the DM rats (P<0.05), however, still more than the controls (P<0.05). The difference of Ccr and 24 h urinary albumin excretion was significant between the DM group and control group with increased urinary albumin excretion and decreased Ccr in the DM group (both P<0.05). With the treatment of the Chinese patent medicine, the diabetic rats of the berberine group had less 24 h urinary albumin excretion and more Ccr than the animals of the DM group (both P < 0.05). The data showed streptozotocin induced renal dysfunction in rats, and berberine had a protective effect on the renal function of the diabetic rats.

# Effects on the expression of AQP 2

Immunofluorescence staining was performed for the expression of AQP 2 in the kidney, which plays a role in maintaining body water balance. As shown in figure 1, the water channel AQP 2 mainly located in the cells of collecting ducts, with no AQP 2 expression in glomeruli and proximal convoluted tubules of the rats in all the three groups (Figure 1B, D, F). Figure 1A showed there was faint AQP 2 expression in the collecting duct principal cells of the control rats. Figure 1C showed much more AQP 2 expression in the cells of the collecting duct, largely located in the apical membrane of the cells and slightly in the cytoplasm, which indicated that the diabetic rats had increased expression of AQP 2. AQP 2 expression of the berberine treated rats was shown in Figure 1E with similar expression level to that of DM group, suggesting that berberine had no distinct influence on the AQP 2 expression in kidney of the diabetic rats.

## Effects on the expression of IL-1 and IL-6

Some cytokines are considered to be involved in the pathogenesis of the diabetic nephropathy. To analyze the effects of berberine on the substances contributing to the kidney lesion, we determined levels of IL-1 and IL-6 in kidney tissue by using ELISA assay. As shown in table 2, a significant increase of concentrations of IL-1 and IL-6 was observed in the kidney tissue homogenate of the diabetic rats in DM group compared to the control animals (both P<0.05). Berberine management reduced the levels of IL-1 and IL-6 in kidney in comparison to the rats of DM group, the differences were statistically significant (both P<0.05).

*Table 1. Urine volume, Urinary albumin and Ccr of the rats* 

Group	Urine volume (ml/24 h)	Urinary albumin (mg/24 h)	Ccr (ml/min)
Control group	17.02±3.61	0.51±0.16	1.25±0.19
DM group	85.50±18.95△	3.29±0.73△	$0.71{\pm}0.12^{ riangle}$
Berberine group	46.37±8.03 <sup>△</sup> ★	1.85±0.31△★	0.99±0.15 <sup>△</sup> ★

Data were expressed as mean  $\pm$  SD.

 $^{\triangle} P < 0.05$  versus Control group; \*P < 0.05 versus DM group.



Figure 1. Immunofluorescence staining for AQP 2 in rat kidneys
However, levels of IL-1 and IL-6 in the berberine treated rats were still much higher than those of the control rats (both P < 0.05), which suggested that berberine could only partially inhibit the over expression of those substances.

*Table 2. Concentrations of IL-1 and IL-6 in kidney in rats* 

Group	IL-1 (pg/ml)	IL-6 (pg/ml)
Control group	35.86±7.13	23.40±4.17
DM group	81.27±9.52△	59.36±7.89△
Berberine group	51.85±11.70 <sup>△★</sup>	35.09±5.73 <sup>△</sup> ★

*Data were expressed as mean*  $\pm$  *SD.* 

 $^{\triangle}P < 0.05$  versus Control group; \*P < 0.05 versus DM group.

### Discussion

The findings in the present study provide evidence for the anti-diabetic-induced renal damage effects of the Chinese patent medicine berberine and some possible pathways by which berberine exerts its protective effects.

We injected streptozotocin to the rats and found the rats had high blood glucose levels, which showed the diabetic animal models were successfully established. Then, we measured the urine volume, the urinary albumin and Ccr of the animals. The results showed that the rats in the DM group had more urine volume, more urinary albumin excretion, and lower Ccr as compared to the control rats, which suggest that the diabetic rats had damaged renal function. In order to detect the protective effects of berberine on the renal function, we administered berberine to diabetic rats, and our results showed the rats with berberine treatment had decreased the urine volume, urinary albumin excretion and increased Ccr. The results indicated that berberine attenuated the renal damage of the diabetic rats.

One of the main questions raised by this study is the mechanism by which berberine attenuates the renal damage of the diabetic rats. Recent studies have suggested that DN is an inflammatory process, and the importance of some inflammatory cytokines in the development and progression of DN has been well documented (3, 4). The renal expression of IL-1 in experimental models of diabetic nephropathy increased (19). Over-expression of IL-1 could enhance the synthesis of ICAM-1 and vascular cellular adhesion molecule-1 by glomerular endothelial cells, which could further promote the renal inflammation. IL-1 is also involved in the development of abnormalities in intraglomerular hemodynamics. Furthermore, IL-1 could increase the generation of hyaluronan by renal proximal tubular epithelial cells and increased production of glomerular hyaluronan could initiate glomerular hypercellularity (20, 21). IL-6 is another important cytokine involved in the pathogenesis of diabetic nephropathy. Studies showed the subjects with diabetes mellitus had high levels of IL-6 than the healthy controls (22), and more interestingly, a study showed that patients with type 2 diabetic nephropathy had even higher levels of IL-6 than the diabetic patients without nephropathy (23). Some other studies demonstrated a significant association between IL-6 and glomerular basement membrane thickening which a crucial lesion of diabetic nephropathy and a strong predictor of renal progression (24, 25). In the present study, we measured the levels of IL-1 and IL-6 in the kidney tissue of the rats. Similarly to the other reports, we found that levels of IL-1 and IL-6 of the rats in the DM group were both higher than those of the control animals. Our results also showed that levels of IL-1 and IL-6 of the rats in the berberine group significantly decreased as compared to those of the rats in the DM group. The data indicated that berberine could decrease the expression of the inflammatory cytokines IL-1 and IL-6 in the kidney tissue.

Some aquaporins also have a role in the pathophysiology of DN. Although the function of AQP2 in DN has been studied (26), the changes of AQP2 expression in diabetic subjects are still debated. Some studies showed increased AQP2 expression in the collecting ducts of the subjects with DN or diabetes and the AQP2 expression could be inhibited if the hyperglycemia was controlled (10, 12, 13, 14); while some other studies showed hyperglycemia might cause down-regulation of AQP2 expression in the STZ-induced diabetic animals and the protein expression of AQP2 was restored to the control level after insulin treatment (15). We detected the expression of AQP2 in kidney of the rats by immunofluorescence staining. We found that AQP 2 mainly located in the cells of collecting ducts, and the rats in the DM group had more AQP 2 expression than the rats in the control group. But our result showed that the AQP 2 expression of the berberine treated rats had no significant difference

from that of DM group. The data suggested that berberine had no distinct influence on the AQP 2 expression in kidney of the diabetic rats.

Taken together, we conclude that berberine improved the renal function of the diabetic rats induced by streptozotocin injection and the drug might exert its effects by inhibiting the local expression of IL-1 and IL-6 in kidney. But the drug had no distinct influence on the AQP 2 expression in kidney of the diabetic rats.

### References

- 1. Whiting D, Guariquata L, Weil C, Shaw J. IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract. 2011; 94: 311-321.
- 2. Bentata Y, Haddiya I, Latrech H, Serraj K, Abouqal R. Progression of diabetic nephropathy, risk of end-stage renaldisease and mortality in patients with type-1 diabetes. Saudi J Kidney Dis Transpl. 2013; 24(2): 392-402.
- Verhave JC, Bouchard J, Goupil R, Pichette V, Brachemi S, Madore F, Troyanov S. Clinical value of inflammatory urinary biomarkers in overt diabetic nephropathy: A prospective study. Diabetes Res Clin Pract. 2013 Jul 20. doi: pii: S0168-8227 (13) 00245-3.
- Mima A. Inflammation and oxidative stress in diabetic nephropathy: new insights on its inhibition as new therapeutic targets. J Diabetes Res. 2013; 2013: 248563. doi: 10.1155/2013/248563. Epub 2013 Jun 3.
- 5. Elmarakby AA, Sullivan JC. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. Cardiovasc Ther. 2012; 30(1): 49-59.
- Navarro-González JF, Mora-Fernández C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol. 2008; 19(3): 433-42.
- 7. Satirapoj B. Review on pathophysiology and treatment of diabetic kidney disease. J Med Assoc Thai. 2010; 93 Suppl 6: S228-241.
- 8. Jung HW, Yoon CH, Kim YH, Boo YC, Park KM, et al. Extract inhibits the release of inflammatory mediators from LPS-stimulated mouse macrophages. J Ethnopharmacol. 2007; 114(3): 439-445.
- 9. Nakamura T, Saito T, Kusaka I, Higashiyama M, Nagasaka S, Ishibashi S, Ishikawa SE. Decrease in urinary excretion of aquaporin-2 associated with impaired urinary concentrating ability in diabetic nephropathy. Nephron. 2002; 92(2): 445-448.

- Choi YE, Ahn SK, Lee WT, Lee JE, Park SH, et al. Soybeans ameliolate diabetic nephropathy in rats. Evid Based Complement Alternat Med. 2010; 7(4): 433-440.
- Yao L, Wang J, Deng A, Liu J, Zhao H. Effect of Micardis on the expression of renal medulla aquaporin-2 in diabetic mice. J Huazhong Univ Sci Technolog Med Sci. 2008; 28(3): 272-275.
- 12. GUO Binghua, LI Feng, LI Xiaomiao, et al. Regulatory effect of Ze-Huang Granule on AQP2 expression in kidney and urine of diabetic nephropathy rats. CJITWN. 2010; 11(2): 121-125. In Chinese.
- 13. HE Cui-e, LI Hua-zhu, LIANG Li-hui. Expression of aquaporin-2 in renal medulla of uncontrolled diabetes mellitus in rats. Journal of China-Japan Friendship Hospital. 2013; 23(1): 28-30. In Chinese.
- 14. Satake M, Ikarashi N, Kagami M, Ogiue N, Toda T, Kobayashi Y, Ochiai W, Sugiyama K. Increases in the expression levels of aquaporin-2 and aquaporin-3 in the renal collecting tubules alleviate dehydration associated with polyuria in diabetes mellitus. Biol Pharm Bull. 2010; 33(12): 1965-1970.
- 15. Lee HS, Li Z, Kim SO, Ahn K, Kim NN, Park K. Effect of hyperglycemia on expression of aquaporins in the rat vagina. Urology. 2012; 80(3): 737.e7-12.
- 16. Cao S, Zhou Y, Xu P, Wang Y, Yan J, Bin W, Qiu F, Kang N. Berberine metabolites exhibit triglyceridelowering effects via activation of AMP-activated protein kinase in Hep G2 Cells. J Ethnopharmacol. 2013 Jul 27. doi:pii: S0378- 8741 (13)00524-2. 10.1016/j.jep.2013.07.025.
- 17. Xie X, Chang X, Chen L, Huang K, Huang J, et al. Berberine ameliorates experimental diabetes-induced renal inflammation and fibronectin by inhibiting the activation of RhoA/ROCK signaling. Mol Cell Endocrinol. 2013 Jul 26. pii: S0303-7207(13)00313-4. doi: 10.1016/j.mce. 2013. 07. 019. [Epub ahead of print]
- Wang M, Wang J, Tan R, Wu Q, Qiu H, Yang J, Jiang Q. Effect of Berberine on PPAR α /NO activation in high glucose- and insulin-induced cardiomyocyte hypertrophy. Evid Based Complement Alternat Med. 2013; 2013: 285489. doi: 10.1155/2013/285489. Epub 2013 Mar 20
- 19. Navarro JF, Milena FJ, Mora C, León C, García J. Renal pro-inflammatory cytokine gene expression in diabetic nephropathy: effect of angiotensin-converting enzyme inhibition and pentoxifylline administration. Am J Nephrol. 2006; 26(6): 562-570.

- 20. Jones S, Jones S, Phillips AO. Regulation of renal proximal tubular epithelial cell hyaluronan generation: implications for diabetic nephropathy. Kidney Int. 2001; 59(5): 1739-1749
- Mahadevan P, Larkins RG, Fraser JR, Fosang AJ, Dunlop ME: Increased hyaluronan production in the glomeruli from diabetic rats: Link between glucose induced prostaglandin production and reduced sulphated proteoglycans.Diabetologia. 1995; 38(3): 298-305.
- 22. Taslipinar A, Yaman H, Yilmaz MI, Demirbas S, Saglam M, et al. The relationship between inflammation, endothelial dysfunction and proteinuria in patients with diabetic nephropathy. Scand J Clin Lab Invest. 2011; 71(7): 606-612.
- 23. Sekizuka K, Tomino Y, Sei C, Kurusu A, Tashiro K, et al. Detection of serum IL-6 in patients with diabetic nephropathy.Nephron. 1994; 68(2): 284-285.
- 24. Nosadini R, Velussi M, Brocco E, Bruseghin M, Abaterusso C, et al. Course of renal function in type 2 diabetic patients with abnormalities of albumin excretion rate. Diabetes. 2000; 49(3): 476-484.
- 25. Dalla Vestra M, Mussap M, Gallina P, Bruseghin M, Cernigoi AM, et al. Acute-phase markers of inflammation and glomerular structure in patients withtype 2 diabetes. J Am Soc Nephrol. 2005; 16 Suppl 1: S78-82.
- Sasaki S. Aquaporin 2: from its discovery to molecular structure and medical implications. Mol Aspects Med. 2012; 33(5-6): 535-46. doi: 10.1016/j.mam. 2012.03.004. Epub 2012 Apr 4.

Corresponding Author Sun Hongri, Department of Nephrology, Affiliated Hospital of Weifang Medical University, P.R. China, E-mail: docsunhongri@163.com

# The prevalence and risk factors associated with otorhinolaryngologic diseases among the students of Hakkari City center

Huseyin Gunizi<sup>1</sup>, Osman Durgut<sup>1</sup>, Derya Seyman<sup>2</sup>

<sup>1</sup> Hakkari State Hospital, Department of Otolarngology, Hakkari, Turkey,

<sup>2</sup> Antalya Education and Research Hospital, Department of Infectious Diseases and Clinical Microbiology, Antalya, Turkey.

### Abstract

**Objective:** To investigate the prevalence and the risk factors associated with otorhinolaryngologic (ENT) diseases among the students of Hakkari city center.

**Method:** A study was made of 1,068 primary school students in Hakkari city center. The students were given ropharynx, anterior rhinoscopy and autoscopic examinations, and details were ascertained of their academic success and the economic and educational status of their families. The results were analyzed using SPSS for Windows 16 software, with chi-square and Fisher's exact tests used for the analyses.

**Findings:** A total of 1,068 primary school students with a mean age of  $7.8 \pm 1.4$  were included in the study. An assessment of their socio-economic status revealed that 65.3 percent of the students were of low level, and the remaining 34.7 percent were of normal or high level. Some 55.1 percent of the students resided with at least one smoker. During the physical examination, 19.5 percent of the students were found to have tonsillar hypertrophy, 4.9 percent had asymmetric tonsils, and 19 (1.8%) had undergone a tonsillectomy. Furthermore, 6 percent of the students were diagnosed with allergic rhinitis and 7.7 percent with rhinosinusitis.

**Result:** Examinations for otorhinolaryngologic disease should be carried out as part of preventative health measures in this age group due to the high risk of otorhinolaryngologic disease in primary school students.

**Key words:** School, Septum deviation, Allergic rhinitis, Rhinosinusitis, Prevalence.

### Introduction

Otorhinolaryngologic (ENT) diseases occur most frequently in childhood, and if left untreated, may lead to growth and developmental problems, while also being detrimental to academic success [1]. Otitis media with effusion, chronic otitis, allergic rhinitis, rhinosinusitis, and OSAS (obstructive sleep apnea syndrome) are among the most common ENT diseases, and are known to cause attention deficit disorders, impaired concentration, sleep disorders, low academic success and a decrease in quality of life, and treatment can prove to be very expensive [2-3]. As a result, many studies have been conducted to find ways of identifying these diseases early [4,5].

Hakkari's population covers the full length of the socio-cultural and economic scale, and is characterized by a low level of development due to terrorism when compared to other regions in Turkey. To date, there have been no studies looking at the prevalence of otorhinolaryngologic disease in the region and the risk factors associated with it, and so it is the intention in this paper to determine its extent, and to make a comparison with the other regions. In this article, the prevalence and risks associated with otorhinolaryngologic disease will be covered in the first part of the paper, while the second part will open a discussion of the prevalence and risk factors associated with otitis media with effusion (EOM).

### Material and Method

A study was made of 1,086 primary school students aged 5–11 in Hakkari city center between April and May 2013, all of which underwent an otorhinolaryngologic examination. Questionnaires were applied to the families of the test subjects to assess the level of family education and income, smokers in the home, any sleep disorders or allergies suffered by the students and any previous operations. Signed consent forms were obtained from the parents, giving permission for the examination, and the academic ability of each student was assessed by their teachers. The schools were visited, and anterior rhinoscopy, oropharynx and autoscopic examinations were made of each student by one doctor, during which the tonsil sizes of the students was evaluated using the Brodsky classification [6]. According to this classification, Stage 3-4 cases are regarded as obstructive tonsillar hypertrophies. Among the students identified with allergic symptoms by their families, those identified with nasal mucosal pallor, congestion or seromucoide discharge during the examination were diagnosed with allergic rhinitis.

The results were analyzed using SPSS for Windows 16 software, with chi-square and Fisher's exact tests used for the analyses. P values <0.05 were considered to be statistically significant.

### Findings

The study was made of 1,068 primary school students aged 5–11 (mean age: 7.8  $\pm$ 1.4 years), 610 of which were (57.1%) male, and 458 (42.9%) were female (Figure 1). Regarding socioeconomic status, 65.3 percent of the students came from lowlevel families, while the remaining 34.7 percent came from normal or high-level families. Some 55.1 percent of the students resided with at least one smoker. During the physical examination, 19.5 percent of the students were found to have tonsillar hypertrophy, 4.9 percent had asymmetric tonsils, and 19 (1.8%) students had undergone a tonsillectomy. In addition, 22 (2.1%) students had a bifid uvula. During the anterior rhinoscopy examination, mucopurulent or seromucoide secretion was the most frequent pathological finding, accounting for 18.1 percent of the sample.

Among the 338 (31.6%) students identified with allergies by their families, 64 (6%) were diagnosed with allergic rhinitis. During the examinations, 82 (7.7%) students were diagnosed with rhinosinusitis, and medical treatments were planned; 189 (17.7%)

of the students had nasal obstructions; and 94 (8.8%) had septum deviations (Table 1). Rhinosinusitis was more common among the students with nasal obstructions and passive smokers than those without (p<0.01, p=0.023), although there was no correlation with allergic rhinitis.

Prominent ear deformity (10.3%) was the most frequent congenital head and neck anomaly in the test subjects, with others being Darwin tubercule (3.3%), pre-auricular skin tag (1.1%), low-set ear (0.2%) and pre-auricular sinus (0.2%) (Figure 2).

Of the total set, 146 (13.7%) students were diagnosed with plugged ear during the autoscopic examination, and underwent a tympanometric examination after clearing the ear. Finally, eight (0.7%) students had chronic otitis, for which medical treatments were planned, and 119 (11.1%) were diagnosed with EOM.

Regarding academic achievement, 75 percent of the students were considered successful, with the remaining 25 percent considered to be unsuccessful. Students diagnosed with sinusitis generally achieved lower academic success than the other students, and this difference was statistically significant (p<0.01 and p<0.05, respectively). There was no correlation between nasal obstructions or sleep disorders and academic success.

The families reported that 49.3 percent of the students had problems sleeping at night (snoring, sleeping with mouth open, sleep apnea), and students with nasal obstructions experienced more sleep problems than other students (p<0.01). No correlation could be identified between sleeping problems and tonsil hypertrophy.

### Discussion

Otorhinolaryngologic diseases are among the most common ailments in childhood, and differ in frequency and diversity among different socio-cultural, economic, ethnic and genetic groups. This has been proven in many epidemiological studies into the prevalence and risk factors associated with otorhinolaryngologic diseases [3-5], both nationally and globally, however to date there has been no study specifically of Hakkari.

With respect to the tonsil size, Stage 3–4 is regarded as obstructive [6]. According to Lam et al., Stage 3–4 tonsils are found in 27 percent [7] of cases, while Kara et al. put the figure at 11 percent, and Egeli and Inankac at 17.6 percent [8-9]. Similarly, the present study identified tonsillar hypertrophy in 19.5 percent of the students.

Upper airway anatomy, adenotonsillar hypertrophy, obesity and neuromuscular tonus are significant factors in the OSAS pathogenesis of children [10]. Yuen-yu et al. found that AHI was significantly high in children with tonsillar hypertrophy [7], however several other studies indicate that there is no correlation between tonsil size and the clinical findings of OSAS [11]. In this study, 49.3 percent of the students were reported to have experienced sleeping problems at night, although there was no correlation between the presence of tonsil hypertrophy and sleep problems, which is conversely associated with the presence of OSAS. The students with nasal obstructions experienced more sleep problems when compared to the others, and this difference was statistically significant (p<0.01).

Benign tumors (papilloma etc.), recurrent infections, lipid storage disorders, granulomatous (tuberculosis, actinomycosis) diseases, and malignity have been reported to cause unilateral asymmetric tonsil enlargement, although consensus has not been reached on the relationship between unilateral asymmetric tonsil enlargement and malignity. Some researchers claim that a histopathological examination should be made through a tonsillectomy, and that the malignity should be excluded in asymmetric tonsil hypertrophy; while others suggest that patients should be monitored due to the low malignity incidence, and a tonsillectomy should be performed only if clinical symptoms occur. Berkowitz et al. report that tonsillar lymphoma results in typical rapid unilateral tonsil enlargement in pediatric age groups; and 86 percent of the pediatric patients experienced neck lymphadenopathies larger than 3 cm [12]. Ridgway et al. identified six cases of lymphoma in 13 years, and recommended that a tonsillectomy be performed in order to exclude malignity in cases of unilateral tonsil hypertrophy [13]. In the study of Reiter et al., 31 of 1,320 adult patients underwent a tonsillectomy for suspected tonsillar asymmetry or lymphoma, two (6.5%) of which were found to have malignity [14]. Younis et al. reported in their study that no malignity was found during their study of the medical records of 2,099 children that had undergone an adenotonsillectomy [15]. In the present study, asymmetric tonsils were observed in 4.9 percent of the students, and their families were advised to undergo future examinations, as no clinical finding could be made during the examination.

Adenotonsillectomies are the most common pediatric procedure in many countries, including the United States, the Netherlands and Scotland [16-17], with the frequency of the procedure changing according to geography, the opinions of experts, socio-cultural differences and access to health services. Mattila et al. reported the frequency of tonsillectomies in childhood to be 8 percent. [18]; the study of Gedikli and Turan found that 0.6 percent of students had undergone a tonsillectomy in Isparta city center, and Polat and Demirören reported this rate to be 4.9 percent of 775 children. [19-20]. In the present study, 19 (1.8%) students had undergone a tonsillectomy, and this low rate may be due to the underdeveloped socio-economic conditions and health services in the region. Polat and Demirören reported that the tonsillectomy rate was higher in students of high socio-economic status [20], however in this study we found this rate to be higher in the families of low socio-economic status, although there was no statistically significant correlation.

Some 82 (7.7%) students were diagnosed with rhinosinusitis during the examination, and the academic success was found to be significantly low in these students among the whole test group (p<0.05). This led to the conclusion that headaches and nasal obstructions, which are symptoms of sinusitis, lead to attention deficit disorders, and consequently, had a negative effect on perception and academic success. Smoking is known to influence mucociliary activity and increase viral infection frequency, and the passive smoking students were found to have more significant levels of rhinosinusitis (p=0.023).

Among the 338 (31.6%) students identified as having allergies by their families, and found to have nasal mucosal pallor, congestion and a seromucoide discharge during the examination, 64 (6%) were diagnosed with allergic rhinitis. In literature, studies have shown the prevalence of allergic rhinitis to be 7 percent in the countries of Northern Europe, 9–21 percent in South America and 27.6 percent in Australia [21]. In their study of childhood, Ones et al. reported allergic rhinitis in 7.9 percent of cases; while Cakır and Cetinkaya put the figure at 9.1 percent [22-23]. In the present study, it was found that the regional allergic rhinitis rate was consistent with literature of the pediatric age group, although slightly lower.

Nasal traumas cause microfractures in the nasal roof and septum in developing children, which lead to permanent nasal and septal deformities in the recovery period [24]. There are only a limited number of studies on the frequency and causes of septum deviation in schoolchildren. In their study of primary school students, Gedikli and Turan reported septal deviation in 10.2 percent of cases; while Keles et al. put the figure at 8.1 percent [19,25]. In world literature, rates vary between 7.58–35 percent [4,26], and in the present study, 189 (17.7%) of the students had nasal obstruction and 94 (8.8%) had septum deviation, which is in accordance with the findings of previous literature. The students with nasal obstructions suffered rhinosinusitis and sleep problems more significantly than those without (p < 0.01).

Chronic otitis media is an infection that can lead to serious intracranial and extracranial complications if not controlled. In developing countries, there is a significant difference in the prevalence of chronic otitis media among different socio-economic classes, ranging between 1.3 and 17.6 percent, while it is known to be less than 1 percent in the United States and United Kingdom [27]. In a study carried out in Turkey, this rate was reported to be 2.5 percent [19]. Shaheen et al. reported chronic otitis media in 5.2 percent of primary school students, and was more common in girls and in families of low socio-economic status [5]. Kuhle et al. found no correlation between socio-economic status and chronic otitis media [28]. In the present study, chronic otitis media was observed in eight patients, which is lower than the rates recorded in previous literature. There was no correlation between chronic otitis media and gender, while more cases were identified in students aged 8 and above, and of low socio-economic status.

Several differences were identified in the prevalence and risks associated with otorhinolaryngologic disease between the students in Hakkari and those in other regions, although there were similarities. Raising awareness among families can help ensure diagnosis and treatment.

*Table 1. Distribution of gender, age and pathological findings* 

	n=1068
Gender	
Male	610 (57.1%)
Female	458(42.9%)
Mean Age	$7.8 \pm 1.4$
Tonsillar Hypertrophy	208 (19.5%)
Asymmetric Tonsil	52 (4.9%)
Allergic Rhinitis	64 (6%)
Sinusitis	82 (7.7%)
Septum Deviation	94 (8.8%)
EOM	119 (11.1%)



Figure 1. Gender distribution



Figure 2. Distribution of ear anomalies.

### References

- 1. Nixon GM, Brouillette RT. Sleep . 8: paediatric obstructive sleep apnoea. Thorax 2005; 60: 511-6.
- 2. Robb PJ, Williamson I. Otitis media with effusion in children: current management. Paediatrics and Child Health. 2012; 22(1): 9-12
- 3. Okur E, Yıldırım I, Akif Kılıç M, Güzelsoy S. Prevalence of otits media with effusion among primary school children in Kahramanmaraş, in Turkey. Int J Pediatr Otorhinolaryngol. 2004 May;68(5): 557-62.

- Jurkiewicz ZB, Sosinska OO. The nasal septum deformities in children and adolescents from Warsaw, Polan. Int. Journal of Pediatric Otorhinolaryngology 2006; 70: 731-6
- Shaheen M, Ahmed Raquib A, Shaikh MA. Chronic Suppurative Otitis Media and Its Association with Socio-Econonic Factors Among Rural Primary School Children of Bangladesh Indian J Otolaryngol Head Neck Surg 2012; 64(1): 36–41
- 6. Brodsky L. Modern assessment of tonsil and adenoid. Pediatr Clin North Am 1989; 36: 1551–1569
- 7. Yuen-yu L, Eric YT, Daniel K, Chung-hong C, Josephine MY, et al. The Correlation Among Obesity, Apnea-Hypopnea Index, and Tonsil Size in Children Chest. 2006; 130(6): 1751-6.
- 8. Kara OC, Ergin H, Koçak G, Kılıç İ, Yurdakul M. Prevalence of tonsiller hypertrophy and associated oropharyngeal symptoms in primary schoolchildren in Denizli, Turkey. İnt. Journal of pediatric otorhinolaryngology 2002; 66: 175-9.
- 9. Egeli E, İrankoç E. Body growth in relation to tonsiller enlargement. Auris Nasus Larynx 1997; 24: 299-301.
- Schechter MS. Section on Pediatric Pulmonology, Subcommittee on Obstructive Sleep Apnea Syndrome. Technical report: diagnosis and management of childhood obstructive sleep apnea syndrome. Pediatrics 2002; 109: 69
- 11. Li AM, Wong E, Kew J, et al. Use of tonsil size in the evaluation of obstructive sleep apnoea. Arch Dis child 2002; 87: 156–9.
- 12. Berkowitz RG, Mahadevan M. Unilateral tonsillar enlargement and tonsillar lynphoma in children. Ann Otol Rhinol Laryngol 1999; 108: 876-9.
- 13. Ridgway D, Wolff LJ, Neerhout RC, Tilford DL. Unsuspected non-Hodgkin's lymphoma of the tonsils and adenoids in children. Pediatrics. 1987; 79: 399-402.
- 14. Reiter ER, Randolph GW, Pilch BZ. Microscopic detection of occult malignancy in the adult tonsil. Otolaryngol Head Neck Surg. 1999;120: 190-4.
- 15. Younis RT, Hesse SV, Anand VK. Evaluation of the utility and cost-effectiveness of obtaining histopathologic diagnosis on all routine tonsillectomy specimens. Laryngoscope. 2001; 111: 2166-9.
- 16. Capper R, Canter RJ. Is the incidence of tonsillectomy influenced by the family medical or social history? Clin Otolaryngol 2001; 26: 484-7.
- 17. Clemen WA, Wales Y. Readability and content of postoperative tonsillectomy instructions given to patients in Scotland. Clin Otolaryngol 2004; 29: 149-52.

- Mattila PS, Tahkokallio O, Tarkhanen J, Pitkaniemi J, Karvonen M, Tuomilehto J. Causes of tonsillar disease and frequency of tonsillectomy operations. Arch Otolaryngol Head Neck Surg 2001; 127: 37-44.
- 19. Gedikli O, Turan A. The incidence of ear nose throat diseases between primary school children in Isparta city center. Journal of Ear Nose Throat and Head Neck Surgery. 1994; 2(3): 236-8.
- 20. Polat C, Demirören K. Frequency of the tonsillectomy and adenoidectomy in children in Elazığ province, in the east of Turkey. Dicle Medical Journal 2010; 37(3): 263-6.
- 21. Van Cauwenberge P, Bachert C, Passalacqua G, Bosquet J, Canonica GW. Et al. Consensus statement on the treatment of allergic rhinitis. Allergy 2000; 55: 1-19.
- 22. Öneş U, Sapan N, Somer A, Disci R, Salman N, et al. Prevalence of childhood asthma in Istanbul, Turkey. Allergy. 1997; 52: 570-5
- 23. Çakır M, Çetinkaya F. Prevalence of Bronchial Asthma and Other Allergic Diseases Among School Children in Samsun. Asthma Allergy Immunology. 2004; 2(3): 139-142
- 24. Brain DJ. The nasal septum. In: Kerr AG, Gleeson M (Editors), Scott-Brown's Otolaryngology. 2. Edition, Oxford: Reed educational and professional Publishing Ltd, 1997: 1-25.
- 25. Keleş E, Yalçın S, Kaygusuz İ, Karlıdağ T, Alpay C, et al. Minor Traumas of First Grade at Elementary School and Nasal Septum Deviation. Fırat Medical journal. 2005; 10(2): 54-8.
- 26. Min YG, Won Ha J, Kim CS, Prevalence study of nasal septal deformities in Korea: result of nation-wide survey. Rhinology 1995; 33: 61-5.
- 27. WHO/CIBA Foundation workshop report, Prevention of hearing impairment from chronic otitis media.UK 19–21 Nov 1996
- 28. Kuhle S, Kirk SF, Ohinmaa A, Urschitz MS, Veugelers PJ. The association between childhood overweight and obesity and otitis media. Pediatr Obes. 2012 Apr; 7(2): 151-7.

Corresponding Author Hüseyin Günizi, Hakkari State Hospital, Department of Otolarngology, Hakkari, Turkey, E-mail: drgunizi@gmail.com

### The prevalence and the nature of violence directed at the medical staff in psychiatric health care in Slovenia

Branko Gabrovec<sup>1</sup>, Ivan Erzen<sup>2</sup>, Branko Lobnikar<sup>3</sup>

<sup>1</sup> Community Health Centre Celje, Slovenia,

<sup>2</sup> University of Ljubljana, Medical Faculty, Slovenia,

<sup>3</sup> University of Maribor, Faculty of Health Sciences & Faculty of Criminal Justice and Security Slovenia.

### Abstract

**Objective:** The degree of violence directed at medical workers is high, especially in intensive care units and closed and/or intensive psychiatric wards. The aim of this study was by using as large a sample as possible, to define the types and frequency of violence medical staff in psychiatric health care are faced with. Representative results can serve as the basis for the development of a comprehensive aggression prevention and employee education program.

**Methods:** The study of the prevalence of violence directed at the medical staff in psychiatric health care was conducted in psychiatric hospitals in Slovenia. 5 psychiatric hospitals participated in the study. 249 questionnaires were distributed among medical workers in closed and/or intensive wards.

Results: 92.6% of examinees experienced patients' verbal aggression in the past year. Patients' physical violence was experienced by 84.2% of examinees in the same period. As many as 63.5% of medical workers were injured by the patients in the past. 40.9% were verbally abused by family members. Verbal aggression of co-workers (13.3%) and superiors (13.8%) is also worth mentioning. Medical workers are also faced with sexual harassment from the patients (24.6 %). Significant positive correlations have been discovered between verbal abuse, physical violence and sexual harassment. The medical workers feel threatened at their workplace (M =2.52, SD = 0.62, on 3 point scale: 1 = low, 3 = high), with fear (2.49±0.60) and insecurity (2.36±0.69) largely present. The understanding of co-workers is significant (1.34±0.57), whereas that of the superiors is somewhat lower (1.74±0.73). The study has shown no correlation between aggression and age, gender, and years of work experience.

**Conclusion:** The occurrence of violence directed at the medical workers in psychiatric health care is high. There is a positive correlation between verbal abuse, physical violence and sexual harassment. Medical workers are threatened and faced with fear and insecurity. The outburst of aggression does not discern between gender, age and work experience of the medical workers. The results show that all medical workers are equally exposed in their work and they reaffirm the need for the development of a systematic approach to control the violence directed at medical staff in psychiatric health care.

Key words: Nursing, patient, violence, psychiatric, hospital.

### Introduction

Ensuring the safety of psychiatric patients and the quality of their treatment is the paramount task at every level of health care and of every medical discipline. With the development of modern society and ensuing trends the incidence of psychiatric disorders is growing. Despite the advancement of psychiatry, violence and aggression are an integral part of psychiatric treatment. Therefore, acute psychoses and various other states where aggression is to be expected will continue to occur.

Despite its humanistic role, psychiatry, in contact with the environment, cannot avoid occasional violent behaviour of its patients. Psychiatrists at times attempt to ignore the potential as well as the actual violence, however, the general assertion that there is no violence -free psychiatry tends to remain valid [1]. The phenomenon of violence is an integral part of psychiatry. As many as 42 % of nursing staff were exposed to physical violence and as many as 73 % are of the opinion that more needs to be done in terms of security [2]. Medical

workers (especially the nursing staff) are the ones most frequently faced with psychiatric patients' violence. However, other groups involved in the treatment process confront it as well: the police, security agencies, paramedics, personnel in retirement homes... Between 35 - 80 % of medical workers were at least once physically assaulted at their workplace, with nurses being the most exposed group [3]. In the USA, as many as 1.7 million workers are injured as a result of workplace violence, 60% of those in health care (U.S. Bureau of Justice Statistics). The percentage of injuries in health care is 6.1 per 10.000, while in other work fields it is 2.1 per 10.000. Despite statistically high percentage of injured in health care, the actual numbers are even higher, mainly due to frequent non-reporting of incidents [4].As many as 70% of incidents or abuses of nurses remain non-reported[5].A Swedish study shows that the majority of the participants (85%) reported having been exposed to violence during their careers, with 57% being victimized in the past 12 months [10]. Findings from a Swiss study revealed that 72% of nurses had experienced verbal patient and visitor violence and 42% physical patient and visitor violence in the past 12 months. Also, 23% were physically injured and 1.4% took one or more days of sick leave. Patient and visitor violence was distressing for the nursing staff [11]. According to the University of Iowa, violence directed towards health professionals is at the highest rate ever. For example, the ratio of violence is more than 100 events per 100 workers in a year in some psychiatric departments [6]. According to a study conducted by the Medical System in Virginia, the health care related violence is getting more dangerous today in various health organizations. In addition, the same study concluded that the medical staff undoubtedly has the highest ratio of attacks in comparison with all other work fields[6].

Medical staff in psychiatric hospitals and institutions is most frequently exposed to violent behaviour. They are neither properly trained nor authorised to manage aggression. However, they are responsible for providing the safety of the aggressive patient, other patients, the surroundings and themselves. The nurses and the assistants who are in direct contact with patients are at the highest risk of being the victims of violence. Other hospital staff, paramedics and hospital safety officers are exposed to increased risk of violence as well [7].Such a situation has been reported in a number of research studies. It was shown that in South Africa, Britain, France and Japan, employees face an increasing frequency of violence [8]. One of the studies shows that exposure to violence is frequent in nursing [9]. The mentioned responsibility is not only important for the providing of safety, but also for the providing of quality medical services. The therapeutic treatment, the therapeutic relationship, etc., are all dependent on the quality of the conduct towards an aggressive psychiatric patient, which is of paramount importance. A psychiatric patient is also a user of the service of psychiatric treatment. Nowadays, users are much more educated and demanding and hence quality-aware. Due to their medical conditions, confidence with psychiatric patients is even harder to achieve and maintain. Those with high degree of confidence in psychiatry tend to return with ease and there are fewer complications when recidivism occurs. In instances of lesser quality and the disruption of the therapeutic process (institution - patient), patients' return to a psychiatric institution is more difficult, rarer and there are more complications.

Studies show that nurses encounter violence more frequently as opposed to other healthcare professionals [17, 18].Among the most exposed are the workers in emergency medical units and especially those working in closed and intensive psychiatric wards. The number of (severe) violent incidents against staff by psychiatric inpatients is high [13]. Nurses, being health workers, are the first to meet the victims of ever increasing violence. In addition, nurses suffer damage from social tolerance of violence [15].

Studies point to a varied but nevertheless high occurrence of all types of violence directed at nursing staff in psychiatric health care. In Taiwan 19.6% indicated that they had experienced physical violence [12]. In a Turkish study 77.2% of the nurses were exposed to verbal abuse while 71.4% were physically assaulted [16]. A Swedish study shows that the majority of the study participants (85%) reported having been exposed to violence during their careers, with 57% being victimized in the past 12 months [10]. A Swiss study revealed that 72% of nurses had experienced verbal pati-

ent and visitor violence and 42% physical patient and visitor violence in the past 12 months. Also, 23% were physically injured and 1.4% took one or more days of sick leave. Patient and visitor violence was distressing for the nursing staff [11].

### Description of methods and sample

The most exposed cadre were chosen to participate in the study: nursing staff in closed and/ or intensive psychiatric units. 5 of 6 Slovenian psychiatric hospitals participated in the study. The sample included male and female employees with secondary, vocational, graduate, and postgraduate education, with varied years of work experience. The sample is representative.

249 questionnaires were distributed among the staff. 203 (81.52%) returned and 46 did not return the questionnaire. The survey was conducted in March and April 2013. The questionnaire consisted of 80 questions divided into five sets: your work and workplace related violence, work management, the influence of various factors on patient safety and the quality of patient treatment, education and demographics. The following scales were utilised in the adopted numerical scale: 1-2x, 3-5x, 6-9x, and more than 10 x. And the following values with descriptive scale: I don't agree, I partially agree, I agree. Cronbach's alpha coefficient of reliability was 0.77.

While composing the questionnaire, we formed a focus group with the students of the master of nursing program. Their remarks and suggestions were entered into the questionnaire.

		No.	%
Candar	Male	95	46,8
Gender	Female	108	53,2
	Secondary	136	67
Educational	Vocational	5	2,5
level	Graduate	54	26,6
	Postgraduate	8	3,9

### Table 1. Demographics statistics of examinees

### Results

203 (81.52%) examinees returned the questionnaires. The survey was conducted in March and April 2013. Cronbach's alpha coefficient of reliability was 0.77. Of all 203 participants, 95 (46.8%) were male and 108 (53.2%) were female. Such male to female ratio can be attributed to the fact that there are more men employed in closed and intensive psychiatric wards than in other wards. Mean age ( $\pm$  SD) of the participants was 37.5  $\pm$  9 years. The oldest participant was 58 years old, the youngest 20 years old. Levels of education: secondary 136 (67%), vocational: 5 (2.5%), graduate: 54 (26.6%), postgraduate: 8 (3.9%). Mean working experience was 16.7 $\pm$ 9.1 years (see Table 1).

In table 2 the frequency of exposure to different types of violent behaviour is shown. The different types were defined as verbal abuse, sexual harassment and physical violence, caused either by patients, relatives, co-workers or superiors. The frequency of patient inflicted injuries is also being determined.

Verbal abuse in the past year is the most common form of violence. It appears with as many as 92.6% of participants. As many as 84.2% of participants experienced physical violence by patients in the past year and 63.5% were injured by a patient in the past. Verbal abuse by relatives is also very frequent (40.9%), with verbal abuse by co-workers (13.3%) and superiors (13.8%) also worth mentioning. The staff is also confronted with sexual harassment by the patients (24.6%).

Medical workers feel threatened at work  $(2.52\pm0.62, \text{ on 3 point scale: } 1 = \text{low, } 3 = \text{high})$ , with fear  $(2.49\pm0.60)$  and insecurity  $(2.36\pm0.69)$  being largely present. Co-workers sympathize strongly  $(1.34\pm0.57)$ , whereas superiors show less support  $(1.74\pm0.73)$  (Table 3).

To continue, a correlation analysis for different types of violence as reported by the study participants was performed. The results are shown in table 1. An important statistic correlation was established between the following variables: patient verbal abuse in the past year and patient physical violence in the past year (r = 0.446; p < 0.01); patient verbal abuse in the past year and injury at workplace (r = 0.216; p < 0.01); patient sexual harassment and patient physical violence (r = 0.216; p < 0.01); patient induced injuries (r = 0.290; p < 0.01) and patient verbal abuse and patient sexual harassment (r = 0.161; p < 0.01). It is evident from the correlation that different types of violent behaviour are not independent from each

		9/	<i>(</i> 0					
Have you been exposed to verbal abus	se by patie	ents at you	ır workpla	ice dur-	VES	= 188	92.0	5%
ing the last year?						100		
If so, how often?	1 - 2x	15	3-5x	51	6-9x	26	10  x >	96
Have you been exposed to verbal abus place during the last year?	Have you been exposed to verbal abuse by patients' relatives at your work- place during the last year?				YES	= 83	40.9	9%
If so, how often?	1 - 2x	42	3-5x	25	6-9x	5	10  x >	11
Have you been exposed to verbal abus	se by co-w	orkers at	your worl	kplace	VEC	- 27	12 2	20/
during the last year?					YES	= 27	13.3	0%0
If so, how often?	1 - 2x	15	3 - 5x	10	6-9x	1	10  x >	1
Have you been exposed to verbal abus	se by supe	riors at yo	our workp	lace	VFS	- 28	13.9	20/2
during the last year?					ILS	- 20	13.870	
If so, how often?	1-2x	11	3 – 5x	17	6-9x	0	10  x >	0
Have you been exposed to sexual hara	ssment by	v patients	at your we	orkplace	VES	= 50	24 (	5%
during the last year?							2-1.0	570
If so, how often?	1-2x	26	3-5x	21	6-9x	1	10  x >	2
Have you been exposed to sexual hara	ssment by	patients'	relatives	at your	YES	S = 1	0.5	%
workplace during the last year?		-				-		
If so, how often?	1-2x	0	3-5x	1	6-9x	0	10  x >	0
Have you been exposed to sexual hara	issment by	/ co-work	ers at you	r work-	<b>YES</b> = 1		0.5	%
If so, how often?	$1-2\mathbf{v}$	1	3 _ 5x	0	6 - 9x	0	10  v >	0
Have you been, exposed to sexual har	$1 - 2\Lambda$	v superio	$\int J = J \Lambda$	work-	$0 - J_{\Lambda}$	0	10 A 2	0
place during the last year		by superio	ns at you	WOIK-	YES	5 = 1	0.5	%
If so how often?	1 - 2x	0	3-5x	0	6 - 9x	1	10  x > 10	0
Have you been exposed to physical vi	olence by	patients a	t vour wo	rkplace				
during the last year?	J	<b>P</b>	<i>j = 12 e</i>	P	YES	= 171	84.2	2%
If so, how often?	1 - 2x	45	3-5x	47	6-9x	31	10  x >	48
Have you been exposed to physical vi-	olence by	patients'	relatives a	t your	VE			
workplace during the last year?		•		5	YES	5 = 4	29	0
If so, how often?	1-2x	0	3-5x	2	6 – 9x	2	10  x >	0
Have you been exposed to physical vi-	olence by	co-worke	ers at your	work-	VE	- <b>- - -</b>	10	/
place during the last year?					YES	5 = 2	17	/0
If so, how often?	1-2x	0	3-5x	1	6-9x	1	10  x >	0
Have you been exposed to physical view	olence by	superiors	at your w	ork-	VF	S – 1	0	5
place during the last year?					I LA	<b>5</b> – 1	U.	.5
If so, how often?	1-2x	1	3 – 5x	0	6-9x	0	10  x >	0
Have you ever been injured by a patie	nt at your	workplac	e?		YES	= 129	63	.5
If so, how often?	1-2x	75	3-5x	45	6-9x	4	10 x >	5

### *Table 2. Work and workplace related violence (n=203)*

Table 3. When treating an aggressive patient medical workers are confronted with:

Claim	I don't agree	I partially agree	I agree	Mean	Std.dev
Fear	5.9 %	38,9 %	55,2 %	2.49	.60
Insecurity	12.3 %	38,4 %	49,3 %	2.36	.69
Helplessness	21.2 %	53,7 %	25,1 %	2.03	.68
Lack of training	48,3 %	36,5 %	15,3 %	1.67	.72
Anger	47,8 %	40,4 %	11,8 %	1.64	.68
Despair	49,3 %	23,6 %	27,1 %	1.77	.84
Vulnerability	6,9 %	34,0 %	59,1 %	2.52	.62
Lack of empathy by co-workers	70 %	25,1 %	4,9 %	1.34	.57
Lack of Empathy by superiors	42,9 %	39,4 %	17,7 %	1.74	.73

other, but are interconnected in that not only one form of violent behaviour occurs, but the occurrence of one (e.g. verbal abuse) triggers the occurrence of another (e.g. sexual harassment).

There are no statistically important correlations between variables "gender", "achieved level of education", "years of work experience" and work and violence at workplace variables, which points to the fact that all workers are exposed to all types of violence regardless of gender, age and years of work experience.

Table 4 shows the most important correlations between emotions and the emotional perception of medical workers facing patient aggression: fear and insecurity, despair and the lack of training, helplessness and insecurity, vulnerability and fear, vulnerability and despair, helplessness and fear, vulnerability and insecurity, despair and vulnerability, lack of empathy by superiors and lack of empathy by coworkers, lack of training and helplessness, lack of training and fear. A connection between emotions and the emotional perception of medical workers facing patient aggression is evident from the correlations. They occur in various forms.

A regression analysis was performed between the dependant variable "fear" and the independent variable "vulnerability". It can be established that the variable "fear" can account for 26.6% of the variance of the variable "vulnerability" (Table 5).

### Discussion

The prevalence of violence directed at medical workers, especially nursing staff, is high.

The aim of our study was to determine the actual level of various types of violence directed at medical workers in psychiatric health care in Slovenia. The following types of violence were defined: verbal abuse, sexual harassment and physical violence. Patients, relatives, co-workers and superiors were defined as the source of violence.

Based on the study's representative sample, a high degree of violence directed at nursing staff was determined. The rate of verbal abuse in the past year is a staggering 92.6%. Physical violence by patients was experienced by as many as 84.2% of study participants. 63.5% were injured by a patient in the past. Verbal abuse by relatives is also very frequent (40.9%), with verbal abuse by co-workers (13.3%) and superiors (13.8%) also worth mentioning. The staff is also confronted with sexual harassment by the patients (24.6%)

An important positive correlation was established between verbal abuse and physical violence, verbal abuse and patient induced injuries, verbal abuse and sexual harassment and physical violence and sexual harassment.

The study also focused on identifying the emotions and emotional perceptions when dealing

Table 4. Correlations between emotions and the emotional perception of medical workers facing patient aggression

	Α	В	С	D	E	F	G	Н
	Fear	Insecurity	Helplessness	Lack of training	Anger	Despair	Vulnerability	Lack of empathy by co-workers
В	,646**							
C	,503**	,598**						
D	,325**	,429**	,356**					
Е	,249**	,260**	,306**	,267**				
F	,434**	,485**	,453**	,612**	,271**			
G	,519**	,490**	,452**	,349**	,256**	,510**		
Η	,029**	,122**	,168**	,195**	,411**	,110**	,027**	
Ι	,211**	,269**	,315**	,260**	,378**	,441**	,329**	,478**

\*\* Correlation is significant at the 0.01 level (2-tailed).

 Table 5. Regression analysis

Model	R	<b>R</b> <sup>2</sup>	$\Delta R^2$	Std. Err.
1	,059ª	,269	,266	,534

a. Predictors: (Constant), Fear, 1 foranalysis 1

with an aggressive patient. It was determined that the staff are predominantly faced with vulnerability, fear, insecurity and helplessness, and to a lesser extent with lack of empathy by co-workers and superiors, which points to a high degree of trust among co-workers and among staff and the management. Important correlations were determined among nearly all these variables.

No statistically important correlation was established between age, gender and years of work experience in relation to the occurrence of any type of aggression directed at medical workers in psychiatric health care. Thus, outbursts of aggression do not choose gender, age or the years of work experience of the employees.

The study has shown that the level of violence directed at the medical staff in psychiatric health care in Slovenia is higher than reported in previously conducted studies (92.6% of examinees experienced patients' verbal aggression in the past year. Patients' physical violence was experienced by 84.2% of examinees in the same time period. As many as 63.5% of medical workers were injured by the patients in the past). The study is comparable with a Swedish one which shows that the majority of the participants (85%) reported having been exposed to violence during their careers, with 57% being victimized in the past 12 months [10].

### Conclusion

The conducted study points to one of the highest rates of aggression directed at medical workers in psychiatric health care. The results can serve as the basis for a systematic approach to managing aggression directed at medical workers.

The occurrence of violence directed at medical workers in psychiatric health care is high. There is a positive correlation between verbal abuse, physical violence and sexual harassment. The staff is faced with vulnerability, fear and insecurity. Outbursts of aggression do not differentiate among gender, age or the years of work experience of the employees. Medical staff in psychiatric hospitals and institutions are most frequently exposed to outbursts of violent behaviour. The study confirms that the staff is at risk at many levels. Medical workers are neither properly trained nor authorised to manage aggression. However, they are responsible for providing the safety of the aggressive patient, other patients, the surroundings and themselves. Current treatment of an aggressive psychiatric patient includes measures within of framework of inadequate or deficient skills.

A set of instructions, guidelines and training in how to safely and efficiently perform a physical restraint of a psychiatric patient are missing for an adequate response in case of a sudden outburst of violence.

The employees require a systematic and comprehensive approach to workplace violence management. They are in need of a recurrent theoretical and practical aggression management training program. The results of the study can therefore be the basis for the development of an organizational model for the control of violence directed at medical workers and the treatment quality provision with the formation of written guidelines. A functional approach to permanent medical staff training would be appropriate.

### References

- 1. Kobal M. Security and Legal Psychopathology. Faculty of Criminal Justice and Security, University of Maribor, Ljubljana, 2009.
- 2. Kolman K. Danger that nurses and medical tehnicians are threatened by in psychiatric hospita l – Diploma thesis. Faculty of Criminal Justice and Security, University of Maribor, Ljubljana, 2009.
- 3. Clements PT, at all. Workplace Violence and Corporate Policy for Health Care Settings. Nurse Econ. 2005; 23(3): 119 124: Jannetti Publications, Inc.
- Gates DM, at all. Violence Against Nurses and its Impact on Stress and Productivity. Nurse Econ. 2011; 29 (2): 59 67: Jannetti Publications, Inc.
- 5. Stokowski LA. Violence: Not in My Job Description. Workplace Violence in Healthcare Settings, 2010.
- 6. American Bar Association Commission on Domestic Violence. A guide for employees: domestic violence in the workplace. Washington DC, 1999.
- 7. Occupational hazards in hospitals. Violence. National Institute for Occupational Safety and Health (NIOSH Publication No.02-101), 2002.
- 8. New model prepared by chappell and di martino, based on poyner and warne: preventing violence to staff. Health and Safety Execute. London, 1998.

- 9. Warshaw LJ, Messite J. Workplace violence: preventive and interventive strategies. J Occup Environ Med. 1996 Oct; 38: 993-1006.
- 10. Soares JJF. The nature, extent and determinants of violence against psychiatric personnel. Work and stress. 2000; Vol. 14. Iss. 2.
- 11. Hahn S. Factors associated with patient and visitors violence experienced by nurses in general hospitals in Switzerland: a cross-sectional survey. Journal of clinical nursing. 2010; Vol. 19. Iss 23-24.
- 12. Pai HC, Lee S. Risk factors for workplace violence in clinical registered nurses in Taiwan. Journal of clinical nursing, 2011; Vol. 20. Iss. 9-10.
- 13. Van Leeuwen ME, Harte JM. Violence against care workers in psychiatry: Is prosecution justified? International journal of law and psychiatry. 2011; Vol. 30. Iss. 5.
- 14. World Health Organization. World report on violence and health. Geneva: WHO, 2002.
- 15. Violence: A world-wide epidemic. http://www.ncbi. nlm.nih.gov/sites/entrez.at . Accessed [December 25, 2010].
- 16. Metin P, at all. The analysis of violence against the nurses who are in employee status in Mugla State Hospital, Turkey. HealthMED. 2012; Vol. 6. No. 11.
- 17. Clerk JM. Introduction to working conditions and environment. 2nd ed. Geneva. International Labor Office, 1989.
- 18. Kiran S. The evaluation of occupational factors levels and relation with expose disease in health workers. Izmir: Dokuz Eylul Univ., 2003.

Corresponding Author Branko Gabrovec, Zdravstveni dom Celje, Celje, Slovenija, E-mail: branko.gabrovec@gmail.com

# The dependence of explosive strength and speed on feet posture

Mladen Zivkovic<sup>1</sup>, Dobrica Zivkovic<sup>1</sup>, Sasa Bubanj<sup>1</sup>, Sasa Milenkovic<sup>1</sup>, Sladjan Karaleic<sup>2</sup>, Zoran Bogdanovic<sup>3</sup>

<sup>1</sup> Faculty of Sport and Physical Education, University of Niš, Niš, Serbia,

<sup>2</sup> Faculty for Sport and Physical Education, University of Kosovska Mitrovica, Serbia,

<sup>3</sup> University of Novi Pazar, Novi Pazar, Serbia.

### Abstract

**Introduction:** The deformity that most frequently occurs after damage to foot statics are flat feet, which in the etymology of their occurrence can be acquired (in a minority of cases) or could be of a congenital character (occurring under the influence of various influences). This deformity is manifested in the loss of normal – physiological arches of the feet.

Aim: The sample of participants consisted of 114 elementary school children, all males, aged 11 and 12, divided int two sub-samples. The first sub-sample (E1) consisted of 57 elementary school children suffering from varying degrees of deformities of the foot, while the second sub-sample consisted of 57 school children with normal arches of the feet. In order to evaluate the status of their feet, the following was used: Thomson's method for the evaluation of the fallen arches (ATPSS), foot width (AŠIS) and foot length (ADUS). In order to evaluate the explosive strength of the lower extremities, the following tests were used: the standing depth jump (MSKUDM), the triple standing jump (MTRSM), the vertical jump (MVKS), the high jump with a flying start (MSUZ), kicking the ball (MSLUD). In order to evaluate speed the following were used: the 20m run with a high start (MT20V), the 40 m run with a high start (MT40V), the 60 m run with a high start (MT60V), the 4x15 m relay (MT4x15), and running in a polygon (MTRUP). For the statistical analysis and interpretation of the results, the statistical package Statistics 13.0 was used. In order to calculate the significant differences between the mean values of the two independent groups, the t-test was used. In order to determine which variables discriminate between the two groups, a discriminant function analysis was used.

**Results:** Participants with normal (healthy) feet were more successful in the applied motor tests for determining explosive strength than the participants with flat feet (difference was statistically significant). Nevertheless, the participants in the E1 sub-sample (those suffering from fallen arches, or flat feet), had better results when completing the motor tests of speed, as compared to the participants from the E2 sub-sample who had normal (healthy) feet (difference was statistically significant in four of totally five variables). In the remaining two variables which defined the longitudinal and transversal dimensionality of the feet (ADUS=-0.071, ASIS= 1.271), the obtained results do not indicate statistically significant differences between the participants. By gaining insight into the coefficients which determine the discriminant function, we can note that except for the variables which define longitudinal dimensionality (ADUS) and transversal dimensionality (AŠIS), as well as the variables for the evaluation of speed (MTRUP), in the case of all the remaining variables, coefficients were obtained which accurately discriminate between the E1 and E2 sub-sample.

**Conclusion:** The results of the research including participants suffering from fallen arches (the El sub-sample) which could be described as very satisfactory in terms of speed, but quite modest in the case of explosive strength, require further detailed analysis.

Key words: Flat feet, motor abilities, children.

### Introduction

From the very first moment that a child takes its first steps, the foot is susceptible to a variety of exogenic and endogenic factors, which can lead to anomalies of the feet, as well as to more complex structural changes to the locomotor apparatus, all of which primarily depends on genetic predispositions. The deformity that most frequently occurs after damage to foot statics are flat feet, which in the etymology of their occurrence can be acquired (in a minority of cases) or could be of a congenital character (occurring under the influence of various influences). This deformity is manifested in the loss of normal - physiological arches of the feet (Živković, 1998, 25). The most frequent risk factors are obesity and hypokinesis (Pfeiffer et al., 2006). According to the research of the authors Mihajlović et al. (2010), in the case of children, the arch of the foot develops past the ages of three and four, and lasts until become of age for school. Support for this hypothesis can be found in the results of the research of the afore mentioned authors which included 270 children, all females, aged 4-7, among whom the incidence of the afore mentioned deformity reached values of up to 90%. It has been suggested that abnormal foot morphology contributes to the excessive occurrence of injuries in athletes (Burns et al., 2005). According to the authors Nigg et al. (1993), although the literature suggests that a relationship exists between the height of the medial longitudinal arch of the foot and athletic injuries to the lower extremities, the functional significance of arch height in relation to injuries has not properly been investigated. Because the controversy about the relation of foot morphology and foot function still persists, authors such as Tudor et al. (2009) found it surprising that there are very few studies published dealing with motor skills and athletic performance of flat-footed school children. Explosive strength represents the ability which allows an athlete maximum acceleration of his body (or part of the body), toward an object or a partner. It is particularly important in the athletic throw, high jump, long jump and sprint. The amount of manifested explosive strength depends on the percentage and composition of the activated motor units in the corresponding muscle group (Bubanj, R. & Branković, M., 1997). Explosive strength is used in a variety of sports activities, or in other words, there are different levels of manifestation of this motor ability in relation to the type of engagement in sport and gender (Gašić, et al., 2011). "What is meant by the term speed most often is the ability of a man to perform a great frequency of movement at a particular point in time or to perform a single movement in the shortest possible period of time

" (Berković, 1978, 35). The same author cites that speed is genetically condition (by approximately 90 %) which leaves relatively little room for its further development under the influence of the training process. The aim of this research was to determine the differences between explosive strength of the feet and speed among participants suffering from flat foot and participants with normal feet.

### The method

The sample of participants consisted of 114 elementary school children, all males, aged 11 and 12. After forming the (E1) sub-sample of participants, which consisted of 57 elementary school children suffering from varying degrees of deformities of the foot, another sub-sample (E2) was formed numbering 57 school children with normal arches of the feet. In order to evaluate the status of their feet, the following was used: Thomson's method for the evaluation of the fallen arches (ATPSS); foot width (AŠIS); foot length (ADUS). Thomson's method presents the extent of fallen arches in percentages (see Živković, 1998, 232) and is the optimal procedure for its determination. What was also calculated was the mathematical value of the arithmetic means between two plantograms of a single foot, and then the same value between two feet.



Image 1. Thomson's for the evaluation fallen arches (from 1 to 30% = I degree; from 30 to 60% = II degree; 60% and upwards = III degree).

The measurements of the width of the feet (ASIS) and length of the feet (ADUS) were carried out according to the International Biological Program (IBP), (see Đurašković, 2001, 12). For the width and length of the feet, 4 anthropometric points were determined (Đurašković, 2001) including: metatarsale fibulare (the point which is located on the outer part of the ball of the heel of the metatarsal bone of the foot); the metatarsale tibiale (the point located on the inner side of the ball of the first metatarsal bone); the point located on the back, most prominent part of the foot, in the region of the heel; the point located on the front part of the foot, representing the most prominent part of the first or second toe. In order to evaluate the explosive strength of the lower extremities, the following tests were used: the standing depth jump (MSKUDM), the triple standing jump (MTRSM), the vertical jump (MVKS), the high jump with a flying start (MSUZ), kicking the

ball (MSLUD) (according to Kurelić et al., 1975). In order to evaluate speed the following were used: the 20m run with a high start (MT20V), the 40 m run with a high start (MT40V), the 60 m run with a high start (MT60V), the 4x15 m relay (MT4x15), and running in a polygon (MTRUP) (according to Kurelić et al., 1975). For the statistical analysis and interpretation of the results, the statistical package Statistics 13.0 was used. In order to calculate the significant differences between the mean values of the two independent groups, the t-test was used. In order to determine which variables discriminate between the two groups, a Discriminant function analysis was used.

### **Results and discussion**

The T-test results shown in the table indicate that differences exist in most of the variables, so it

*Table 1. The results of the applied t-test for each of the variables individually, between the participants of the E1 and E2 sub-sample.* 

	Sub-sample	Mean	Std.Dv.	N	Diff.	Std.Dv.	t	df	Р
MERTIDM	E1	133.8182	22.77026	55	27.000	21 5 42 (2	( 249	54	0.00000
MSKUDM	E2	160.8182	19.94817	33	-27.000	31.34302	-0.348	54	0.000000
MTDOM	E1	391.7273	35.76179	55	50.001	71 45625	5 100	51	0.000002
IVI I KSIVI	E2	441.8182	58.74745	35	-30.091	/1.43033	-3.199	54	0.000005
MUKS	E1	21.7636	3.85484	55	6.045	6 46502	6.024	54	0.00000
IVI V KS	E2	27.8091	5.06119		-0.043	0.40392	-0.934	54	0.000000
MOUT	E1	67.0909	10.78798	55	14 626	16 19240	6 707	51	0.00000
MSUZ	E2	81.7273	10.14790	55	-14.030	10.18340	-0./0/	54	0.000000
MSLID	E1	22.7873	3.64931	55	1 562	5 9/115	5 702	54	0.00000
MSLUD	E2	27.3491	4.07489		-4.302	3.84113	-5.792	34	0.000000
MT20V	E1	4.6560	0.43438	55	0.450	0.54900	6.084	54	0.00000
W1120V	E2	4.2056	0.31646	55	0.450	0.34699			0.000000
MT40V	E1	8.3555	0.61178	55	0.700	0 71126	71136 7.298	54	0.00000
W1140 V	E2	7.6555	0.53972	55	0.700	0.71130			0.000000
MTGOV	E1	12.5107	0.81428	55	1.247	1 1 5 2 0 7	8 661	54	0.00000
W1100 V	E2	11.1642	0.93685	55	1.34/	1.13297	8.001	54	0.000000
MT4-15	E1	18.8742	1.57241	55	1.062	1 02500	7 072	51	0.00000
IVI 14XI3	E2	16.9113	0.78027		1.905	1.82389	1.975	54	0.000000
ATDSS	E1	20.5255	16.70614	55	20.525	16 70614	0.112	54	0.00000
AIF55	E2	0.0000	0.00000	55	20.323	10./0014	9.112	54	0.000000
	E1	20.1020	1.56509	55	0.566	12 01000	0.204	51	0 762225
MIKUP	E2	20.6684	14.07694	33	-0.566	13.01090	-0.304	54	0.702555
	E1	9.0527	0.68282	55	0.156	0.01242	1 271	51	0.200100
A515	E2	8.8964	0.54771	55		0 0.91242	1.2/1	54	0.209199
	E1	23.8782	1.69178	55	0.022	2 26074	0.071	54	0.042421
ADUS	E2	23.9000	1.50591	33	-0.022	2.269/4	-0.0/1		0.943431

	Wilks*	Partial	F-remove	p-level	Toler.	1-Toler.
MSKUDM	0.30	1.00	0.03	0.86	0.55	0.45
MTRSM	0.30	1.00	0.01	0.93	0.70	0.30
MVKS	0.30	0.99	0.56	0.46	0.54	0.46
MSUZ	0.32	0.95	5.70	0.02	0.65	0.35
MSLUD	0.31	0.98	2.39	0.13	0.71	0.29
MT20V	0.30	0.99	1.28	0.26	0.82	0.18
MT40V	0.31	0.98	2.39	0.13	0.79	0.21
MT60V	0.31	0.97	3.27	0.07	0.77	0.23
MT4x15	0.32	0.93	7.29	0.01	0.90	0.10
MTRUP	0.31	0.97	3.35	0.07	0.89	0.11
ATPSS	0.35	0.86	15.69	0.00	0.93	0.07
ASIS	0.30	1.00	0.17	0.68	0.76	0.24
ADUS	0.30	1.00	0.06	0.80	0.78	0.22

Table 2. The results of the discriminant analysis.

*Wilks*\* *Lambda: .30 approx. F* (13,98)=17.54 pO.OO

can be concluded that the motor skills of the participants with flat and normal (healthy) feet differ in a statistically significant manner. For each of the variables which defined explosive strength as a motor skill, statistically significant values of the T-test were obtained (MSKUDM = -6.348, MTRSM = -5.199, MVKS = -6.934, MSUZ = -6.707, MLUD = -5.792), with a negative value, which means that the participants who made up the E2 sub-sample achieved better results than the participants from the E1 sub-sample. In other words, the participants with normal (healthy) feet were more successful in the applied motor tests for determining explosive strength than the participants with flat feet. Nevertheless, by analyzing the values of the T-test for the second set of motor variables (speed), we can conclude that statistically significant differences exist in the case of four of the manifest variables (MT20V = 6.084, MT40V = 7.298, MT60V = 8.661, MT4x 15 =7.973), while in the case of the fifth variable (MTRUP = -0.304), the obtained value is not statistically significant. The values obtained on the T-test for the first four variables have a positive value, which means that the participants in the E1 subsample (those suffering from fallen arches, or flat feet), had better results when completing the motor tests of speed, as compared to the participants from the E2 sub-sample who had normal (healthy) feet. For the variable used to define flat feet, the expected value of the T-test was obtained, which indicates the statistical significance of the difference that exists between the two sub-samples. The obtained positive value (ATPSS=9.112) confirms that the participants from the E1 sub-sample suffered from flat feet, while the participants from the E2 sub-sample had normal (healthy) feet. In the remaining two variables which defined the longitudinal and transversal dimensionality of the feet (ADUS=-0.071, ASIS= 1.271), the obtained results do not indicate statistically significant differences between the participants. This indicates that irrespective of the presence of flat feet, no statistically significant difference exists in terms of the width and length of the feet of the participants.

Table 3. Matrix structure.

<b>Group Function</b>	Root 1
MSKUDM	0.42
MTRSM	0.35
MVKS	0.45
MSUZ	0.46
MSLUD	0.40
MT20V	-0.38
MT40V	-0.41
MT60V	-0.51
MT4x15	-0.53
MTRUP	0.02
ATPSS	-0.57
ASIS	-0.09
ADUS	-0.02

Table 4. Group centroids

G 1:1	-1.49
G2:2	1.54

Tables 2-4 show the results of the canonical discriminant analysis. The obtained statistical significance was at the (p=0.00) level. By means of the transformation and condensation of the variables in motor space (explosivity, speed) and in the space of the variables used to evaluate flat feet (ATPSS, AŠIS, ADUS), a single discriminant function was isolated which separates the participants of the E1 and the E2 sub-sample, on the basis of the obtained dsicriminant coefficients. By gaining insight into the coefficients which determine the discriminant function, we can note that except for the variables which define longitudinal dimensionality (ADUS) and transversal dimensionality (AŠIS), as well as the variables for the evaluation of speed (MTRUP), in the case of all the remaining variables, coefficients were obtained which accurately discriminate between the E1 and E2 sub-sample. The highest coefficient was obtained for the flat foot variable (ATPSS), while in the case of all the remaining motor variables, high or similar coefficients were obtained. By analyzing the group centroids, their extent and value, we can conclude that the sample of participants which made up the E2 sub-sample is statistically discriminated in relation to the participants in the E1 sub-sample in motor space, or in other words in terms of explosive strength, while the participants from the E1 sub-sample are statistically discriminated in relation to the participants of the E2 sub-sample in terms of motor skill abilities and postural deformities such as flat feet.

The results obtained during the current research are not in accordance with the results of research conducted by authors Tudor et al. (2009). The afore mentioned authors aimed to determine if there is a relation between the degree of fallen arches and several motor skills that are necessary for sport performance in the research which included of 218 children aged 11 to 15. The value of the arch index was corrected for the influence of age, and then the entire sample was categorized into 4 groups according to the flatness of the participants' feet. The children were tested for eccentric-concentric contraction and hopping on a Kistler force platform, speed-coordination polygon (Newtest system), balance (3 tests), toe flexion (textile crunching), tiptoe standing angle, and repetitive leg movements. Altogether, 17 measures of athletic performance were measured. No significant correlations between the arch height and 17 motor skills were found. Categorizing the sample into 4 groups did not reveal any differences between the groups in terms of athletic performance. In addition, several multivariate analyses of variance sets of multiple independent variables referring to a particular motor ability were not found to be significant. No difference was found even after comparing only the 2 extreme groups, meaning children with very low and children with very high arches, nor a disadvantage in sport performance originating from flat-footedness were confirmed. Children with flat and children with "normal" feet were equally successful at accomplishing all motor tests; thus, the afore mentioned authors suggested that there is no need for treatment of flexible flat feet with the sole purpose of improving athletic performance, as traditionally advised by many.

Concerning other studies related to the foot postural status and sport activities, the authors Kaufman et al. (1999) tended to determine whether an association exists between foot structure and the development of musculoskeletal overuse injuries. The study group was a well-defined cohort of 449 trainees at the Naval Special Warfare Training Center in Coronado, California. Before beginning training, measurements were made related to ankle motion, subtalar motion, and the static (standing) and dynamic (walking) characteristics of the foot arch. The subjects were tracked prospectively for injuries throughout training. The afore mentioned authors identified the risk factors that predispose people to lower extremity overuse injuries. These risk factors include dynamic pes planus, pes cavus, restricted ankle dorsiflexion, and increased hind foot inversion, all of which are subject to intervention and possible correction.

The authors Nigg et al. (1993) aimed to determine the influence of arch height on kinematic variables of the lower extremities that have been associated with the incidence of injury in running in an attempt to gain some insight into a functional relationship between arch height and injury. The three-dimensional kinematics of the lower extremities was measured during running for 30 subjects using high-speed video cameras. A joint coordinate system was used to calculate the threedimensional orientation of the ankle joint complex for a single stance phase. Simple, linear regre-

ssion analyses showed that arch height does not influence either maximal eversion movement or maximal internal leg rotation during running stance. However, assuming that knee pain in running can result from the transfer of foot eversion to internal rotation of the tibia, a functional relationship between arch height and injury may exist in that the transfer of foot eversion to internal leg rotation was found to increase significantly with increasing arch height. A substantial (27%), yet incomplete, amount of the variation in the transfer of movement between subjects was explained by arch height, indicating that there must be factors other than arch height that influence the kinematic coupling at the ankle joint complex. Additionally, the transfer of movement is only one factor of many associated with the etiology of knee pain in running. Therefore, according to the results of research, it is suggested that a running-injury-related foot typology based on arch height is not possible. The authors Živković, D. et al. (1991) carried out a study on the relations between flat feet and static balance. The obtained results corresponded with their assumption of the statistical significance of the status of the feet for this type of motor skill. The higher the percentage of the fallen arches, the more the values of the tests for the evaluation of static balance indicated "poorer" results. Levajac (1991) used a sample of preschool children aged three to seven to study the differences in the level of postural disorders in relation to gender and age by using eight variables for the determination of postural. The research indicated that in the case of children in this age group, postural deformities of the spinal column could be found in the frontal and sagittal plain (to a lesser extent), while the majority of children suffering from postural disorders had deviations of the feet.

### Conclusion

The results of the research including participants suffering from fallen arches (the El subsample) which could be described as very satisfactory in terms of speed, but quite modest in the case of explosive strength, require further detailed analysis. The probable cause of such a state could be sought in the static and dynamics of not only the muscles of the feet but also of the muscle groups of the lower and upper leg and pelvic region. The muscles of the feet evidently play and more significant part in determining balance, as well as in motor activities related to explosivity (startability), so the range of motor tests for the evaluation of explosivity confirmed the significance of the muscles of the longitudinal arch of the foot and thus confirmed the lack of explosivity of the participants suffering from this type of weakness (flat feet). Nevertheless, once speed as a cyclical motor activity is analyzed, we obtain significantly better results, and this in turn leads us to consider the degree of significance of the muscles of the feet, their influence in activities such as running, in relation to the significance and importance of the muscles of the m. quadriceps femoris, m. biceps femoris, m. triceps sure or the flexors and extensors of the upper leg. It is evident that the afore mentioned muscles take on a significant function in running so that their function in cyclical movements of the lower extremities is more significant than the activity of the muscles of the feet. Thus better results were obtained for the participants with flat feet on the motor tests for the evaluation of speed.

#### References

- 1. Berković L. Metodika fizičkog vaspitanja. Beograd: Partizan. Barrow, M.H.&, 1978.
- 2. Bubanj R, Branković M. Athletics-techniques and methodics (Atletika-tehnika i metodika). Autonomous edition of authors, Niš, In Serbian, 1997.
- 3. Burns J, Keenan AM, Redmond A. Foot Type and Overuse Injury in Triathletes. J Am Podiatr Med Assoc, 2005; 95(3): 235-241. http://www.japmaonline.org/ cgi/content/abstract/95/3/235
- 4. Đurašković R. Biology of human development with medicine of sport (Biologija razvoja čoveka sa medicinom sporta). Niš: S.I.I.C. In Serbian, 2001.
- 5. Gašić T, Bubanj S, Živković M, Stanković R, Bubanj R, Obradović B. Difference in the explosive strength of upper extremities between athletes in relation to their sport activity, type of engagement in sport and gender. Sport Science, 2011; 4(1): 63-67.
- Kaufman K, Brodine S, Shaffer R, Johnson C, Cullison C. The Effect of Foot Structure and Range of Motion on Musculoskeletal Overuse Injuries. Am J Sports Med, 1999; 27(5): 585-593.

- Kurelić N, Momirović K, Stojanović M, Sturm J, Radojević D, Viskić-Stalec N. Structure and development of morphologic and motor dimensions in youth (Struktura i razvoj morfoloških i motoričkih dimenzija omladine). Beograd Institut za naučna istraživanja fakulteta za fizičku kulturu. In Serbian, 1975.
- 8. Levajac R. Razlike u nivou motoričkih sposobnosti, morfoloških karakteridtika i posturalnih devijacija predškolske dece s obzirom na pol i uzrast. Unpublished doctoral dissertation. Niš: Faculty of Physical Culture, University of Niš, 1991.
- Mihajlović I, Smajić M, Sente J. Frequency of foot deformity in preschool girls. Vojnosanit Pregl, 2010; 67(11): 923-927.
- 10. Nigg BM, Cole GK, Nachbauer W. Effects of arch height of the foot on angular motion of the lower extremities in running. J Biomech, 1993; 26(8): 909-916.
- 11. Pfeiffer M, Koty R, Ledl T, Hauser G, Sluga M. Prevalence of flat foot in percolated children. Pediatrics 2006; 118 (2): 634–639.
- 12. Tudor A, Ružić L, Sestan B, Sirola L, Prpić T. Flat-Footedness Is Not a Disadvantage for Athletic Performance in Children Aged 11 to 15 Years. Pediatrics, 2009; 123(3): e386-e392.
- 13. Živković D. Theory and methodics of corrective gymnastics (Teorija i metodika korektivne gimnastike). Autonomous edition of authors. Niš, In Serbian, 1998.
- 14. Zivković D, Petrović M, Petković D. Relations between flat foot and static balance (Relacije između ravnog stopala i statičke ravnoteže). X Letnja škola pedagoga fizičke kulture Jugoslavije. Ohrid. In Serbian. Book of proceedings, 1991.

Corresponding Author Mladen Zivkovic, University of Nis, Faculty of Sport and Physical Education, Nis, Serbia, E-mail: profzile@gmail.com

### Effect of WIN51,708, a NK<sub>1</sub> receptor antagonist, on the signal transmission between two endings of primary afferent nerves from adjacent spinal segments\*

Qi-Xin Sun<sup>1</sup>, Yan Zhao<sup>2</sup>, Shi-Hong Zhang<sup>3</sup>, Wen-Chun Shi<sup>2</sup>, Hui-Sheng Wang<sup>2</sup>

- <sup>1</sup> Department of Biological Medical Engineering, College of Life Science and Technology, Xi'an Jiaotong University, Xi'an, China;
- <sup>2</sup> Department of Physiology, Medical College, Xi'an Jiaotong University, Xi'an, China;

<sup>3</sup> Pharmacological Department of Pharmacological School, Zhejiang University.

### Abstract

WE reported previously that the signal transmission between the endings of adjacent spinal segments could produced by the electric stimulation across spinal segments and proposed the involvement of the endogenous SP through evoking NK<sub>1</sub> receptor in peripheral nerve and other tissue. The present study aimed to determine the possible involvement of the NK<sub>1</sub> receptor in the process of signal transmission between the endings of adjacent spinal segments. The discharge increase of Afibers, used as the index of the signal transmission between endings of adjacent spinal segments, were induced by the antidromic electric stimulation of nerve stem from adjacent spinal segment and observed. The signal transmission was significantly attenuated a micro-injection of WIN51,708 (0.57micromol/L, 10µl), a selective non-peptide antagonist of NK, receptors into the receptive fields of recorded A-units recorded. Before the blockage of NK, receptor, a moderate increase of the discharge of A-fibers was evoked by a microinjection of SP (0.74micromol/L, 10µl), a NK, receptor agonist, showing a facilitating effect of SP on the signal transmission. WIN51,708, administered i.c. 30 min prior to SP micro-injection into receptive field antagonized the facilitating effect of SP on the signal transmission. The blockage duration of WIN51,708 on the signal transmission was longer than 67.16±8.33min. These findings suggest that the effects of both of endogenous SP released from endings of nerve stem stimulated antidromically, and exogenous SP injected into receptive field of recorded A-fibers are mediated

through NK<sub>1</sub> receptive sites. Endogenous SP and its receptor bond mechanism may be an essential principle of the signal transmission between peripheral endings from adjacent spinal segments. That may also be an important neurobiological base of sensory transmission along meridian.

**Key words:** WIN 51,708; antagonist; NK<sub>1</sub> Receptor; Primary Afferent A-fibers; Discharge; Signal Transmission between Peripheral Endings from Adjacent Spinal Segments; Meridian

### 1. Introduction

Substance P (SP) is widely distributed in the central and peripheral nerve system. There is growing body of evidence that support the implication of tachykinin involvement in the signal transmission among peripheral nerve. After stimulation of nociceptive-peptidergic afferents, SP is released peripherally and exerts various pro-inflammatory effects such as vasodilation, increased vascular permeability and the release of some inflammatory substances [35]. The contribution of SP to peripheral nerve was shown to be mediated by the neurokinin-1 (NK<sub>1</sub>) tachykinin receptor <sup>[18,25]</sup>. By cutting dorsal root and stimulating the distal end, the content of SP in the dermic perfusing liquid on the stimulated side was markedly higher than that on the control side while the contents of other kind of transmitters were much lower than that on control side <sup>[21]</sup>. Similarly, with a radioimmunoassay of method, SP was revealed significantly increased release under the condition of dorsal root reflex in receptive field of dorsal cutaneous nerve stimulated <sup>[4]</sup>. By the efferent and local effector function of primary affe-

HealthMED - Volume 8 / Number 2 / 2014

rent nerve, some vasoactive peptide transmitters or modulators were released from the peripheral endings and a following neurogenic inflammation wad found in the receptive field of stimulated nerves. It has also been turned out that in this way the released bioactive substances can activate the endings of adjacent spinal segment, and finally, cause the discharge change there. That is called the signal transmission between peripheral endings from adjacent spinal segments or the signal transmission across spinal segments [8, 15, 40]. Without any involvement of central nerve system, a significant change of mechanical and electro-physiological properties of primary afferent A-fibers was evoked by an antidromical electrical stimulation of cutaneous nerve stem from adjacent spinal cord <sup>[28]</sup>. This could be verified by some research results of morphology: SP-like immunoreactivity (SP-LI) was observed in Aδ-fibers in dorsal root ganglia of cats <sup>[30]</sup>. A synapse-like connection between SP-containing axon endings and mast cell was found in human skin<sup>[34]</sup>. The gap between SP-ergic or calcitonin gene-related peptide (CGRP)- ergic fiber ending and mast cell is less than 100 nm in periphery, similar to that of synapse<sup>[6]</sup>. But up to now, the mechanism of signal transmission across spinal segments is maintained unknown. The present study aimed to determine the effect of WIN 51,708, a selective nonpeptide NK, receptor antagonist, on the discharge and signal transmission between peripheral endings of A-fibers from dorsal cutaneous branch of spinal nerve from adjacent spinal segments in anaesthetized rats and to assess the involvement of local SP release and its mechanism in the signal transmission across spinal segments on the meridian in the Traditional Chinese Medicine.

### 2. Methods and materials

### 2.1. Animals

The experiments were carried out in sixty-two Sprague–Dawley rats from experimental animal center of Xi'an Jiaotong University in China. These animals, without sexual distinguish weighing  $200 \sim 230$ g, were kept under standard laboratory conditions (12:12 h light: dark cycle at  $22 \pm 2^{\circ}$  C) and given free access to food and water before the experiment.

### 2.2. Surgery

The animals were deeply anesthetized with urethane (1.0 g/kg, i.P.). Supplemental doses were given to maintain constant anaesthesia and to prevent any reaction to electrical or mechanical stimulation. A paramedian incision on back skin was made longitudinally to separate the cutaneous branch of dorsal ramus (CBDR) of  $T_{0}$  and  $T_{10}$ spinal nerves for 2.5~3.5 cm from the surrounding tissues. With the exposed tissues kept at 37°C in liquid paraffin, the separated nerve branches of T9 and T10 CBDR were transected proximally and the discharge of single A $\delta$ - and A $\beta$ -fibers of the distal branches responding to mechanical stimulation were selected for recording by the use of conventional teased-filament techniques<sup>[3]</sup>.



Figure 1. Schematic drawing of the experimental set-up in the experiment. Cross-excitation between two intact neighboring spinal nerves was studied by recording the spontaneous discharge of single  $A\delta$ - and  $A\beta$ -fibers in the cutaneous branch of the dorsal ramus (CBDR) of T10, and antidromically electronical stimulating the neighbor spinal nerve of T9 CBDR (20 Hz for 10 sec, at a strength of 1.0mA, 0.2ms in shock duration). Since both nerves were cut proximally, any change in the recorded activity must be due to a cross-talk in the periphery. The scheme shows our hypothesis: activated antidromically traveling action potentials of T9 CBDR caused the release of SP and other peptide from their peripheral terminals. SP diffused to neighboring mechanoceptive  $A\delta$ -fibers and  $A\beta$ -fibers, binding to NK-1 receptors, sensitizing the terminals of  $A\delta$ -fibers in T10 CBDR and causing them to increase their spontaneous discharge.

Both of the filaments and nerve branches of adjacent spinal segment, T9 and T10 CBDR were put on platinum bipolar electrodes in proper order for discharge recording of A-fibers of T10 and antidromical electrical stimulation. Schematic drawing of the experimental set-up in the experiment is shown in Figure 1.

### 2.3. Recording and Stimulation

The recording was started when the unit activity had become stable 1h after surgery. The adaptation characteristic was measured by a set of calibrated von Frey's hair (0.02~16.6 milli-Newton, mN). On the basis of their adapting speed to mechanical stimulus, the units were divided into two types: rapidly adapting (RA) units and slowly adapting (SA) units.

The recording electrode was connected to a VC-10 Oscilloscope (Nihon Kohden) to display the bioelectric signal of the units and take photos. The signal was synchronously fed to a desktop computer (Apple II) that sketched the sequence histogram with 1 min spontaneous firing as control. The discharge change following antidromical electrical stimulation (0.45 mA, 0.1 ms, 20 Hz, for 10 s) to the nerve stem of adjacent spinal segment was recorded for 3 min or longer to record a response of recorded unit as a indicator of signal transmission between peripheral endings from adjacent spinal segments.

Before an experimental order on a unit was finished, a single pulse with progressively increased intensity was delivered to the center of the receptive field of the unit to read out its electrical stimulation threshold and the latency of action potential to calculate the conduction velocity (CV) of the unit. The units with the CVs 2.1m/s to 29.9m/s were put under primary afferent A $\delta$ -fibers while the ones with CV faster than 30m/s were put in the group of primary afferent A $\beta$ -fibers. The body temperature of animals was kept at 38~39 °C.

### 2.4. Drug administration

The NK-1 receptor antagonist, WIN 51,708 (from Sigma RBI, St. Louis. MO) was administrated via subcutaneous micro-injection into the skin of the receptive field of recorded unit after

the introduction of signal transmission between peripheral endings from adjacent spinal segments. Shortly before subcutaneous micro-injection, WIN51, 708 was dissolved in a solution of phosphate buffered saline (PBS; PH 7.2) containing 0.3% dimethylsulfoxide at a concentration of 0.57 micromol/L. Phosphate buffered saline (PBS; PH 7.2) containing 0.3% dimethylsulfoxide was used as vehicle. A volume of 10µl of WIN, 51,708 solution or its vehicle was injected into the receptive filed of recorded unit over 8 min. An antidromical electrical stimulation was delivered to the cutaneous nerve stem from adjacent spinal segment again to observe the effect of WIN51,708 on the signal transmission between peripheral endings from adjacent spinal segments.

SP (0.74micromol/L, 10 $\mu$ l), a NK<sub>1</sub> receptor agonist, was injected into same point to confirm the facility role of NK<sub>1</sub> receptor on the signal transmission between peripheral endings from adjacent spinal segments before and after the microinjection of WIN51,708.

A minimum time of 40 min was allowed between micro-injection of WIN51,708 and SP as our earlier study showed that the interval is adequate for evoked changes in afferent activity to return to control level <sup>[26]</sup>.

### 2.5. Statistical analysis and illustration

The Spearman Rank Order Correlation of SigmaStat.2.03 software was employed to analyze all experiment data. All values are given as mean  $\pm$ S.E. *P*<0.05 was considered to be statistically significant. The SigmaPlot 8.02 and Photoshop 6.0 were used to treat all figures illustrated in the paper.

### 3. Results

### 3.1. The mechanical receptive properties of A-fibers

Of all 66 recorded units, 58 A $\delta$ -fibers and 8 A $\beta$ -fibers, there were 77.3% (51/66) SA units and 22.7% (15/66) FA units. The adapting duration of all A-units lasted for one second to a few minutes that mean value was 16.82 ± 3.12s. The duration was significantly shorter than that of C-fiber (Wilcoxon signed rank test, *P*<0.05) <sup>[26]</sup>. Except

9 units having a high mechanoceptive threshold from 1.2 mN to 16.6 mN, the rest 57 A-fibers belonged to low-threshold A-mechanoreceptors fibers and nociceptors having a lower mechanoceptive threshold ranging from 0.01 mN to 0.7 mN (mean 0.31  $\pm$  0.08) than that of C-fibers <sup>[39]</sup>. Similar to the reported result<sup>[26,28]</sup>, most receptive fields of A-fibers were smaller than that of C-fibers. 8.7% (4/46) of them were even only a single dot and 65.2% (30/46) were ellipse. The rest 26.1% (12/46) were circle. The area of receptive fields varied in a range from 0.28 mm<sup>2</sup> to 150.75  $mm^2$ . The mean value was  $17.75 \pm 4.11 mm^2$ . There were one to two dots within each receptive field at which a number of immediately increasing discharges could be induced by a suprathreshold mechanical stimulation. That was so-called the center, or the sensitive point of receptive field. 79.0% (60/76) of the sensitive points distributed along the belt between two branches of the Bladder meridian of Foot Taiyang on the dorsum.

### 3.2. The spontaneous discharge of all recorded A-units and the changes evoked by the micro-injection of WIN51,708

Sixty-six units, 58 A $\delta$ -fibers and 8 A $\beta$ -fibers, were observed. A spontaneous discharge was recorded at 39.4% (n=66) A-units in a single, scattered and irregular pattern with a mean of  $4.98 \pm 1.08$  per 30s.



Figure 2. An example of mechanoceptive  $A\delta$ fibers in T10 CBDR that were cross-excited by antidromically electrical stimulating the neighbor spinal nerve of T9 CBDR, whose conduction velocity is 8.9 m/s. Con. is the spontaneous discharge level record of the unit control discharge level for 1 minute before antidromically electrical stimulating of T9 CBDR. Black bar indicates the onset and duration of tetanus (stimulus intensity - 1.0 mA, pulse width - 0.2 ms, shock frequency - 20 Hz, tetanus duration - 10 sec). A following evoked discharge was recorded for 4 minutes to show the recovering process of the unit.

The amplitude of spikes was bigger than that of C-fibers <sup>[39]</sup>. The electrical stimulation threshold of A-fibers was 0.08 to 0.2 mA and the mean was 0.14  $\pm$  0.01 mA. These electro-physiological properties were similar to those previous reports <sup>[27~28]</sup>. The spontaneous discharge and change of an A $\delta$ -fiber of T10 CBDN reduced by an antidromically electric stimulation on T9 CBDN was shown in Figure 2. The average discharge changes of all recorded units induced by antidromical electrical stimulation on T9 CBDN were shown in Figure 3.



Figure 3. The time course of firing frequency changes of mechanoceptive A-fibers in T10 CBDR that were cross-excited by tetanization of T9 CBDR in intact rats (n=90 units). The break on the abscissa indicates the onset and duration of antidromically electrical stimulating (stimulus intensity - 1.0mA, pulse width - 0.2 ms, shock frequency – 20 Hz, tetanus duration - 10 sec). \*\* indicates P < 0.01 compared to control level and \* represent P < 0.0, respectively.

Immediately WIN51, 708 or vehicle was injected into the center of receptive field of recorded units, an appreciable increasing discharge was found at A-units at varies level. Having been given a micro-injection of WIN51,708, 33.3% (22/66) units displayed a exciting firing for 3 s to 5 min (mean  $3.12 \pm 0.68$  min). The mean values of discharge numbers were significantly higher than that of its control level for 3 min. Similar response occurred at the units that were given a vehicle micro-injection. But there is a lack of any significant difference between discharge mean values of per minute of two groups units that were treated

with WIN51,708 or the vehicle. The discharge number changes of all recorded units caused by the micro-injection of vehicle or WIN51,708 was illustrated in Figure 4.



Figure 4. The time course of firing frequency changes of mechanoceptive A-fibers in T10 CBDR following local intradermal microinjection of Win51,708 (A, n = 46) and its vehicle (B, n = 23) into the center of the RFs. The zero point of abscissa indicates the onset of microinjection and its duration (5min). \*\* and \* represent P < 0.01 and P< 0.05, respectively, when compared to the spontaneous discharge lever before microinjection.

3.3. The introducing of the signal transmission between peripheral endings from adjacent spinal segments before and after the micro-injection of WIN51,708

With 1 min recording of spontaneous discharge for control, an antidromical electrical stimulation (0.45 mA, 0.1 ms, 20 Hz, for 10 s) was delivered continuously to T9 CBDR. A notable increasing spike was found in 81.8% (54/66) A-fibers. Meanwhile, the activity of the rest 18.9% units was unaffected or inhibited. The mean number of discharge of all A-fibers reached at peak during 0~30s and then, declined gradually and retuned to its control level 90s after the antidromical electrical stimulation of T9 CBDR. The spike number of the units increased significantly from the control level of  $4.98 \pm 1.08$  to  $8.24 \pm 1.89$ ,  $7.21 \pm 1.53$  and  $6.33 \pm 1.27$  respectively during  $0 \sim 30$  s,  $30 \sim 60$ s and  $60 \sim 90$  s after the stimulation (Wilcoxon signed rank test, P < 0.01, 0.05). There is a significant change evoked by antidromical electrical stimulation of T9 CBDR before microinjection of WIN51,708 like illustrated in Figure 3.

On the background of exciting discharge after the micro-injection of WIN51,708, an antidromical electrical stimulation of T9 CBDR did not produce a increasing discharge of A-fibers. Only 30.3% (20/66) A-fibers was slightly activated, 24.2% (16/66) was inhibited and the rest 45.5% (30/66) remained unchanged.



Figure 5. The time course of firing frequency changes of mechanoceptive A-fibers in T10 DBCR following the antidromically electrical stimulating of T9 CBDR after the local intradermal microinjection of WIN 51,708, a non-peptide NK-1 receptor antagonist (n = 46 units) into the RFs. The break on the abscissa indicates the onset and duration of antidromically electrical stimulating (stimulus intensity - 1.0 mA, pulse width - 0.2 ms, shock frequency – 20 Hz, tetanus duration - 10 sec). \*\* and \* represent P < 0.01 and P < 0.05, respectively, when compared to the control level.

No more significant increasing discharge was seen in discharge numbers of the units treated with the micro-injection of WIN51,708. The mean values of discharge per 30s were mild higher than the control level. As illustrated in Figure 5B, WIN 51,708 had significantly attenuated the signal transmission between peripheral endings from adjacent spinal segments. Discharge number of most A-fibers was not increased again following a antidromical electrical stimulation of T9 CBDR. The exiting of antidromical electrical stimulation of T9 CBDR on discharge of A-fibers was blocked by the WIN51708 microinjection. But vehicle did not produce the attenuating effect.

3.4. The effect of SP on the signal transmission between peripheral endings from adjacent spinal segments before and after the micro-injection of WIN51,708

When a SP microinjection (0.74micromol/L,  $10\mu$ l, i.c., n =49) was given into the RFs, the discharge of units increased significantly. Similar to our previous report <sup>[26]</sup>, the discharge of primary afferent nerve was significantly activated for 9 minutes and the signal transmission between peripheral endings from adjacent spinal segments was promoted for 120 s by the micro-injection of SP in the receptive field of recorded T10 CBDR. The evoked discharge changes of A-fibers induced by SP microinjection are illustrated in Figure 6.

On the background of increasing discharge induced by SP microinjection, an antidromical electrical stimulation of T9 CBDR was delivered. A facilitating effect of SP was detected on the activating effect of an antidromical electrical stimulation of T9 CBDR. The facilitating effect is shown in Figure 7.

After a pre-treatment of Win51,708 microinjection, SP microinjection was given into the centre of RFs, antidromical electrical stimulation of T9 CBDR did not evoke any significant exciting change of Afibers. The discharge number was found only a transient increase and then gradually recovering to its' control level. The facilitating effect of SP on the signal transmission between peripheral endings from adjacent spinal segments was significantly attenuated by the pre-treatment of NK<sub>1</sub> receptor antagonist WIN51,708. The blocking effect of WIN51,708 on NK1 receptor is shown in Figure 8.



Figure 6. The time course of firing frequency changes of mechanoceptive A-fibers in T10 CBDR following local intradermal microinjection of substance P(n = 46) into the center of the RFs. The zero point of abscissa indicates the onset of microinjection and its duration (5min). \*\* and \* represent P < 0.01 and P < 0.05, respectively, when compared to the spontaneous discharge lever before microinjection.



Figure 7. The time course of firing frequency changes of mechanoceptive A-fibers in T10 CBDR following antidromical electrical stimulation of T9 CBDR after a local intradermal microinjection of substance P (n = 46 units for each group) into the center of the RFs. The black bar indicates the onset of antidromical electrical stimulation of T9 CBDR. \*\* and \* represent P <0.01 and P <0.05, respectively, when compared to the control level of spontaneous discharge.



Figure 8. The blocking effect of WIN51,708 on NK1 receptor. The time course of firing frequency changes of mechanoceptive A-fibers in T10 DBCR following an antidromical stimulation of T9 CBDR after the local intradermal microinjection of WIN 51,708 and substance P, (n=46 units) into the RFs. The facilitating effect of substance P on the exciting action of tetanization of T9 CBDR was blocked. The break on the abscissa indicates the onset and duration of tetanus (stimulus intensity - 1.0 mA, pulse width - 0.2 ms, shock frequency - 20 Hz, tetanus duration - 10 sec). \*\* and \* represent P < 0.01 and P < 0.05, respectively, when compared to its' control level.

### Discussion

The present study showed that WIN51, 708, belongs to the heterosteroid series of selective non-peptide antagonist of NK, receptors, did not markedly affect the spontaneous firing activity of primary afferent A-fibers in anaesthetized rats while the antagonist suppressed apparently the signal transmission between peripheral endings from adjacent spinal segments. The results reveal the involvement of endogenous SP in the process of signal transmission between peripheral endings from adjacent spinal segments and also substantiate our hypothesis about the mechanism of the signal transmission between peripheral endings from adjacent spinal segments without any influence of central nervous system that endogenous SP released from primary afferent endings induced by electrical stimulation, evokes the signal transmission between peripheral endings from adjacent spinal segments mainly through its NK<sub>1</sub> receptor  $[4, 8, 12, 21, 28, 35 \sim 37, 40]$ .

WIN51,708 possesses moderate affinity for the rat NK<sub>1</sub> receptor, interacting competitively with the receptor, and blocks the excitatory action of SP and neurokinin A (NKA)<sup>[29]</sup>. It has shown species-selective interaction with this receptive site, being more potent in rat than in human or guinea pig tissue in binding to and blocking of the tachykinin NK<sub>1</sub> receptor<sup>[2]</sup>. Thus, with regard to the receptor-specificity of the antagonist it is likely that the observed facilitating effect of endogenous or exogenous SP on the signal transmission between peripheral endings from adjacent spinal segments are mediated through NK<sub>1</sub> receptive sites. When NK<sub>1</sub> receptor was blocked by WIN51, 708 micro-injection in same point, an exogenous SP micro-injection failed in inducing the signal transmission between peripheral endings from adjacent spinal segments. This was significantly different from the facilitating effect of exogenous SP or NKA before the treatment of WIN51,708. These inhibitory effects of WIN51,708 were identical with the latest reports<sup>[5]</sup>.

SP has been speculated as a transmitter of mechanical and chemical signal transmission and was proved an excitatory effect on the potential of A-fibers through the mediating of NK, receptor <sup>[20,</sup> <sup>24, 37]</sup>. There exists widely NK<sub>1</sub> receptor in peripheral nervous tissue [9, 16, 19-20, 22-23]. By the studies of immunocytochemical methods with light and electron microscopy, NK, receptor, a receptor preferentially activated by SP, was found in peripheral tissue, not only dorsal root ganglia, nerve endings and primary afferent fibers partially or completely covered by a Schwann cell sheath, but also throughout various cells in the connective tissue (endothelial cells, neutrophils, macrophages) at invaginations of the plasma membrane and in vesicular and granular structures that are probably endosomes and are found close to both the plasma membrane and the nucleus <sup>[16]</sup>. Repetitive, low-frequency group I/II Aβ-fiber stimulation evoked a novel wind-up response after NGF injection. The novel Aβ-fiberevoked wind-up response was reduced by selective NK<sub>1</sub> receptor antagonist RP67580<sup>[11, 31]</sup>.

A $\beta$  sensory neurons appeared to contribute to inflammatory allodynia in the rats and cats. The number of A $\beta$  neurons with spontaneous firing was enhanced significantly and DRG A $\beta$  neurons were less depolarized 2-4 h following carrageenan injection. SP-LI was then detected at A $\beta$  sensory neurons. Repeated application of SP depolarized the membrane potential in most A $\beta$  neurons and did not induce obvious desensitization of A $\beta$  neurons. The SP-induced responses was completely blocked by L668,169, another antagonist of SP receptor, suggesting that peripheral inflammation increased the excitability, SP level and sensitivity of SP receptor of A $\beta$  neurons <sup>[20, 36]</sup>.

A number of details have been given to show the function mechanism of tachykinin NK, receptor, existing widely in A $\beta$ - and A $\delta$ -fibers<sup>[10, 29, 32]</sup>. By using whole-cell patch-clamp methods, NKA and SP caused an inward current in a concentration-dependent manner. These excitatory effects, producing inward currents, were reduced by non-selective peptide antagonist Spantide or selective nonpeptide antagonist CP-99,994 for NK<sub>1</sub> receptor. The inward current produced by tachykinins was associated with decrease in K<sup>+</sup> conductance. They suppressed both a voltage-dependent K<sup>+</sup> current and a voltage-independent background K<sup>+</sup> current. Depletion of intracellular ATP depressed the inward current. These results suggest that the tachykinininduced inward current is mediated through the NK, receptor that mainly couples to pertussis toxininsensitive G-protein in bullfrog primary afferent neurons<sup>[1]</sup>. Superfusion of SP depolarized 42% of tonic neurons and inhibited afterhyperpolarizations in 66% of long afterhyperpolarizing of dorsal root ganglion neurons without significant desensitization. These effects of SP were abolished by the selective NK<sub>1</sub> receptor antagonist, SR140333, suggesting that exogenous SP activated a receptor with NK, pharmacology. All neurons responding to SP had SP immunoreactive fibers within one cell diameter, indicating good spatial matching between SP release sites and target neurons <sup>[13]</sup>. SP depolarizes peripheral nerve endings, also possibly through inositol trisphosphate-gated Ca2+ influx, followed by induction of action potentials in the peripheral axons of primary afferent neurons <sup>[11,]</sup> <sup>19]</sup>. WIN51,708 could antagonize SP effects mainly through blocking NK<sub>1</sub> receptor in this mechanism.

Some evidences were given to reveal that NK<sub>1</sub> tachykinin receptor antagonists produce an inhibitory effect by blocking NK<sub>1</sub> receptor cooperating with other transmitters and their receptors <sup>[13, 21]</sup>. They attenuated the suppressant effect of the  $\alpha_2$ -adrenoceptor agonist clonidine on the firing acti-

vity of serotonin (5-HT) and noradrenaline (NA) neurons. These findings suggest that NK<sub>1</sub> receptor antagonists affect markedly the NA system via an attenuation of the function of  $\alpha_2$ -adrenoceptors on the cell body of NA neurons and, consequently, may also modulate 5-HT neurotransmission [7]. The triple combination of NMDA glutamate receptor antagonist MK-801, the NK<sub>1</sub> tachykinin receptor antagonist GR-205,171 and the NK, tachykinin receptor antagonist SR-144,190 reduced significantly the nucleus tractus solitarii response to intragastric HCl, showing that glutamate acting via NMDA receptors and tachykinins acting via NK1 and NK2 receptors cooperation in the vagal afferent input from the acid-threatened stomach to the nucleus tractus solitarii and participate in the processing of afferent input to the AP in a different and complex manner <sup>[14]</sup>. Intrathecal administration of RP67580 attenuated the flexor reflex evoked in adjuvant-treated animals. Intravenous or intraplantar injection of RP67580 did not affect the flexor reflex in adjuvant-treated animals indicating a spinal action of the drug following intrathecal administration. But these effects were not mediated entirely by its action at NK, receptors <sup>[23]</sup>. NK<sub>1</sub>, NK<sub>2</sub>, and NMDA receptor antagonists also significantly reduced the summated ventral root potential responses evoked by repeated A-fiber stimulation in UV-treated animals. The evoked response and the expression of an NK<sub>1</sub> receptor component were associated with behavioral hyperalgesia to thermal and mechanical stimuli. The enhanced ventral root responses and changes in receptor sensitivity may contribute to the phenomenon of central sensitization and may be directly related to the behavioral hyperalgesia observed <sup>[32]</sup>. The cooperation manner of several transmitters and their receptors can be employed to explain that only part of the discharge of A-fibers involving in the signal transmission was blocked by WIN51,708 in present experimental data, indicating the existence of other mechanism.

The expression of NK<sub>1</sub> receptors was studied in the primary afferent nerves of young rats using immunohistochemical and electrophysiological techniques. Use of a specific immunoserum raised against the C-terminal fragment of rat NK<sub>1</sub> receptor revealed immunoreactivity in 32% of dorsal root ganglion neurons. The diameter of the majority of the NK<sub>1</sub> receptor immunostained neurons was smaller than 30 µm. Double immunohistochemical labeling using NK<sub>1</sub> receptor and SP antibodies revealed that about 1/3 of the NK<sub>1</sub> receptor expressing neuron contains SP. Likewise, about 1/3 of the SP producing DRG cells expressed the NK<sub>1</sub> receptor. Superfusion of SP to an in vitro preparation of the fourth lumbar dorsal root ganglion induced a reversible long-lasting depolarization as measured by extracellular suction electrodes attached to the dorsal roots. This response to SP was only partially antagonized by the selective NK, receptor antagonist RP67580. Intracellular recordings distinguished between A $\alpha$ -/ $\beta$ -, A $\delta$ - and C-sub-types of ganglion neurons. Superfusion of SP evoked excitatory responses in A $\delta$ - and C-type neurons. These results demonstrate the expression of functional NK<sub>1</sub> receptors on a subpopulation of A $\delta$ - and C-type sensory ganglion neurons, suggesting the possible physiological importance of peripheral NK<sub>1</sub> receptors located on dorsal root ganglion neurons and primary afferent fibers<sup>[1, 10]</sup>.

Some morphological data was shown that A-fibers were involved in the composing and activation of meridian<sup>[34, 38]</sup>. In present work, both of nerves stimulated electrically and A-fibers recorded in the experiment distributed along the Bladder Meridian of Foot-Taiyang. The responding latency of A-fibers to electrical stimulation across spinal segment was a few seconds to a minute, similar to the speed of sensory transmission along meridian (1~10 cm/s), a special phenomenon in human body according to the Traditional Chinese Medicine [4, 21, 28~29, <sup>38-40]</sup>. According to the evidence mentioned above, we inferred the signal transmission between endings of primary afferent nerves can be an important neurobiological basis of the sensory transmission along meridian. A-fibers is an important part of the neurobiological basis. Primary afferent A-fibers, A $\delta$ - and A $\beta$ -fibers recorded belong to low- threshold mechanoceptor and nociceptors, were proposed to be the essential morphological proportion involving the process of the meridian action [17, 21, 28, 40].

### Conclusion

Overall, primary afferent A-fibers were involved in the process of the signal transmission between the endings of adjacent spinal segments through the release of endogenous SP induced by the antidromical electric stimulation of adjacent spinal nerve. This effect of endogenous SP could be enhanced by the micro-injection of exogenous SP and was essentially blocked by the microinjection of WIN51, 708, a selective non-peptide antagonist of NK<sub>1</sub> receptors, indicating that SP induces the signal transmission across spinal segments mainly through NK<sub>1</sub> receptor in primary afferent A-fibers. That may also be an important neurobiological base of some endogenous nervous disease and the sensory transmission along meridian in the Traditional Chinese Medicine.

#### References

- 1. Akasu T, Ishimatsu M, Yamada Y. Tachykinin causes inward current through NK<sub>1</sub> receptors in bullfrog sensory neurons, Brain Res, 1996; 713(1-2): 160-167
- 2. Applle KC, Fragale BJ, Losci J, Singh S, Tomczuk BE, Antagonists that demonstrate species differences in neurokinin-1 receptors. Mol Pharmacol. 1992; 41: 772-778
- 3. Bruce L, Carpenter SE. Primary afferent units from the hairy skin of the rat hind limb. Brain Res., 1982; 238: 29-43.
- 4. Cao DY, Niu HZ, Tang XD, Li Q. Dorsal root reflex from A-delta and C afferent fibers induced by electrical stimulation of the sural nerve in rats. Acta Physiologica Sinica. 2003; 55(1): 105-109
- De Araujo JE, Huston JP, Brandao ML. Opposite effects of substance P fragments C (anxiogenic) and N (anxiolytic) injected into dorsal periaqueductal gray. Eur J Pharmacol. 2001; 432(1): 43-51
- 6. Dimitriadou V, Rouleaou A, Trung Tuong MD, et al. Functional relationships between sensory nerve fibers and mast cells of dura mater in normal and inflammatory conditions. Neuroscience, 1997; 77(3): 829-839.
- 7. Haddjeri N, Blier P. Effect of neurokinin-I receptor antagonists on the function of 5-HT and noradrenaline neurons. Neuroreport. 2000; 11(6): 1323-1327
- 8. Holzer P. Local effector functions of capsaicin-sensitive endings: involvement of tachykinins, CGRP and other neuropeptides. Neuroscience, 1988; 24(3): 739-768.
- 9. Hu HZ, Li ZW. Substance P potentiates ATP-activated currents in rat primary sensory neurons. Brain Res. 1996; 739(1-2): 163-168

- 10. Hu HZ, Li ZW, Si JQ. Evidence for the existence of substance P autoreceptor in the membrane of rat dorsal root ganglion neurons, Neuroscience, 1997; 77(2): 535-541
- 11. Inoue M, Tokuyama S, Nakayamada H, Ueda H. In vivo signal transduction of tetrodotoxin-sensitive nociceptive responses by substance P given into the planta of the mouse hind limb. Cell Mol Neurobiol. 1998; (5): 555-561
- 12. Jia J, Zhao Y, Shi WC, Wang HS, Guo Y. Effects of electrical stimulation of the dorsal cutaneous branches of spinal nerves on the discharge activity of remote mechanoreceptive units in rats. Acta Physiologica Sinica, 2002; 54(2): 125-128
- 13. Jobling P, Messenger JP, Gibbins IL. Differential expression of functionally identified and immunohistochemically identified NK(1) receptors on sympathetic neurons. J Neurophysiol. 2001; 85(5): 1888-1898
- 14. Jocic M, Schuligoi R, Schoninkle E, Pabst MA, Holzer P. Cooperation of NMDA and tachykinin NK (1) and NK (2) receptors in the medullary transmission of vagal afferent input from the acid-threatened rat stomach. Pain. 2001; 89(2-3): 147-157
- 15. Kajander KC, Bennet GJ. Onset of a painful peripheral neuropathy in rat: a partial and differential deafferentation and spontaneous discharge in  $A\beta$  and  $A\delta$  primary afferent neurons. J.Neurophysiol, 1992; 68(3): 734-744.
- 16. Kido MA, Yamaza T, Goto T, Tanaka T. Immunocytochemical localization of substance P neurokinin-1 receptors in rat gingival tissue. Cell Tissue Res. 1999; 297(2): 213-222
- 17. Knieestal M, Vallbo AB. Single unit analysis of mechanoreceptor activity from the human glabrous skin. Acta Physiol. Scand, 1970; 80(1): 178-195
- 18. Lam FY, Ferrell WR. Specific neurokinin receptors mediate plasma extravasation in the rat knee joint, Br.J.Pharmacal., 1991; 103: 1263-1267
- 19. Li HS, Zhao ZQ. Small sensory neurons in the rat dorsal root ganglia express functional NK-1 tachykinin receptors, Eur J Neurosci, 1998; 10(4): 1292-1299
- 20. Li XH, Zhao Y, Shi WC, Wang HS. The effect of antidromic electric stimulation on preprotachykinin mRNA expression in adjacent dorsal root ganglions of rats. J Xi'an Med Univ, 1999; 20(1): 97-102
- 21. Liu XCh, Shi J, Li LL, Guan XM, Ouyang XB. The influence of transecting dorsal root of rat on the contents of SP, NE and E in soak liquor of skin innerva-

ted by the transected nerve. Acta Univ Med Tongji, 1997; 26(1): 4-6.

- 22. McCarson KE. Central and peripheral expression of neurokinin-1 and neurokinin-3 receptor and substance P-encoding messenger RNAs: peripheral regulation during formalin-induced inflammation and lack of neurokinin receptor expression in primary afferent sensory neurons, Neuroscience, 1999; 93(1): 361-370
- 23. Parsons AM, Honda CN, Jia YP, Budai D, Xu XJ, et al. Spinal NK<sub>1</sub> receptors contribute to the increased excitability of the nociceptive flexor reflex during persistent peripheral inflammation. Brain Res. 1996; 739(1-2): 263-275
- 24. Renback K, Inoue M, Ueda H. Lysophosphatidic acid-induced, pertussis toxin-sensitive nociception through a substance P release from peripheral nerve endings in mice, Neurosci Lett, 1999; 270(1): 59-61
- 25. Seung Kil Hong, Jeong Seok Han, Sun Seek Min, Jong Moon Hwang, et al. Local neurokinin-1 receptor in the knee joint contributes to the induction, but not maintenance, of arthritic pain in the rat. Neuroscience Letters, 2002; 322: 21-24
- 26. Shi WC, Zhao Y, Zhang BZ. The effect of substance P and histamine in information transmission meridian. J Xi'an Med Univ, 1995, 18(2): 149-151.
- Simone DA, Kajander KC. Responses of cutaneous A-fiber nociceptors to noxious cold. J Neurophysiol, 1997, 77(4): 2049-2060. Cain DM, Khasabov SG, Simone DA. Response properties of mechanoreceptors and nociceptors in mouse glabrous skin: an in vivo study. J Neurophysiol. 2001; 85(4): 1561-1574.
- 28. Sun QX, Zhao Y, Zhang SH, Shi WC, Wang HS. Changes of Mechano-receptive Properties of Aβ-fiber Induced by Antidromical Electrical Stimulation to Cutaneous Nerve from Adjacent Spinal Segment. Acta Physiologica Sinica, 2002; 54(6): 501-507
- 29. Szucs P, Polgar E, Spigelman I, Porszasz R, Nagy I. Neurokinin-1 receptor expression in dorsal root ganglion neurons of young rats. J Peripher Nerv Syst. 1999; 4(3-4): 270-278
- Tao YX, Shu YS, Wang GD, Zhao ZQ. Observation on the effects of SP and CGRP on afferent neurons with C- and Aδ -fibers in dorsal root ganglia of cats. Chin J Neuroanat, 1997; 13(1): 15-18
- 31. Thompson SW, Dray A, McCarson KE, Krause JE, Urban L. Nerve growth factor induces mechanical allodynia associated with novel A fibre-evoked spinal reflex activity and enhanced neurokinin-1 receptor activation in the rat. Pain. 1995; 62(2): 219-231

- 32. Thompson SW, Dray A, Urban L. Injury-induced plasticity of spinal reflex activity: NK<sub>1</sub> neurokinin receptor activation and enhanced A- and C-fiber mediated responses in the rat spinal cord in vitro. J Neurosci. 1994; 14(6): 3672-3687
- 33. Venepalli BR, Aimone LD, Appell KC, Bell MR, Dority JA, etal. Synthesis and substance P receptor binding activity of androstano [3,2-b] pyrimido [1,2-a] benzimidazoles. J Med Chem. 1992; 35: 374-378
- 34. Wang JM. The morphological evidence intermediary effect of mast cell between axon endings of peripheral nerve. J Xi'an Med Univ, 1989; 10(3): 209-211
- 35. White DM, Helme RD. Release of substance P from peripheral nerve terminal following electrical stimulation of sciatic nerve, Brain Res., 1985; 336: 27-31
- 36. Xu GY, Zhao ZQ. Change in excitability and phenotype of substance P and its receptor in cat Abeta sensory neurons following peripheral inflammation. Brain Res. 2001 Dec 27; 923(1-2): 112-9.
- 37. Zanchet EM, Cury Y. Peripheral tackykinin and excitatory amino acid receptors mediate hyperalgesia induced by Phoneutria nigriventer venom. Eur J Pharmacol. 2003; 467(1-3): 111-118
- 38. Zhang BZ. The morphology and function of meridian lines. Xi'an. Science and Technical Press in Shaanxi, 1992: 19-25
- 39. Zhang SH, Zhao Y,Sun QX,Wang HS, Shi WC. The effect of electrical stimulation of the cutaneous nerve of the adjacent spinal segment on afferent discharge of C-mechanoreceptive units in rats. Acupunct Res., 2001; 26(1): 5-9.
- 40. Zhao Y, Shi WC, Wang HS Huang QE, Jia FY. The information conduction between endings of peripheral afferent nerves across spinal segments. J Xi'an Med Univ, 1996; 17(2): 140-142.

Corresponding Author Yan Zhao, Department of Physiology, Medical school of Xi'an Jiaotong University, Xi'an, Shaanxi, The People's Republic of China E-mail: zhaoy502@xjtu.edu.cn

# Factors related to the frequency of citation of the Journal of Orthopaedic & Sports Physical Therapy

Bayram Unver<sup>1</sup>, Fatma U. Kocak<sup>2</sup>, Mehmet Erduran<sup>3</sup>

<sup>2</sup> School of Sports Sciences and Technology, University of Pamukkale, Kinikli, Denizli, Turkey,

<sup>3</sup> Department of Orthopaedics, School of Medicine, University of Dokuz Eylul, Balcova-Izmir, Turkey.

### Abstract

**Objective:** We conducted a review of the highest impact physical therapy journal [Journal of Orthopaedic & Sports Physical Therapy (JOS-PT)] to determine the factors associated with subsequent citations within five years of publication.

**Methods:** We conducted the citation counts for all the original articles published in JOSPT 2006 (12 issues). We used a logistic regression analysis to identify the factors associated with the citation counts.

**Results:** We identified 76 original articles in the JOSPT. There were 878 subsequent citations within five years of publication of these articles. The type of the article and the participant, the level of evidence and the number of the authors were the variables associated with the subsequent citation rate. The research reports, the topical reviews, the studies made with the asymptomatic adults, the studies not including any participants, the level A studies, the multi-centered articles and those with more authors articles are citated more.

**Conclusions:** We found significant correlations between the citation rates and the type of the article and the participant, the level of evidence, and the number of the authors. We consider that this information may help the readers of the JOS-PT, the authors, the reviewers, the librarians and the promotion committees to plan their studies, and also to analyze and evaluate the articles.

**Key words:** Citation analysis, citation, bibliometrics, sports, orthopedics, physical therapy (specialty).

#### Introduction

The article that has been referenced by another peer-reviewed article receives what is known as a

citation [1]. An important part of the research process is the publication in the peer-reviewed journals. An equally important outcome, however, is the use and the citation of these published articles by other researchers and authors [2]. One way to measure the academic importance of a journal, or the articles within it, is the rate at which the work is quoted or referenced by the other authors [3]. Citation analyses within specific journals and specific subject areas have become a popular method of assessing the citation impact of a journal, an article, or an author [1-4]. The citation and the other academic impact information have been collected by and available from the Institute for Scientific Information (ISI; Philadelphia, PA, USA) since 1945, and electronically since 1979 [1-4]. In 1955, the impact factor (IF) was proposed by Eugene Garfield as a simple method to calculate the relative frequencies of the citations between the journals. Subsequently, the IF was used to select the journals for the Science Citation Index (SCI), a commercial property of the ISI and founded by Garfield in 1961 [4].

The citation analyses were performed to assist the librarians, authors, practitioners, and others in identifying the important journals for acquisition, publication, and reference. Citation analysis is also widely used for impact assessment of the individual scientists, clinicians, institutions, and entire nations for the determination of awards, rankings, and even promotion and tenure decisions [2,5-8]. Moreover, to increase the visibility of their research, the researchers want to have their work published in highimpact journals. Publishing manuscripts with high citation potential is also of interest to scientific journals, as doing this can improve the journal's credibility, relevance, and financial independence. In this regard, it seems to be very important to identify the manuscript characteristics associated with a higher

<sup>&</sup>lt;sup>1</sup> Department of Orthopedic Physiotherapy, School of Physical Therapy and Rehabilitation, University of Dokuz Eylul, Izmir, Turkey,

number of citations, as well as more views from the journal readers [9].

The Journal of Orthopaedic & Sports Physical Therapy Journal (JOSPT) strives to be the premier journal for orthopaedic and sports physical therapy research, and is the official journal of the Orthopaedic and Sports Physical Therapy Sections of the American Physical Therapy Association (APTA) [10-12]. It has been published since 1979 [10-12], and it is one of the most widely circulated (The Journal reaches more than 32,000 individuals and institutions in the United States and 55 countries around the world. The Journal's website, www. jospt.org, receives more than 47,000 unique visitors each month and the readers rank JOSPT first in usefulness among 12 leading journals) [11], and is currently the most frequently cited clinical specialty physical therapy journal [10,11,13,14], with a reported impact factor of 3.000 [11]. JOSPT is the top specialty physical therapy journal [10,14], and listed in the rehabilitation category in SCI journal lists [11,13,15,16].

The studies investigated the citation analysis and the factors affecting the citation rates have been conducted in various fields of medicine [1-7,9,17-44]. There is also citation studies related to the physical therapy field in the literature [8,10,13,14,45]. However, the factors affecting the citation rates have not been investigated in the top specialty physical therapy journal (JOSPT). Therefore, we undertook a study to determine what factors were associated with an increased rate of citation using a cohort of articles published in 2006 in JOSPT.

### Methods

We included all original scientific articles published in the JOSPT in 2006. All research or review articles were considered for the analysis, whereas the studies of the editorials, the letters to the editor, the report of the expert committee, and the congresses proceedings were excluded. From each eligible article, we evaluated the articles using a standardized evaluation form the included variables that have been previously reported to predict the rates of citations in similar studies [2,3,6,8,9,17,18,20-28,31-35,37-39,41,43,44,46-48]. These were:

1) the month of the publication (January to June, July and December); 2) Type of the article (research report, topical review and case report); 3) level of evidence (level A: consistent level 1 studies, Level B: consistent level 2 or 3 the studies or extrapolations from level 1 studies, Level C: level 4 the studies or extrapolations from level 2 or 3 studies, Level D: level 5 evidence or troublingly inconsistent or inconclusive studies of any level); 4) geographical location of the study in which the research was performed (defined as the country or countries in which the research participants were recruited or, for research which did not use research participants, e.g., systematic review, the country of the corresponding author); 5) sample size of the study (None: no participants included in the study, 1-25, 26-99,  $\geq$  100); 6) Type of the participant (Symptomatic adults: Includes or refers to humans aged 18 and older with a current symptomatic clinical condition, Asymptomatic adults: Includes or refers to normal or asymptomatic human adults, Combined adults; Includes both a clinical and healthy adult cohort, Tissue: Includes or refers to removed tissue or fluid, None: no participants included in study; 7) Number of authors (1-3, 4-6, >6); 8) Number of institutions (1, >1); 9) Number of pages (1-10, >10); 10) First citation time (the number of months from the date of publication to the first citation); 11) Language of the first author (from English speaking countries, from non- English speaking countries); 12) Number of references (1-46, >47); and 13) the length of the title; title word counts (1-13, >14).

### Citation counts

Using the first author's name, both of us (BU, FUK) queried the ISI Web of Science database (http: //isiknowledge.com) to ascertain, as of December 31, 2011, the number of the subsequent citations for each article after publication. If entering the first author's name failed to yield any citations for an article, we searched for the second and last author to limit misclassification of an article as having zero subsequent citations. We chose a 5-year period after publication (2006–2011) to assess citations, on the basis of previous reports (see Okike K, et al) [39].

Two independent reviewers performed the data extraction and compared their results. There was good agreement between the reviewers for their results (ICC values were between 0.98 and 1.00).

### Data analysis

We analysed categorical variables using proportions and continuous variables using the median and interquartile range (IQR). We further performed Mann-Whitney U test, kruskal-wallis test, and binary logistic regression analysis to assess for associations between predictor and outcome variable. We included variables in the logistic regression analysis if their level of significance was p <0.05. *p*-values less than 0.05 were accepted as significant.

### Results

We identified 76 original articles in JOSPT published in 2006. The characteristics of the studies are presented in Table 1.

Tuble 1 C al Cl

Table 1. Sumple Characteristics						
Characteristics	N (%)					
The month of the publication						
January to June	33 (43.4)					
July to December	43 (56.6)					
Type of the article						
Research report	45 (59.2)					
Topical review	16 (21.1)					
Case report	15 (19.7)					
Level of evidence						
Level A	10 (13.2)					
Level B	30 (39.5)					
Level C	7 (9.2)					
Level D	29 (38.2)					
Geographical location of the study						
North America	58 (76.3)					
Other	18 (23.7)					
Sample size of the study						
None	17 (22.4)					

1-25	36 (47.4)				
26-100	16(21.0)				
>100	7 (9.2)				
Type of the participants					
Symptomatic adults	29 (38.2)				
Asymptomatic adults	14 (18.4)				
Combined adults	12 (15.8)				
Tissue	4 (5.3)				
None	17 (22.4)				
Number of the authors					
1-3	33 (43.4)				
4-6	40 (52.6)				
>6	3 (3.9)				
Number of the institutions					
1	14 (18.4)				
>1	62 (81.6)				
Number of the pages					
1-10	51 (67.1)				
>10	25 (32.9)				
First citation time					
First year	22 (28.9)				
Second year	34 (44.7)				
Third year and after	16 (21.1)				
Not cited	4 (5.3)				
Language of the first author					
English speaking countries	66 (86.8)				
Non- English speaking countries	10 (13.2)				
Number of the references					
1-46	42 (55.3)				
>47	34 (44.7)				
The length of the title					
1-13 words	36 (47.4)				
>14 words	40 (52.6)				

Table 2. Results of binary logistic regression analysis

	Sig. Exp(B)	95,0% C.I.for EXP(B)					
		Ехр(Б)	Lower	Upper			
Level of evidence	,004	,151	,042	,538			
Type of the participants	,006	2,077	1,231	3,502			
Type of the article	,004	10,800	2,105	55,412			
Number of the authors	,018	2,712	1,186	6,202			
Table 3.	Median	citation	rates	by	study	charac	eteristics
----------	--------	----------	-------	----	-------	--------	------------
				~	~		

Variables	Median citation (IQR)	P value
The month of the publication		0.183
January to June	10 (4-18.5)	
July to December	9 (2-14)	
Type of the article		0.023
Research report	9 (4-20)	
Topical review	13 (8-14)	
Case report	2 (0-10)	
Level of evidence		0.069
Level A	19 (7-25)	
Level B	9,5 (4-18)	
Level C	10 (4-16)	
Level D	6 (2-13)	
Geographical location of the study		0.696
America	10(4-16)	
Europe	5 (2,5-12,5)	
Australia/New-Zealand	14 (4-23)	
Asia	7 (5-9)	
Sample size of the study		0.369
None	13 (9.5-15)	
1-25	4.5 (2-14)	
26-100	11 (4-19)	
>100	9 (5-28)	
Type of the participants		0.005
Symptomatic adults	5.5 (2-15)	
Asymptomatic adults	14 (6.5-21.5)	
Combined adults	9 (4-21)	
Tissue	3 (1-4)	
None	13 (9.5-15)	
Number of the authors		0.011
1-3	5 (2-13)	
4-6	12 (5.5-19)	
>6	14 (10-20)	
Number of the institutions		0.049
1	4 (2-10)	
>1	11(4-16)	
Number of the pages		0.199
1-10	8 (3-14)	
>10	13 (4.5-16)	
First citation time		0.000
First year	13 (7-20)	
Second year	13 (6-18)	
Third year and after	2 (1.5-4)	
Language of the first author		0.465
English speaking countries	10(4-16)	
Non- English speaking countries	7 (5-12)	
Number of references		0.100
1-46	6 (3-14)	0.130
>47	11.5 (6-16)	0.670
The length of the title	0.00014.0	0.658
1-13 Words	9 (3.5-14.5)	
>14 words	10 (4-17)	

*IQR: Interquartile range* 

#### Subsequent citations

We identified 878 citations of the 76 original articles. The first citation time after the publication ranged from 1 to 49 months (mean 17.2). The number of the citations after the publication ranged from 0 to 53 (mean 11.5); of these, 4 articles (5.3%) had received no citations up to December 31, 2011.

The result of the logistic regression, the type of the article, the type of the participant, the level of evidence, and the number of the authors were the variables associated with subsequent citation rate (Table 2).

The research report and the topical review articles have been citated more in a statistically meaningful level compared to the case report studies (p=0,023). There was not a statistically meaningful difference between the number of the citations of the research report and the topical review articles. There was not a meaningful difference between the numbers of the citations of the studies made with the asymptomatic adults, and the studies not including any participants; however, the numbers of the citations of the studies made with the asymptomatic adults and the studies not including any participants were meaningfully more than the numbers of the citations made to the other studies (Symptomatic adults, combined adults and tissue) (p=0,005). The studies with more number of authors (> 3 authors) have received statistically more citations compared to the studies with fewer number of authors (< 3authors), (p=0.011). The numbers of the citations made to the multi-centered studies were more in a statistically meaningful level than the numbers of the citations made to the studies performed with a single center (p=0,049). There is a meaningful difference between the numbers of the citations made to the studies whose time slice when it was first citated is different (p=0,000). The studies which received their first citation in the 1st and 2nd year after they were published have received statistically more citations in a meaningful level compared to the articles that were citated in the 3<sup>rd</sup> year and later (p=0,000). There was not a statistically meaningful difference between the numbers of the citations of the studies which were citated in the 1<sup>st</sup> and 2<sup>nd</sup> year after they were published, (Table 3).

The studies which were citated most were the level A studies, and the studies which were citated least were the level D studies (median 19 and 6 respectively). The articles that received the first citation in the first year and the articles that were citated first in the second year were citated more than the articles citated first in the third year and later (median 13, 13 and 2 respectively). The topical reviews and the research report articles were the most received citations (median 13 and 9 respectively), whereas the case report articles were the least (median 2). Whereas the asymptomatic adults and the studies not including any participants were citated most (median 14 and 13 respectively), the symptomatic adults and the tissue articles were those that were citated least (median 5.5 and 3 respectively). The articles with more numbers of authors were citated more than the articles with fewer numbers of authors (median 14 and 5 respectively). The articles with more numbers of institutions were citated more than the articles with fewer numbers of institutions (median 11 and 4, respectively). There was no significant difference between the citation rates in terms of the level of evidence, the language of the first author, the geographical location, the number of the pages, the sample size, the number of the references, the length of the title and the month of publication (Table 3).

#### Discussion

The prestige and standing of a scientific journal within its discipline can be judged in a number of ways. However, the first and the foremost effective peer-review of all submitted manuscripts is the paramount to guarantee the quality and validity of the work eventually published [29]. Second, the bibliometric methods (such as the journal citation rates, the impact factors, the circulation, the manuscript acceptance rate, and indexing on MEDLINE) may be useful in evaluating the quality of a journal [33]. Therefore, we investigated the parameters affecting the citation rate in peer-reviewed JOSPT indexed in SCI which is one of the most cited journals in physical therapy field with the highest impact factor, and the most circulation rate. The results of this study show which factors were associated with an increased rate of citation in JOSPT. According to our results, the type of the article, the type of the participant, the level of the evidence, and the number of the authors were the variables associated with the subsequent citation rate.

Evidence-based medicine continues to guide clinical decision making based on the best available evidence in the literature [15]. In the hierarchy of the research study designs, the prospective randomized controlled trials (RCTs) and the metaanalysis of several RCTs are considered to provide the highest quality of evidence [15]. Recently, there has been a progressive effort by the researchers and the journal editors to assess and improve the quality of the published studies [15]. One obvious factor that affects the citations of an article is the level of evidence [26]. The studies evaluating the influence of the level of evidence of the study on the citation rates have reported conflicting results [2,3,6,8,18,33-35,38,39,44,48]. Some studies found that the articles of higher level of evidence with clearly documented research methods did receive more citations [18,33-35,38,39,44]. Other studies found that the level of evidence or the quality of the studies has been shown to be very poor or moderately related to citation counts [2,3,6,48]. Also, Shadgan et al, when they analyzed the topcited articles in rehabilitation, reported that there was no correlation between the number of citations and the level of evidence [8]. We did find a correlation between the level of evidence and the number of citations (Table 2). The studies which were citated most were the level A studies, and the studies which were citated least were the level D studies (median 19 and 6 respectively). Hence, increasing the level A studies and decreasing the level D studies (4 of which have not received any citations in a 5-year period) might affect the number of citations positively. Review articles (such as; meta-analysis, systematic reviews) are all recognized as having a higher average rate of citations than the original research papers [7,18,22,24,32,43]. Our results also supported the idea that the systematic reviews and the topical reviews were the most citated articles (Table 3). The review articles often have a greater impact on the physical therapists than the other types of articles. Physical therapists can reduce the time required to find and read the evidence by reading systematic reviews instead of individual trials. A systematic review summarizes the results of multiple studies that address a particular question. Unlike narrative literature reviews, however, the systematic reviews use explicit methods designed to minimize bias. The systematic reviews also frequently use a statistical method called meta-analysis to combine the results of multiple similar studies to give a single summary result [49]. Also, authors of such reviews, naturally, may prefer to submit their reviews to journals with large circulation and impact [37]. In addition to this, editors may have a tendency to publish systematic reviews and RCTs because they are cited more often than the other study designs [33], thereby positively influencing their own journal's IF. Accordingly, increasing the number of meta-analysis and systematic reviews could affect the number of citations positively.

The textbooks of the medical statistics require that the sample size should be large enough (or as large as possible), and that some justification for the size chosen should be given [38]. It has been claimed that the researchers prefer to cite large studies rather than small ones [2,6,39,44]. Our data did not support this hypothesis: the sample size was not associated with the frequency of the citations (Table 3). Nieminen et al. came to the same conclusion when they analyzed a set of psychiatric articles [38]. Although larger sample size does not necessarily indicate better research, it may serve as a surrogate for the sample size sufficiency (i.e., power of the study), which could be considered a quality measure [39].

The studies made with asymptomatic adults and those without any participants have been citated most, and the studies made with symptomatic adults, combined adults and the tissue studies have been citated the least (Table 3). Due to the fact that the studies made with asymptomatic adults rather contain metric (reliability and validity studies) and diagnostic studies, they might have attracted the attention of the readers more. Nevertheless, the studies that do not contain any participants comprise of review articles, hence they are citated more. Therefore, the authors should be encouraged in terms of presenting metric, diagnostic and review articles.

Authorship on scientific publications has become the issue on modern science and a measure of a scientist's participation in the international scientific community [19]. In a competitive environment where appointments, promotions, and grant applications are strongly influenced by the publication and citation records, the scientists are under intense pressure to publish. Undoubtedly,

academic careers are to a great extent related to the number of publications and citations [19]. The studies evaluating the relation between the number of the authors and the citation rates have reported conflicting results [6,20,22,24,26,31,32,34,35,39, 44,47]. A number of studies found that multi-authorship increases, above all, the probability to be cited by others [6,20,22,24,26,31,32,34,35]. The other studies did not find a statistically significant correlation between the number of the authors and the citation counts [39,44,47]. In our study, we did find a significant relationship between the citation rate and the number of the authors (Tables 2 and 3). In theory, the more authors a paper have the higher number of citations of this paper that can be expected. Bornmann et al. suggested four reasons for this association; first, each additional author increases the probability of self-citations, second, the papers with many authors are most probably multidisciplinary papers, so citations in various disciplines can be expected, third; the more authors a paper has, the larger the network in which the paper will become known through personal contacts, and fourth, not only informal, but also formal communication in the scientific community can contribute to the greater visibility (and thus to a higher citation count) of a multi-authorship paper [47]. The articles with many authors are most probably multidisciplinary articles, so citations in various disciplines can be expected [20]. Figg et al [20], Lokker et al [34], Okike et al [39], and Willis et al [44] reported that the number of times an article was cited correlated significantly with the number of institutions. In contrast, Loonen et al [36] did not find any correlation between the citation counts and the number of the institutions. Contrary to Loonen et al [36], but similar to Figg et al [20], Lokker et al [34], Okike et al [39]. and Willis et al [44], we did find a significant relationship between the citation rate and the number of institutions (Table 3). Okike et al [39] suggest that the authors who are open to collaboration with investigators from other institutions may be able to produce articles that have a higher impact on the field. We agree with the suggestions of Okike et al; if the researchers who will send articles to JOSPT perform multidisciplinary studies and those carried out with various centers, their studies might be citated more.

It is not possible to publish new material whatever is its quality without demonstrating a minimal overlap with the status quo by including relevant references to reach this aim [47]. Therefore, references are essential components of the published articles [16]. The articles with a long reference list may have several attributes that promote the citation impact [26]. As Webster et al [43], Bornmann et al [47], Lokker et al [34], Haslam and Koval [26] showed, there was a positive correlation between the citation counts and the number of the cited references: the more cited references a paper contains, the higher the citation counts a paper will be expected to have. Webster et al [43] concluded that one of the reasons for this connection was that "the tit-for-tat nature of 'I cite you, you cite me," may be at work (/may be at issue): the more people you cite in your paper, the more people are likely to cite your paper (the paper they were cited in) in the future. We did not find a significant relationship between the number of references and the citation rates in our study (Table 3). Another possible predictor of article citation impact is the length. Long articles have been found to receive more citations [19,22,26,41,46], because they have more opportunities than the short articles to develop arguments and present replicated and integrated findings [26]. In contrast, Lokker et al compared the citation counts of the journal articles and the Cochrane reviews and Health Technology Assessment reports which are typically lengthy articles. They found that there is a negative correlation between the citation counts and the number of pages [34]. Also, Haslam examined the publications from three major psychology journals for the number of citations and the length of the article. He found that although longer articles received more citations on average, the shorter articles received more citations per page than the longer articles [27]. In our study, we did not find a relationship between the citation rates and the number of the pages (Table 3).

The titles hold a special place of prominence in the scientific literature. They hold a place on top of every article; they are searchable by every major indexing service. The titles inform the readers exactly what information will be presented in the paper. The titles attract the readers to a paper and weigh strongly in the computer-based literature searches and information retrieval [50]. It is generally

the first information obtained from the published article [9]. Perneger reported that the readers judge the scientific value of an article from the title and the abstract, and if this assessment is favorable, they access the full paper and then use it for their study [41]. Jacques and Sebire investigated the effect of the length of the title on the citation rate in three medical journals (Lancet, BMJ and Journal of Clinical Pathology) [28]. They found that the number of citations was positively correlated with the length of the title, with the highest-scoring articles having more than twice as many words in the title than the lowest-cited articles. Also, Habibzadeh and Yadollahie showed that the articles with longer titles obtained more citations. They think that the longer titles are mainly those which include the study methodology and/or the results in more details and thus, attract more attention and citations [25]. In contrast, Paiva et al found that the short-titled articles had higher viewing and citation rates than those with the longer titles [9]. In our study, we did not find a relationship between the citation rate and the length of the title (Table 3).

The scientific quality of a publication can be determined not only based on the number of the citations, but also based on the citation speed [46]. Whereas the citation count is a bibliometric standard indicator in the assessment of the research, the amount of time up to the first citation is an indicator which has been scarcely used in the bibliometric studies. The time at which an article receives its first citation (t1) is important for an article since at this time, the article shifts its status from the 'unused' to the 'used', and the smaller t1 is, the more we can say – in general – that the article under study is important and visible early in the scientific world [46]. Bornmann et al reported that there was a correlation between the first citation time and the citation rate [46]. Our results also supported this idea. The articles that received the first citation in the first year and in the second year were citated more than the articles citated first in the third year or later (Table 3). Another important challenge in examining the citations is the effect of time. The calendar time can affect the citation of an article in two important ways. First, the articles published in the first month or in the first issue of the year compared to the last month or the last issue of the year become advantageous almost with an extra year to come to the attention of the authors, and being also citated in that extra year, the citation counts or the impact factors of the journal are affected [4,21]. Second, there is a latency period between the decision to cite an article and the publication of the citing article. This latency period can be highly variable, depending on the number of times the article is submitted, different review times, and the duration of the 'in press' period [21]. We did not find a significant relationship between the citation rate and the calendar time (Table 3).

In some studies, it was indicated that the articles which were citated more originated from North America [5,8,21,26,30,32,40]. This can be explained by the large girth of the American scientific community, their higher research budgets [5,8] and the fact that the American authors tend to cite the American articles preferably, and tend to publish their works in the American journals [17]. The latter might be related to a preference of the American reviewers to accept the American articles [8,30,40]. Unlike these findings, Willis et al [44] reported that there is no correlation between the citation rate and the continent of origin. In our study, we found that the 76.3 % of the articles published in JOSPT originated from North America whereas 23.7 % from other countries (Europe, Asia and Australia -New Zealand). In our study, similar to the findings of Willis et al [44] we did not find a relationship between the citation rate and the income level of the corresponding author's country (Table 3). The reason for this might be the fact that a great majority of the articles published at JOSPT are published by the countries of North America (USA, Canada).

The fact that English is the lingua franca of today's science is an indisputable fact. Publication in English in the international journals is a prerequest for a research paper to gain visibility in academia [42]. An article written by the authors from the countries where English is a national language attract significantly more citations than do the articles written by the authors from non-native English speaking countries [23,26,32]. It is an indisputable fact that poor writing skills may be considered the Achilles's heel of many non-native English-speaking scientists [42]. Also, eloquence and English language fluency may also improve the chances of the research being ranked more

highly by the reviewers and the editors [17]. In the JOSPT, the majority of the papers came from English-speaking countries (86.8 %), with Netherlands, Swiss, Spain, Finland, Taiwan, Japan, Hungary, and Sweden being the only other representatives. In our study we did not find a relationship between the citation rate and the language of the corresponding author (Table 3). The reason for this might be the fact that a great majority of the articles published at JOSPT are published by the English-speaking countries.\_

There are several implications for preparing articles for JOSPT, based on the findings from this study. The studies which were citated more were the level A articles, the review articles and the research articles. If the researchers want their studies to be read more and citated more, they should prepare well-designed and high quality research articles and review articles. Case reports are the least citated studies (4 of these were never citated in a 5-year period), therefore, decreasing the number of these might affect the number of the citations positively. The studies made with the asymptomatic adults and the studies not including participants are citated the most. However, the studies of the symptomatic adults, the combined adults and the tissues have been citated the least. Due to the fact that the studies made with asymptomatic adults rather contain metric (reliability, validity studies) and diagnostic studies, they might have attracted the attention of the readers more. Consequently, the authors should incline towards metric and diagnostic studies more. The articles with many authors and the multi-centered ones are citated more. If the physiotherapists carry out their studies related to orthopedics and sports injuries with different professional groups (such as doctors, dieticians, nurses, psychologists, coaches, athletics trainers) and different centers in a multidisciplinary way, their studies can be citated more.

#### Limitations

There are limitations to consider when interpreting the results of this bibliometric study. First, we did not assess self-citation, which has been associated with an increased frequency of subsequent citation [3,4,31]. Second, we evaluated only the level of evidence of the study and the quality assessment (such as the clear reporting of the research question, presence or absence of controlling, blinding, prospectiveness and appropriateness of data analysis) of the articles was not performed.

#### Conclusion

In our study in which we investigated the factors affecting the citation rates in JOSPT, we found significant correlations between the citation rates and the type of the article, the type of the participant, the level of evidence, and the number of the authors. We consider that this information could be used by the readers of JOSPT, the authors, the reviewers, the librarians and the promotion committees to plan the studies, and also to analyze and evaluate the articles. Furthermore, JOSPT editors may consider citation potential when deciding which manuscript to accept in order to maintain or increase the overall impact of their journal.

#### Acknowledgements

We would like to thank Assistant Professor Dr. Sinan Aytekin from Balıkesir University for the support he has given to the statistics in this study.

#### References

- Lefaivre KA, Guy P, O'Brien PJ, Blachut PA, Shadgan B, Broekhuyse HM. Leading 20 at 20: top cited articles and authors in the Journal of Orthopaedic Trauma, 1987-2007. J Orthop Trauma. 2010; 24(1): 53-8.
- 2. Callaham M, Wears RL, Weber E. Journal prestige, publication bias, and other characteristics associated with citation of published studies in peer-reviewed journals. JAMA. 2002; 287(21): 2847-50.
- 3. Mehlman CT, Wenger DR. The top 25 at 25: citation classics in the Journal of Pediatric Orthopaedics. J Pediatr Orthop. 2006; 26(5): 691-4.
- 4. Mavrogenis AF, Ruggieri P, Papagelopoulos PJ. Selfcitation in publishing. Clin Orthop Relat Res. 2010; 468(10): 2803-7.
- 5. Baltussen A, Kindler CH. Citation classics in anesthetic journals. Anesth Analg. 2004; 98(2): 443-51
- 6. Kulkarni AV, Busse JW, Shams I. Characteristics associated with citation rate of the medical literature. PLoS One. 2007; 2(5): e403.

- 7. Kurmis AP. Understanding the limitations of the journal impact factor. J Bone Joint Surg Am. 2003; 85(12): 2449-54.
- 8. Shadgan B, Roig M, Hajghanbari B, Reid WD. Topcited articles in rehabilitation. Arch Phys Med Rehabil. 2010; 91(5): 806-15.
- 9. Paiva CE, Lima JP, Paiva BS. Articles with short titles describing the results are cited more often. Clinics (Sao Paulo). 2012; 67(5): 509-13.
- Coronado RA, Wurtzel WA, Simon CB, Riddle DL, George SZ. Content and bibliometric analysis of articles published in the Journal of Orthopaedic & Sports Physical Therapy. J Orthop Sports Phys Ther. 2011; 41(12): 920-31.
- 11. http://jospt.org
- 12. Simoneau GG. Thirty years of publishing and progress. J Orthop Sports Phys Ther. 2009; 39(1): 1-3.
- 13. Costa LO, Moseley AM, Sherrington C, Maher CG, Herbert RD, Elkins MR. Core journals that publish clinical trials of physical therapy interventions. Phys Ther. 2010; 90(11): 1631-40.
- 14. Fell DW, Burnham JF, Buchanan MJ, Horchen HA, Scherr JA. Mapping the core journals of the physical therapy literature. J Med Libr Assoc. 2011; 99(3): 202-7.
- 15. Kocak FU, Unver B, Karatosun V. Level of evidence in four selected rehabilitation journals. Arch Phys Med Rehabil. 2011; 92(2): 299-303.
- 16. Unver B, Senduran M, Unver Kocak F, Gunal I, Karatosun V. Reference accuracy in four rehabilitation journals. Clin Rehabil. 2009; 23(8): 741-5.
- 17. Akre O, Barone-Adesi F, Pettersson A, Pearce N, Merletti F, Richiardi L. Differences in citation rates by country of origin for papers published in top-ranked medical journals: do they reflect inequalities in access to publication? J Epidemiol Community Health. 2011; 65(2): 119-23.
- 18. Bhandari M, Busse J, Devereaux PJ, et al. Factors associated with citation rates in the orthopedic literature. Can J Surg. 2007; 50(2): 119-23.
- 19. Bornmann L, Daniel H. Multiple Publication on a single research study: does it pay? The influence of number of research articles on total citation counts in biomedicine. Journal of The American Society for Information Science and Technology 2007; 58(8): 1100-7.

- 20. Figg WD, Dunn L, Liewehr DJ, et al. Scientific collaboration results in higher citation rates of published articles. Pharmacotherapy. 2006; 26(6): 759-767.
- 21. Filion KB, Pless IB. Factors related to the frequency of citation of epidemiologic publications. Epidemiol Perspect Innov. 2008; 5: 3.
- 22. Frosch DL, Saxbe D, Tomiyama AJ, et al. Assessing the scholarly impact of health psychology: a citation analysis of articles published from 1993 to 2003. Health Psychol. 2010; 29(5): 555-62.
- 23. Fung IC. Open access for the non-English-speaking world: overcoming the language barrier. Emerg Themes Epidemiol. 2008; 5: 1.
- 24. Gargouri Y, Hajjem C, Larivière V, et al. Self-selected or mandated, open access increases citation impact for higher quality research. PLoS One. 2010; 5(10): e13636.
- 25. Habibzadeh F, Yadollahie M. Are shorter article titles more attractive for citations? Cross-sectional study of 22 scientific journals. Croat Med J. 2010; 51(2): 165-70.
- 26. Haslam N, Koval P. Predicting long-term citation impact of articles in social and personality psychology. Psychol Rep. 2010; 106(3): 891-900.
- 27. Haslam N. Bite-Size Science: Relative Impact of Short Article Formats. Perspectives on Psychological Science. 2010; 5(3): 263-264.
- 28. Jacques TS, Sebire NJ. The impact of article titles on citation hits: an analysis of general and specialist medical journals. JRSM Short Rep. 2010; 1(1): 2.
- 29. Jones AW. Which articles and which topics in the forensic sciences are most highly cited? Sci Justice. 2005; 45(4): 175-182.
- 30. Kelly JC, Glynn RW, O'Briain DE, Felle P, McCabe JP. The 100 classic papers of orthopaedic surgery: a bibliometric analysis. J Bone Joint Surg Br. 2010; 92(10): 1338-43.
- 31. Kulkarni AV, Aziz B, Shams I, Busse JW. Comparisons of citations in Web of Science, Scopus, and Google Scholar for articles published in general medical journals. JAMA. 2009; 302(10): 1092-6.
- 32. Lansingh VC, Carter MJ. Does open access in ophthalmology affect how articles are subsequently cited in research? Ophthalmology. 2009; 116(8): 1425-31.
- *33. Lee KP, Scotland M, Bacchetti P, Bero LA. Association of journal quality indicators with methodological*

*quality of clinical research articles. JAMA. 2002;* 287(21): 2805-8.

- 34. Lokker C, McKibbon KA, McKinlay RJ, Wilczynski NL, Haynes RB. Prediction of citation counts for clinical articles at two years using data available within three weeks of publication: retrospective cohort study. BMJ. 2008; 336(7645): 655-7.
- 35. Lokker C, Haynes RB, Chu R, McKibbon KA, Wilczynski NL, Walter SD. How well are journal and clinical article characteristics associated with the journal impact factor? a retrospective cohort study. J Med Libr Assoc. 2012; 100(1): 28-33.
- Loonen MP, Hage JJ, Kon M. Plastic Surgery Classics: characteristics of 50 top-cited articles in four Plastic Surgery Journals since 1946. Plast Reconstr Surg. 2008; 121(5): 320e-327e.
- 37. Montori VM, Wilczynski NL, Morgan D, Haynes RB; Hedges Team. Systematic reviews: a cross-sectional study of location and citation counts. BMC Med. 2003; 1: 2.
- 38. Nieminen P, Carpenter J, Rucker G, Schumacher M. The relationship between quality of research and citation frequency. BMC Med Res Methodol. 2006; 6: 42.
- 39. Okike K, Kocher MS, Torpey JL, Nwachukwu BU, Mehlman CT, Bhandari M. Level of evidence and conflict of interest disclosure associated with higher citation rates in orthopedics. J Clin Epidemiol. 2011; 64(3): 331-8.
- 40. Paladugu R, Schein M, Gardezi S, Wise L. One hundred citation classics in general surgical journals. World J Surg. 2002; 26(9): 1099-105.
- 41. Perneger TV. Relation between online "hit counts" and subsequent citations: prospective study of research papers in the BMJ. BMJ. 2004; 329(7645): 546-7.
- 42. Vasconcelos SM, Sorenson MM, Leta J. Scientistfriendly policies for non-native English-speaking authors: timely and welcome. Braz J Med Biol Res. 2007; 40(6): 743-7.
- 43. Webster GD, Jonason PK, Schember TO. Hot Topics and Popular Papers in Evolutionary Psychology: Analyses of Title Words and Citation Counts in Evolution and Human Behavior, 1979 – 2008. Evolutionary Psychology. 2009. 7(3): 348-362.
- 44. Willis DL, Bahler CD, Neuberger MM, Dahm P. Predictors of citations in the urological literature. BJU Int. 2011; 107(12): 1876-80.

- 45. Bohannon RW. Core Journals of Physiotherapy. Physiotherapy. 1999; 85: 317-21.
- 46. Bornmann L, Daniel H. Citation speed as a measure to predict the attention an article receives: An investigation of the validity of editorial decisions at Angewandte Chemie International Edition. Journal of Informetrics. 2010; 4(1): 83-8.
- 47. Bornmann L, Schier H, Marx W, Daniel H. What factors determine citation counts of publications in chemistry besides their quality? Journal of Informetrics. 2012; 6(1): 11-18.
- 48. West R, McIlwaine A. What do citation counts count for in the field of addiction? An empirical evaluation of citation counts and their link with peer ratings of quality. Addiction. 2002; 97(5): 501-4.
- 49. Elkins MR, Herbert RD, Moseley AM, Sherrington C, Maher C. Rating the quality of trials in systematic reviews of physical therapy interventions. Cardiopulm Phys Ther J. 2010; 21(3): 20-6.
- 50. Hays JC. Eight recommendations for writing titles of scientific manuscripts. Public Health Nurs. 2010; 27(2): 101-103.

Corresponding Author Bayram Unver, Dokuz Eylul University, School of Physical Therapy and Rehabilitation, Department of Orthopedic Physiotherapy, Izmir, Turkey, E-mail: bayram.unver@deu.edu.tr

# The correlation of a postural status and isometric endurance with rowers of school age

Sasa Milenkovic<sup>1</sup>, Mladen Zivkovic<sup>1</sup>, Dobrica Zivkovic<sup>1</sup>, Sasa Bubanj<sup>1</sup>, Zoran Bogdanovic<sup>2</sup>, Sladjan Karaleic<sup>3</sup>

<sup>1</sup> Faculty of Sport and Physical Education, University of Nis, Nis, Serbia,

<sup>2</sup> University of Novi Pazar, Novi Pazar, Serbia,

<sup>3</sup> Faculty for Sport and Physical Education, Univesity of Kosovska Mitrovica, Kosovska Mitrovica, Serbia.

#### Abstract

By measuring of a postural status in children who practice rowing it was concluded that out of their total number of 30, even 16 participants have a postural disorder (53.3%). 6 participants in the research have kyphotic incorrect body posture (20%), 7 participants have lordotic body posture (23.3%), and there were 3 cases of scoliotic body posture (10%). Other participants had results which are within normal ranges. A certain negative correlation was noticed by correlating a postural status and isometric endurance of abdominal and lumbar muscles, which indicates that a higher degree of deformity causes lower isometric endurance results. A statistic value exists only between scoliotic body posture and trunk flexors. In this particular case this would mean that the higher result of isometric trunk flexor endurance, the lower or even degree of deformity. Sport activities help the development of motor skills and thus the maintenance of the correct postural status. This is valid only if children work with experts who would be able to introduce suitable corrective exercises in order to remove the deficiencies of a certain sport, so that a complete muscular structure would be properly engaged.

**Key words**: A postural status, isometric endurance, rowers.

#### Introduction

Rowing exercise may be an important strategy to promote bone health and reduce vertebral fracture risk (McNamara, 2005), or a rehabilitation exercise used after injuries (Hagerman, Lawrence and Mansfield, 1998). Morris, Smith, Payne, Galloway and Wark, (2000) completed a test to exhaustion on a rowing ergometer simulating competition, in order to estimate the shear and compressive forces experienced by the spine. They found that the spinal forces vary according to forces applied on the oar and that the peak compressive force on the spine was approximately four to five times body weight. Rowing exercise is also related to increased back strength in rowers compared to non-athletes. This outcome is important considering that an imbalance of trunk muscles strength and the strength of back muscles influences a lumbar lordosis and may be one of the risk factors for a lumbar syndrome (Ho-Jun Kim et al., 2006; Alexiev, 1994). Contrary to expectations, McGregor, Anderton and Gedroyc (2002) suggested that lower back pain in rowers' isn't the consequence of the muscle weakness. While investigating the trunk strength in elite rowers with reported back pain, they found no differences between oarside and non-oarside in terms of muscle cross sectional area and no left-right asymmetries were noticed. While working with young rowers, trainers must possess the knowledge about the structure of basic sport movements and the way of training procedures application, for if otherwise, they can consciously or unconsciously provoke scoliosis and kyphosis spine deformities and speed up the very processes of further deformations (Kosinec et al., 2001). A muscular imbalance is one of the basic causes of spinal column instability, according to the latest researches (Norris, 2000). Shortening or weakening of a certain musculature leads to a muscular imbalance and presents the main cause of the incidence and development of incorrect body postures or body deformities (Dejanović and Bošnjak, 2005). Past researches have shown that during isometric endurance testing the relation of the left and right lateral trunk musculature must not be higher than 0.05, and the relationship of lumbar extensors and abdominal flexors during isometric endurance testing must be in a 1:1 ratio (McGill, 2002). According to Dejanović and Fratrić (2007), correct and healthy posture is that state of a body which can hold an upright posture for a longer period of time without effort, pain or fatigue. Physical activity influences changes in the basic motoric and anthropometric areas, and especially in postural disorders area (Milenković, 2000). The main aim of actual study was to determine the correlation of a postural status and isometric endurance with rowers of school age.

#### Materials and methods

The method used for the evaluation of a postural status in a sagittal and frontal plane was developed at the Faculty of Sport and Physical Education, University of Belgrade. It is a modified Smout and McDomel method for which application is needed: a ruler, a dermographic pen, a plummet and a level. For variables that define incorrect postures evaluation a somathoscopy method was used (anthroscopy- an observation method) and somathometry (anthropometry-a measuring method). The evaluation of an isometric endurance was performed by measuring the time that an examinee spent doing a certain task (with a stopwatch). The data procession was performed in a statistic package SPSS (Version 17). Basic descriptive statistics and a correlation analysis were made.

#### The examinees sample

The examinees sample was composed of male children rowers of a rowing club ``Smederevo`` from Smederevo. The examinees are members of a junior club team, and 30 members have undergone the testing. The examinees were 14 years  $\pm$  6 months old.

#### Variables sample

The following postural disorders were examined:

- *Kyphotic incorrect posture (KYPH)*-the deviation from the seventh cervical vertebra and the rope was measured, which is 2.5-4cm in the case of a normal physiology kyphosis.
- *Lordic incorrect posture (LOR)*, the distance in the area of lumbar curve is measured , from the centre of the curve to the plummet rope, which is 3.5-4.5 in the case of a normal physiological lordosis.

- *Scoliotic incorrect posture (SCO)*-it is determined by the deviation of spinal extensions from the rope and by measuring the angle of shoulder blades by a level.

The state of isometric endurance of a trunk was also evaluated:

- *trunk flexors (T.F)* –Out of a starting position (laying on their back), an examinee bends till the angle of 60° and tries to keep that position as long as possible
- *trunk extensors (T.E)*-out of the starting position (laying on their stomach), an examinee bends backwards and tries to keep that position as long as possible
- *left lateral trunk flexors (LLTF)*-Out of the starting position of laying on his left hip, an examinee lifts his body in that way that he pushes off the floor with his left forearm, his right arm is besides his body, legs are together and he tries to keep the position.
- *right lateral trunk flexors (RLTF)* Out of the starting position of laying on his right hip, an examinee lifts his body in that way that he pushes off the floor with his right forearm, his left arm is besides his body, legs are together and he tries to keep the position.

#### **Results and discussion**

Out of the total number of examinees of the rowing club "Smederevo" that were the subjects of examination, 16 of them (53.3%) had some of the researched postural disorder. Six examinees had kyphotic incorrect body posture (20%), seven examinees had a lordotic incorrect body posture (23.3%), and 3 cases of scoliotic incorrect body posture were notified (10%). The rest of the examinees had results that are within a normal range.

The results of a descriptive statistics calculated on the sample of 30 rowers (Table 1) show a normal distribution according to the achieved results (Skewnees and Kurtosis). The results of a Correlation Analysis show how the postural disorders correlate with the results of isometric endurance (Table 2). There is a negative correlation between postural disorders and isometric endurance, which indicates that higher degrees of deformity cause lower values of isometric endurance. That pattern

	Valid N	Mean	Min	Max	Variance	Std.Dev	Skewness	Kurtosis
Sport internship	30	193.37	30.00	730.00	35707.1	188.96	1.50	1.37
KIPH.	30	3.90	2.00	8.00	1.52	1.23	1.39	3.20
LOR.	30	2.53	0.00	8.00	3.98	2.00	1.40	1.90
SCO.	30	3.37	1.00	7.00	3.34	1.83	-0.04	-0.94
T.F.(s)	30	209.77	88.00	330.00	4342.60	65.90	0.42	-0.49
E.T.(s)	30	136.10	20.00	728.00	15401.20	124.10	3.95	18.75
L.L.T.F(s)	30	92.57	26.00	138.00	1068.60	32.69	-0.46	-1.04
D.L.T.F(s)	30	103.80	15.00	167.00	1509.89	38.86	-0.40	-0.40

Table 1. Descriptive Statistics

Table 2.Correlation Analysis

		КҮРН.	LOR.	SCO.
	Pearson Correlation	116	161	-,368*
<b>T.F.</b>	Sig. (2-tailed)	.543	.396	.045
	Ν	30	30	30
	Pearson Correlation	040	106	256
T.E.	Sig. (2-tailed)	.833	.576	.173
	Ν	30	30	30
	Pearson Correlation	284	197	.128
L.L.T.F.	Sig. (2-tailed)	.128	.296	.501
	Ν	30	30	30
	Pearson Correlation	278	238	.071
D.L.T.F.	Sig. (2-tailed)	.137	.206	.709
	Ν	30	30	30

is present in all variables. The statistic value exists only between scoliotic incorrect posture and trunk flexors. In this particular case it means that the higher result of isometric trunk flexors endurance is, the degree of deformity is lower or even absent.

The results of a somatic examination of 27 junior rowers and 24 younger junior rower competitors conducted by Kosinec et al. (2001) showed that paramorphic changes of a spinal column, chest and feet are more frequent with younger junior rowers. About 26% younger junior rowers have laterally curved spine with the following symptoms of asymmetry of certain body parts, while junior rowers usually have a kyphotically curved spine (37%). A predictor set of 11 indicators of a body posture significantly explains the impact of the way of rowing on a deviation of orthostatic and body postures, so it can be a base for a prediction of its impact on the spine paramorphosis incidence in a phase of a rapid growth. The results achieved during this research provided a completely plausible explanation of a complex mechanism functioning during a specific strain in rowing, concerning a rowing method and the etiology of paramorphic incidences with rowers in a growing-up period. It is a devastating fact that a high percentage of primary school children in Serbia do not participate in sporting activities, and it is known that a rapid and asymmetric growth in a childhood period with other environmental factors are extremely important elements in spinal column deformity incidence. The reasons for non-participation are mostly a high club membership fee and a distance of a child's residence from the sports centre where chosen sport disciplines are practiced (Bogdanović and Marković, 2010). Certainly, a modern life-style that includes a lowered locomotor's apparatus activity has a negative impact on a correct body posture as well as on a correct development of a spinal column. Kyphosis, lordosis and scoliosis are the direct consequences of unhealthy habits and life conditions. (Karalejić, 2006). But how to explain such an incidence of mentioned postural deformities found in actual study among rowers who are actively engaged in sport activities for a relatively long period? No matter how high the intensity of rowing in the

population of school-age children is, rowing loads repeated in a longer duration seem to be of high enough intensity to offset the postural deformities. There is a strong need for the experts who would be able to introduce suitable corrective exercises in order to remove the deficiencies of a certain sport, so that a complete muscular structure in children would be properly engaged. The ideal situation would be if already nursery teachers would notice the first indicators of an incorrect body posture (Milošević and Obradović, 2008). Stable head position maintenance with children during various activities is followed by complex motor skills that are gradually developed during a growing-up period. Balance is very important for keeping a correct body posture, and it depends on a body's ability to harmonize body movements with a required balance, and all this in order to achieve the most efficient performance of a certain task (Assainte et al., 2005). Kosinec (1994) suggested that in domain of motor skills, people without scoliosis achieve better results on tests which are influenced by synergetic regulation mechanism. It leads to a conclusion that people with a functional scoliosis in puberty have notable disharmony in controlling mechanism functioning.

#### Conclusion

Out of the total number of examinees from the rowing club ``Smederevo``, a postural disorder was detected with 16 of them, which certainly indicates that they need to be engaged in a program of corrective exercises. By correlating a postural status and isometric endurance we came to a conclusion that with a higher value of isometric endurance lower values of postural disorders were notified, but in this particular case, the most important correlation was between trunk flexors and scoliotic incorrect body posture. It is possible to lower the risk of postural disorder incidence by strengthening of abdominal and lumbar musculature. It is particularly evident in the case of isometric trunk endurance and scoliotic incorrect body posture.

#### References

- Assainte C, Mallau S, Viel S, Jover M, Schmitz C. Development of postural control in healthy children: a functional approach. Neural Plasticity, 2005; 12(2-3): 109-118.
- 2. Alexiev AR. Some differences of the electromyographic erector spinae activity between normal subjects and low back pain patients during the generation of isometric trunk torque. Electromyography and Clinical Neurophysiology, 1994; 34: 495-499.
- 3. Bogdanović Z, Markovič Ž. Presence of lordotic poor posture resulted by absence of sport in primary school children. Acta Kinesiologica, 2010; 4(1): 63-66.
- Dejanović A, Bošnjak S. Some possibilities in application of isometric exercises in prevention of muscular disbalance as the cause of instability in the lumbar region of the spinal column. 1st International Conference "Management in Sport", Belgrade, Faculty of Management in Sport, Proceedings, 2005; 13-19. ISBN 86-86197-07-8. COBISS.SR-ID 213180423. In Serbian
- 5. Dejanović A, Fratić F. Spinal column, (non) training and children. Novi Sad: "ABM Ekonomik". In Serbian, 2007.
- 6. Hagerman FC, Lawrence RA, Mansfield MC. A comparison of energy expenditure during rowing and cycling ergometry. Medicine & Science in Sports and Exercise, 1998; 20(5): 479-488.
- 7. Ho-Jun K, Chung S, Kim S, Shin H, Lee J, et al. Influences of trunk muscles on lumbar lordosis and sacral angle. European Spine Journal, 2006; 15(4): 409-414.
- 8. Karalejić S. Postural status in students of higher elementary-school stature. Journal of the Anthropologycal Society of Serbia, 2006; 41: 257-260.
- 9. Kosinec Z, Bižaca J, Kučić R. Relations between the paramorphic and dismorphic changes in the system of movement and manner of rowing of the junior competing rowers. Fizička kultura, 2001; 55(1-4): 67-72. In Serbian
- 10. Kosinec Z. Morphologic and motor characteristics of girls with different scoliotic level. Kineziologija, 1994; 26(1-2): 22-26. In Croatian
- 11. McGill SM. Low back disorders–Evidence-based prevention and rehabilitation. Human Kinetics, USA, 2002.
- 12. McGregor AH, Anderton L, Gedroyc WMW. The trunk muscles of elite oarsmen. British Journal of Sports Medicine, 2002; 36: 214-216. doi:10.1136/ bjsm.36.3.214

- McNamara AJ. Bone mineral density and rowing exercise in older women. Master Thesis, Oregon State University. Retrived on the World Wide Web, 2005: http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/29622/McNamaraAdrieneeJ2005.pdf?sequence=1
- 14. Milenković S. Determining differences in postural, anthropometric and kinesics area at the beginning and the end of a school year. Facta Universitatis series Physical Education and Sport, 2000; 1(7): 39-48.
- 15. Milošević Z, Obradović B. Posture of preschool boys and girls of Novi Sad at the age of 7. Journal of the Anthropologycal Society of Serbia, 2008; 43: 301-309. In Serbian
- 16. Morris FL, Smith RM, Payne WR, Galloway MA, Wark JD. Compressive and shear force generated in the lumbar spine of female rowers. International Journal of Sports Medicine, 2000; 21: 518-523.
- 17. Norris CM. Back Stabillty. Leeds: Human Kinetics Publishers, 2000.

Corresponding Author Mladen Zivkovic, University of Nis, Faculty of Sport and Physical Education, Nis, Serbia, E-mail: profzile@gmail.com

# Species distribution and Caspofungin susceptibility of *Candida* spp. isolated from blood cultures

Yesim Cekin<sup>1</sup>, Nevgun Sepin Ozen<sup>1</sup>, Nilgun Gur<sup>1</sup>, Hamit Yasar Ellidag<sup>2</sup>

<sup>1</sup> Antalya Antalya Reseach and Training Hospital, Clinical Microbiology, Antalya, Turkey,

<sup>2</sup> Antalya Reseach and Training Hospital, Central Laboratory, Antalya, Turkey.

#### Abstract

Candidemia remains associated with high mortality rates and with increased costs of care and duration of hospitalization. This study aims to determine the distribution and caspofungin susceptibility of candida species isolated from blood cultures in Antalya Training and Research Hospital. Between June 2011 to December 2012, totally 71 candida spp isolates of 68 patients were included into the study. Candida albicans is the most prevelant isolated species (% 40,9) from blood cultures followed by Candida tropicalis (% 17,9), Candida glabrata (% 17,9), Candida parapsilosis (% 17,9), Candida lusitaniae (% 17,9), Candida krusei (% 17,9). Caspofungin susceptibilities of all isolates were determined by E test method. E-test minimal inhibitory concentration values of caspofungin for C. albicans isolates were 0.002 and 0.125 mg/L, respectively. All of 71 candida spp isolates were susceptible to caspofungin. This study demonstrates that C. albicans remains the predominant species isolated from candida blood stream infections and caspofungin demonstrated an excellent potency against Candida spp isolates. Using knowledge of local epidemiologic trends in *Candida* spp to establish therapeutic and preventive strategies.

Key words: Candida, Candidemia, Caspofungin.

#### Introduction

Nosocomial blood stream infections (BSI) due to *Candida* spp are important cause of mortality and morbidity in hospitalized patients [1, 2]. *Candida* spp is reported between the fourth and the sixth common cause of BSI and *Candida albicans* is the most responsible pathogen [1-5]. However *non albicans Candida* spp has been documented increasingly from different geographical locations [3, 5]. For appropriate control measures it is important to understand the differences in the epidemiology of candidemia between different regions, the need for continuous surveillance in incidence, species distribution and antifungal susceptibility profiles [6, 7]. This study aims to determine the species distribution and caspofungin susceptibility of *Candida* spp. isolated from blood cultures in Antalya Research and Trainig Hospital respectively.

#### **Materials and Methods**

A total of 71 candida spp isolates recovered from blood cultures were collected from 68 patient (35 male and 33 female) between 01 June 2011 to 31 December 2012. Identification of *Candida* spp. was performed with conventional microbiological procuderes and Phoenix (Becton Dickinson, US) automated system. The MICs of caspofungin were performed by using E-test (AB Biodisk, Sweeden) method according to manufacturers recommendations. MIC data are presented as the range, MIC<sub>50</sub> and MIC<sub>90</sub> for each species. Statistical data were analysed by using Kruskal Wallis test. Quality control was ensured by testing *C.albicans ATCC 90028 ve C.parapsilosis ATCC 22019* strain.

#### Results

From June-2011 to December 2012, totally 71 *Candida* spp isolates (29 *Candida albicans*, 14 *Candida tropicalis*, 14 *Candida glabrata*, 11 *Candida parapsilosis*, 2 *Candida lusitaniae*, 1 *Candida krusei*) of 68 patients were included into the study. 35 male (51,5%) and 38 female (48,5%) patients mean age was 58,5±16,5. Table 1 summarizes the species distribution and in vitro activities of caspofungin against 71 candida isolates. Overall all isolates were classified as susceptible to caspofungin.

In our study the most common isolate was *C. albicans* (29%) in accordance with recent reports and the average MIC range is less than other *Can-dida* spp. (p=0.004).

	Sample	Sample Caspofungin (mg/L)				
	number (%)	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Avarage range	р
C. albicans	29 (40.9)	0.002-0.125	0.010	0.034	25.19	
C. parapsilosis	11 (15.5)	0.002-0.500	0.120	0.274	47.27	
C. tropicalis	14 (19.7)	0.002-0.230	0.050	0.128	40.50	0.004*
C. galbrata	14 (19.7)	0.002-0.500	0.120	0.278	37.75	0.004
C. krusei	1 (1.4)		0.030	0.064		
C.lusitaniae	2(2.8)		0.050	0.125		

Table 1. Distribution of Candida species and caspofungin susceptibility

\*statistical analysis performed by using Kruskal Wallis test, except C. krusei and C. lusitaniae (because of the insufficient number of these organisims)

#### Discussion

Candidemia infections increase patient mortality, both the length of stay and cost associated with hospitalization. Approximately 95% of candidaemia is caused by 4 species: C. albicans, C. glabrata, C. parasilosis and C. tropicalis especially in Europe and United States [8, 9]. The distribution of species and susceptibility patterns of Candida isolates play an important role on the management of invasive candidiasis and surveillance strategies for epidemiological evaluation of antifungal resistance. [1, 6, 7]. Despite a wide range of antifungal agents, emerging resistance paterns are reporting from all over the world. A new novel of ecinocandin, caspofungin is water-soluble glucan synthesis inhibitor that was approved by FDA (Food Drug Administration) for the management of invasive candidiasis and invasive aspergillosis. The reference tests internationally accepted standarts for caspofungin are broth dilution methods (BMD) developed by the CLSI (Clinical Laboratory Standards Institute) and European Committee on Antibiotic Susceptibility Testing (EUCAST) [10]. These reference methods are expensive, time consuming and poorly suitable for clinical laboratories. Caspofungin E-test is commercially agar-based diffusion method which is easy to perform and reliable for susceptibility testing of Candida spp [3] But there is insufficient data at present for interpretive breakpoints for caspofungin [5]. Many investigators showed that E-test results for caspofungin demonstrated good correlation between BMD recommended by CLSI and EUCAST [11-13]. In a study which 726 candida isolates were used by Pfaller et al, the agreement between the Etest and microdilution MICs was

found >90 % for all species except C. tropicalis and C. parapsilosis [9]. The E-test MIC<sub>50</sub> values reported in this study are in agreement with those reported previously [5-7,12]. Arendrup et al. showed that caspofungin E-test results of 65/496 echinocandin- susceptible isolates (13.1%) were misclassified as intermediate or resistant where as misclassifications were most commonly observed for C. krusei (73.1% misclassified as I) and for C. glabrata (31.6% misclassified as intermediate and 1.5% as resistant), but only a single C. albicans isolate (0.4%) was misclassified as intermediate and no isolates belonging to the other species were misclassified [13]. Lockhart et al found echinocandin resistance was low (1% of isolates) but was higher for C. glabrata isolates [7]. In our study no resistance was determined for caspofungin and C. albicans (40,9%) is the most prevelant isolate responsible for candidiasis in accordance with many reports. There were differences in species distribution between the regions. C. parapsilosis and C. glabrata was significantly more frequently isolated non candida albicans isolate published from all over the world [1, 5-7, 14]. C. glabrata and C. tropicalis were isolated at the same percent (19,7%) in our study.

This report has some limitations. Clinical isolates were collected from only one hospital and thus do not represent the general antifungal distribution and susceptibility pattern in our country. However antifungal susceptibility is a rapidly changing field of knowledge. Owing to this surveillance studies must perform to follow the epidemiologic changes, changing levels of resistance and use these data to update future treatment managements.

#### References

- Conde-Rosa A, Amador R, Pérez-Torres D, Colón E, Sánchez-Rivera C, Nieves-Plaza M, et al. Candidemia Distribution, Associated Risk Factors, and Attributed Mortality at a University-Based Medical Center. P R Health Sci J. 2010; 29: 26–29.
- Manual of Clinical microbiology. 9<sup>th</sup> Edition. Patrick R Murray, Ellen Jo Baron, James H Jorgensen, Marie Louise Landry, Michael A Pfaller. Candida, Cryptococcus ve Tibbi Onemi Olan Diger Mayalar.
- 3. Belet N, Ciftçi E, Aysev D, Güriz H, Uysal Z, Taçyildiz N, et al. Invasive Candida infections in children: the clinical characteristics and species distribution and antifungal susceptibility of Candida spp. Turk J Pediatr. 2011; 53: 489-98.
- 4. Bassetti M, Ansaldi F, Nicolini L, Malfatto E, Molinari MP, Mussap M, et al. Incidence of candidaemia and relationship with fluconazole use in an intensive care unit. J Antimicrob Chemother. 2009; 64: 625-629.
- 5. Tan TY, Tan AL, Tee NW, Ng LS. A retrospective analysis of antifungal susceptibilities of Candida bloodstream isolates from Singapore hospitals. Ann Acad Med Singapore. 2008; 37: 835-840.
- 6. Bassetti M, Taramasso L, Nicco E, Molinari MP, Mussap M, Viscoli C. Epidemiology, species distribution, antifungal susceptibility and outcome of nosocomial candidemia in a tertiary care hospital in Italy. PLoS One. 2011; 6(9): e24198.
- 7. Lockhart SR, Iqbal N, Cleveland AA, Farley MM, Harrison LH, Bolden CB, et al. Species identification and antifungal susceptibility testing of Candida bloodstream isolates from population-based surveillance studies in two U.S. cities from 2008 to 2011. J Clin Microbiol. 2012;50: 3435-3442.
- Comert F, Kulah C, Aktas E, Eroglu O, Ozlu N. Identification of Candida species isolated from patients in intensive care unit and in vitro susceptibility to fluconazole for a 3-year period. Mycoses. 2007; 50: 52-57.
- 9. St-Germain G, Laverdie M, Pelletier R, René P, Bourgault AM, Lemieux C, et al. Prevalence and antifungal susceptibility of 442 Candida isolates from blood and other normally sterile sites: results of a 2-year (1996 to 1998) multicenter surveillance study in Quebec, Canada. J Clin Microbiol. 2001; 39: 949-953.
- 10. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A3, 3rd ed. Clinical Laboratory Standards Institute, Wayne, Pa. 2008.

- Pfaller MA, Messer SA, Mills K, Bolmström A, Jones RN. Evaluation of Etest method for determining caspofungin (MK-0991) susceptibilities of 726 clinical isolates of Candida species. J Clin Microbiol. 2001; 39: 4387-4389.
- Serefko A, Los R, Biernasiuk A, Malm A. Comparison of microdilution method and E-test procedure in susceptibility testing of caspofungin against Candida non-albicans species. New Microbiol. 2008; 31: 257-262.
- 13. Arendrup MC, Pfaller MA. Danish Fungaemia Study Group. Caspofungin Etest susceptibility testing of Candida species: risk of misclassification of susceptible isolates of C. glabrata and C. krusei when adopting the revised CLSI caspofungin breakpoints. Antimicrob Agents Chemother. 2012; 56: 3965-3958.
- 14. Çekin Y, Pekintürk N, Çekin AH. Evaluation of Species Distribution and Antifungal Resistance of Candida Isolates From Hospitalized Patients. J Clin Anal Med. 2013 March 01. Doi:10.4328/JCAM.1638

Corresponding Author Yesim Cekin, Antalya Reseach and Training Hospital, Clinical Microbiology, Antalya, Turkey, E-mail: yesimcekin@hotmail.com

# Diabetic kidney disease and its associated complications

Azreen Syazril Adnan<sup>1</sup>, Fauziah Jummaat<sup>2</sup>, Yusra Habib Khan<sup>3</sup>, Amer Hayat Khan<sup>3</sup>

- <sup>1</sup> Chronic Kidney Disease (CKD) Resource Center, School of Medical Sciences, University Science Malaysia, Kota Bharu, Kelantan, Malaysia,
- <sup>2</sup> Department of Obstetrics and Gynecology, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Malaysia,
- <sup>3</sup> Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia.

#### Abstract

Chronic kidney disease secondary to diabetic nephropathy is referred as diabetic kidney disease (DKD). The progression of DKD to end stage renal failure has been shown to increase dramatically over the past few years. However, this progression can be slowed or reserved by providing adequate treatment at early stages with a special focus on clinical complications.

Following is a case of DKD patient diagnosed with acute kidney failure and uncontrolled hypertension accompanied by complications like anaemia and oedema. Treatment goal in such patient is to control blood pressure by eliminating oedema and maintaining kidney function. Such patients' need extra care to control blood sugar level as fluctuations in blood sugar level will aggravate secondary complications.

Key words: Diabetic kidney disease, hypertension, anaemia, oedema

#### Introduction

Chronic kidney disease secondary to diabetic nephropathy is referred as diabetic kidney disease (DKD). Diabetic kidney disease is the leading cause of End stage renal failure with a prevalence of 35 to 40 % among diabetic patients [1].

Diabetic nephropathy is a pathological condition in which albumin (a blood protein) begins to leak into urine. Initially kidneys filtration function remains normal and only small amount of albumin is present in urine. This stage is known as microalbuminuria. As disease progresses, more amount of albumin becomes evident in urine leading to macroalbuminuria. At this stage, kidneys are unable to filter waste products [2]. As a result of poor kidney function, arterial blood pressure rises significantly leading to uncontrolled blood pressure. High blood pressure along with elevated blood glucose and hyperlipidemia is the main cause of diabetic nephropathy which leads to diabetic kidney disease. In diabetic kidney disease high blood glucose level damages blood vessels in the kidneys, thus affecting ability of kidneys to filter blood properly. Without any treatment, kidneys will eventually fail resulting in oedema and numerous other complications especially uncontrolled blood pressure and anaemia [3].

#### **Case presentation**

A 61 years old Malay male diagnosed with Diabetes mellitus (DM) 2 was admitted to Hospital Universiti Sains Malaysia (HUSM). Patient has been suffering from DM 2 for past 7 years but his condition was quite stable all this while until he started to experience generalized oedema (noted especially on lower limbs), scrotum swelling with facial puffiness and abdominal distension. Patient complained of breathlessness and orthopnoea (shortness of breath while lying straight) to his General practitioner and was given Tablet Furosemide 60 mg OD (taken once daily). His condition became better for few days but symptoms of oedema recur. Within one week, he was admitted to hospital due to severe nausea, vomiting. Patient was lethargic with clear signs of oedema with shortness of breath.

Upon admission to hospital, physical examination of patient showed presence of ascites in abdomen with pedal oedema up to knee especially on lower right limb. Examination of lower limbs revealed chronic bilateral ulcer. Vitals of patient showed nor-

Parameters	Normal value	Day 1	Day 2	Day 3
Sodium, Na <sup>+</sup>	136-146 mmol/L	135	135	134
Potassium, K <sup>+</sup>	3.5 – 5.1 mmol/L	3.3	3.2	3.6
Urea	2.8 – 7.2 mmol/L	13.5	12.7	12.3
Creatinine	72 – 127 μmol/L	278	274	180
Creatinine clearance (CrCl)	105 – 150 ml/min	26.17	26.55	37.72

#### Table 1. Renal Functioning tests

#### Table 2. Hydration status of patient

Day	Intake (ml)	Output (ml)	Balance (ml)
Day 1	250	500	- 250
Day 2	400	1500	-1100
Day 3	800	900	- 100
Day 4	600	2600	-2000

Table 3. Medical profile of patient

Drug	Indication				
T. Felodipine 10mg stat then OD	Calcium-channel blocker. Used to manage uncontrolled hypertension				
T. Calcium carbonate 500mg BD	As an electrolyte supplement				
IV Metoclopramide 10mg stat, then TD	Anti-emetic				
T. Unasyn 375 mg stat then BD	Treatment of diabetic dermopathy				
T. Losartan 100mg OD	Angiotensin receptor blocker (ARB). Treatment of diabetic				
1. Losartan Toonig OD	nephropathy in Type 2 diabetes & HTN				
S/C Actrapid 8 unit TD	Insulin. Used to manage the diabetes mellitus.				
T. Vitamin B complex 1/1 OD	Supplement				
IV Furosemide 60 mg TDS	Loop diuretic. Used to remove excess fluid (oedema)				
T. Fe Fumarate 200mg OD	Prevention and treatment of iron-deficiency anemia				
T Folate 1/1 OD	Treatment of megaloblastic and macrocytic anemia due to folate				
	deficiencies				
T. Perindopril 4 mg OD	ACE inhibitor. Used to manage high blood pressure.				
T. Felodipine 15 mg OD	Calcium-channel blocker. Used to manage uncontrolled hypertension				

*OD:* once daily, *BD:* bis in die (twice daily), *TD:* thrice daily, stat: , *T:* tablet, *HTN:* hypertension, stat: medication to be administered immediately

mal body temperature and pulse rate but blood pressure was quite high 185/115 mmHg. Next haematological, renal and liver tests were done. Reports of haematological test showed abnormally high uric acid 622 mmol/L (normal value: 98-106mmol/L). Furthermore Activated partial thromboplastin time (aPTT) was very high i.e. 96.2 s (normal value: 26-42 s). Liver profile of patient showed slightly low albumin i.e. 32 (normal value: 35-52 g/L). The renal profile and hydration status of patient is shown in Table 1. and Table 2. respectively.

After being admitted to ward and all basic tests, patient was prescribed following medications: (Table 3)

#### Discussion

A diabetic patient (type 1) was admitted to HUSM due to his complain of breathlessness, severe oedema and pain in lower limbs. After physical examination and detailed blood tests, patient was diagnosed with chronic kidney disease (CKD) stage IV secondary to diabetic nephropathy, uncontrolled hypertension, anaemia secondary to CKD and generalized oedema secondary to decompensate heart failure.

Patient suffered from uncontrolled high blood pressure. Initially he was given Tablet Felodipine 10 mg stat but his hypertension remained uncontrolled. Thus, the dose of felodipine was increased to 15mg OD and other anti-hypertensive agent

Tablet Losartan 100mg OD was added to the regimen on second day. Still on fourth day, patient blood pressure was 180/107 mmHg and so physician decided to add Tablet Perindopril 4mg to control high blood pressure. Meanwhile diuretic (I.V Furosemide 60 mg) was given to patient to drain out excess water from the body as a result of oedema. As evident from patient hydration profile, patient was able to urinate excess fluid out of his body but still physician were unable to control his blood pressure. High blood pressure worsen diabetic nephropathy that is evident from higher level of albumin (3+) in urine. Moreover patients' haemoglobin and hematocrit level was lower than normal range clearly indicating anaemia secondary to chronic kidney disease stage IV.

One of the functions of kidney is to filter blood and to produce red blood cells by generating hormone erythropoetin. However, in stage 4 kidney disease, 85% to 90% of kidney function is lost. Thus, the kidneys become unable to filter blood and produce red blood cells that ultimately results in anaemia [4]. In order to treat anaemia in this patient, Tablet Folate and Ferrous fumarate were prescribed by physician.

As seen by renal functioning test reports, extremely high urea level suggests careful intake of protein. This would help to decrease work load on kidney and ultimately slows progression of CKD by controlling uremia.

Lastly patient was suffering from diabetic dermopathy. It is a type of skin lesions (cutaneous) seen in patients with DM and results due to poor supply of oxygenated blood to the effected part. Deoxygenated blood will pool in effected part and leads to painful cutaneous skin lesions [5].

#### Conclusion

Diabetic kidney disease (DKD) is a complicated medical condition that itself increases risk of other complications. As seen in our patient uncontrolled hypertension, oedema and extremely low creatinine clearance clearly shows end stage renal failure. Treatment of all these complications requires extreme care by physician as far as dosage adjustment, selection of drug and appropriate diet plan is concerned. Main priority in this case is to control blood pressure by eliminating oedema and to maintain blood sugar level to avoid further complications.

#### References

- 1. Reutens A, Atkins R. "Epidemiology of diabetic nephropathy." Contributions to Nephrology, 2011; 170: 1-7.
- 2. Ayodele OE, Alebiosu CO, et al. "Diabetic nephropathy--a review of the natural history, burden, risk factors and treatment." Journal of the National Medical Association, 2004; 96(11): 1445-1454.
- 3. Dronavalli S, Duka I, et al. "The pathogenesis of diabetic nephropathy." Nature Clinical Practice Endocrinology & Metabolism, 2008; 4(8): 444-452.
- 4. Rahman M, Smith MC. "Chronic Renal Insufficiency. A Diagnostic and Therapeutic Approach." Arch Intern Med, 1998; 158(16): 1743-1752.
- 5. Adams SP. "Dermacase. Diabetic dermopathy." Can Fam Physician. 2001 April; 47: 725–729.

Corresponding Author Azreen Syazril Adnan, Chronic Kidney Disease (CKD) Resource Center, School of Medical Sciences, University Science Malaysia, Kota Bharu, Malaysia, E-mail: drazreenadnan@gmail.com

# White matter hyperintensities and related risk factors in Chinese normal aging : A community based study

Guoxing Zhu<sup>1</sup>, Shuguang Chu<sup>2</sup>, Ding Ding<sup>3</sup>, Chi-Shing Zee<sup>4</sup>

<sup>1</sup> Department of Neurology, Huashan Hospital, Fudan University, Shanghai, P.R. China,

<sup>2</sup> Department of Radiology, Huashan Hospital, Fudan University, Shanghai, P.R. China,

<sup>3</sup> Department of Biostatistics and Epidemiology, Institute of Neurology, Fudan University, Shanghai, P.R. China,

<sup>4</sup> Department of Neuroradiology, Keck School of Medicine, University of Southern California, Los Angeles, USA.

#### Abstract

**Background**: White Matter Hyperintensity (WMH) -related findings in normal aging are rarely reported in China. This study investigated the prevalence and anatomical location of WMH and related risk factors in Chinese population of normal aging.

**Methods:** A resident group of 156 healthy subjects aged over 60 years old were enrolled in a community in Shanghai. All participants received physical and neurological examinations and Magnetic Resonance Imaging. Fazekas rating scales were used to describe the severity of periventricular hyperintensity (PVH) and deep white matter hyperintensity (DWMH). Multivariate logistic regression model were performed to examine the association of WMH and potential related factors.

**Results:** PVH and DWMH appeared in 77.6% and 88.5% of the subjects respectively. Adjusted by other variables, increasing age (OR, 1.102; P=0.003) and hypertension (OR, 2.707; P=0.015) were found as the independent risk factors of PVH. Independent risk factors for DWMH were increasing age (OR, 1.189; P<0.001), obesity (OR, 2.977; P=0.015), and cigarette smoking (OR, 4.125; P=0.049).

**Conclusions**: Among Chinese normal aging, WMH are common and are associated with increasing age. Consistent with numerous prior studies, vascular risk factors such as hypertension, obesity and cigarette smoking were also significantly associated WMH burden suggesting a possible vascular etiology.

**Key words:** aging, white matter hyperintensity, magnetic resonance imaging

#### Introduction

White matter hyperintensities (WMH) refer to areas of hyperintense signal on T2- or proton density-weighted brain magnetic resonance imaging (MRI). They are thought to reflect ischemic brain changes [1], but advancing age and other etiologies may also contribute [2]. Magnetic resource-pathologic studies suggest that deep white matter and periventricular hyperintensities represent primarily ischemic tissue damage, although Wallerian degeneration secondary to a degenerative process such as Alzheimer's disease as well as blood-brain barrier dysfunction are likely to contribute as well [3-6].

Increasing evidence supports the view that WMH have detectable clinical implications even in healthy elderly subjects [7-11]. In western countries, WMH are reported to occur in 30%-96% of the elderly population [12,13], and the incidence of these lesions even approaches 100% by the age of 85 [14]. Significant risk factors for more severe WMH include older age, history of hypertension, stroke, smoking, and clinically silent stroke on MRI [15]. One study found an association between severity of WMH and history of diabetes [16], but others have not [12,17].

In China and even in Asia in general, WMH-related findings in normal aging are rarely reported. The aim of the current study was to investigate the prevalence and anatomical location of WMH in a community-living population of normal elderly. Furthermore, we also examined the risk factors related to WMH in this population.

#### Methods

#### Subject population

Subjects were recruited using a government maintained "name list", which includes the name, sex, age, address and telephone number of every resident in Jing'an Temple community in urban Shanghai. This list is kept current by the neighborhood administration office, which keeps track of all individuals living in that neighborhood, including in- and out-migration, as well as deaths. We enrolled subjects focusing on a resident group in a defined geographic area, consisting of five resident units in the community. Potential participants were approached at the door. The recruitment rate in the community was 81.6%.

The inclusion criteria were as follows: 1) age over 60 years old; 2) neurologically asymptomatic by comprehensive neurological evaluation; 3) without history of stroke; 4) education-adjusted Chinese Mini-Mental State Exam score  $\geq=26$ ; 5) without any contraindications to MR scanning (pacemakers or other metal objects).

All the participants were requested to provide their written informed consent after reading the study information. This study was approved by the Medical Ethics Committee of Huashan Hospital, Fudan University, Shanghai.

#### **Clinical Evaluation**

All participants received physical and neurological examinations in Huashan Hospital, Fudan University. Besides age and gender, height and weight were also measured for each enrolled participants. The body mass index (BMI) was calculated as a person's weight in kilograms (kg) divided by their height in meters (m) squared. BMI of  $\geq$ 25 was defined abnormal according to the WHO standard. Blood pressure was measured with the subject in the sitting position for over 5 min. Risk factors, such as history of diabetes mellitus (DM), hypertension, atrial fibrillation (AF), hyperlipidemia, and coronary artery diseases (CADs) were recorded. DM was defined as type I or II DM treated with antidiabetic therapy. Hypertension was defined as a systolic/diastolic blood pressure of ≥140/90 mm Hg or self-reported hypertension that was being treated with antihypertensive therapy. Patients were considered to have hyperlipidemia based on self-report and if they exhibited any of the following criteria: 1) total cholesterol (TC) level  $\geq$  5.9 mmol/l; 2) low-density-lipoprotein cholesterol (LDL-C) level  $\geq$  2.6 mmol/l; 3) high-density-lipoprotein cholesterol (HDL-C) level < 0.9 mmol/l (male), HDL-C < 1.0 mmol/l (female); or 4) triglyceride (TG) level  $\geq$  1.8 mmol/l. CADs included stable angina, unstable angina, and myocardial infarction.

#### **MRI** Acquisition

Brain images were obtained at Huashan Hospital in Shanghai. For MRI acquisition, we used a series of image acquisition protocols developed at the UCD Imaging of Dementia & Aging Laboratory (IDeA Lab), which are suitable for the GE 1.5T MRI system. Imaging parameters were as follows: 1) Axial spin echo, T2 weighted double echo image with TE1 equal to 20 ms, TE2 equal to 90 ms, TR equal to 2420 ms, a field of view of 24 cm and a slice thickness of 3 mm. 2) Coronal 3D spoiled gradient recalled echo (IR-prepped SPGR) acquisition, T1 weighted image with TR equal to 9.1 ms a flip angle of 15 degrees, a field of view of 24 cm and a slice thickness of 1.5 mm. 3) Axial high resolution Fluid Attenuated Inversion Recovery (FLAIR) image with a TE of 120 ms, a TR of 9000 ms, a TI 2200 ms, a 24 cm field of view, and a slice thickness of 3 mm. The scan was checked and stored at the Radiology Department of Huashan Hospital.

#### Image analysis

A modification of suggested Fazekas rating scales was used to describe the severity of hyperintense signal abnormalities surrounding the ventricles and in the deep white matter [18]. Periventricular hyperintensity (PVH) was graded as 0=absence, 1 (mild)= "casps" or pencil-thin lining, 2 (moderate)=smooth "halo", 3 (severe)=irregular PVH extending into the deep white matter. Periventricular caps are hyperintense regions around the anterior and posterior pole of the lateral ventricles and are associated with myelin pallor and dilated perivascular spaces. Periventricular bands or 'rims' are thin linear lesions along the body of the lateral ventricles and are associated with subependymal gliosis. Separate deep white matter hyperintense signals (DWMH) were rated as 0=absence, 1 (mild)=punctuate foci, 2

(moderate)=beginning confluence of foci, 3 (severe)= large confluent areas. The presence of lacunar infarct, defined as small cavitated lesions no larger than 2 cm in diameter, were assessed on T1weighted and T2-weighted FLAIR images. Image analysis was performed by an experienced rater (Dr. Chu) who was blinded to age, gender, and other risk factors.

#### Data analysis

Data analysis was performed with SPSS 10.0 (SPSS Inc, Chicago, USA). Prevalence of hyperintense signal abnormalities were described as percentage stratified by gender and age group. Differences in hyperintense signal abnormalities between risk factor subgroups were compared by chi-squared test or fisher's exact test. To examine the association of potential risk factors with PVH and DWMH, we performed multivariate logistic regression models, with age, gender, BMI, cigarette smoking, alcohol intake, hypertension, DM, AF, hyperlipidemia, and CADs as independent co-variables. In the model, participants who had grade 0 or 1 PVH and grade 0 or 1 DWMH were regarded as hyperintense signal normality. Participants who had grade 2 or 3 PVH and grade 2 or 3 DWMH were regarded as hyperintense signal abnormality. Adjusted Odds Ratio (OR) along with 95% CI was presented as the risk measurement for the hyperintense signal abnormality. All P values and CIs were estimated in a two-tailed fashion. Difference was considered to be statistically significant at P < 0.05.

#### Results

#### Characteristics of enrolled study subjects

We recruited 156 subjects (61 male and 95 female) with mean age of  $69.4\pm6.2$  years (range, 60-83). The average BMI of the subjects was  $24.3\pm3.6$ (range, 16.6-35.4). High BMI ( $\geq$ 25) was observed in 38.3% of the patients. Subjects with habit of cigarette smoking and alcohol drinking were 23 (14.7%) cases and 20 (12.8) cases. Hypertension, hyperlipidemia and DM were found respectively in 48.1%, 45.5% and 11.5% of subjects. Patients with AF and CADs accounted 2.6% and 10.3%. Lacunar infarct was detected on MRI in 6 (3.8%) subjects without any clinical symptoms (Table 1).

## Prevalence of hyperintense signal abnormality

The prevalence of each grade of PVH and DWMH in study subjects is shown in Table 2. Among 156 sujects, 35 (22.4%) and 60 (38.5%) were evaluated as Fazekas grade 0 and 1 of PVH; 18 (11.5%) and 87 (55.8%) were evaluated as Fazekas grade 0 and 1 of DWMH. Fifty eight (37.2%) and 3 (1.9%) subjects were evaluated as Fazekas grade 2 and 3 of PVH, whereas 36 (23.1%) and 15 (9.6%) subjects were evaluated as Fazekas grade 2 and 3 of DWMH. Individuals older than 70 years old had more WMH burden than those aged 60-69 (p<0.05). WMH burden did not differ by gender.

 Table 1. Demographic and clinical characteristics of study subjects

01	
Variable	Study subjects (n=156)
Gender [male,n (%)]	61(39.1)
Age, years (mean±SD)	69.4±6.2 (60-83)
BMI (mean±SD)	24.3±3.6 (16.6-35.4)
Smoking [n (%)]	23(14.7)
Alcohol drinking [n (%)]	20(12.8)
Medical history	
Hypertension [n (%)]	75(48.1)
Diabetes mellitus [n (%)]	18(11.5)
Hypercholesterolaemia [n (%)]	71(45.5)
Atrial fibrillation [n (%)]	4(2.6)
Coronary artery diseases [n (%)]	16(10.3)
Lacunar infarct on MRI [n (%)]	6(3.8)

## *Risk factors related to hyperintense signal abnormality*

Univariate analysis showed that cigarette smoking (p=0.006), alcohol consumption (p=0.004), hypertension (p<0.001), CAD (p=0.043), and lacunar infarct on MRI (p=0.024) were related to PVH abnormality; lacunar infarct (p=0.001) were also related to DWMH abnormality (Table 3). Multivariate logistic regression model indicated that, adjusted by other variables, increasing age (OR, 1.102; 95% CI, 1.033 to 1.177; P=0.003) and hypertension (OR, 2.707; 95% CI, 1.211 to 6.050; P=0.015), were found as the independent risk factors of PVH; while alcohol consumption (OR, 0.137; 95% CI, 0.024 to 0.771; P=0.024) was found to be an independent protective factor of PVH (Table 4). Independent risk factors for DWMH were increasing age (OR, 1.189; 95% CI, 1.101 to 1.284; P<0.001), high BMI (OR, 2.977; 95% CI, 1.236 to 7.166; P=0.015), as well as cigarette smoking (OR, 4.125; 95% CI, 1.007 to 16.893; P=0.049).

*Table 2. Prevalence of hyperintense signal abnormalities among study subjects stratified by gender and agegroup* 

			Fazekas	scale of P	VH		Fazekas scale of DWMH				
0			I	<u>1 (%)</u>					<u>n (%)</u>		[
		1	2	3	р	0	1	2	3	р	
Candar	Male	16(26.2)	26(42.6)	18(29.5)	1(1.6)	0.754	7(11.5)	35(57.4)	15(24.6)	4(6.6)	0.798
Gender	Female	19(20.0)	34(35.8)	40(42.1)	2(2.1)	0.734	11(11.6)	52(54.7)	21(22.1)	11(11.6)	
	60-69	30(41.7)	24(33.3)	18(25.0)	0(0)	<0.001	14(19.4)	47(65.3)	10(13.9)	1(1.4)	< 0.001
Age(yis)	>=70	5(6.0)	36(42.9)	40(47.6)	3(3.6)	<0.001	4(4.8)	40(47.6)	26(31.0)	14(16.7)	
Total		35(22.4)	60(38.5)	58(37.2)	3(1.9)		18(11.5)	87(55.8)	36(23.1)	15(9.6)	

Table 3. Univarate analysis of hyperintense signal abnormalities

Variable	PVH*	р	DWMH*	р
BMI abnormal [n=58]	26 (44.8)	0.260	23(39.7)	0.154
Cigarette smoking [n=23]	3(13.0)	0.006	8(34.8)	0.817
Alcohol drinking [n=20]	2(10.0)	0.004	4(25.0)	0.195
Medical history				
Hypertension [n =75]	40(53.3)	< 0.001	30(40.0)	0.061
Diabetes mellitus [n=18]	9(50.0)	0.314	6(33.3)	0.951
Hypercholesterolaemia [n =71]	30(42.3)	0.461	22(31.0)	0.678
Atrial fibrillation [n=4]	2(50.0)	0.651	1(25.0)	0.740
Coronary artery diseases [n=16]	10(62.5)	0.043	6(37.5)	0.665
lacunar infarct on MRI [n=6]	5(83.3)	0.024	6(100)	0.001

\* Fazekas scale grade 2 or 3

Table 4.	Odd ratios (OR) f	for hyperintense	signal a	bnormalities	among s	study s	ubjects	associated	with
different	risk factors								

	PVH				DWMH			
	OR	95% CI		Р	OR	95% CI		Р
Age	1.102	1.033	1.177	0.003	1.189	1.101	1.284	< 0.001
Gender (female)	1.009	0.405	2.513	0.985	0.627	0.229	1.713	0.362
BMI (>=25)	1.777	0.783	4.030	0.169	2.977	1.236	7.166	0.015
Cigarette smoking	0.417	0.089	1.942	0.265	4.125	1.007	16.893	0.049
Alcohol drinking	0.137	0.024	0.771	0.024	0.232	0.046	1.158	0.075
Hypertension	2.707	1.211	6.050	0.015	1.705	0.707	4.111	0.235
Diabetes mellitus	2.029	0.651	6.329	0.223	1.328	0.385	4.582	0.654
Hypercholesterolaemia	0.911	0.407	2.040	0.821	0.545	0.228	1.306	0.173
lacunar infarct	13.747	0.521	362.880	0.117	0.000	0.000	0.000	0.999
Atrial fibrillation	2.406	0.192	30.073	0.496	0.763	0.060	9.682	0.834
Coronary artery diseases	1.521	0.427	5.414	0.518	0.674	0.184	2.472	0.552

#### Discussion

The current study provides evidence suggesting that WMH are common among cognitively normal Chinese aged 60 and older. Increasing age was significantly associated with severity of WMH. In addition, hypertension was significantly associated with risk of PVH, whereas high BMI and cigarette smoking were significantly associated with risk of DWMH, after adjusting for the effects of gender and other risk factors.

The use of visual rating scales is the most common approach to quantifying WMH on MRI [19]. Such scales yield ordinal-level data at best, and are beset by variable inter- and intra-rater reliability, ambiguous terminology, and inconsistent analyses of lesion size/quantity, location, and configuration [20-23]. Visual rating of WMH, however, is easy, and several scales are available with good reproducibility [24]. A study of 3 established rating scales (Manolio; Fazekas and Schmidt; Scheltens) studied reflect the actual volume of age related white matter changes well from 5 European centers [25]. Although visual scales often do not detail size and location, and most are not linear, they may still be preferable to other, more timeconsuming quantitative measurements in epidemiologic studies with relatively large sample size, especially in developing areas with scarce resources of computer-relied image analysis software.

The prevalence of WMH is varied with different target population and age spectrum [12, 26-31]. The Framingham Offspring Study reported 12% of extensive WMH prevalence among stoke-free subjects, among which only 52% of the study subjects were over 60 years old [12]. The LADIS study reported that 56% of European subjects aged 65-84 years old were evaluated as Fazekas score 2 or 3 [31], and 27% of moderate and severe white matter lesions were reported among 111 subjects aged 65-84 years old in Roterdam study. However, these studies did not exclude subjects with the history of stroke [27]. Moreover, data from Asian populations is sparse. Cerebral WMH was found in about 65% of healthy Korean subjects over 60 years old even subjects with stroke and lacunar infarct were excluded [30]. The current study reported 35% of the prevalence of moderate to severe WHM. The data of Chinese elderly subjects is lower than that of the Korean study. The Korean study, however, was based on the retrospective review of brain MRI and had no predefined criteria for cerebral WMH.

The presence of traditional cardiovascular risk factors (eg, increasing age, smoking, hypertension) have a well-recognized impact on vascular function and have been associated with increased WMH [29]. Interestingly in the current study, besides increasing age, hypertension was found as an independent risk factor for PVH, while high BMI and smoking were found as independent risk factors for DWMH, suggesting that PVH may arise from an underlying vascular cause, rather than DWMH. Presence of PVH may have more value to predict further occurrence of cerebrovascular diseases as well as neurodegenerative diseases.

Lacunar infarcts are attributed most commonly to deep penetrating arterial occlusion of the lenticulostriate arteries, anterior choroidal artery, paramedian branches of the basilar artery, and thalamoperforator branches of the posterior cerebral artery [32]. Lacunar infarcts are seen relatively commonly, and it is thought that most of these infarcts are thrombotic manifestations of atheromatous disease. In two large cohorts, approximately 20% of people with a mean age in the early 70s were found to have one or more silent brain infarcts [33,34] Compared to Westen studies, we found a lower prevalence (6%) of lacunar infarct on MRI in our stroke-free subjects. MRI infarcts were also found within 83.3% of subjects with PVH and 100% of subjects with DWMH (table 3). Since cerebrovascular risk factors, such as hypertension, DM, CAD, etc., were found to be significant associated with the prevalence of MRI infarcts, the impact of cerebrovascular risk factors should be adjusted to measure the net risk of MRI infarct to hyperintense signal abnormalities. Although a significant p value was undetected within this study population, we still found 13.747 of OR (Table 4), which means adjusted for other factors, an individual with MRI infarct would have 13.7 times of risk of WMH than one without MRI infarct. Similar to studies in Japan, Austria, and America [35-37], a significant association between MRI infarct and severity of WMH would be expected in Chinese population with larger sample size, and suggest that lacunar infarction and WMH share a common etiologic background.

The current cross-sectional study illustrated the prevalence data of WMH and related risk factors. A longitudinal study with large sample of study population will provide an opportunity to clarify the incidence and long-term outcome related to WMH.

#### References

- Fazekas F, Schmidt R, Scheltens P. Pathophysiologic mechanisms in the development of age-related white matter changes of the brain. Dementia and Geriatric Cognitive Disorders. 1998; 9: 2–5.
- 2. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke. 1997; 28: 652–659.
- 3. Suenaga T, Ohnishi K, Nishamura M, Nakamura S, Akiguchi I, Kimura J. Bundles of amyloid precursor protein-immunoreactive axons in human cerebrovascular white matter lesions. Acta Neuropathology. 1994; 87: 450-455.
- 4. Matsusue E, Sugihara S, Fujii S, Ohama E, Kinoshita T, Ogawa T. White matter changes in elderly people: MR-pathologic correlations. Magn Reson Med Sci. 2006; 5: 99-104.
- 5. Young VG, Halliday GM, Kril JJ. Neuropathologic correlates of white matter hyperintensities. Neurology. 2008; 71: 804-811.
- 6. Simpson JE, Hosny O, Wharton SB, Heath PR, Holden H, Fernando MS, et al. Microarray RNA expression analysis of cerebral white matter lesions reveals changes in multiple functional pathways. Stroke. 2009; 40: 369-375.
- DeCarli C, Miller BL, Swan GE, Reed T, Wolf PA, Carmelli D. Cerebrovascular and brain morphologic correlates of mild cognitive impairment in the National Heart, Lung, and Blood Institute twin study. Arch Neurol. 2001; 58: 643–647.
- Garde E, Mortensen EL, Krabbe K, Rostrup E, Larsson HB. Relation between age-related decline in intelligence and cerebral white-matter hyperintensities in healthy octogenarians: a longitudinal study. Lancet. 2000; 356: 628–634.
- 9. Gunning-Dixon FM, Raz N. Neuroanatomical correlates of selected executive functions in middle-aged and older adults: a prospective MRI study. Neuropsychologia 2003; 41: 1929–41.
- 10. Koga H, Yuzuriha T, Yao H, Endo K, Hiejima S. Takashima Y, et al. Quantitative MRI findings and cognitive impairment among community dwelling

elderly subjects. J Neurol Neurosurg Psychiatry. 2002; 72: 737–741.

- Leaper SA, Murray AD, Lemmon HA, Staff RT, Deary IJ, Crowford JR, et al. Neuropsychologic correlates of brain white matter lesions depicted on MR images: 1921 Aberdeen Birth Cohort. Radiology. 2001; 221: 51–55.
- 12. Seshadi S, Wolf PA, Beiser AS, Selhub J, Au R, Jacques PF, et al. Assocation of plasma homocysteine levels with subclinical brain injury: cerebral volumes, white matter hyperintensity ad silent brain infarcts on volumetric MRI in the Framingham offspring study. Arch Neurol. 2008; 65: 642-649.
- 13. Award IA, Spetzler RF, Hodak JA, Awad CA, Williams F, Carey R. Incidental lesions noted on magnetic resonance imaging of the brain: Prevalence and clinical signoficant in various age groups. Neurosurgery. 1987; 20: 222-227.
- 14. Ovbiagele B, Saver JL. Cerebral White Matter Hyperintensities on MRI: Current Concepts and Therapeutic Implications. Cerebrovasc Dis 2006; 22: 83-90
- Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, et al. Pathologic correlates of incidental white matter signal hyperintensities. Neurology. 1993; 43: 1683-1689.
- 16. Lazarus R, Prettyman R, Cherryman G. White matter lesions on magnetic resonance imaging and their relationship with vascular risk factors in memory clinic attenders. Int J Geriatr Psychiatry. 2005; 20: 274-279.
- Kumar R, Anstey KJ, Cherbuin N, Wen W, Sachdev PS. Association of type 2 diabetes with depression, brain atrophy, and reduced fine motor speed in a 60- to 64-year-old community sample. Am J Geriatr Psychiatry. 2008; 16: 989-998.
- 18. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5T in Alzheimer's dementia and normal aging. AJNR Am J Neuroradiol. 1987; 8: 421–426.
- 19. Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjögren M, et al. A new rating scale for age-related white matter changes applicable to MRI and CT. Stroke. 2001; 32: 1318-1322.
- 20. Bigler ED, Kerr B, Victoroff J, Tate DF, Breitner JCS. White matter lesions, quantitative magnetic resonance imaging, and dementia. Alzheimer Disease & Associated Disorders 2002; 16: 161–170.

- 21. Garrett KD, Cohen RA, Paul RH, Moser, DJ, Malloy PF, Shah P, et al. Computer-mediated measurement and subjective ratings of white matter hyperintensities in vascular dementia: Relationships to neuropsychological performance. Clinical Neuropsychologis. 2004; 18: 50–62.
- 22. Pantoni L, Garcia J. The significance of cerebral white matter abnormalities 100 years after Binswanger's report: A review. Stroke. 1995; 26: 1293–1301.
- 23. Wardlaw JM, Ferguson KJ, Graham C. White matter hyperintensities and rating scales-observer reliability varies with lesion load. Journal of Neurology. 2004; 251: 584–590.
- 24. Scheltens P, Erkinjunti T, Leys D, Wahlund LO, Inzitari D, del Ser T, et al. White matter changes on CT and MRI: an overview of visual rating scales. European Task Force on Age-Related White Matter Changes. Eur Neurol. 1998; 39: 80–89.
- 25. Kapeller P, Barber R, Vermeulen RJ, Adèr H, Scheltens P, Freidl W, et al. Visual Rating of Age-Related White Matter Changes on Magnetic Resonance Imaging: Scale Comparison, Interrater Agreement, and Correlations With Quantitative Measurements. Stroke. 2003; 34: 441-445.
- 26. Liao D, Cooper L, Cai J, Toole JF, Bryan NR, Hutchinson RG, et al. Presence and severity of cerebral white matter lesions and hypertension, and its control. The ARIC Study. Atherosclerosis risk in communities study. Stroke. 1996; 27: 2262-2270.
- 27. Breteler MMB, van Swieten JC, Bots ML, Grobbee DE, Claus JJ, van den Hout JHW, et al. Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: The Rotterdam Study. Neurology. 1994; 44: 1246-1252.
- 28. Longstreth WT, Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, et al. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Stroke. 1996; 27: 1274-1282.
- 29. Jeerakathil T, Wolf PA, Beiser A, Massaro J, Seshadri S, D'Agostino RB, et al. Stroke risk profile predicts white matter hyperintensity volume: The Framingham Study. Stroke. 2004; 35: 1857–1861.
- 30. Choi HS, Cho YM, Kang JH, Shin CS, park KS, Lee HK. Cerebral white matter hyperintensity is mainly associated with hypertension among the components of metabolic syndrome in Koreans. Clinical Endocrinology. 2009; 71: 184-188.

- 31. Korf ESC, van Straaten ECW, de Leeuw FE, van der Flier WM, Barkhof F, Pantoni L, et al. Diabetes mellitus, hypertension and medial temporal lobe atrophy: the LADIS study. Diabeticmedicine. 2007; 24: 166-171.
- 32. Ishii N, Nishihara Y, Imamura T. Why do frontal lobe symptomes predominate in vascular dementia with lacunes? Neurology. 1986; 36: 340-345.
- *33. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MMB. Silent brain infarcts and the risk of dementia and cognitive decline. N Engl J Med. 2003; 348: 1215-1222.*
- 34. Longstreth WT, Bernick C, Manolio TA, Bryan N, Jungreis CA, Price TR. Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: The Cardiovascular Health Study. Arch Neurol. 1998; 55: 1217-1225.
- 35. Kobayashi S, Okada K, Koide H, Bokura H, Yamaguchi S. Subcortical silent brain infarction as a risk factor for clinical stroke. Stroke. 1997; 28: 1932– 1939.
- 36. Schmidt R, Fazekas F, Hayn M, Schmidt H, Kapeller P, Roob G, et al. Risk factors for microangiopathyrelated cerebral damage in the Austrian Stroke Prevention Study. J Neurol Sci. 1997; 152: 15–21.
- 37. Van Zagten M, Boiten J, Kessels F, Lodder J. Significant progression of white matter lesions and small deep (lacunar) infarcts in patients with stroke. Arch Neurol. 1996; 53: 650–655.

Corresponding Author Shuguang Chu, Department of Radiology, Huashan Hospital, Fudan University, Shanghai, China, E-mail: chushu1018@hotmail.com

## Radiological classification of newly formed alveolar bone: A Cone Beam CT study

Usman Haider Uzbek<sup>1</sup>, Shaifulizan Ab. Rahman<sup>1</sup>, Mohammad Khursheed Alam<sup>2</sup>

<sup>1</sup> Department of Oral and Maxillo-Facial Surgery, Universiti Sains Malaysia, Kelantan, Malaysia,

<sup>2</sup> Orthodontic Unit, Universiti Sains Malaysia, Kelantan, Malaysia.

#### Abstract

**Objectives:** To evaluate the bone density using cone beam computed tomography scans in the region of maxillary sinus augmentation carried out using an-organic bovine bone graft and to compare the bone density values with the radiological classification of alveolar bone in the same region proposed by Norton and Gamble 2001.

Methods: Sterile freeze dried bovine bone graft produced by National Tissue Bank, Universiti Sains Malaysia was used for 1-stage implant placement with maxillary sinus augmentation in a total of 19 subjects with 19 implants. After a period of one and a half year, all subjects underwent a follow up CT scan using Planmeca Promax 3D <sup>®</sup>Cone beam computed tomography scanner .The collected data was then analyzed to evaluate bone density in Hounsfield Units using Planmeca Romexis<sup>™</sup> Imaging Software 2.2 ®

**Results:** There was bone formation at the site of the augmented sinus. Bone quality had improved one grade based upon the radiological classification by Norton and Gamble 2001 from grade 4 to grade 2/3.

**Conclusion:** An-organic bovine bone graft is a viable osteoconductive grafting material which forms a better quality of bone from what previously existed in the posterior maxillary region.

**Key words:** Cone beam CT, An-organic bovine bone graft, Bone density, Hounsfield Units.

#### Introduction

Maxillary sinus is the largest of the 4 paranasal sinuses. Its growth is progressive as the skull matures. Due to the presence of teeth, they prohibit the inferior growth of the sinus (1,2). Studies have shown that the maxillary sinus undergoes pneumatization in adults after extraction of posterior teeth (3,4). Prosthetic rehabilitation of a severely atrophic maxilla poses a challenging therapeutic problem, because bone augmentation is required to enable placement and ensure stability of a sufficient number and length of implants. To provide primary anchorage, and installation of dental implants in the posterior maxillary region where there is a lack of bone to accommodate the implants, the procedure of maxillary sinus augmentation is carried out.

There are different materials that can be used for maxillary sinus augmentation. Grafting with autogenous bone has shown the best results and a high success rate. They are often used as a baseline for the comparison of other grafting materials (5,6). The absolute biocompatibility of autogenous bone avoids the issue of graft rejection within the sinus, and exhibits osteoconductive and osteoinductive properties and vital osteogenic cells (7). Its limitations however are patient discomfort, use of general anaesthesis in cases, donor site morbidity, increased surgical time and frequently the reconstruction of large osseous defects like bilateral maxillary sinus lift may require large amounts of bone graft material (8).

This has led researchers to consider alternate grafting materials including allografts; materials derived from the same species however with a different genetic composition, xenografts; materials derived from a different species and alloplastic materials; inorganic materials such as metal, ceramic or plastic which are biocompatible, with varying degrees of success (9).

An-organic bovine bone is a xenograft which has a chemical composition and architectural geometry similar to that of human bone and can support new bone formation in direct contact to the graft An-organic bovine bone graft or Bovine bone mineral (BBM) (Bio-Oss®, Geistlich Biomaterials, Wolhusen, Switzerland) is an excellent biocompatible and osteoconductive material (1014), and has proved to be an appropriate scaffold in ridge deficiencies, periimplant destruction, and sinus augmentation procedures (15-24).

Studies have shown that an organic bovine bone mineral is a material with osteoconductive properties (16,18). In numerous experimental studies, it has been shown to facilitate growth of new bone and it has also been used successfully in humans to repair defects, augment ridges, and to raise the floor of the sinus. Host bone quality can be a decisive factor to ensure long-term clinical success of dental implants. In dental practice the surrounding bones can be qualitatively classified into four distinct classes (25). Norton and Gamble, (26) have upgraded this classification of Lekholm and Zarb, (25) by utilizing computed tomography to establish a quantitative range in Hounsfield units (HU). Details of both classifications are described in tble 1.

Although these classifications exist in literature but there is no radiological study that relates the density of new bone formed as a result of osteoconduction by the xenograft with these existing classifications. Cone-beam computed tomography has to be considered as a giant leap forward with regards to the field of dental radiology. Cone beam computed tomography provides us a medium through which we can evaluate this new bone without any invasive procedure.

#### **Materials and Methods**

Our study consisted of a total of 19 subjects with the mean age of  $51\pm4.70$ . There were 11 male and 8 female patients. All patients had undergone a maxillary sinus augmentation using the bovine bone xenograft carried out with immediate placement of dental implants in the region of the augmented sinus. All subjects were indicated for dental implants and each patient had a missing tooth in the posterior maxillary region with a bone height of less than 4mm. A total of 19 implants were placed in our region of interest in the posterior maxilla with one implant per patient. All implants had a uniform length of 10 mm

A Single/1- stage surgical protocol was carried out in which the implant placement was done in conjunction with the maxillary sinus augmentation (27). After a graft maturation period of 6 months the implant was loaded with the prosthesis. Follow up CT scans using Planmeca Promax 3D ® (Planmeca Oy, Finland) were taken 18 months after placement of the dental implant housed in the grafted region. The scanning conditions were: tube voltage 84kV, tube current 12 mA, and slice thickness 1 mm. CT images were stored in DICOM format. Patient scans analyzed using the Cone Beam CT machines computer based software; Planmeca Romexis<sup>™</sup> Imaging Software 2.2 <sup>®</sup> by which the data is analyzed in all 3 dimensions. The scans are analyzed in the 3D X-ray volume view mode.

Pre-measurement preparations included a 30 min warm-up time for the liquid crystal display (LCD) screen to attain its maximum performance, room lighting control to eliminate reflections on the screen having comfortable seat in place for the examiners (Practice guideline for digital radiography, The American College of Radiology 2007).

Our region of interest was divided into 2 sections the grafted region and the existing alveolar bone below the grafted region. The implant was divided into 2 parts of 5 mm each using the length measurement tool of the software by drawing a 10 mm line from neck of the implant embedded the alveolar bone towards the apical end of the implant embedded in the grafted region. Since a One stage /single stage sinus augmentation was carried out, 5 mm of residual bone was already present at the site on top of which the grafting material was placed to lift the maxillary sinus. Hence the measurement above 5 mm of the embedded im-

Table 1. Classification of bone density in the jaw bone according to (Lekholm and Zarb 1989) and (Norton and Gamble, 2001)

Quality (Lekholm & Zarb)	Bone density range (HU) ( Norton & Gamble )	Region of interest
Quality 1	>+850	Anterior Mandible
Quality 2/3	+500  to + 800	Posterior mandible/Anterior Maxilla
Quality 4	0 to + 500	Posterior Maxilla
Quality 4 *	< 0	Tuberosity region

Journal of Society for development in new net environment in B&H

plant apically (towards the maxillary sinus) is considered to be alveolar bone formed as a result of osteoconduction by the graft and was termed as the grafted region. To standardize the cut on which every time the measurement is taken on different subjects, a set of steps are to be made for each implant in every case. The axial view is set to the level where all implants are seen, the coronal and the sagittal reference lines are adjusted to intersect at the center of the implant to be measured. The implant then is adjusted by rotation of the view in order to obtain an image of the implant of its long axis parallel to sagittal and coronal reference lines in coronal and sagittal views respectively. In the coronal and sagittal using the length measurement tool in the software a 10 mm tangential line is drawn along the long axis of the implant. In the coronal and sagittal views the axial line is set at the 3 levels (10, 9 & 8 mm) for the grafted region. The radiographic measurements were taken at these 3 different lengths per Implant with 4 readings (palatal, buccal, mesial and distal) per point making a total of 12 readings per subject. The mean and SD of these 12 readings were then calculated.

The bone density was measured in Hounsfield unit displayed on the screen was made using the automatic option for density measurement incorporated in the software. The bone densities at the buccal and palatal bone surfaces are measured on the sagittal view screen. While the bone densities at the mesial and distal surfaces are measured on the coronal view screen.

#### Statistical analysis

For statistical analysis the latest version of  $IBM^{\ensuremath{\mathbb{R}}}$  SPSS<sup>®</sup> 20 was used. Data are presented as Mean  $\pm$  standard deviation (SD). All the recorded density readings were in Hounsfield units.

#### Results

In the grafted region for each subject the bone density values ranged of 600 HU to 900 HU. The highest mean bone density was  $832 \pm 169.73$  HU whereas the lowest bone density was  $605 \pm 226.76$  HU, illustrated in Table 2. According to these values the bone quality in the grafted region of all the subjects was classified as type 2/3 bone, shown in Table 3.

Patient	Mean (HU) SD	
1	$648 \pm 128.43$	
2	$680 \pm 241.23$	
3	$659 \pm 174.35$	
4	$773 \pm 147.20$	
5	$809 \pm 199.68$	
6	$537 \pm 174.09$	
7	$754 \pm 170.74$	
8	832 ± 169.73	
9	$765 \pm 173.95$	
10	$815 \pm 235.78$	
11	$722 \pm 222.26$	
12	$730 \pm 178.78$	
13	$605 \pm 226.76$	
14	$693 \pm 162.22$	
15	$699 \pm 158.18$	
16	$718 \pm 245.27$	
17	$710 \pm 325.80$	
18	821 ± 276.18	
19	$710 \pm 209.56$	

 Table 2. Mean Bone density of the grafted region

 for each subject in Hounsfield Unit (HU)

#### \*Hounsfield Unit

Table 3. Bone quality classification of the grafted region based on the jaw bone classification by (Norton and Gamble 2001)

Patient	Donsity	Quality		
(HU)	Density	Classification		
1	648	2/3		
2	680	2/3		
3	659	2/3		
4	773	2/3		
5	809	2/3		
6	537	2/3		
7	754	2/3		
8	832	2/3		
9	765	2/3		
10	815	2/3		
11	722	2/3		
12	730	2/3		
13	605	2/3		
14	693	2/3		
15	699	2/3		
16	718	2/3		
17	710	2/3		
18	821	2/3		
19	710	2/3		
Mean = 720				
$SD = \pm 76.22$				

\*Hounsfield Unit

#### Discussion

An-organic bovine bone is widely used in dentistry for the purpose of sinus augmentation. Various authors have reported the material to be suitable for sinus augmentation (28,29). The most commonly used product that has been reported in literature comes under the proprietary name of Bio-Oss ® (Geistlich Pharma Switzerland) (30,31). Mainly studies on the an-organic bovine bone graft have focused primarily on histomorphometric analysis of biopsy specimens (23,32).

Cone beam computed tomography is an emerging tool and is being used in dentistry for the determination of bone density in the implant recipient sites. The mainstay of studies conducted previously focused on the evaluation of bone density prior to implant placement (33). The assessment of jaw bone tissue for the purpose of dental implant serves the purpose of being a diagnostic tool to assess whether there exist sufficient amount of bone for implant treatment and as a prognostic tool to predict the probability of success or failure, as alveolar bone quality, quantity and density are important with regard to the outcome of the procedure.

A classification system for jaw bone quality referred to in frequent publications on dental implant treatment was proposed by Lekholm & Zarb (25). The system is presented as drawings of the jaws accompanied by text, and assessment methods. There also exist other classification systems that have been used for the purpose of bone assessment with regards to dental implants (34,35). These differing classification systems for bone tissue characteristics may lead to confusion and interfere with attempts to compare the results of various studies. One of the most frequently referred and compared to publication is a classification system proposed by Norton and Gamble (26). Many recent publications that have studied the alveolar bone and reported on bone density in Hounsfield unit using computed tomography scans have compared their results with this publication (36-38). We chose to compare our results with the Norton and Gamble (26) study, the reason being that it is a publication that is frequently used as reference in literature and that the authors utilized a interactive software program to measure the bone density in Hounds field units. The author's also established quantitative ranges are applied to the subjective quality classification as previously described (25).

Based upon the Norton and Gamble classification system the bone density range for the posterior maxilla is 0 to +500 Hounsfield Units which is classified as Type 4 bone. Our results are in agreement to these findings as our recorded bone density values for the alveolar bone that lies beneath the augmented region with the graft was between 261 & 398 Hounsfield Units. Clinical studies have also indicated that region of the posterior maxilla exhibits the highest failure rate for dental implantation due to the fact that this region frequently lacks adequate volume and/or has a lower density of bone compared to other regions of the human jaw (39,40). In our study although the sample size had the limitation of being relatively small, there was no implant failure. This can be attributed to one of the fact that the one part of the implant was embedded in the existing alveolar bone of low density and the other half was embedded in the new bone formed as a result of the osteoconduction nature of our graft. The bone density of this 'newly formed alveolar bone' was recorded to be in the range of 537 and 832 Hounsfield Units. This is our pilot finding, and according to the classification of Norton and Gamble it falls in the category of Type 2/3 bone that is present in the posterior mandible and anterior maxilla. Irrespective of gender, age group or the implant diameter used the bone quality improved vastly in the region of the augmented sinus. The bone density ranged jumped up from a quality 4 to a quality 2/3 at the same site i.e. the posterior maxillary bone. Bone density is a key factor to take into account when predicting implant stability. This improvement of quality can be related to the success rate of our study as all 19 subjects had functioning, osseointegrated dental implants. Osseointegration is the mode of tissue integration around a healed functioning implant in which the primary load bearing tissue at the interface is bone (41).

These results agree with a recent study by (42)2011 on the relation between bone density and primary implant stability which reports that a higher bone density value (HU) leads to a higher primary implant stability, Hounsfield Units are considered to be a valid diagnostic parameter to possibly predict the stability of implants.

#### Conclusion

In the posterior maxillary region, the existing alveolar bone was of a low quality as compared to the rest of the human jaw bone as proposed by the Norton and Gamble classification. However, post maxillary augmentation by the xenograft has significantly produced a higher bone quality in the posterior maxillary region irrespective of the existing bone quality which translates into a better implant stability.

#### References

- 1. Misch CE. Maxillary sinus augmentation for endosteal implants: organized alternative treatment plans. Int J Oral Implantol. 1987; 4(2): 49-58.
- 2. Sharan A, Madjar D. Maxillary sinus pneumatization following extractions: a radiographic study. Int J Oral Maxillofac Implants. 2008; 23(1): 48-56.
- 3. Kosko JR, Hall BE, Tunkel DE. Acquired maxillary sinus hypoplasia: a consequence of endoscopic sinus surgery? Laryngoscope. 1996; 106(10): 1210-3.
- 4. Ikeda A, Ikeda M, Komatsuzaki A. A CT study of the course of growth of the maxillary sinus: normal subjects and subjects with chronic sinusitis. ORL J Otorhinolaryngol Relat Spec. 1998; 60(3): 147-52.
- 5. Barone A, Crespi R, Aldini NN, Fini M, Giardino R, Covani U. Maxillary sinus augmentation: histologic and histomorphometric analysis. Int J Oral Maxillofac Implants. 2005; 20(4): 519-25.
- 6. Stavropoulos A. Deproteinized Bovine Bone Xenograft. In: Pietrzak, W. (ed.), Musculoskeletal Tissue Regeneration: Humana Press. 2008: 119-151.
- 7. Regev E, Smith RA, Perrott DH, Pogrel MA. Maxillary sinus complications related to endosseous implants. Int J Oral Maxillofac Implants. 1995; 10(4): 451-61.
- 8. Gerressen M, Hermanns-Sachweh B, Riediger D, Hilgers RD, Spiekermann H, Ghassemi A. Purely cancellous vs. corticocancellous bone in sinus floor augmentation with autogenous iliac crest: a prospective clinical trial. Clin Oral Implants Res. 2009; 20(2): 109-15
- Seong WJ, Barczak M, Jung J, Basu S, Olin PS, Conrad HJ. Prevalence of sinus augmentation associated with maxillary posterior implants. J Oral Implantol. 2011, Jun 8. [Epub ahead of print] http: // dx.doi.org/10.1563/AAID-JOI-D-10-00122
- 10. Spector M. Anorganic bovine bone and ceramic analogs of bone mineral as implants to facilitate bone regeneration. Clin Plast Surg. 1994; 21(3): 437-44.

- 11. Berglundh T, Lindhe J. Healing around implants placed in bone defects treated with Bio-Oss. An experimental study in the dog. Clin Oral Implants Res. 1997; 8(2): 117-24.
- 12. Hammerle CH, Olah AJ, Schmid J, Fluckiger L, Gogolewski S, et al. The biological effect of natural bone mineral on bone neoformation on the rabbit skull. Clin Oral Implants Res. 1997; 8(3): 198-207.
- 13. Skoglund A, Hising P, Young C. A clinical and histologic examination in humans of the osseous response to implanted natural bone mineral. Int J Oral Maxillofac Implants. 1997; 12(2): 194-9.
- Artzi Z, Nemcovsky CE. The application of deproteinized bovine bone mineral for ridge preservation prior to implantation. Clinical and histological observations in a case report. J Periodontol. 1998; 69(9): 1062-7.
- 15. Smiler DG, Johnson PW, Lozada JL, Misch C, Rosenlicht JL, Tatum OH Jr, Wagner JR. Sinus lift grafts and endosseous implants. Treatment of the atrophic posterior maxilla. Dent Clin North Am. 1992; 36(1): 151-88.
- Wetzel AC, Stich H, Caffesse RG. Bone apposition onto oral implants in the sinus area filled with different grafting materials. A histological study in beagle dogs. Clin Oral Implants Res. 1995; 6(3): 155-63.
- 17. Dies F, Etienne D, Abboud NB, Ouhayoun JP. Bone regeneration in extraction sites after immediate placement of an e-PTFE membrane with or without a biomaterial. A report on 12 consecutive cases. Clin Oral Implants Res. 1996; 7(3): 277-85.
- Hurzeler MB, Quinones CR, Kirsch A, Gloker C, Schupbach P, et al. Maxillary sinus augmentation using different grafting materials and dental implants in monkeys. Part I. Evaluation of anorganic bovine-derived bone matrix. Clin Oral Implants Res. 1997; 8(6): 476-86.
- 19. Valentini P, Abensur D, Densari D, Graziani JN, Hammerle C. Histological evaluation of Bio-Oss in a 2-stage sinus floor elevation and implantation procedure. A human case report. Clin Oral Implants Res. 1998; 9(1): 59-64.
- Piattelli M, Favero GA, Scarano A, Orsini G, Piattelli A. Bone reactions to anorganic bovine bone (Bio-Oss) used in sinus augmentation procedures: a histologic long-term report of 20 cases in humans. Int J Oral Maxillofac Implants. 199; 14(6): 835-40.
- 21. Artzi Z, Nemcovsky CE, Tal H, Dayan D. Histopathological morphometric evaluation of 2 different hydroxyapatite-bone derivatives in sinus augmentation procedures: a comparative study in humans. J Periodontol. 2001a; 72(7): 911-20.

- 22. Artzi Z, Tal H, Dayan D. Porous bovine bone mineral in healing of human extraction sockets: 2. Histochemical observations at 9 months. J Periodontol. 2001b; 72(2): 152-9.
- 23. Hallman M, Hedin M, Sennerby L, Lundgren S. A prospective 1-year clinical and radiographic study of implants placed after maxillary sinus floor augmentation with bovine hydroxyapatite and autogenous bone. J Oral Maxillofac Surg. 2002a; 60(3): 277-86.
- 24. Hallman M, Lundgren S, Sennerby L. Histologic analysis of clinical biopsies taken 6 months and 3 years after maxillary sinus floor augmentation with 80% bovine hydroxyapatite and 20% autogenous bone mixed with fibrin glue. Clin Implant Dent Relat Res. 2001; 3(2): 87-96.
- 25. Lekholm U, Zarb GA. Patient selection and preparation, in tissue integrated Prostheses: Osseointegration in Clinical Dentistry, Branemark PI, Zarb GA, Alberktsson T. Eds. Quintessence, Chicago, Ill, USA, 1985: 199–209.
- 26. Norton MR and Gamble C. Bone classification: an objective scale of bone density using the computerized tomography scan. Clin Oral Implants Res. 2001; 12(1): 79-84.
- 27. Cordioli G, Mazzocco C, Schepers E, Brugnolo E, Majzoub Z. Maxillary sinus floor augmentation using bioactive glass granules and autogenous bone with simultaneous implant placement. Clin Oral Implants Res. 2001; 12(3): 270-278.
- 28. Degidi M, Piattelli M, Scarano A, Iezzi G, Piattelli A. Maxillary sinus augmentation with a synthetic cell-binding peptide: histological and histomorphometrical results in humans. J Oral Implantol. 2004; 30(6): 376-83.
- 29. Schlegel KA, Rupprecht S, Petrovic L, Honert C, Srour S, et al. Preclinical animal model for de novo bone formation in human maxillary sinus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009; 108(3): e37-44.
- 30. Araujo M, Linder E, Lindhe J. Effect of a xenograft on early bone formation in extraction sockets: an experimental study in dog. Clin Oral Implants Res. 2009; 20(1): 1-6.
- 31. Froum SJ, Wallace S, Cho SC, Rosenburg E, Froum S, Schoor R, Mascarenhas P, Tarnow DP, Corby P, Elian N, Fickl S, Ricci J, Hu B, Bromage T, Khouly I. A histomorphometric comparison of bio-oss alone versus bio-oss and platelet-derived growth factor for sinus augmentation: a postsurgical assessment. Int J Periodontics Restorative Dent. 2013; 33(3): 269-79.
- 32. Maiorana C, Beretta M, Battista Grossi G, Santoro F, Scott Herford A, Nagursky H, Cicciu M. Histomorphometric evaluation of anorganic bovine bone coverage to reduce autogenous grafts resorption: preliminary results. Open Dent J. 2011; 5: 71-8.

- *33.* Shapurian T, Damoulis PD, Reiser GM, Griffin TJ, Rand WM. Quantitative evaluation of bone density using the Hounsfield index. Int J Oral Maxillofac Implants. 2006; 21(2): 290-7.
- 34. Misch CE. Density of bone: effect on treatment plans, surgical approach, healing, and progressive boen loading. Int J Oral Implantol. 1990; 6(2): 23-31.
- 35. Gaucher H, Bentley K, Roy S, Head T, Blomfield J, Blondeau F, Nicholson L, Chehade A, Tardif N, Emery R. A multi-centre study of Osseotite implants supporting mandibular restorations: a 3-year report. J Can Dent Assoc. 2001; 67(9): 528-33.
- 36. Turkyilmaz I, Tozum TF, Tumer C. Bone density assessments of oral implant sites using computerized tomography. J Oral Rehabil. 2007; 34(4): 267-72.
- 37. Hiasa K, Abe Y, Okazaki Y, Nogami K, Mizumachi W, Akagawa Y. Preoperative computed tomographyderived bone densities in hounsfield units at implant sites acquired primary stability. ISRN Dent. 2011; 2011: 678729.
- 38. Ribeiro-Rotta RF, Lindh C, Pereira AC, Rohlin M. Ambiguity in bone tissue characteristics as presented in studies on dental implant planning and placement: a systematic review. Clin Oral Implants Res. 2011; 22(8): 789-801.
- 39. Jemt T, Lekholm U. Implant treatment in edentulous maxillae: a 5-year follow-up report on patients with different degrees of jaw resorption. Int J Oral Maxillofac Implants. 1995; 10(3): 303-11.
- 40. Kaptein ML, De Lange GL, Blijdorp PA. Peri-implant tissue health in reconstructed atrophic maxillae--report of 88 patients and 470 implants. J Oral Rehabil. 1999; 26(6): 464-74.
- 41. Branemark PI, Svensson B, Van Steenberghe D. Tenyear survival rates of fixed prostheses on four or six implants ad modum Brånemark in full edentulism. Clin Oral Implants Res. 1995; 6(4): 227-231.
- 42. Farre-Pages N, Auge-Castro ML, Alaejos-Algarra F, Mareque-Bueno J, Ferres-Padro E, Hernandez-Alfaro F. Relation between bone density and primary implant stability. Med Oral Patol Oral Cir Bucal. 2011; 16(1): e62-7.

Corresponding Author Mohammad Khursheed Alam, Senior Lecturer, Orthodontic Unit, School of Dental Sciences, Universiti Sains Malaysia, Kelantan, Malaysia, E-mails: dralam@gmail.com, dralam@kk.usm.my

### Relevance of evaluation for quality assurance in the field of health education work - evaluation of midwifery studies

#### Anita Jug Dosler, Metka Skubic

Faculty of Health Sciences, Department of Midwifery, University of Ljubljana, Slovenia.

#### Abstract

The article gives an overview and theoretical foundations about the relevance of evaluation in the field of health education work and presents empirical research that deals with the existing practice in the field of midwifery education at university level. Evaluation survey is based on an empirical survey research approach and quantitative research paradigms. An causal and non-experimental method of empirical research is used. The quantitative data analysis has been carried out on the descriptive statistics level. Factor analysis has been used to examine the relationship between the observed variables. Results show that a relatively large percentage of students suggested to change the study programme, especially in the following areas: (1) carrying out practical work and internships at the clinic, (2) organisation of studies, (3) integration of students in research activities at the Faculty of Health Sciences and at the clinic, (4) giving feedback on personal progress (5) preparing students on how to deal with psychological consequences and effects of traumatic experiences, (6) interdisciplinary approach in the midwifery education process. The research presents that evaluation in the field of health education work is important and efficient in supporting and improving teachers' s educational work and their professional development, especially on a process level, fields and quality indicators. In the field of health education work, a system of internal and external evaluating of educational programs could be one of the future options, where educational institutions could evaluate their education programs and clinical practices for students from the perspective of different target groups in different fields and in different quality levels.

Key words: Education, evaluation, studies, midwife.

#### Introduction

Developing the ability of "deliberate learning" from practical work, self-questioning and selfreflexion, developing the ability of examining and coping with subjective theories, ideas and concepts about one's own position and the integration of all these subjects into new findings in the profession and research achievements is becoming an ever more important task in the process of ensuring higher quality in the field of health education work for future midwives/nurses and must begin already during their studies. The long tradition of midwifery education in Slovenia and the clearly defined role of a midwife, according to international definitions in the public and the private health care sector, present a good starting point for examining and researching the quality and efficiency of midwifery education in Slovenia. The evaluation of educational programmes and educators, which was in our case done, can show educators ability to overcome traditional educational methods and introduce new, modern approaches in the process of midwifery students' education, irrespective of health policy and educational system. In that way evaluation allows us the internal quality control of an institution and reflection on the extent to which the goals and objectives have been achived, and provides feedback on the implementation of the educational program, enabling further actions to be planned.

#### Goal

The article gives an overview and an analysis of theoretical foundations and definitions about the evaluation process and presents an empirical research which is focused on the students' view and their perception of midwifery studies with the existing clinical and education practice in the field of midwifery education at university level. The research presents that evaluation in the field of health education work is important and efficient in supporting and improving teachers' s educational work and their professional development.

## *Relevance of evaluation in the field of health education work*

Different definitions of the word evaluation can be found in literature. Many of them, which are presented in this article, define evaluation as a systematic collection of data about a phenomenon with the aim to evaluate it. Some authors added to this definition by saying that an evaluation is a systematic and critical analysis with the basic intention of assessment, planning and improvement (Patton, 1987; Norris, 1993; Fereday, Collins, Turnbull, Pincombed, Osterd, 2009). Information gathering is linked to individual criteria, measurements and statistic procedures with the purpose of developing a rational basis for judgement in crucial situations (Stufflebeam, Madaus, Scriven, 2000; Dunt 2009; Busari, 2012). Evaluation in the field of health education work is usually part of any learning programme or education, as it is a part of the midwifery/nursing care process cycle (Sekelani, 2008; Dunt, 2009; Busari, 2012; Fawcett, 2009; Fereday, Collins, Turnbull, Pincombed, Osterd, 2009).

Quality and education of health professionals is one of the important concerns in health worldwide (Busari, 2012; WHO, 2011; Knapp, Bennett, Plumb, Robinson, 2000). Studies (Busari, 2012; McCann et al., 2012; Fentahun and Molla, 2012; WHO, 2011; McClain et al., 2012; Engbers, De Caluwé, Stuyt, Fluit, Bolhuis, 2013) in the wider international context have shown that reforms in education must be informed by community health and education needs and evaluated with respect to how well they serve these needs. Stronger collaboration between the education and health sectors, other national authorities, and the private sector can improve the match between health professional education and the realities of health service delivery. Educational institutions need to increase capacity and reform recruitment, teaching methods and curricula in order to improve the quality and the social accountability of graduates (Sekelani, 2008; Barrow, Lyte, Butterworth, 2002; McCann et al., 2012). The international community has an important role to play by partnering to support country-led efforts.

This can bring about a new era for health professional education (WHO, 2011).

To raise the quality of health education work with the help of an evaluation, it is necessary to examine the work of a health education institution as a whole from the perspective of different target groups (the medical, higher management and educational staff, health education service users, public and private health care etc.), in different fields and in different quality levels. The purpose of an evaluation is to have an influence on the following fields based on data that has been collected as objectively as possible: (1) decision making, formulation of rules and functioning of institutions; (2) evaluation of the health and socioeconomic efficiency of the programmes carried out by an institution; and (3) quality assurance of health education work (Licqurish and Seibold, 2008; Hughes and Fraser, 2011; Dykeman and Cruttenden, 2009). The first phase encompasses an examination of the situation in an establishment or an institution (e.g. where are the problems arising and in which areas and levels of health education work quality are they arising). Afterwards we define the research problem (what is going to be pursued). Quality assurance, in a narrower sense is only possible when we have evaluated the situation and acquainted ourselves with the whole topic from different points of view. Due to the fact that the evaluation object is adapting itself to the sociocultural context in which the evaluation is taking place, it is difficult to precisely define universally applicable standards for its planning and evaluation implementation in advance. The explicitness of planning and evaluating the effects of our own health education work requires us to have the cognitive capabilities of planning, tracking and selfevaluating the quality of our own activities. In this way an evaluation process is also very important for medical staff's own professional development.

#### Methods

The study was based on an empirical survey research approach and quantitative research paradigms. An causal and non-experimental method of empirical research was used.

In our evaluation survey we especially focused on the students' perception of midwifery studies and therefore, the following assertions and research questions have been addressed: (1) What is the students' overall evaluation of the educational programme?, (2) How do students feel about and evaluate the study programme, its organisation and realisation, its content and quality?, (3) Advantages and disadvantages of the study programme, or of such types of education for future midwives.

#### Instrument description

Beside general data about the respondents the evaluation questionnaire contained a total of 30 Likert-type statements and one descriptive evaluation scale. The evaluation scales had five response categories, where category 1 meant I disagree completely, category 2 mostly disagree, category 3 neither disagree nor agree, category 4 mostly agree and category 5 completely agree. In the course of this evaluation, experts have, on the ground of previous theories and findings, evaluated whether if the categories or questions were clear, comprehensive and unambiguous (Polit et al., 2001; Dunt, 2009; Dykeman and Cruttenden, 2009).

Measured on the basis of Cronbach's alpha coefficient, the evaluation scales have a sufficient reliability rate (the internal consistency coefficient ranges from 0.70 to 0.87) and validity (in all cases a minimum of 27.74 % variance was explained with the first factor). Reliability was also controlled with a factor analysis. According to the equation  $r_{tt} \ge \sqrt{h^2}$  the aforementioned part of the questionnaire reached a good reliability rate ( $r_{tt} > 0.71$ ). Validity has also been achieved with the method of expert evaluations. Objectiveness was achieved by giving the respondents clear instructions prior to filling out the questionnaire.

#### Ethical aspects of the study

The students voluntarily participated in the study on the basis of a declaration of voluntary participation and personal data protection. In line with the confidentiality agreement, the researchers have bound themselves to use the collected data for research purposes only. The study was approved by the departmental faculty ethics committee.

#### Sample description

The questionnaire was completed by 52 fulltime degree students (100.0 %) in their second (n=28) and third (n=24) year of Midwifery at the Faculty of Health Sciences in Ljubljana (Slovenia). Five (9.6 %) of the sample were male and 47 (89.4 %) were female. The average age was 20.3 years (standard deviation 1.31). 22 (42.3%) were single, 26 (50.0 %) lived in a cohabitation relationship and 4 (7.7%) were married.

The midwifery study programme at the Faculty of Health Sciences in Ljubljana is the only midwifery study programme in Slovenia. Each year the number of students admitted to the programme is limited to 30.

#### Data processing

Data have been processed with the SPSS 20.0 software package. The quantitative data analysis has been carried out on the descriptive statistics level, where the frequency distribution (f. f%) for attributive variables and the basic descriptive statistic for numeric variables (measures of central tendency, measures of dispersion) has been used. To determine the validity of the measure instrument a factor analysis has been used (% of explained variance by the first factor) and for the determination of reliability, Cronbach's alpha coefficient has been applied. Factor analysis has also been used to examine the relationship between the observed variables. It has been carried out with variables that met the criteria of factorisation. We used the Varimax rotation method. The adequacy of a correlation matrix for factorisation was assessed with the KMO test that has a value of 0.807 and the Bartlett's test (a value of 743.432, g=465, p=0.000) that is of high statistical importance. Both test results showed that a factor analysis is reasonable.

#### **Results and Discussion**

We wanted to find out if the relationships between the observed variables could be explained by a smaller number of directly observed variables or factors, and on this basis, explain the content of common factors, which best explain the relationships between the observed variables. Factors were determined by different numbers of questions that bring to light the chosen aspects of each concept. Factor analysis results were also incorporated in the findings presentation and proved that the questionnaire terms were well defined.

In the factor analysis, six factors have been defined: (1) carrying out practical work and internships at the clinic (explained variance 27.7%), (2) organisation of studies (explained variance 19.7%), (3) integration of students in research activities at the Faculty of Health Sciences and at the clinic (explained variance 9.8%), (4) giving feedback on personal progress (explained variance 6.1%), (5) preparing students on how to deal with psychological consequences and effects of traumatic experiences in form of individual therapies and supervisions (explained variance 4.3%), (6) interdisciplinary approach in the midwifery education process (explained variance 2.6%). These explain 70.3 % of the common variance, where by communalities for most variables are higher than 0.65 and no variable value is lower than 0.60. If the condition is set that the eigenvalues have to be higher than 1, the variables form 6 factors with a 70.3 % common variance explanation rate.

In the following discussion, an overview of factors is given according to the average score height, as well as a content analysis of the results according to corresponding factors that have been conducted through the factor analysis.

New variables or factors were arranged into three categories according to the average score height into three categories. Table 1 shows that in category I, which has the highest average score (M > 4.40), the factors "interdisciplinary approach in the midwifery education process" and "organisation of studies" are listed. Category II, with an average score of 3.90 < M < 4.40, comprises three factors: preparing students on how to deal with psychological consequences and effects of traumatic experiences in form of individual therapies and supervisions, carrying out practical work and internships at the clinic and giving feedback on personal progress. In category III, the interval with the lowest average score (3.00 < M < 3.90), the factor "integration of students in research activities at the Faculty of Health Sciences and at the clinic" is listed.

Table 2 shows that most students are of the opinion that, in the framework of the study programme, more foreign and reputable professors from the midwifery field and other related medical disciplines should be invited to hold lectures (M=4.59, R=1).

Table 3 shows that in the category II, second and third year full-time degree students gave the highest score to the variable: *My wish is that the practical work at the clinic would be organised in one unit (e. g. in one semester)* (M=4.39, R=1).

Data shown in table 4 indicate that, out of all observed variables ranked in category III on the basis of the factor analysis results, midwifery students prefer to see themselves in the field of research activities at the Faculty of Health Science (M=3.87, R=1).

#### Conclusions

What our study exposes in the nursing/midwifery education and nursing/midwifery practice in the international context?

In our research we have measured how second and third year full-time degree students evaluated the undergraduate Midwifery study programme at the Faculty of Health Science, which is the only study programme in Slovenia. Our goal was to examine how students feel about and evaluate the

Category	Average (mean) score interval	Factors	
I more than 4.40	more than 1.40	6	interdisciplinary approach in the midwifery education process
	2	organisation of studies	
II from 3.90 to 4.40	5	preparing students on how to deal with psychological consequences and effects of traumatic experiences in form of individual therapies and supervisions	
		1	carrying out practical work and internships at the clinic
		4	giving feedback on personal progress
III	from 3.00 to 3.90	3	integration of students in research activities at the Faculty of Health Sciences and at the clinic

Table 1. Overview of the results and factors according to the average score height
Statement	Mean	Std. deviation	Rank
The published learning programme contains insufficient information.	4.40	1.174	7
It bothers me that professors who do not give lectures are listed in the learning programme.	4.41	1.236	5.5
At enrolment the midwifery profession was presented as an established profession regulated by law.	4.45	1.340	3
I am of the opinion that all midwifery studies candidates should be obliged to take aptitude or admission tests.	4.41	1.098	5.5
It is necessary to define clear rules about the fulfilment of study obligations, non-attendance at courses, practical work and internships.	4.52	0.874	2
In the framework of the study programme more foreign and reputable professors from the midwifery field and other related medical disciplines should be invited to hold lectures.	4.59	0.863	1
I would like to have more information about further education possibilities in Slovenia or abroad and get more support from the coordinator for international affairs concerning working abroad during my studies.	4.42	1.257	4

Table 2. Variables distribution of frequencies that according to the average score interval have been classified as category I: interdisciplinary approach in midwifery educational process and organisation of studies

Table 3. Variables distribution of frequencies that according to the average score interval have been classified as category II: preparing students on how to deal with psychological consequences and effects of traumatic experiences in form of individual therapies and supervisions, carrying out practical work and internships at the clinic, giving feedback on personal progress

Statement	Mean	Std. deviation	Rank
Students should be prepared on how to deal with possible psychological consequences or effects of traumatic experiences before the start of practical work and internships at the clinic.	4.32	0.913	4
Each student should be given more opportunities for independent work under the supervision of a mentor during practical work and internship at the clinic.	4.38	0.776	2
Individual therapies in form of inter- and supervisions should be provided for students who need or wish it.	3.94	1.789	9
I would like to have more feedback on my personal progress from university lecturers – professors.	4.31	1.326	5
I would like to have more feedback on my personal progress from my mentor at the clinic.	4.35	1.034	3
I would like to have more feedback on my personal progress from the university staff – assistants.	4.02	1.210	7
My wish is that the practical work at the clinic would be organised in one unit (e. g. in one semester)	4.39	1.377	1
I think that the mentors at the clinic are not well informed about the newest approaches in midwifery.		1.265	8
I wish the premises with simulation machines would be better equipped.	4.24	1.256	6

Table 4. Variables distribution of frequencies that according to the average score interval have been classified as category III: integration of students in research activities at the Faculty of Health Sciences and at the clinic

Statement		Std. deviation	Rank
I wish to be integrated into research activities at the Faculty of Health Science.	3.87	0.762	1
I wish to be integrated into research activities at the clinic.	3.09	0.983	3
I wish to do more research work	3.01	1.334	2

programme, its organisation and implementation, its content and quality and how they experience the approachability and responsiveness of professors and clinical mentors. The reasons to research this topic were findings of some foreign surveys (Barrow, Lyte, Butterworth, 2002; Fawcett, 2005; Fereday, Collins, Turnbull, Pincombed, Osterd, 2009) that, as opposed to our research, focused on specific aspects of midwifery studies and not directly on the student population. Key findings of such surveys have shown that the authors were too much focused on theories and models, clinical experiences, programme contents, students' stress and problems of inequality. They paid too little attention on understanding the viewpoints, positions, interests and wishes of students in the educational process itself. Therefore, our survey contains a wide variety of themes and presents an attempt to describe the experiences with, and the evaluation of, the educational system in Slovenia from the viewpoint of students undergoing midwifery education at the time of the evaluation. At the same time, we point out that although our survey sample is not representative, a certain trend regarding students' evaluation can nevertheless be observed. Results have shown that a high percentage of students suggest programme changes referring to: (1) carrying out practical work and internships at the clinic, (2) organisation of studies, (3) integration of students in research activities at the Faculty of Health Sciences and at the clinic, (4) giving feedback on personal progress (5) preparing students on how to deal with psychological consequences and effects of traumatic experiences in form of individual therapies and supervisions, (6) interdisciplinary approach in the midwifery educational process. The present findings represent a starting point for the preparation of a new educational programme for midwives in Slovenia. They show the necessity for a more detailed implementation of positive changes. Ability to critically evaluate the education programme and use evidence in practice was regarded highly. Student outcomes are showing some evidence of possible divergence of grades in the study programme. All these changes have been pointed out by students and recognised by evaluation and analysis as well as a wider discussion about this topic, that would lead to the profession in form of the preparation of a new Midwifery bachelor study programme in Slovenia. The manner and context of implementation of the educational change may have important implications for students' learning experiences and also has the potential to contribute to the profession by providing evaluation information for educatiors, about how to provide an optimal education and clinical learning environment. So, in the field of health education work, a system of internal and external evaluating of educational programs could be one of the future options, where educational institutions could evaluate their education programs and clinical practices for students from the perspective of different target groups in different fields and in different quality levels.

Based on our results the evaluation research have several possibilities for further investigation and interpretation in the international context. Research of nursing/midwifery education and practice is one of the important factors for establishing and ensuring the quality of educational process. This is one of the main reasons why the evaluation is have to be formally recognized and standardized. It (1) allows us the internal quality control of an institution and reflection on the extent to which the goals and objectives have been achived, and (2) provides feedback on the implementation of the educational program (education and practice), enabling further actions to be planned. The effectiveness of teaching would be substantially improved if teaching were a research-based profession and if educational practioners were to play an active role in carrying out evaluation process. The discourse of the reflective practitioner emphasizes the particular skills needed to reflect constructively upon ongoing experience as a way of improving the quality and effectiveness of one's work. The discourse encourages teachers to take into account the whole picture – analysing the effectiveness of study programme through an attempt to evaluate what was learned, by whom, and how more effective nursing/midwifery education and nursing/midwifery practice might take place in the future. In conjunction with this, our results of the evaluation research have also shown on factors of »hidden curriculum« in correlation to the quality of educational process, which can simply not be disregarded. They must be professionally devised and regularly as well as reflectively applied to the

course of planning, implementation and evaluation of educational process. We can concluded that evaluation in the field of health education work is important and efficient in supporting and improving teachers' s educational work and their professional development, especially on a process level, fields and quality indicators.

# References

- 1. Barrow JE, Lyte G, Butterworth T. An evaluation of problem based learning in a nursing theory and practice module. Nurse Education in Practice. 2002; 2: 55-62.
- 2. Busari JO. Comparative analysis of quality assurance in health care delivery and higher medical education. Adv Med Educ Pract. 2012; 3: 121-127.
- 3. Dunt D. Levels of project evaluation and evaluation study design. In: Jirojwong S, Liamputtong P (Eds). Population Health, Communities and Health Promotion. Melbourne: Oxford University Press; 2009; 267-283.
- Dykeman M, Cruttenden K. Frameworks of project Evaluation. In: Jirojwong S, Liamputtong P (Eds). Population Health, Communities and Health Promotion. Melbourne: Oxford University Press; 2009; 253-267.
- 5. Engbers R, De Caluwé LI, Stuyt PM, Fluit CR, Bolhuis S. Towards organizational development for sustainable high-quality medical teaching. Perspect Med Educ. 2013; 2: 28-40.
- 6. Fawcett J. Contemporary nursing knowledge: Analysis evaluation of nursing models and theories. Philadelphia: UPA; 2009; 13-49.
- 7. Fentahun N, Molla A. Determinants of and opportunities for continuing education among health care professionals in public health care institutions in Jimma township, Southwest Ethiopia. Adv Med Educ Pract. 2012; 18: 89-96.
- 8. Fereday J, Collins C, Turnbull D, Pincombed J, Osterd C. An evaluation of Midwifery group practice and educational process. Women and Birth. 2009; 22: 11-16.
- 9. Hughes A, Fraser DM. The experience of newly qualified midwives in England. Midwifery. 2011; 27: 382-386.
- 10. Knapp ML, Bennett NM, Plumb JD, Robinson JL. Community-based quality improvement education for the health professions: balancing benefits for communities and students. Journal of Interprofessional Care. 2000; 14: 119-130.

- 11. McCann E, Higgins A, Maguire G, Alexander J, Watts M, et al. A survey of pedagogical approaches and quality mechanisms used in education programs for mental health professionals. Journal of Interprofessional Care. 2012; 26: 383-389.
- 12. McClain EK, Babbott SF, Tsue TT, Girod DA, Clements D, et al. Use of a structured template to facilitate practice-based learning and improvement projects. J Grad Med Educ. 2012; 4: 215-219.
- 13. Licqurish S, Seibold C. Bachelor of Midwifery students' experiences of achieving competencies: The role of the midwife preceptor. Midwifery. 2008; 24: 480-489.
- 14. Norris N. Understanding Educational Evaluation. London: Kogan Page; 1993; 9-52.
- 15. Patton M. How to Use Qualitative Methods in Evaluation. London, New Delhi: Sage Publications; 1987; 8-49.
- 16. Polit DF, Beck CT, Hungler BP. Essentials of nursing research: methods, appraisals and utilization. Philadelphia: Lippen-cott; 2001; 7-42.
- 17. Sekelani SB. Teaching and Teacher Education for Health Professionals: Perspectives on Quality and Outlook of Health Professionals Education in Zambia. Medical Journal of Zambia. 2008; 35: 70-74.
- Stufflebeam DL, Madaus GF, Scriven M. Evalvation models: Viewpoints on Educational and Human Services Evaluation. Boston: Kluwer-Nijhoff Publishing; 2000; 24-45.
- 19. World Health Organization. Transformative scale up of health professional education. Switzerland: WHO Press; 2011; 1-20.

Corresponding Author Anita Jug Dosler, Faculty of Health Sciences, Department of Midwifery, University of Ljubljana, Ljubljana, Slovenia, E-mail: anita.jug@zf.uni-lj.si

# Imported visceral Leishmaniasis in western Romania: Report of four cases

# Iosif Marincu, Nicoleta Bertici, Livius Tirnea

Department of Infectious Diseases, Pneumology and Parasitology, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania.

#### Abstract

The imported tropical pathology is frequently associated with diagnosis and treatment difficulties. We present 4 cases of imported visceral leishmaniasis (VL) from Western Romania within the period 2004-2011. The diagnoses were made on epidemiologic elements, clinical symptoms and biological tests. The confirmation of the clinical diagnoses was performed by Giemsa-stained blood smears obtained via bone marrow biopsy. The period between the first medical consultation and confirmation of VL diagnosis was 4–10 weeks. It requires the application of rigorous epidemiological control measures and early diagnosis of import diseases from all patients returning to the country from different geographical areas.

**Key words:** Visceral leishmaniasis, amastigotes, diagnosis, symptoms.

# Introduction

Leishmaniasis is an important vector-borne disease and is the only tropical disease that has been endemic to 88 countries worldwide and southern European regions for decades. In southern Europe, most reported cases are due to zoonotic VL, which is the most dangerous form and proves lethal when left untreated (1).

VL is endemic in more than 60 countries in tropical and subtropical areas and in Mediterranean countries (2). About 350 million people are at risk of contracting leishmaniasis, one of the most neglected tropical diseases (3). It is known that VL is a broad clinical spectrum disease, so atypical forms of presentation are relatively frequent (4).

In Romania, the imported tropical pathology is associated with diagnosis and treatment difficulties and doctors have no experience in this field. The increase in the number of Romanians travelling after the year 2000 throughout the Mediterranean has led to a growing number of imported leishmaniasis cases. Here we report the clinical and epidemiological peculiarities of imported VL from Western Romania within the period 2004-2011.

#### **Cases report**

The authors have retrospectively studied 4 cases of imported visceral leishmaniasis investigated in the Infectious Disease Clinic of Timiş County, Romania within the period 2004-2011. The positive diagnosis was based on epidemiological (cases of leishmaniasis in specific geographical regions), clinical (fever, sweating, headache, asthenia, loss of appetite, dizziness, abdominal discomfort, weight loss, hepatomegaly, splenomegaly, jaundice, etc.) and laboratory (erytrocyte sedimentation rate (ESR), blood counts, alanine transaminase (ALT), aspartate aminotransferase (AST), conjugated bilirubin, total bilirubin, electrophoresis, alkaline phosphatase, Gamma-glutamyl transferase (GGT), etc) characteristics. The final laboratory confirmation of the disease was by Giemsa-stained blood smears obtained by bone marrow biopsy which revealed the presence of amastigotes. Data of the epidemiological survey were collected from the Institute of Public Health from Timisoara. The data was statistically processed using Epi Info software.

Of the 4 VL cases investigated (3 men and 1 woman; average values  $\pm$  standard deviation ( $\pm$  SD) of age = 31.75  $\pm$  4.75 years), 3 patients were from rural areas and one was from an urban area. All possessed rudimentary knowledge of sanitation and individual hygiene, having completed only primary level of education. All worked as agricultural labourers in Spain (3 patients) and southern Italy (1 patient) over a period of 2–6 years during which they returned to Romania for 3–5 months/year.

All patients presented with fever, sweating, headache, asthenia, anorexia and dizziness. In addition, 2 patients complained of abdominal pain and polyarthralgia. Objective examinations identified 3 patients with tegumentary pallor and facial hyperpigmentation, 1 patient with red-violet nodes in the calves, 2 patients with tachycardia, 1 patient with jaundice and 1 patient with necrotizing vasculitis of the left fifth toe. All patients had hepatomegaly and splenomegaly and experienced severe weight loss (Table 1).

Results of biological tests were as follows: average values  $\pm$  SD of white blood cell count, 5.775  $\pm$  8.55 × 10<sup>3</sup>/µL; haemoglobin concentration, 9.37  $\pm$  1.25 g/dL; platelets, 61.250  $\pm$  19.31 × 10<sup>3</sup>/µL; alanine aminotransferase, 134.25  $\pm$  145.85 U/L and gamma globulin, 2.03  $\pm$  0.57 g/dL. All patients tested negative for human immunodeficiency virus (HIV). The confirmation of the clinical diagnoses in all the patients was performed by Giemsa-stained blood smears obtained via bone marrow biopsy which revealed the presence of amastigotes.

A positive diagnosis is challenging for majority of specialists confronted with such cases, and the period between the first medical consultation and confirmation of VL diagnosis was 4–10 weeks.

The confirmation of VL diagnosis in the first case took 6 weeks. The patient was admitted twice to our infectious disease clinic and then subsequently transferred to the haematology department. Here the patient's condition worsened and he fell into a coma and died two days later.

The second patient was diagnosed while in ambulatory care in Spain with prolonged fever syndrome. After arriving back in Romania, he was hospitalised in Bucharest and treated with corticosteroids; however, his general condition worsened and was admitted to the infectious disease clinic of Timişoara. A confirmation of VL was made after 4 weeks of hospitalisation.

The third patient was initially admitted to the Department of Rheumatology and treated with corticoids. Subsequently, he was diagnosed with peritoneal tuberculosis and treated with tuberculostatic agents for 4 weeks; however,2 months later he was admitted again at the infectious disease clinic.

The fourth patient was previously examined at a private healthcare facility and intended to follow the recommended treatment prior to admission at our hospital. Two patients were treated with amphotericin-B, with favourable evolution; however, one patient refused further treatment and the second patient fell into coma 2 days after admission and eventually died. An autopsy revealed blood stasis in the lungs, a reddish–brown liver, splenomegaly (mass, 2 kg; area,  $29 \times 17 \times 7$  cm) and blood stasis in the kidneys with infarcts.

#### Discussion

Reportedly, there has been a sharp increase in imported leishmaniasis cases over the past decade in industrialized, non-endemic countries and in association with increasing international travel, military activities and immigration (5).

Data from the German Surveillance System for Imported Diseases collected during 2001–2004 indicated that the vast majority (81%) of VL infections were acquired in southern European countries (6). Similarly, cases of imported VL to the UK during 1985–2004 had reportedly contracted the disease in Italy, Spain, Greece, Cyprus and Malta (7).

Visceral leishmaniasis is a severe and potentially fatal vector-borne disease (8). Typically, the patient develops fever, weakness, anorexia, weight loss, pallor, hepatosplenomegaly (predominantly splenomegaly), lymphadenopathy and progressive deterioration (9). In VL, anaemia, leucopenia or thrombocytopenia and hypergammaglobulinaemia are characteristically observed (10).

The clinical symptoms and the biological test parameters in these cases were specific to VL and we noted the presence of polyarthralgia in 2 patients

Table 1. Reported symptoms and physical signs

Symptom	No of cases	Physical signs	No of cases
Fever	3	Hepatomegaly	3
Sweating	3	Splenomegaly	3
Headache	3	Jaundice	1
Asthenia	3	Tegumentary pallor	2
Loss of appetite	3	Facial hyperpigmentation	2
Dizziness	3	Red-violet nodes in the calves	1
Abdominal pain	2	Tachycardia	2
Polyarthralgia	2	Necrotizing vasculitis of the left fifth toe	1

and jaundice in the third patient. The persistence of the prolonged fever syndrome, associated with polymorphic symptoms led to multiple diagnosis errors (poliarticular rheumatism, fibromialgy, peritoneal tuberculosis) and also to the delayed diagnosis confirmation. Furthermore, not even one patient didn't bring any medical letter from the medical personnel of those geographic areas, letters that could help the Romanian doctors with no clinical experience concerning the tropical infectious pathology.

The first Romanian VL case was reported by Manicatide in 1912. In 1934, 24 VL cases were reported in the province of Oltenia (11). During the period between 1999 and 2006, the "Dr. V. Babes" Clinic of Infectious and Tropical Diseases (Bucharest, Romania) reported 5 VL cases diagnosed in Romanian agricultural labourers returning from Spain, Italy and Greece (12).

Notably, the patients were young (average values of age =  $31.75 \pm 4.75$  years) and left the country for employment. None of the patients contacted their family physician or any specialist before leaving Romania, indicating that they were not aware of the risk of VL infections in epidemic areas. Furthermore, they were not aware of the risks presented by daily exposure to unsanitary working and living conditions for several years.

All patients lived in rural agricultural areas under unsanitary conditions in highly infectious, risk areas inhabited by many stray dogs. Further, all had limited sanitary knowledge, failed to comply with individual or collective hygiene standards and did not have medical insurance. When they were ill, they interrupted their work and returned to Romania for diagnosis and treatment.

The extended period of time necessary to diagnose VL was due to differentially diagnosing VL from other disorders that are characterized by fever of an undetermined aetiology, particularly in tourists returning from countries in the Mediterranean Basin. It requires the application of rigorous epidemiological surveillance, along with early diagnosis of import diseases in all patients returning home from various geographic areas.

# Acknowledgements

The authors would also like to thank Enago (www.enago.com) for the English language review.

#### References

- 1. Dujardin JC, Campino L, Capavate C, Dedet JP, Gradoni L, Soteriadou K, et al. Spread of vector-borne diseases and neglect of leishmaniasis, Europe. Emerg Infect Dis. 2008; 14: 1013–8.
- 2. Maltezou HC. Leishmaniasis. In: Maltezou HC, Gikas A, editors. Tropical and emerging infectious diseases. Research Signpost. 2010; 163–85.
- 3. Lima IP, Müller MC, Holanda TA, Harhay M, Costa CHN, Costa DL. Human immunodeficiency virus/ Leishmania infantum in the first foci of urban American visceral leishmaniasis: clinical presentation from 1994 to 2010. Revista da Sociedade Brasileira de Medicina Tropical, 2013: 00-00.
- 4. Souza GF, Biscione F, Greco DB, Rabello A. Slow clinical improvement after treatment initiation in Leishmania/HIV coinfected patients. Revista da Sociedade Brasileira de Medicina Tropical. 2012; 45: 147-150.
- 5. Pavli A, Maltezou HC. Leishmaniasis, an emerging infection in travelers. Int J Infect Dis. 2010; 14(12): e1032-e1039.
- 6. Weitzel T, Muhlberger N, Jelinek T, Schunk M, Ehrhardt S, Bogdan C, et al. Imported leishmaniasis in Germany 2001–2004: data of the SIMPID surveillance network. Eur J Clin Microbiol Infect Dis. 2005; 24: 471–476.
- Stark D, van Hal S, Lee R, Marriott D, Harkness J. Leishmaniasis an Emerging Imported Infection: Report of 20 Cases From Australia. J Travel Med 2008; 15: 351–354.
- 8. Diniz LMO, Duani H, Freitas CR, Figueiredo RM, Xavier CC. Neurological involvement in visceral leishmaniasis: case report. Revista da Sociedade Brasileira de Medicina Tropical. 2010; 43: 743-745.
- 9. Kafetzis DA, Maltezou HC. Visceral leishmaniasis in paediatrics. Curr Opin Infect Dis. 2002; 15: 289–294.
- 10. Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. Lancet 2005; 366: 1561–1577.
- Găman A, Dobrea C, Găman G. A case of visceral leishmaniasis in Oltenia region (Romania).Rom J Morphol Embryol. 2010; 51: 391–394.
- 12. Florescu S, Popescu C, Cotiga M, Raduta L, Botgros R, Voinea C, et al. Visceral leishmaniasis cases in Romania. ESCMID, 2007; Abstract number: 1733.

Corresponding Author Iosif Marincu, Department of Infectious Diseases, Pneumology and Parasitology, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania, E-mail: imarincu@umft.ro

# Instructions for the authors All papers need to be sent to e-mail: healthmedjournal@gmail.com Preparing Article for HealthMED Journal

First Author<sup>1</sup>, Second Author<sup>2</sup>, Third Author<sup>3</sup>

- <sup>1</sup> First affiliation, Address, City, Country,
- <sup>2</sup> Second affiliation, Address, City, Country,
- <sup>3</sup> Third affiliation, Address, City, Country.

# Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

Key words: Camera ready paper, Journal.

# Introduction

In order to effect high quality of Papers, the authors are requested to follow instructions given in this sample paper. Regular length of the papers is 5 to 12 pages. Articles must be proofread by an expert native speaker of English language. Can't be accepted articles with grammatical and spelling errors.

# Instructions for the authors

Times New Roman 12 points font should be used for normal text. Manuscript have to be prepared in a two column separated by 5 mm. The margins for A4 (210×297 mm2) paper are given in Table 1. *Table 1. Page layout description* 

Paper size	A4	
Top margin	20 mm	
Bottom margin	20 mm	
Left margin	20 mm	
Right margin	18 mm	
Column Spacing	5 mm	

Regular paper may be divided in a number of sections. Section titles (including references and acknowledgement) should be typed using 12 pt fonts with **bold** option. For numbering use Times New Roman number. Sections can be split in subsection, which should be typed 12 pt *Italic* option. Figures

should be one column wide. If it is impossible to place figure in one column, two column wide figures is allowed. Each figure must have a caption under the figure. Figures must be a resolution of 300 DPI, saved in TIFF format, width 10 cm min. For the figure captions 12 pt *Italic* font should be used. (1)



Figure 1. Text here

# Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

# Acknowledgements (If any)

These and the Reference headings are in bold but have no numbers.

# References

- 1. Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. N Engl J Med 1999; 341: 1284–1291.
- 2. Stewart SM, Lam TH, Beston CL, et al. A Prospective Analysis of Stress and Academic Performance in the first two years of Medical School. Med Educ 1999; 33(4): 243- 50.

Corresponding Author Name Surname, Institution, City, Country, E-mail: