Volume 1 / Number 3 / 2013 ISSN 2303-4092

Balkan Journal of Health Science

design by Almir Rizvanovic

Volume 1 / Number 3 / 2013

Balkan Journal of Health Science

Address: Sarajevo, Hamdije Kresevljakovica 7A, Bosnia and Herzegovina

E-mail: balkanjournal@yahoo.com
Web page: http://www.drunpp.ba/bjhs.html

Published by DRUNPP, Sarajevo
Volume 1 *Number 3, 2013*
ISSN *2303-4092*

Balkan Journal of Health Science is indexed in:

INDEX COPERNICUS INTERNATIONAL

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Transient loss of consciousness: Is it an epileptic seizure?

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Abstract

Objectives: Assessment and diagnose of patients presenting with transient loss of consciousness (T-LOC) is not always easy because of the presence of clinical conditions, such as epileptic seizure, syncope or pseudoseizures, with similar presentations. In this study, we aimed to evaluate the diagnostic values of a scoring system, serum PRL and CPK parameters in order to determine their usability in diagnosing epileptic seizure in the ED.

Methods: Patients who presented with non-traumatic T-LOC and who were not diagnosed during the initial assessment during the six month period were included in the study. PRL and CPK values were obtained and scoring forms were filled out for each one of the patients.

Results: Total of 45 patients were diagnosed with epileptic seizure during the three month follow-up and the median age of the patients was 24±3.4. There was no statistically significant relationship present between the high PRL and CPK values and scoring system value ≥ 1 and diagnosis of epileptic seizure compared to the other conditions diagnosed. The specificity value was calculated as 90% in the presence of high PRL and scoring system value ≥ 1, in which case the likelihood of epileptic seizure diagnosis is 5.8 times greater.

Conclusion: The PRL and CPK had low sensitivity and specificity values whereas the scoring system had high sensitivity and increasing specificity with higher PRL values. It is recommended that the suggested parameters, scoring system, PRL and CPK values are used in larger studies to validate their usability as diagnostic tools.

Key words: Loss of consciousness, epileptic seizure, syncope, PRL, CPK.

Introduction

The transient loss of consciousness (T-LOC) is a self-limited and short-lived event that includes syncope and epileptic seizures, and psychogenic or other rare disease processes (1). The prompt evaluation and diagnosis of patients presenting to the emergency department (ED) with T-LOC is not always easy, as their conditions may be confused with spontaneously resolving convulsive syncope (2,3) that results from global cerebral hypoperfusion, and presents very similarly to seizures or with pseudoseizures. The main problem with diagnosing patients with a loss of consciousness is the lack of a laboratory test that can be considered as a gold standard (4). Other than a detailed patient history and witnessing the seizure, the presence of easy-to-use laboratory tests is limited in the ED. Even though computed cranial tomography (CCT) and electrocardiography (ECG) studies do not provide an exact diagnosis for such conditions, they are invaluable tools to determine the underlying causes in the ED. While electroencephalographies (EEG) are helpful, they are not conclusive in the majority of epileptic seizure patients. There are studies concluding that the EEG is an unpredictable tool for diagnosing seizures, with abnormal EEG findings in as many as 50% of the patients during the first 24 hours, and abnormal findings in 21% during the first 48 hours after the seizures (5-8). Video recording, on the other hand, is recommended as a last resort in patients for whom the EEG does not provide a conclusive diagnosis (2).

If a conclusive diagnosis cannot be reached during the initial evaluation, a thorough patient history may provide valuable information for the diagnosis. Sheldon et al. developed a 94% sensitive and specific scoring system based on the symptoms (2,9). Moreover, there are studies using serum pro-

lactin (PRL) and creatine phosphokinase (CPK) as the parameters to form a differential diagnosis for seizures, pseudoseizures, and syncope (5,10-16).

In this study, we aimed to evaluate the diagnostic value of the scoring system (9), PRL, and CPK values for epileptic seizures in patients presenting to the ED with a transient loss of consciousness, and without a known cause.

Material and Methods

The present study was planned after obtaining approval from the University Clinical Study Ethics Board and informed consent from patients or the patients' relatives. During the six-month study period, patients older than 16 that presented to the ED with non-traumatic T-LOC, and in whom a definite diagnosis between seizures, syncope, or pseudoseizure could not be made during the initial evaluation, were included in the study. Exclusion criteria were set as: a) patient history and physical exam consistent with epileptic seizure (presence of neurological deficit, seizure witnessed by a healthcare professional, presence of typical tonic-clonic contractions, and prolonged post-ictal episodes with confusion) in which case the patients were treated as seizure, a neurology consultation was requested, and they were excluded from the study; b) patient history, physical exam, and laboratory findings consistent with syncope (sudden onset and spontaneously resolved loss of consciousness with preceding chest pain or palpitation, with the presence of cardiac pathology) in which case the patients were treated as convulsive syncope, a cardiology consultation was requested, and they were excluded from the study; c) patient history and physical exam consistent with a pseudo seizure (no loss of consciousness, able to hear and sense surroundings, irregular, asymmetrical, and asynchronized convulsions especially with pelvic pushing movement) in which case the patients were referred to the psychiatry clinic, and were excluded from the study (17). Patients in whom syncope, epileptic seizure, or a pseudo seizure diagnosis could not be made following the initial evaluation, or for whom a thorough patient history could not be obtained, were included in the study.

The patients' demographic information, time of onset of symptoms, time of ED visit, and information regarding the survey score were recorded (Table 1). Questions of survey score were asked to the patients and the people who have witness the event. EEGs for all of the patients were performed within the first 12 hours of the ED arrival (Nihon Kohden, EEG 9100K, Nihon Kohden Korporation, Tokyo, Japan) (6). A CCT and ECG were obtained to rule out underlying pathologies that would result in a loss of consciousness. The CPK values were measured using Abbott Laboratories' Architect ci8200 (Illionis, USA) (0-190U/L normal range), and PRL values were measured with the Immulite 2000 (Siemens Laboratories, Germany) (normal range for males between 16 and 18 is 2.7-15.2ng/mL, for females between 16 and 18 is 2.1-18.4ng/mL; normal range for adult males is 4.6-21.4ng/mL, and for adult females is 6-29.9ng/mL). The ED discharge diagnoses were recorded following the laboratory and imaging findings and treatment. The patients' 3-month follow-up records at the neurology, cardiology, and psychiatry clinics were evaluated for a final diagnosis. The patients who did not present for their follow-up visits, or whose hospital records were missing were reached via telephone, and their final diagnosis information as well as the possible recurrence of loss of consciousness following the hospital discharge were requested. Patients in whom epileptic discharges were noted in the EEGs performed at least once during the 3-month period, and those who were started on epileptic treatment were diagnosed as epileptic by a neurologist.

Table 1. Questionnaire and scoring system for symptoms according to loss of consciousness

Symptoms	Puan
Wake with tongue cutting?	2
Déjà vu or jamais vu?	1
Emotional stress associated with loss of consiousness?	1
Head turning during a spell	1
Any one of these Unresponsive, unusual posture, limb movement, or amnesia 1 of spells?	1
Confusion after a spell	2
Sweating before spell	-2
Spell associated with prolonged sitting or standing	-2
Lightheaded spells point score <1 the likelihood is syncope, score ≥1 the likelihood is seizure	-2

Statistical Analyses

The SPSS 17.0 for Windows (SPSS Inc., Chicago, IL) software was used for the statistical analyses. The frequencies for the variables with normal distribution, and the median ± standard errors for the variables that were not distributed normally (95% CI, min/max values), were calculated for demographic properties. The accuracy, sensitivity, specificity, and the positive likelihood ratios (LR^+ ratio) for the survey scores, PRL, and CPK values were determined. The categorical values were tested using chi-square tests. A p-value of less than 0.05 was considered to be significant.

Results

A total of 122 patients with non-traumatic T-LOC were presented at the ED during the 6-month study period. Of those, 45 who met inclusion criteria, and in whom the diagnosis of seizure, pseudoseizure, or syncope could not be determined, were included in the study. Patients who were not diagnosed at the end of the 3-month follow-up period (n=2, 4.4%) and those who did not come in for a follow-up or those who could not be reached (n=12, 26.7%) were not included in the analyses investigating relationship between CPK, PRL, survey scores and epilepsy. Those analyses were performed on patients who were diagnosed with any conditions at the end of the 3-month follow-up period (n=31).

Demographic Properties

The median age of the 45 study participants was 24±3.49 years (95% CI, 30.94-45.01). Of those, 30 (66.7%) were between 16-40 years old, and twenty six (57.8%) of the patients were females. The median of the period of time between the onset of loss of consciousness and admission to the hospital was 30±35.77 minutes (95% CI, 38.4-188.6). When the distribution of the time of day of the hospital admissions was evaluated, 24 (53.3%) patients presented to the hospital between 12: 00-18: 00, 14 (31.1%) between 06: 00-12: 00, 4 (8.9%) between 00: 00-06: 00, and 4 (8.9%) between 18: 00-24: 00 hrs. Ten (58.8%) of the patients who were diagnosed with epilepsy at the end of 3-month follow-up period were found to arrive at the hospital between 12: 00-18: 00, 4 (23.5%) between 06: 00-12: 00, and 3 (17.6%) between 00: 00-06: 00 hrs.

Following the evaluation and treatment, 18 (40%) patients were discharged from the ED with a diagnosis of epilepsy, 11 (24.4%) with syncope, and

Table 2. The patients with ED discharge diagnosis and the diagnosis according to 3-month follow-up at related departments

ED discharge diagnosis	n	%	Diagnosis (3 –month follow)	n	%
Seizure			Seizure	15	83.3
			Without follow up	2	11.1
			Other *	1	5.6
Total	18	40		18	100
Syncope			Cardiac syncope	6	54.5
			Without follow up	3	27.3
			Indefinite diagnosis	1	9.1
			Other **	1	9.1
Total	11	24.4		11	100
Indefinite diagnosis			Without follow up	7	63.6
			Seizure	2	18.2
			Indefinite diagnosis	1	9.1
			Other **	1	9.1
Total	11	24.4		11	100
Other *	5	11.4	Other ***	5	100
Total	**45**	**100**		**45**	**100**

** Other: hyponatremia*

*** Other: anxiety disorder*

**** Other: anxiety disorder, Cerebrovascular disease (2 case), hypoglycemia, hypoprolactinemia*

5 (11.1%) with other diagnoses (1 with psychiatric disorders, 2 with stroke, 1 with hypoglycemia and 1 with hyperprolactinemia). Eleven (24.4%) were discharged without a known diagnosis for the loss of consciousness they experienced. After the 3-month follow-up results were evaluated, 15 patients who were discharged from the ED with the diagnosis of epilepsy, and 2 patients initially discharged without a definite diagnosis were found to be diagnosed with epilepsy. The 3-month follow-up diagnoses and ED diagnoses of the study patients are given in Table 2.

We found that 21 (46.7%) of the patients had no prior history related to their T-LOC, and 7 (33.3%) of those were diagnosed with epilepsy after the follow-up evaluation. Nine (20%) of the patients had experienced a similar episode of T-LOC within the previous 6 months, and 4 (44.4%) of them were diagnosed with epilepsy. Seven (15.6%) had similar complaints during the previous year, and 4 (57.1%) were diagnosed with epilepsy on follow-up visits. Finally, 8 (17.7%) had experienced similar episodes for more than a year, and 2 (25%) of them were diagnosed with epilepsy. There was no significant difference between the presence of similar episodes in the past, and receiving a final diagnosis of epilepsy during the follow-up visits (p=0.623).

Fifteen (33.3%) patients in the study group had not have an episode of LOC following the hospital discharge, 1 (2.2%) had 1 episode, 1 (2.2%) had 2 episodes, and 3 (6.7%) had more than 3 episodes of epilepsy before the 3-month follow-up period. All of the patients with repeating episodes of LOC were diagnosed with epilepsy during their follow-up visits.

Evaluation of Imaging Studies

When the ECG of the patients were considered, 14 out of 40 (88.9%) with a normal sinus rhythm, 2 out of 4 (8.9%) with atrial fibrillation, and 1 with sinus bradycardia were found to be diagnosed with epilepsy on their follow-up visits.

Seventeen (37.8%) patients had epileptic activity detected in their initial EEG, 15 (33.3%) had normal EEGs, and 13 (28.8%) had not received an EEG evaluation during their stay in the hospital. Of the 17 who had epileptic activity during the initial EEG, 14 (82.4%) were diagnosed with epilepsy, 1 (5.9%) with syncope, and 2 (11.9%) with an anxiety disorder at the end of the 3-month follow-up period.

Thirty-five (87.8%) of the patients had a cranial computed tomography (CT) examination performed in the ED and 32 (71.1%) had normal findings, while 1 (2.2%) had signs of cerebral ischemia, 1 (2.2%) had a cerebral hemorrhage, and 1 (2.2%) had signs of chronic changes. Of the patients who were diagnosed with epilepsy during their follow-up visits, 13 (76.5%) had normal CTs, 1 (5.9%) had signs of chronic changes, and 3 (17.7%) had not undergone CT evaluation.

Evaluation of PRL, CPK, and Survey Scores

The median value for the CPK was found to be 83±180.53 (CI -39.25- 698.16) U/L. In 57% (n=4) of the patients with high CPK values, the

Table 3. Comparison of initial PRL and CPK values of patients with their diagnosis after the 3-month follow up period

	Level		Seizure	Other**	Total	p *
PRL	Low/normal	n	10	10	20	0.707
		%	50	50	100	
	High	n	7	4	11	
		%	63.6	36.4	100.0	
	Total	n	17	14	31	
		%	54,8	45,2	100,0	
CPK	High	n	13	11	24	0.617
		%	54.2	45.8	100	
	Low	n	4	3	7	
		%	57.1	42.9	100	
	Total	n	17	14	31	
		%	54.8	45.2	100	

** Fisher's Exact Test*

***Other: Non-seizure patients*

final diagnosis was seizure, while it was "other disease processes" in 42.9% (n=3) of the patients. Fifty-four percent (n=13) of the patients with normal CPK values were diagnosed with seizure. There was no statistically significant relationship found between high CPK values and patients being diagnosed with epilepsy (p=0.617) (Table 3). The accuracy of the high CPK values was 48.3%, sensitivity was 23.5%, specificity was 78.7%, and the negative predictive value was 45.8%. Having a high CPK value had very low positive LR^+ ratio (1.093) in being diagnosed with epilepsy

The average PRL level upon arrival to the ED was 22.4±20.4 ng/mL (min=1, max=93). When variations of the PRL values were based on sex and age, 7 (43.8%) patients who were diagnosed with seizures during the 3-month follow-up visit had normal values, 7 (43.8%) had high, and 2 (12.5%) had low values. There was no statistically significant relationship found between high PRL values and patients being diagnosed with epilepsy (p=0.707) (Table 3). The accuracy of the high PRL values was 54.8%, sensitivity was 41.1%, specificity was 71.4%, and the negative predictive value was 50%. Having a high PRL level had LR^+ ratio of 1.4 in being diagnosed with epilepsy Having a high PRL level had positive LR of 1.4 in being diagnosed with epilepsy. All three patients who were diagnosed with a psychiatric disorder had normal PRL values.

The distribution of the survey questions scored by the patients, or by those who witnessed the seizure, is given in Figure 1. In 21 (46.7%) of the surveys the score was above 1, in 16 (35.6%) it was below 1, in 5 (11.1%) it was 1, and in 3 (6.7%) the score was zero.

When survey scores and 3-month follow-up results were compared, there was no statistically significant relationship found between the patients with scores greater than 1 and those who were diagnosed with epilepsy (p=0.063) (Table 4). The accuracy of the seizure diagnosis score of ≥1 was 67.7%, sensitivity was 82.3%, specificity was 50%, and the negative predictive value was 70%. Having a survey score of ≥1 had positive LR^+ ratio 1.6 in being diagnosed with epilepsy.

Eigth out of 9 patients with high PRL values and survey scores of ≥1 were diagnosed with epilepsy during the follow-up visit. On the other hand, there was only one patient not diognosed with epilepsy with high PRL levels and survey scores of ≥1. There were 9 patients who were diagnosed with epilepsy after the 3-month follow-up period and who had not have high PRL levels or survey scores of ≥1. There were 13 patients not diagnosed with epilepsy who do not have high PRL scores or survey scores of ≥1. The accuracy was 67,7%, sensitivity was 47%, and specificity was 92,8%. Having a high PRL level and a survey score of ≥1 had LR^+ ratio of 5,8 in being diagnosed with epilepsy.

Discussion

Epileptic seizures can be mistaken for non-epileptic seizures due to the similarities in symptoms. Even though a thorough patient history and witnessing the seizure are important parameters for the differential diagnosis of epilepsy, this is not always possible in clinical practice. There are studies in the literature that report the high costs and low rates of diagnostic accuracy (11% to 50%) of the tests for diagnosing adult LOC (18,19,20). We found that the diagnostic accuracy of our tests was higher (68.8%) over the period of 3 months. This higher accuracy can be attributed to the limitation imposed by our inclusion criteria (unknown causes during the first presentation at the hospital).

Conditions such as hallucinations, changes in taste and smell, the feeling of déjà vu, and amnesia support epilepsy, whereas not feeling well, nausea/vomiting, defecation, incontinence, and provocation by pain support syncope as a diagnosis (2). The survey scores found to have high specificity and sensitivity by Sheldon et al. consisted of questions for which we can obtain the answers using a thorough patient history. In our study, the survey scores had a high sensitivity (82.3%) but low specificity (50%). The inclusion of patients whose condition cannot be differentiated between syncope or epilepsy, and enrolling low numbers of patients into the study might have caused the low rates of sensitivity and specificity.

One-fourth of the patients who were admitted to the clinics for syncope presented with pseudoseizures (21,22). There are numerous studies reporting that PRL values can be used to support a pseudoseizure diagnosis instead of syncope or seizures (17,22,23). Other studies report the limited effect of the changes in PRL values (0-15

minutes-2 hours) to differentiate between seizures and pseudoseizures (11) (sensitivity of 20%, specificity of 40%), and the questionable use of PRL values in diagnosing epilepsy (10). We found that 3 patients who were diagnosed with a psychiatric disorder had PRL values within normal limits, and that there was no relationship between high PRL values and the diagnosis of epilepsy. On the other hand, when we evaluated the high PRL values and survey scores of ≥1 together, we had low sensitivity (47%) but high specificity (92%) and LR^+ ratio 5.8. Further studies enrolling larger numbers of patients are required to confirm these results.

In a study which evaluated serum CPK levels to differentiate generalized tonic-clonic seizures from psychogenic and vasovagal syncopes, the researchers found that the CPK levels were significantly high 12 to 15 hours after the onset of seizure (mean ± SD: 286 ± 236). However these high levels were maintained only in patients with tonic-clonic seizures 24 to 26 hours after the onset, while it was lower in the others (15). The sensitivity of high levels of CPK during the initial presentation of generalized seizures was 69%, and the specificity was 94% (15). Goksu et al. reported no significant difference in the CPK values between seizure and syncope patients in their study that enrolled 63 patients (14). Similarly, we found no difference in CPK values between the patients who were diagnosed with seizures and those who were not. The sensitivity of the CPK values in patients who were diagnosed with seizure during the follow-up was 23.5% and the specificity was 78.7%. This result can be explained by the early evaluation of the CPK values (during the ED course), and limiting the enrollment to those with unknown causes of LOC. Patients in whom the causes of generalized tonic-clonic seizures were known were not included in our study.

Since ECGs are considered as one of the cornerstone tests to rule out cardiological causes, performing ECGs in patients with LOC is important to detect cardiogenic syncopes. Kapoor reported that the 5-year incidence of sudden death in patients with cardiogenic syncope was 33% (18). There are other studies reporting tachycardia in all (24) and 98% (25) of the patients during the seizure (25). In another study, Petkar et al. found that in 69% of the patients who were diagnosed with epilepsy by a neurologist, and out of which only 4% had tonic-clonic activity, the implantable ECG recorder (ILR) device detected bradycardia and asystole in 21% of the patients during the convulsive seizure (3). We found that 88.9% of our patients who were diagnosed with epilepsy had a sinus rhythm on their arrival to the ED. Other studies also stated that ECGs are not effective in differential diagnoses, and only long term ECG monitoring can be valuable for supporting a diagnosis (3,26). The evaluation of the ECG was performed in order to rule out life threatening conditions in our study, but not as a diagnostic tool for seizures.

Conclusion

The best approach to diagnose seizures is obtaining a thorough patient history or performing related tests following a witnessed seizure. Using diagnostic scales and tests will nonetheless shorten the time for a definite diagnosis and the initial treatment in the ED. We found that high survey scores and high PRL values seen together have a relatively significant specificity for diagnosing epileptic seizures. Studies with larger patient groups are required to draw more accurate conclusions.

Limitations

The first limitation of our study was to limit the observation time for the patients in order to determine the diagnostic parameters that would assist the clinicians to rapidly diagnose an epileptic seizure. The second limitation is not conducting detailed cardiological examinations. For example, we did not perform electrophysiological studies to differentiate electrical and connective tissue disease to diagnose syncope. Finally, we assumed that the patient, patient's relatives, or the bystanders who witnessed the seizure provided an accurate and complete patient history.

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Brucella and importance of the fight against brucellosis

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Abstract

Brucellosis is one of the most commonly encountered zoonotic diseases, reported from all parts of the world. However, it continues to be an important health concern in developing countries. About 500.000 new cases are reported every year throughout the world, according to World Health Organization figures. One of the most important scientific achievements has been a significant improvement in the average life expectancy of humans. Many reasons have been noted for this achievement in life expectancy; however, the most important of these has been the prevention of death due to infection. Accomplishments in the prevention and control of infectious diseases have not evenly occurred in developed and developing countries. Despite the high morbidity rate reported due to brucellosis, the mortality rate has been low. Many humans suffer from this disease every year, and in addition to physical disability, it also causes great loss in the work force. On the other hand, brucellosis leads to a decrease in milk production from animals, which are the source of the disease, and as a consequence a negative effect in the economy of the country. In this article, the importance of the fight against brucella has been tackled.

Key words: Brucella, epidemiology.

Introduction

Brucellosis is a disease with nonspecific signs and symptoms greatly resembling other inflammatory diseases and may progress undiagnosed due to lack of medical knowledge and interest. The incubation period is about two to three weeks in humans; however, this period may be as short as one week or as long as one month (Young, 2005). The disease mostly presents as malaise, lack of appetite, body pain and subfebrile fever. Complications such as osteoarticular involvement, as well as meningeal, cardiovascular and central nervous system infections are difficult to treat (Dilmener, 1990). Skin involvement, complications of the genitourinary system and hematologic signs can also be observed (Kaya, 2006).

Brucella species

Agents of brucellosis come from the family of Brucellacea. They have been classified into six classical species (together with their biotypes) according to the host preference, and their antigenic and phenotypic characteristics (without taxonomic considerations) (Yaman, 2011; Osterman et al., 2006). *Brucella melitensis* (biovars 1-3) is the most contagious of the human species and has been found in goats, sheep and camels. *Brucella abortus* (biovars 1-6 and 9) is the second most commonly encountered and has been found in cattle, camels, deer, oxen, and water buffaloes. *Brucella suis* (biovars 1-3) has been found in pigs, *Brucella suis* (biovars 4) in reindeers, while *Brucella suis* (biovars 5) has been found in wild rodents (Yaman, 2011; Conner et al. 2008). Brucella canis found in dogs (Shin and Carmichael, 1999; Köylü et al., 2009) and Brucella suis (biovars 2) found in wild rabbits may cause sporadic infections in humans. *Brucella ovis* found in goats and *Brucella neotomae* found in desert rats are non-pathogenic in humans. Three species have recently been isolated. These include, *Brucella microti* isolated in field rats, and the other two species *Brucella ceti* (or Brucella cetaceae species; in wales and dolphins) and *Brucella pinnipedialis* (in fox fish, sea lions and sea horses) isolated in sea mammals (Scholz et al., 2008; Dawson et al. 2008). On the other hand, Brucella strains have also been isolated from humans and monkeys (Schlabritz-Loutsevitch et al.,

2009; De Barun et al., 2008). The pathogenicity and virulence of agents of brucellosis is still complicated due to variations in the host organisms. Recent genetic maps of *Brucella melitensis*, *Brucella abortus* and *Brucella suis*, together with that of the non-zoonotic *Brucella ovis* (Tsolis et al., 2009) have thrown more light on the understanding of interactions between host and pathogen. Human pathogens of brucellosis include *Brucella melitensis*, *Brucella abortus*, *Brucella suis* biovars 1-4, *Brucella canis* and *Brucella spp.* isolated from sea mammals (Anonymous, 2009).

Routes of brucella transmission

Brucellosis may present as a severe, malaise-causing chronic disease in humans, and may affect many body organs. From the public health point of view, most cases are reported to arise due either to direct contact with infected animals or contaminated from unpasteurized milk and milk products (Yaman, 2011; Altindiş, 2001). However, other sources of infection in humans may include inadequately cooked meat or other uncooked meat products, bone marrow (Erten et al.,2000), laboratory culture samples and other tissue samples, and accidental injection of live attenuated vaccines developed to combat brucellosis. Although human-to-human transmission (Ruben et al., 1991; Mesner et al., 2007) is rare, transmission through blood transfusion, bone marrow transplantation (Akçakuş et al., 2005; Doganay et al., 2001; Wood, 1995), or transmission by sexual route (Ruben et al., 1991; Mantur et al., 1996; Vandercam et al., 1990) has been reported. On the other hand, passage through the placenta (Giannacopoulos, 2002) or breast-feeding (Al-Mofada et al., 2006; Çetin et al., 2009) may lead to development of the disease in babies. Infections which develop in newborns following birth (Lubuni et al., 1988; Dogan, 2010) are due mostly to exposure of the baby to the micro-organism while in the mother's womb, urine, or feces (Anonymous, 2009; Singer et al., 1991).

Diagnosis of brucella

Clinical signs of brucella infection are varied and the disease may progress asymptomatically, making diagnosis very difficult. As a result, demonstration of bacteria through culture or polymerase chain reaction (PCR), or detection of the immune response that develops against bacteria antigens is very necessary. Brucella culture sample are made using blood, bone marrow or tissue products. Blood cultures should be performed in the case of acute infections. Despite the late and difficult culturing nature of the bacteria, they are known to be cultured within the first seven days by automated blood-culture systems. Serologic tests are known to play an important role in the diagnosis (Altay, 2008).

With PCR, subtyping is performed using the BCS P31 gene (encoding 31kDa), and genes encoding 16 S rRNA and membrane protein. Approximately 700 cfu/mL bacteria can be detected using PCR. Its sensitivity and specificity is 98.3-100%. The fact that positive results of PCR may be obtained five months after recovery from the disease, should be taken into consideration (Navarro et al., 2004; Pakzad et al., 2012).

During the disease period antibodies start forming after two weeks; the IgM type antibodies appear within approximately one week, reaching its peak within three months. It becomes negative after recovery from the disease, or may remain at low titers for several years. On the other hand, IgG antibodies appear within three weeks, and reach their highest level within six-to-eight weeks. Their titers gradually fall following clinical recovery. Re-surge of IgG levels after subsidence of persistent infection indicate recurrence of the disease. Tests used for serologic diagnosis include, Rose Bengal, Standard tubal agglutination (STA) (Wright), Mercaptoethanol Wright, Coombs standard tubal agglutination, Immunocapture (Brucellacapt) and ELISA (www.biruni.com.tr).

Treatment of brucellosis

Preoperative treatment of about three weeks is recommended in cases with a clinically stable condition. Valve insufficiency and a possible abnormality in hemodynamic stability are known to arise in patients with brucella endocarditis. Under such conditions surgical operation is recommended at an earlier stage. The suggested medical (conservative) treatment involves doxycycline+ rifampicin, and a triple combination with streptomycin for the first three weeks, as well as an alternative with trimethoprim-sulfamethoxazole (TMP-SMZ) (Baktir and Baktir 2002). Patients who continued long-term treatment with rifam-

picin+ ciprofloxacin combination have also been reported. In addition to the three-month post-operative treatment, post-operative treatment for a three-six months period is also possible when necessary. However, caution should be taken when administering conservative therapy. Sudden changes in the patient's hemodynamic stability may be recorded during treatment. Close follow-up with conservative therapy is recommended in patients with no signs of left ventricular failure, those with no prosthetic heart valves, and in patients who experience a short period of symptoms of the disease (< 2 months) (Çokca, 2006).

Prevalence of brucella and methods of prevention

Deaths due to diseases have been reported to be associated with infection in 53% of cases in Africa, 7% in the United States of America and 2% in Europe. Brucellosis is primarily a disease of herbivorous animals caused by the brucella bacteria. Transmission of the disease to humans is generally by direct contact with secretions of the infected animal. The disease can be found in every part of the world; however, it has been reported to be highly prevalent in some countries (Seleem, et al., 2010; Mantur and Amarnath, 2008; Karadzinska-Bislimovska et al., 2010; Cutler et al., 2005). Animal breeding is not conducted by modern means, but by classical methods in less developed or developing countries alike. Instead of using machine, extraction of milk from animal is performed by hand, and adequate hygiene is not maintained in animal barns. Brucellosis continues to pose as a great health problem in less developed and developing countries, unlike in developed countries where the disease has been eradicated through vaccination programs and pasteurization of milk. The most effective method of the fight against brucellosis lies primarily in correct detection of the prevalence of the disease in the community and to rightly implement preventive measures.

Prevalence of Brucella

Brucellosis is considered as the most prevalent zoonotic disease by the United Nation's Food and Agricultural Organization (FAO), the World Health Organization (WHO) and the Office of International Epizootics (OIE). It has greatly been eradicated in Northern Europe, North America, Australia, and New Zealand through long standing efforts of many years. However, it is still greatly prevalent in some countries of Southern Europe particularly the Mediterranean regions, the developing countries of Middle East and Western Asia, Indian Subcontinent, Africa, and some parts of Central and South America. The annual incidence of brucellosis has been reported to vary between 1-78 cases per every 100.000 individual, in the Mediterranean and Middle Eastern countries. However, more than 550 cases have been reported in highly prevalent areas where control programs of the disease are not implemented in animals. Although brucellosis in human is considered a notifiable disease in many countries, official bodies do not publish the correct number of infected individuals. The disease is considered to be 10-15 times more prevalent than the published figures. The most important reason behind this lies in the incorrect diagnosis of the disease (WHO, 2004; OIE, 2008; Yarsan 2009).

Many studies on brucellosis have been reported, with variations in the rate from country to country and even from region to region within the same country. Turutoglu et al. conducted a study to determine the prevalence of this infectious disease in the Burdur region of Turkey, through detection of the presence of brucella and antibodies that developed against these agents in cow and sheep milk in the infected regions. In the said study, although Brucella species were not isolated through bacteriological examinations, 12 (3%) of the cow milk samples and 40 (17.7%) of the sheep milk sample were detected by the Milk Ring Test (MRT), whereas nine (2.2%) and 31 (13.7%) of samples were detected with the milk agglutination test (Whey-AT) in cow and sheep milk, respectively. Accordingly, it was demonstrated that brucellosis was more prevalent in sheep compared to cows in the Burdur region, and that bacteriological and serologic tests used in the diagnosis of the disease may reveal different results (Türütoğlu et al., 2003). In the exploratory spatial analysis performed by Demirel et al., human brucellosis was reported as a very big health problem in South Eastern Turkey, in terms of prevalence, and indicated that the region should be selected as the primary region in the fight against brucellosis through preventive measures. They also stated that the analytic methods used may contribute in under-

standing the epidemiology of brucellosis, and that further research for risk factors should be conducted in areas of where the disease was detected en masse (Demirel et al., 2009). Mukhtar et al. conducted a study on workers of the slaughterhouse in Pakistan, using the ELISA method to investigate anti-Brucella IgG, and demonstrated 21.7% seropositivity (Mukhtar and Kokab, 2008). Ofukwu et al. also investigated Brucella antibodies in the sera of a total of 1040 patients who presented at the Federal Medical Center of Makurdi city of Nigeria between March 2003 and February 2004, using the Rose-Bengal Plate Test (RBT) and Serum Agglutination Test (SAT) methods. The prevalence of Brucella was found to be 7.6% with RBT. In the positive results of the study, 77.2% of the cases were Brucella abortus whereas the remaining 22.8% were found to be Brucella melitensis. On the other hand, the seroprevalence in workers of slaughterhouses, those in contact with animals and in those involved in the production of animal products was reported to be higher (Ofukwu et al., 2010). Ahmed et al. investigated the seroprevalence of Brucella on animals and the human populations, in Libyan's Western Mountain region. Brucella was tested in the sera samples obtained periodically from 561 animals (sheep, goats, cattle and camels) and human sera from 546 voluntary participants, within a period of more than 13 months, between December 2006 and January 2008. Test methods used were the Rose-Bengal Plate Test, Tubal Agglutination Test and the ELISA test. Of the positive result rate obtained from the domestic animals 31% was detected in goats and 42% in cattle. On the other hand, the positive result rate of 40% was obtained from human sera. A total of 95 (43%) of these were found to be IgM positive, and suggested the presence of active or recurrent infection (Ahmed et al., 2010). Nasir et al. also conducted a seroprevalence study on 1473 cattle and 481 buffaloes, using the Rose-Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) methods. Of these animals, 286 cattle and 223 buffaloes were from domestic animal farms owned by different individuals. The positive result rate obtained from these cattle and buffalo animals for the seroprevalence RBPT and SAT were 18.53% and 35.40%, respectively, whereas those obtained from the other domestic animals for the tests was found to be 7.19% and 2.91%, respectively. On the other hand, positive results of samples obtained from 9% of cattle and 23.7% of buffaloes were detected with SAT more than with RBPT (Nasir et al., 2004). In a seroprevalence study conducted by Abo-Shedada et al. on a individuals with a high risk for brucellosis in Northern Jordan, the sera of 1236 individuals (636 high risk carriers and 600 controls) was investigated using the RBT and ELISA test methods. The Brucella seroprevalence rate (8.2%) detected in the high-risk group was found to be higher than that of the control group. This high seroprevalence rate in the high-risk group was detected in sheep rearers and meat processing workers. However, no positive rates for the ELISA and RBT tests were reported (Abo-Shehada et al., 1996). In the study by Ebrahimpour et al. from the Mazandaran region of Iran, 25.7% of the 337 positive cases were reported to originate from the city center, whereas 74.28% were detected from originate rural areas (Ebrahimpour et al., 2012).

Prevention of Brucellosis

Brucella spp. are known as microorganisms found in the WHO Risk Group 3, and are considered as microorganisms which cause disease in humans and can be found in a veterinary and microbiology laboratory (Yaman, 2011; Corbel, 2006). Only trained laboratory personnel are advised to manipulate Brucella spp. and the detection of species types and studies geared towards the detection of antimicrobial sensitivity should be performed only in experience public health laboratories. In addition, the container of culture media should tightly be closed when not in active use. Moreover, in the event of any accident, all workers at the time should be scrutinized periodically for agglutination antibodies, and individuals detected to have positive results are recommended to receive appropriate treatment, by so doing an obscure disease process can easily be overcome (Yaman, 2011; Lim and Rickman, 2004; Singh, 2009; Yagupsky et al., 2000).

Food products (meat, milk and milk products) should be cooked before eating and fruits and vegetable which are rawly consumed should be washed with microorganism-sensitive disinfectants, since the microorganism is sensitive to the heat of pasteurization. It is important to separate infected animals from the lot, in order to control the disease. The most important of the preventive measures

is vaccination of animals, thus controlling spread of the disease. Vaccination is higher recommended since treatment cost in animals is very high.

Vaccination is one of the most important and cost saving measures for prevention from the disease. Inactive and attenuated vaccines are very effective measures for the fight against specific and zoonotic infections in the economically important livestock farming sector. However, with developments in molecular biology and immunology, studies are underway to develop effective and safe vaccines, and products based on current technology are being marketed for use.

Vaccines for brucellosis, which confer active immunity, are the live Brucella abortus S-19 vaccine and the live Brucella melitensis Rev-1 vaccine. There are two separate Brucella abortus S-19 vaccines administered in calves and adult cows. The Brucella abortus S-19 vaccine is used on 4-8 months old healthy female calves; it provides protection for at least seven years, and it is not used on adult cows and males. On the other hand, the Brucella abortus S-19 adult vaccine is used on female cattle of more than eight-months-old, and is administered twice every 24 month. It provides protection for one years and can also be used for the unvaccinated and those vaccinated while young (http://www.corumtb.org.tr/hayvancilik/Brusella.html; Büyüktanir, 2010).

Conclusion

The rapid spreading nature of the disease, difficulty in control and management, long duration of the disease, and the high cost involved in its management are of great importance. The negative effects surrounding the sources of animal protein is a hindrance to trading in animals and animal products and is detrimental for the socio-economic development of individuals dealing in livestock farming, mostly from rural areas and with very limited facilities. These factors show the difficulties involved and the importance in the fight against the disease. One of the most important features in the fight against brucellosis is to first make scientific analyses to determine the level of disease in the community, and then investigate reasons for the disease. Thousands people acquire the disease every year through consumption of milk and milk products during the lactation period from infected and inadequately treated animals, leading to physical disability and a great loss in the work force. The real incidence of global brucellosis has not been identified due to inadequate publication of results and also due to the presence of subclinical cases. On the other hand, economic loss is also reported due to production losses and abortions in animals. Provision of global collaboration and the development of international projects would help in identification of the prevalence of brucella and the fight against brucellosis. The presence serious health problems due to the disease in some countries, while being only limited in other countries is a factor, which reduces the importance entrusted in the disease in the global world of today. The seriousness of this disease should be considered in international scientific platforms, due to the possibility of using certain Brucella species as agents of bioterrorism (Young, 2005; Dilmener, 1990). On the other hand, effective methods should be developed in the fight against the disease.

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Predictors of drug-drug interactions among geriatric inpatients who received potentially inappropriate medications in a prospective cohort study in Penang Hospital, Malaysia

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Abstract

Aim: To determine the incidence and predictors of drug-drug interactions (DDIs) among geriatric inpatients who received potentially inappropriate medications (PIMs).

Methods: A prospective cohort design with convenient sampling was conducted in Penang General Hospital in Malaysia. Data were collected from 400 geriatric inpatients admitted to Penang Hospital. Beers' criteria were used to assess PIMs among geriatric inpatients. Additionally, causality of DDIs was determined using a drug interaction probability scale (DIPS). Statistical analysis, with univariate and multivariate analysis, was applied to identify the predictors of DDIs.

Results: The mean age of 400 geriatric inpatients was 74.2 ± 6.5 years, 214 (53.5%) patients were women and the majority were Chinese (58%) patients. PIMs were used in 211 (52.8 %) of the geriatric inpatients during hospitalization, and the incidence of DDIs was 13%. PIMs (p=0.001), polypharmacy (p=0.001), polymorbidity (p=0.020) and length of hospital stay (p=0.001) were the main predictors of DDIs.

Conclusion: The incidence of DDIs during hospitalization of geriatric inpatients increased when PIMs were used. Beers' criteria of PIMs were useful in geriatric inpatients to decrease DDIs. The predictors suggest that medications that were listed in Beers' criteria should be discontinued to avoid risk of DDIs.

Key words: Geriatric; drug-drug interaction; potential inappropriate medications.

Introduction

The percentage of the population over 65 years of age has doubled in the last century. Furthermore, it is predicted that this acceleration process will double over the next twenty years and that the geriatric population in the world may to reach 1100 million by the year 2025 [1]. Geriatric patients have been defined as patients who are 65 years or above [1,2,3,4]. Age-related physiological and pathological changes can influence the pharmacokinetics and pharmacodynamics of medications [2]. With advancing age, diseases become more common and tend to occur concurrently due to physiological, pathological and anatomical changes [2,4]. Accordingly, polypharmacy is a logical result of the concurrence of multiple diseases among geriatric patients [3]. Polypharmacy has been defined as the use of five or more different prescription medications at the same time in the same patient [3,4,5,6]. In addition, there is a significant danger of certain specific medications when used by geriatric patients which is termed potential inappropriate medications (PIMs) [8,9]. The consequences of overuse of PIMs among geriatric patients include increase in drug costs, polypharmacy and drug-drug interactions (DDIs) [10]. Therefore, a list was designed to identify drugs that are considered inappropriate for use in the geriatric population. This list was later known as

the Beers' criteria [8]. Beers' criteria are useful in geriatric patients to decrease adverse outcomes such as DDIs [11,12]. In the geriatric population, DDIs are common, especially in those receiving polypharmacy, and 50% of DDIs can ultimately lead to adverse events [1,6]. DDI has been defined as a change in the action of one drug caused by the presence of another [6]. DDIs can alter pharmacokinetics and pharmacodynamics of a drug [16]. This may enhance (a synergistic effect) or reduce (an antagonistic effect) the efficacy of one or both of the drugs, or a new effect may appear [6]. Data on PIMs used and their consequences in geriatric hospitalized patients are quite rare [13]. Moreover, the predictors of DDIs have not been well studied especially in Malaysia. Hence, the main objective of this study was to estimate the incidence of DDIs as a consequence of PIMs prescribed to geriatric inpatients in Penang Hospital, Malaysia.

Methods

A prospective cohort design with convenient sampling was used. Data were collected from 400 geriatric inpatients in Penang Hospital, Penang, Malaysia for 9 months which spanned from December 1st, 2007 until August 31, 2008. The inpatient units selected were general medicine, endocrinology, nephrology, cardiology and cardiac rehabilitation. Penang Hospital is the main governmental and referral hospital in Pulau Pinang. The population of Pulau Pinang is approximately 1, 5 million, and the major ethnic groups include Malay (40.5%), Chinese (41.8%), Indian (10.4%), and others (7.3%)[14]. The study was approved by the Clinical Research Committee (CRC) and supported from medical staffs and staff nurses at medical wards of Penang Hospital. Data were collected from admission records, patients' progress notes and investigational medical charts all in the inpatient setting and from cumulative medication records at the satellite pharmacy. Geriatric patients were eligible if they were aged 65 years or above, admitted to one of the medical inpatient units of Pulau Pinang General Hospital with any chronic medical disease or condition, had received at least one medication and void of any adverse drug reactions. For the cohort design, geriatric inpatients were followed from the time of their admission until they were discharged, transferred to another ward or dead. Additionally, the patients were monitored daily to determine if PIMs were prescribed and to determine the occurrence of DDIs. The 2003 version of the Beers' criteria of PIMs were used to assess appropriateness of prescribing among geriatric inpatients[15]. Based on Beers' criteria, PIMs are divided in two groups. The first group is medications or a medication class that should be avoided in geriatric patients because they are ineffective or because of an unnecessarily high risk of a potential undesired effect or because a safer drug as an alternative is available. The second group is medications that should be avoided if the patient is having specific medical diseases or conditions. ADRs, according to the WHO definition, include DDIs [15,26]. Therefore, in practice, it is difficult to recognize whether an adverse outcome found in patients has resulted from DDIs or ADRs [24]. In this research, we used a drug interaction probability scale (DIPS) to assess the probability of the causal relationship between DDIs and adverse events [15]. DDIs were common in geriatric patients who received multiple medications, and 50% of DDIs caused adverse outcomes [37]. Undesired clinical outcomes as consequences of DDIs were determined using a drug interaction probability scale (DIPS) scale. This scale is based on the modification of Naranjo adverse drug reactions scale. These modifications were performed [19]. A DIPS algorithm was designed to assess the association between DDIs and the adverse event [15]. The severity of DDIs was identified using the updated MICROMEDEX ® databases. In addition, the severity of DDIs was further categorized as major (life-threatening with required medical intervention), moderate (an exacerbation of the patient's health with required drug alteration) or minor (does not require drug alteration) [16,17,29]. The causality of DDIs was determined using a drug interaction probability scale (DIPS). A DIPS algorithm was designed to assess the association between DDIs and the adverse event [16]. The severity of DDIs was identified using the updated MICROMEDEX ® databases [17]. In addition, the severity of DDIs was further categorized as major (life-threatening, required medical intervention), moderate (an exacerbation of the patient's health, required drug alteration) or minor

(not required drug alteration) [17,18]. Collected data were analyzed using the Statistical Package for Social Sciences (SPSS) software version 16. All reported p-values were two tailed, and the result was significant if p-value was <0.05. Binary and multiple logistic regression models were applied to identify the most important predictors associated with DDIs.

Results

The mean age of geriatric inpatients was 74.2 ± 6.5 years, and more than half (53.5%) were females. The largest ethnic group was Chinese (58.0%), followed by Malay (25.8%) and Indian (14.0%). The mean length of hospital stay was 5.52±5.9 days. Only 7.5% of the hospitalized geriatric inpatients were admitted from nursing homes in Penang (Table 1). The mean number

Table 1. Comparison of the proportion of Sociodemographic profile of patients with and without DDIs among geriatric inpatients (n=400)

Characteristic	Category	Patients with ≥ 1 DDIs (n=52)	Patients without DDIs (n=348)	p-value*
Age	65-69	(14.3)17	(85.7)102	0.460
	70-74	(8.3)11	(91.7)121	
	75-79	(17.1)13	(82.9)63	
	80-84	(15.8)6	(84.2)32	
	85-89	(13.8)4	(86.2)25	
	≥ 90	(16.7)1	(83.3)5	
Gender	Male	(11.8)22	164(88.2)	0.562
	Female	(14)30	184(86)	
Ethnicity	Malay	(13.6)14	89(86.4)	0.799
	Chinese	(13.8)31	(86.2)201	
	Indian	(9.2)6	(90.8)59	
	Others	1(11.1)	8(88.9)	
Nursing residency	Yes	(10)3	(90)27	0.002
	NO	(13.2)49	(86.8)321	
Hospital readmission	Yes	9(17.3)	26(7.5)	0.019
	No	43(82.7)	322(92.5)	
LOHS	Mean ± SD	6.42±1.32	3.69±1.10	<0.001[a]
Smoking consumption	Smoker	6(11.5)	36(10.3)	0.140
	Ex-smoker	2(3.8)	47(13.5)	
	Non-smoker	44(84.6)	265(76.1)	
Alcohol consumption	Drinker	10(19.2)	53(15.2)	0.675
	Ex-drinker	10(19.2)	81(23.3)	
	Non-drinker	32(61.5)	214(61.5)	
Body systems	Cardiovascular	(13)47	(87)314	0.972
	Renal	(13)13	(86.9)86	0.964
	GIT	(19.1)22	(80.9)93	0.021
	Haematological	(12)6	(88)44	0.822
	Skin	(21.9)7	(78.1)25	0.120
Diagnosis	Arrhythmia	11(24.4)	34(75.6)	0.015
	Diabetes mellitus	35(15.9)	185(84.1)	0.038
	GIT diseases	25(17.5)	118(82.5)	0.047
	Renal diseases	14(14.1)	85.9)	0.697
	Heart Failure	9(13.0)	60(87.0)	0.991
	Skin diseases	6(22.2)	21(77.8)	0140

[a] *Independent t-test*

Table 2. Distribution of drug-drug interactions (DDIs) and adverse outcomes developed in geriatric inpatients during hospitalization

Drug-drug interactions	Frequency (n=52)	Outcome	Incidence (%)
Warfarin + NSAID	5	Bleeding	9.6
ASA + Perindopril	4	Hypotension	7.7
ASA + Warfarin	4	Bleeding	7.7
ASA + Clopidogrel + Enoxaparin	3	Bleeding	5.7
Diltiazem + Lovastatin	2	Lovatoxicity	3.8
Digoxin + Metoprolol	2	Bradycardia	3.8
ASA + Furosamide + Digoxin	2	Digoxin toxicity, hypokalemia	3.8
Perindopril + KCl	2	Hyperkalemia	3.8
Ticlopidine + Warfarin	2	Bleeding	3.8
Diltiazem + Digoxin	2	Digoxin toxicity	3.8
Warfarin + Clopidogrel	2	Bleeding	3.8
Warfarin + Enoxaparin	2	Bleeding	3.8
Metoprolol + Diltiazem	2	Bradycardia	3.8
Perindopril + KCl + Furosemide	2	Nephrotoxicity	3.8
ASA + Clopidogrel + Ticlopidine + Enoxaparin	2	Bleeding	3.8
ASA + Warfarin + Clopidogrel + Enoxaparin	2	Bleeding	3.8
Digoxin + Spironolactone	1	Digoxin toxicity	1.9
Chlorothiazide + digoxin	1	Digoxin toxicity	1.9
Amiodarone + Clopidogrel	1	Bleeding	1.9
Amiodarone + Warfarin	1	Bleeding	1.9
Amiodarone + Atenolol	1	Bradycardia, arrhythmia	1.9
Amiodarone + Digoxin	1	Digoxin toxicity	1.9
Diltiazem + Atenolol	1	Hypotension	1.9
Metoprolol + Insulin	1	Hypoglycemia	1.9
Metoprolol + Diphenhydramine	1	Bradycardia	1.9
Metformin + Enalapril	1	Metabolic acidosis	1.9
ASA + Clopidogrel + Enoxaparin	1	Bleeding	1.9
ASA+ Perindopril + Furosemide + Spironolactone	1	Nephrotoxicity	1.9

NSAID: non sterioidal antinflammatory drugs, ASA: acetyl salicylic acid, KCl: potassium chloride

of medications was seven per geriatric inpatient, which was similar to that reported by previous studies [24,30]. There was a significant association between length of hospitalization and hospital readmission of the geriatric inpatients and DDIs (p=0.019). Additionally, the incidence of DDIs among those who lived in nursing homes was significantly increased (p=0.002). In this study, 9% of geriatric inpatients taking 1-4 drugs had at least one DDI, whereas 34% of those taking 5-8 drugs had DDIs, and 59% of patients taking 9-18 drugs had DDIs. Based on the second group of Beers' criteria, the PIMs that should be avoided when geriatric inpatients have specific diseases or conditions in this study included the following: the prescribing of acetylsalicylic acid and/or NSAIDS for geriatric inpatients receiving antithrombotic medications such as clopidogrel, ticlopidine and dipyridamole (69.25%) and the prescribing of calcium channel blockers and/or anticholinergics for geriatric inpatients diagnosed with chronic constipation (3.75%). Additionally, the most frequently prescribed PIMs were high doses of ferrous sulfate (22%), short acting nifedipine (15.3%), ticlopidine (13.6%), digoxin (12.2%), chlorpheniramine (5.4%) and diphenhydramine (5.1%). The severity of PIMs received by geriatric inpatients ranged from 186 (63.04%) classified as high risk versus 109 (36.95%) as low risk. A significant association between PIMs used and the occurrence of DDIs

Table 3. Predictors of DDIs among geriatric inpatients (n=52) using Binary Logistic Regression Model

Category	Predictor	B[a]	OR[a]	95% CI[a]	p-value*
Patient related	LOHS	0.06	1.12	1.02-1.24	0.001
	Nursing homes	1.18	3.25	1.40-7.57	0.006
Diseases related	GIT disease	0.59	1.80	1.0-3.25	0.040
	GIT symptoms	0.35	1.42	1.10-1.82	0.006
Drug related	Haemorrhage	0.94	2.57	1.08-6.09	0.032
	Hypoglycemia	1.64	5.12	2.58-10.26	<0.001
	GIT system	0.69	2.01	1.10-3.66	0.022
	Warfarin	2.23	9.33	3.98-21.87	<0.001
	Anticoagulants	0.41	1.50	1.07-2.11	0.018
	Diltiazem	1.38	3.99	1.41-11.32	0.009
	OHA	0.95	2.58	1.42-4.70	0.002
	Gliclazide	1.07	2.91	1.60-5.27	<0.001
	Metformin	0.64	1.90	1.03-3.50	0.038
	Antidiabetics	1.08	2.95	1.59-5.47	0.001
	Antipsychotics	0.76	2.13	1.11-4.09	0.023
	Tramadol	1.12	3.07	1.46-6.45	0.003
	Nutrition	0.73	2.08	1.12-3.85	0.020

**Binary logistic regression test, [a]OR: Odds ratio, [a]CI: Confident interval, [a]B: regression coefficient value. LOHS: length of hospital stay, GIT: gastrointestinal, OHA: oral hypoglycemic agents.*

Table 4. Predictors of DDIs among geriatric inpatients (n=52) using Multiple Logistic Regression Model

Category	Predictor	B[a]	OR[a]	95% CI[a]	p-value*
Patient related	LOHS	0.07	1.14	1.01-1.24	0.001
Disease related	Polymorbidity	0.83	2.20	1.15-9.10	0.020
Drug related	Warfarin	2.45	8.35	2.90-19.80	<0.001
	Polypharmacy	1.58	10.4	3.10-35.40	0.001
	PIMs	1.20	2.45	1.30-8.50	0.003

**Backward stepwise logistic regression test. [a] Crude OR: Odds ratio, [a]CI: Confident interval,*
[a]B: regression coefficient value. PIMs: Potential inappropriate medications, LOHS: Length of hospital stay.

was observed (p=0.012). Approximately 69% of patients with DDIs had received PIMs. Based on the DIPS, approximately 27 (52%) of the geriatric inpatients were classified as having the probable risk of developing adverse events and 22 (42.3%) of patients were classified under the possible rating. Other patients were classified as unlikely. With respect to the severity of DDIs, approximately 19 (36.5%) of patients were rated as having major adverse events of DDIs, 23 (44.2%) as moderate in severity and 10(19.3%) experienced minor severity of adverse outcomes. Additionally, 24 (46.2 %) of patients had recovered from adverse events that were caused by DDIs. However, 11 (21.1%) of patients did not recover, 9 (17.3%) had unknown outcomes and 8 (15.4%) of patients died. The most frequent adverse clinical outcomes caused by DDIs among geriatric inpatients were hypoglycemia (36.2%), hypotension (33.3%), GIT problem (28.2%), and hemorrhage disorders (25.8%). The common drug-drug combinations resulting in DDIs were warfarin with NSAIDs, ACE inhibitors with diuretics or NSAIDs, digoxin with metoprolol, ASA, furosemide and digoxin and others (Table 2). From Table 3, the binary logistic model identified the following predictors of DDIs: arrhythmia, renal disease and nursing home residency, elevated INR and elevated serum creatinine. Moreover, the prescribing of diuretics, warfarin, amiodarone, digoxin, diphenhydramine, chlorpheniramine, tramadol, antiarrhythmics, cephalosporins, antihistamines, gastrointestinal

drugs, antipsychotics, nitrates, and poor nutrition are most likely responsible for the DDIs in geriatric inpatients. These predictors suggest that medications that are listed in Beers' criteria should be discontinued to avoid risk of DDIs. Using logistic regression analysis, we confirmed that the incidence of DDIs correlated with polymorbidity and the use of PIMs. Similar to previous studies, we found a strong correlation between polypharmacy and the risk of DDIs, and polypharmacy was found to be a significant predictor of DDIs among geriatric inpatients at Penang Hospital [37,41,42,44] (Table 4).

Discussion

Controversy continues regarding the use of polypharmacy in the elderly and the risk of DDIs . Unlike previous studies, an increased risk of DDIs with age was not confirmed in this study [29,35,36]. This study provides additional findings on the assessment of the appropriateness of prescribing medications among geriatric inpatients. Beers' criteria have been considered as a reliable tool for assessing the appropriateness of prescribing medications among geriatric patients [15,20]. In the present study, the prevalence of PIMs was identified in 52.8 % of geriatric inpatients who were admitted to Penang Hospital, and this rate is considered higher than rates found in previous studies [20,32]. For instance, the prevalence rates of PIMs in U.S. studies in outpatient settings using the 1997 Beers' criteria found that the PIMs rate was between 14.3% and 28% [21]. In agreement with this study, it was reported that ferrous sulfate (dose ≥325 mg/day) and nifedipine (short acting) were among the more common PIMs prescribed to geriatric patients [34]. Ferrous sulfate was commonly prescribed to geriatric patients because approximately half the patients in this group had anemia, and 30% of them had renal disease. In fact, there are many safer alternative medications belonging to that same class that are available for use in geriatric inpatients. Similar to previous studies, the occurrence of DDIs during hospitalization of geriatric inpatients increased with PIMs [24,25,26]. In the present study, the incidence of DDIs was 13% among geriatric inpatients. Actually, this rate is double the rate reported in a previous study conducted by Grönroos et al. (1997) [27]. However, our finding is reasonable compared with previous studies that found that the incidence rates of DDIs among geriatric patients ranged from 7.4% to 46% [27,28,29]. We found a significant association between mortality rate and DDIs (p=0.004) RR: 3.54; 95%CI: 1.43-8.69, which is in agreement with previous studies [22,23]. Unfortunately, DDIs noted in this study may be an underestimate because only prescribed drugs were assessed and not nonprescription drugs, herbal remedies or food supplements. In fact, it was difficult to estimate the actual incidence of DDIs among hospitalized geriatric inpatients due to the difficulty of detecting a difference between clinically significant and clinically non-significant DDIs. Additionally, it was unclear whether the adverse events found were the result of DDIs or of one of the other drugs used. Therefore, we used the drug interaction probability scale (DIPS) to assess the probability of the causal relationship between DDIs and an adverse event [16]. According to the DIPS, the higher the scores on the DIPS algorithm, the higher association between the adverse event and DDIs. It was found that warfarin and digoxin are the two of the most common medications associated with in DDIs with considerable morbidity or fatal outcomes. Other important DDIs that need to be avoided because they led to adverse outcomes included NSAIDs with antithrombotic agents and digoxin with diuretics and/or beta blockers. Additionally, prescribers should be aware of prescribing diuretics for patients taking ACE inhibitors because this combination was associated with an increased the risk of hypotension for many patients in this study. Diuretics, NSAIDs, and ACE-inhibitors accounted for the highest number of DDIs, which is similar to the findings reported in previous studies [29,33]. Although the potential negative impact of PIMs among geriatric inpatients is still not well documented in any previous studies in Malaysia, this study attempted to address this critical question. Limitations of our study included the inability to prevent PIMs from being prescribed and the occurrence of DDIs. Although the prospective nature of PIMs and the detection of DDIs were the major strengths of this study, our results may still be an underestimate of the incidence of DDIs and drug-disease interactions. The main reason is that the

effects of non-prescription medications and herbal remedies were not assessed in this study.

In conclusion, geriatric patients on polypharmacy, having polymorbidity and taking PIMs should be monitored closely because they are at increased risk for DDIs. Therefore, we are suggesting that frequent monitoring of geriatric inpatients should be continued, and PIMs should be discontinued over the period of hospitalization because of the high risk of DDIs. The predictors of DDIs can help health care professionals to identify patients with a high risk of DDIs.

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Correlation analysis between cerebral micorbleeds and severity of hypertension by susceptibility - weighted imaging

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Abstract

Objective: To explore the diagnostic value of susceptibility-weighted imaging (SWI) for cerebral micorbleeds (CMBs), and to study the correlation between CMBs and severity of hypertension by SWI.

Methods: Clinical data on routine MR and SWI of 150 hypertension patients combined with CMBs was retrospectively analyzed. The correlation of the number of CMBs with the severity and duration of hypertension and the control of blood pressure was analyzed.

Result: A total of 526 CMBs was detected in the 150 patients. The CMBs were mainly multiple, and mainly distributed in the deep areas such as basal ganglia (35.6%) and thalamus (30.8%). There was significant difference in the number of CMBs among the patients with different severity and duration of hypertension and the control of blood pressure ($P<0.05$). The grade of CMBs had significant positive correlation with grade of lacunar infarction ($r=0.269$, $P=0.0009$).

Conclusions: SWI has high sensitivity to CMBs, the number of which is significantly positively correlated with the severity of hypertension.

Key words: Susceptibility-weighted imaging, cerebral micorbleeds, hypertension, correlation.

Introduction

Cerebral microbleeds (CMBs), which is a kind of subclinical damage of brain parenchyma, mainly manifests as local deposition of hemosiderin induced by cerebral small vessel lesions and minor bleeding [1, 2]. Recent literatures report that CMBs is significantly correlated with hypertension, lacunar infarction, white matter lesions and cerebral hemorrhage, and hypertension may be one of the risk factors for CMBs. The presence of CMBs indicates severe cerebral microangiopathy, and the involved vessels are prone to recurrent bleeding. Lack of specific clinical symptoms, not be diagnosed by conventional magnetic resonance imaging (MRI), and the presence of induced relaxation with magnetic sensitivity result in the difficulty in CMBs diagnosis[3]. Magnetic susceptibility-weighted imaging (SWI), which is a new MRI method developed in recent years, greatly increases the detection rate of CMBs through the application of high resolution scanning, phase image masking and minimum intensity projection, and currently has been widely used for the examination of central nervous system [4,5]. In this study, we applied SWI to detect CMBs in patients with hypertension, aiming to explore the correlation of the number of CMBs with the grading of hypertension.

Material and Methods

General information

One hundred and fifty hypertension patients complicated with CMBs in the Neurology Department of our hospital from October 2009 to October 2012 were enrolled. All the subjects underwent conventional MRI and SWI examination. Inclusion criteria: (1) clinical hypertension; (2) complete records of medical history; (3) patients' condition allowing MRI examination; (4) clinical features and cranial CT and MRI imaging features supporting the diagnosis of CMBs. There were 86 male patients and 66 female patients with the age from 31 to 75 years old and (51.4 ± 8.2) years on average. All patients signed informed consents before receiving the examination.

Diagnostic criteria and classification

Hypertension was in accordance with the diagnostic criteria of "Chinese Hypertension Prevention and Treatment Guide, 2005" [6], namely systolic blood pressure ≥ 140 mmHg and (or) diastolic blood pressure ≥ 90 mmHg without taking antihypertensive drugs. According to the 1999 WHO criteria, the hypertension was divided into three grades: 140~159/90~99 mmHg for hypertension grade I, 160~179/100~109 mmHg for hypertension grade II, and above 180/110 mmHg for hypertension grade III. Hypertension duration was divided into three periods including less than 5 years, 5-10 years and more than 10 years. Patients' blood pressures controlled in the normal range were regarded as well-control, while those controlled above the normal range were as poorly-control. The SWI performance of CMBs were defined as round and oval low signal with diameter <2~5 mm or lack of signal in brain parenchyma, clear boundary and without edema in surrounding tissues [7, 8].

Imaging methods

Siemens Magnetom Avanto 1.5 T superconducting MR scanner and head 8-channel phased array coils were applied for MRI image acquisition. The scanning sequences of all the patients comprised of at least cross-sectional T1WI, T2WI, T2 FLAIR and sagittal T1WI. T1WI (TR/TE = 500/8.4), T2WI (TR/TE = 5000/89), T2 FLAIR (TR/TE = 9000/89), layer thickness 6 mm, interlayer spacing 4 mm, FOV 23 × 20 cm, matrix 256 × 177, and 16-layer scanning. SWI (TR/TE = 49/40), flip angle 15°, layer thickness 2 mm, FOV 23 × 20 cm, matrix 256 × 177, and 56-layer scanning. All the images were delivered to a workstation for minimum intensity projection (MinIP) post-processing, and were evaluated by two experienced physicians of our hospital to determine the location and number of CMBs: determining the position of CMBs (cortex-subcortical area, basal ganglia, thalamus, brainstem and cerebellum) and calculating the number of CMBs in different positions. Patients with CMBs were classified according to the number of CMBs: Grade I: 1~3; Grade II: 4~6; Grade III: 7~9; Grade IV: more than 9. According to the MRI features, the patients with CMBs were also classified as: Grade 0: 0; Grade I: 1~3; Grade III: 4~10; Grade IV: more than 10.

Statistical analysis

SAS 8.1 software package was applied for statistical analysis. Measurement data was represented as ($\bar{x} \pm$ s), and correlation of CMBs number with the grading, duration and control of hypertension was analyzed by rank sum test. $P<0.05$ was considered statistically significant.

Results

SWI sequence performance and distribution of CMBs

All the 150 patients had CMBs, which appeared as oval and uniform very low signal area with clear boundary and solitary and discontinuous distribution between the upper and lower layers. Thirty-four patients had single CMB and 116 patients had multiple CMBs. A total of 526 CMBs were detected with a diameter of about 0.3~0.5 cm, and mainly distributed in the deep areas of basal ganglia (187, 35.6%) and thalamus (162, 30.8%), following by cortex- subcortical area (53, 10.1%), brainstem (61, 11.5%) and cerebellum (63, 12.0%).

Correlation of CMBs number with grading, duration and control of hypertension

According to rank sum test, the average number of CMBs showed significant difference in patients with different grading, duration and control of hypertension ($P<0.05$) Table 1.

Correlation of CMBs grade with lacunar cerebral infarction

Spearman rank correlation analysis showed that CMBs grade was significantly positively correlated with the degree of lacunar cerebral infarction (r=0.269, $P=0.0009$) Table 2.

Discussions

The prevalence of CMBs among population is approximately 5% ~ 6%, and significantly increases along with the aging of society, the gradual increase of the prevalence of hypertension and stroke, and unreasonable use of anticoagulants [9, 10]. It is generally believed that cerebral hemorrhage, lacunar infarction and white matter lesions are associated with hypertension-induced cerebral microvascular diseases. Pathological findings demonstrate that CMBs are mostly located around those arteriloes

Table 1. Correlation of CMBs number with grading, duration and control of hypertension ($\bar{x} \pm s$)

Variables	n	CMBs number	Average CMBs number	Z	P
Hypertension grading				3.78	<0.05
Grade I	36	81	2.25±0.84		
Grade II	85	316	3.72±0.92		
Grade III	29	129	4.45±1.37		
Hypertension duration				4.04	<0.05
< 5 years	48	99	2.06±0.74		
5-10 years	74	282	3.81±0.85		
> 10 years	28	145	5.18±1.22		
Hypertension control Well-control Poorly-control	 89 61	 279 247	 3.13±1.04 4.05±1.51	3.05	<0.05

Table 2. Correlation of CMBs grade with lacunar cerebral infarction

CMBs grade	Degree of lacunar cerebral infarction				Sum
	0	1	2	3	
1	22	33	15	12	82
2	6	15	14	17	52
3	2	3	2	1	8
4	1	0	3	4	8
Sum	31	51	34	34	150

Spearman r=0.269, P=0.0009

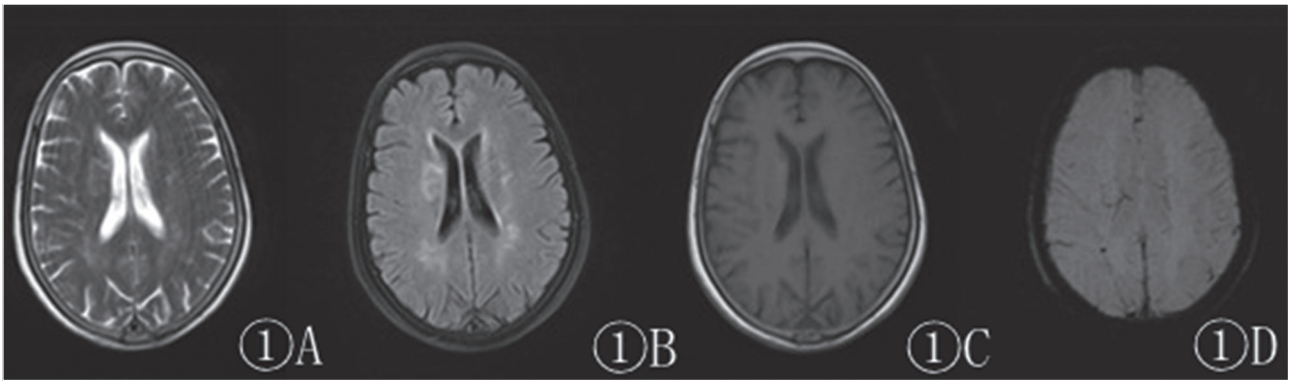

Figure 1. A female patient (71 years of age) with hypertension for 20 years, whose blood pressure was poorly controlled, and the maximum of blood pressure was 180/100 mmHg

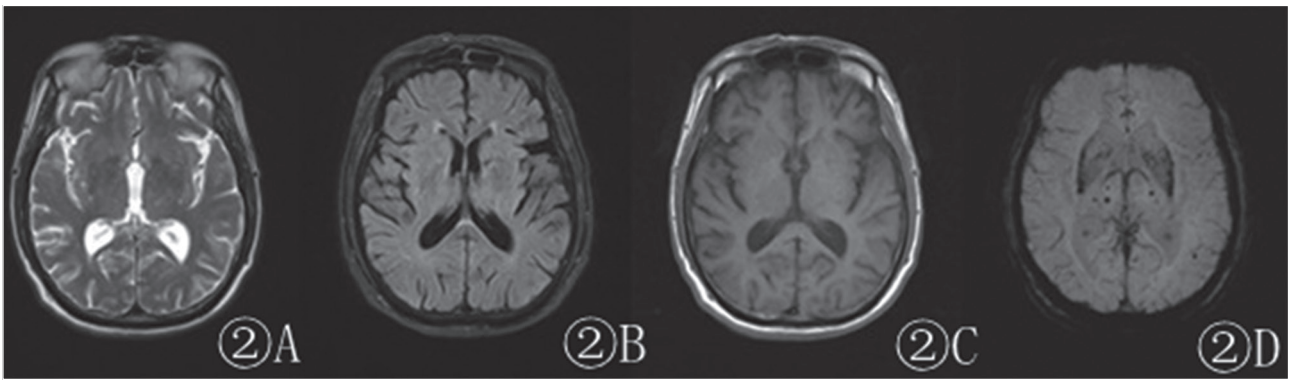

Figure 2. A male patient (70 years of age) with hypertension for 7 years, whose blood pressure was controlled at 130/90mmHg, and different sizes of CMBs were observed in bilateral basal ganglia

or microaneurysms, indicating hypertension is independent risk factors for CMBs. Well control of hypertension can effectively reduce the occurrence of CMBs lesions [11-13]. However, there is still not very clear that whether the presence and number of CMBs are correlated with the grading, duration and control of hypertension.

Due to less hemosiderin deposition in local CMBs lesions (diameter <5 cm) and no edema in surrounding brain tissues, the conventional MRI sequences such as T1WI, T2WI and FLAIR show negative appearance because of lack of sensitivity. SWI, as a high-resolution and completely flow empty compensating 3-dimensional gradient echo sequences, the images of which can fully demonstrate the difference of intrinsic magnetic susceptibility between tissues, especially small venous bleeding and iron ion deposition. Recently, SWI is mainly used in examining the lesions of central nervous system [14], and the sensitivity is 4 to 6 times higher than that of conventional MRI sequences [15, 16]. A total of 526 CMBs lesions were found in the 150 patients with the diameter of 0.3~0.5 cm. Most of CMBs were multiple, and mainly distributed in the deep regions of basal ganglia (35.6%) and thalamus (30.8%). Considering feeding arteries are mostly deep perforating arteries in deep brain, the latter is more likely to form CMB lesions due to hypertension blood flow induced curving, spiral and eventually bleeding [17,18].

The results of our study found that patients with different grading, duration and control of hypertension had significantly different numbers of CMBs (P<0.05), suggesting the CMBs number was correlated with the grading, duration and control of hypertension, and along with the aggravation of hypertension, the extension of hypertension duration, and the improper control of hypertension, the number of CMBs markedly increased, which further illustrated that hypertension was one of the important risk factors for CMBs. Chronic hypertension can seriously damage cerebral tiny perforating arteries, resulting in vascular stenosis, brittle or tiny aneurysm, which may also be etiological basis of CMBs [19]. Some studies have pointed out that the incidence of symptomatic hemorrhagic transformation in acute cerebral infarction patients with CMBs during intravenous thrombolytic treatment was significantly higher than that of acute cerebral infarction patients without CMBs [20]. Our data showed that CMBs patients usually complicated with different degrees of lacunar cerebral infarction, which was significantly positively correlated with CMBs (r=0.269), suggesting lacunar cerebral infarction, as one of the severe complications of hypertension, may have the same pathophysiological mechanism with CMBs. Both of the diseases are caused by the increase of cerebral microvascular permeability, which is probably induced by long-term hypertension [21]. Therefore, CMBs can be used as a predictor of the risk of cerebral hemorrhage to a certain extent in the long-term anticoagulation or antiplatelet therapy. Screening the patients with high risk of bleeding in clinical treatment and giving individualized treatment can effectively reduce the incidence of symptomatic hemorrhagic transformation.

Taken together, SWI has high sensitivity to CMBs, and can significantly improve the detection rate of CMBs. CMBs number is significantly positively correlated with the grade of hypertension, and strict control of blood pressure in hypertension patients complicated with CMBs has great significance in reducing the formation of CMBs and preventing the damage of small blood vessels.

Acknowledgement

The study was supported by the medical funds of the Science and Technology Department of Zhejiang Province. (project number: 2012C33099)

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Effect of different scoring systems on mortality rates and length of hospitalization in cardiac surgery patients

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Abstract

Objective: Precise risk estimation of mortality in cardiac surgery patients is often difficult. Main objectives of this study is to calculate mortality probability by two different risk score system and to inquire effects of informing anesthesiologist and surgical team; of score of the patient; on length of hospitalization and intensive care and also on actualized mortality.

Methods: We have studied retrospectively and prospectively for estimation of mortality by two different scoring systems.(ASA and EuroSCORE II) This study is carried out in one center, designed at the university hospital. It includes 438 cardiac surgery patients in between December 2010 and May 2012 period. During this study we have given international scores (ASA and EuroSCORE II) to the patients before cardiac surgery.

Results: The group which was applied ASA and EuroSCORE II had a significant shorter hospitalization length, and in this group predicted mortality and observed mortality were in a linear relationship.

Conclusion: Preoperative determination of the mortality risk of patient by anesthesiologists and surgeons can be used as an early warning for the estimation of treatment period and course of the disease. By these means length and cost of hospitalization can be minimized.

Key words: ASA, euroSCORE, cardiac surgery, mortality, hospitalization.

Introduction

Through the advances of technology and surgical technique; cardiac surgery and anesthesia become dealing with high risk patient population. (1) In anesthesia for predicting mortality of patients preoperatively ASA (American Society of Anesthesiologists) staging system or alike are used and these provide some level of standard and quality at the follow up; but none of them incorporates patients risk profile information and can only give coarse mortality rates and values.

In 1995 a new scoring system, EuroSCORE; by patients from 8 European countries and including 97 parameters is constructed (2). In 2011 it is revised as EuroSCORE II. (3) By this risk score system, mortality, morbidity, length of hospitalization and cost estimations can be made. (4) Aim of this study is to calculate mortality rate of the patients preoperatively with two different scoring system (ASA, ASA and EuroSCORE II combination) and to determine the effects of informing anesthesiologist and surgical team of the scores on length of hospitalization and intensive care and also on actualized mortality.

Materials and Methods

This study involves a single centre. It includes cardiac surgery patients between December 2010 and May 2012 period; which are studied retrospectively and prospectively. As the result of statistical power analysis when α=0.05, β=0.20, (1-β)=0.80, control group event rate: (Group A) 4,3% , study group event rate (Group AE): %0,04 is taken 180 patients were enrolled for each of the group, for the study and as a result at least 360 patients were planned to be included totally. After ethical committee approval and informed consents of the patients; ASA anesthesia risk group 2-5 patients between 18-70 years of age and with planned cardiac sur-

gery are included. In the study mortality estimates of ASA system is standardized according to the means of international standard and predicted mortality values are as follows: ASA 1: 0.07%, ASA 2: 0.33%, ASA 3: 3.05%, ASA 4: 15.4% and ASA 5: 30.2%. It was conducted on 341 male (78%) and 97 female (22%) patients. Total number of patient was 438. For EuroSCORE II calculated values and predicted mortalities online computerized program on EuroSCORE II website is used.

All scoring process is conducted by a single anesthesiologist whom has not attended anesthesia or surgery of the patients and was not planned to follow up the patient postoperative. Terminal malignant diagnosis, multiple organ failure, confirmed brain dead, mechanical ventilation dependent or planned transplantation patients were excluded from the study.

Patients were divided into two groups; preoperative "A" group only has ASA staging system and "AE" group had both ASA and EuroSCORE II system. Patients are followed up in intensive care, in ward and first 30 days after surgery for mortality (observed mortality). Identified risk groups according to EuroSCORE II risk score system is as follows: 0-2 low risk group, 3-5 medium risk groups and 5 or more high risk group. All the medical care professionals providing care to the patients (anesthesiologists, surgical team, cardiologists, intensive care nurses, ward nurses) were informed for determined scores and expected mortalities. Patients were monitored for mortality starting from preoperative period to intensive care and wards up to 30 days after operation.

Data were analyzed with SPSS (ver.20.0) and Kolmogorov-Smirnov test was performed to all the parameters for compliance with normal distribution. For parameters fitting normal distribution independent sample t test and for parameters non fitting Mann Whitney U test is used with a confidence interval of 0.05.

Results

Study was conducted on 341 male (78%) and 97 female (22%) demographically, total of 438 patients. Cardiac surgery procedures which were applied to patients are shown in the table 1.

There was no statistically significant relationship between the groups and the sexes. ($p>0.05$) Mean age of Group A was 62.2±10.4, and Group AE was 61.9±10.1 and difference between two groups was not statistically significant ($p>0.05$). Males in A group were 81%. There was no significance according to gender and age ($p>0.05$).

AE groups length of hospitalization was found to statistically significant ($p<0.05$).

Length of hospitalization for AE group was found to be 8±1.89 days, and A group was 8.93±1.95 days. In AE group predicted mortality and observed mortality were in a linear relationship.

Predicted mortality rate, observed mortality rate and length of intensive care were not different for patient groups and were not statistically significant ($p>0.05$).

All parameters and gender were not statistically relevant ($p>0.05$).

Table 1. Distribution of cardiac surgical procedures

	No.of patients	Percentage
CABG	303	69.2
AVR	51	11.8
MVR	17	3.8
CABG+AVR	40	9.1
CABG+MVR	8	1.8
CABG+DOUBLE VALVE	3	0.6
CABG+TRIPLE VALVE	1	0.2
CABG+AORTIC ANEURYSM	2	0.4
DOUBLE VALVE	5	1.1
ANEURYSM ASCENDING AORTA	8	2
Σ	**438**	**100**

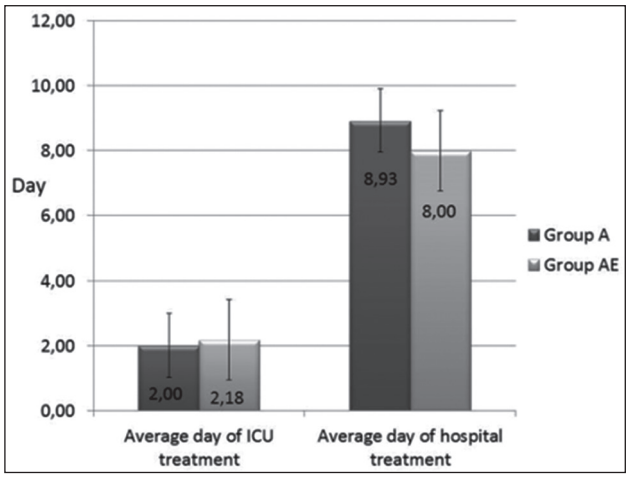

Figure 1. Average day of ICU and hospital treatment

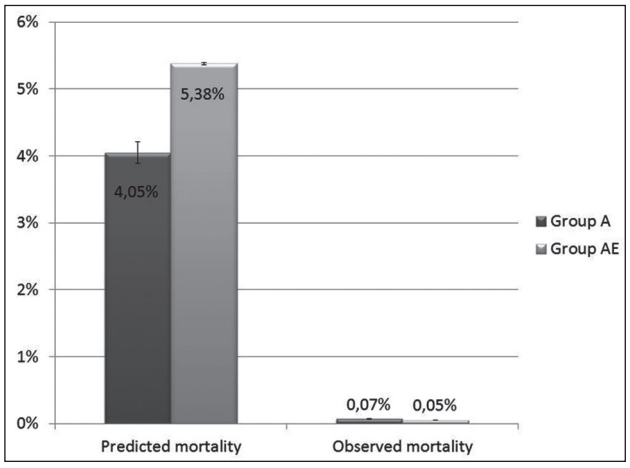

Figure 2. Mortality rates

Discussion

Open heart surgery procedure being performed widespread around the world; made determination of preoperative mortality and morbidity risks imperative. Statistical analysis of patient outcomes is in great importance for institutes, health care professionals and patients in determination of risks and prognosis and constructing specific treatment and procedures. (5) For our knowledge the study of us is the first comparison about the ASA alone and ASA with EuroSCORE combinaton. For leveling difference between patient populations and comparing previous and recent outcomes many different scoring systems has been developed. (2,6-14) Operative and hospital mortality are accepted as indicators of quality of life in cardiac surgery. Nevertheless, considerable variation in the ASA classification allocation has been reported in previous studies as it neither does consider the patient age, sex, weight, and pregnancy nor the nature of the planned surgery, the skill of the anesthesiologist or surgeon, the degree of pre-surgical preparation or the facilities for postoperative care.(15–17) The definitions are based on severity of disease and may result in inconsistent application. The measure of surgical complexity in the ASA classification system is less clear. The terms minor, intermediate and major are used to categorize the complexity of surgery. However, the assumption is that these definitions are intuitive and self-explanatory. In addition, the ASA score had been found in some studies to be a strong predictor of postoperative resource utilization and mortality in numerous surgical fields. It was significantly related to the incidence of postoperative death in a group of 3,438 elective total hip and total knee arthroplasty (TKA) patients with class III patients were more likely to encounter postoperative death as compared to patients with lower ASA scores.(18)

ASA (The American Society of Anesthesiologists (ASA) Physical Status (PS) Classification) is the most common used preoperative classification by anesthesiologists worldwide. For some authors and also for us, it must not use for risk stratification alone, because this classification is only a physical status indicator. In ASA classification the published absolute mortality rates of the individual classes showed considerable variation, with 0-0.3% for ASA I, 0.3-1.4% for ASA II, 1.8-4.5% for ASA III, 7.8-25.9% for ASA IV and 9.4-57.8% ASA V. (19) Because of these wide range variable values anesthesiologists and surgeons could hesitate about the mortality rates.

Most of the high risk patients are also the group that is going the benefit the most from the surgery. Mortality in the first 30 days after surgery, with regarding the risk factors, is one of the most important indicators of the quality of the service of the center. (2) In EuroSCORE risk scoring system, for calculating mortality; there are 8 preoperative patient related parameters, 4 heart related parameters, 4 operative parameters. It can be concluded that EuroSCORE risk scoring system is not much affected from operative variables. (14)

Risk scoring system in open heart surgery; in preoperative period; helps to provide a safer environment for the patient and family, anesthesiologist, surgeon and surgical team while de-

ciding upon surgery. In a study of Geissler et.al. six different risk scoring systems were compared prospectively with 504 patients. The actual 30-day mortality was 4%. The Cleveland, French and OPR scores predicted mortality between 3.5 and 4.9%, whereas mortality was considerably overestimated by the predictions of the Parsonnet, Euro, and Pons scores. Concordance of predicted and observed mortality was found to be highest in EuroSCORE.(14) In our study a similar result is also obtained. Predicted mortality and observed mortality in the group where ASA and EuroSCORE II is applied together is in a linear relationship and predicted mortality was increase as observed mortality in a linear fashion.

Predicted mortality in "AE" group being higher than "A" group did not affect the observed mortality in both groups in 30 days postoperative period. Early and precise determination of the risks by anesthesiologists and informing the other health care professionals about it seem to have no effect on observed mortality. Length of hospitalization in "AE" group is statistically significant. In case of ASA and EuroSCORE II are co-administered (AE group) length of hospitalization is shorter. This can be interpreted as in cases risks are well known; patients are monitored closely and treatments are made promptly.

Both ASA and EuroSCORE II are cheap, objective and modern risk scoring systems. Using these two systems together may be beneficial for treatment and follow up of high risk cardiac surgery patients.

Among adult patients that have undergone open heart surgery in this clinic; results of the "A" group which was scored with ASA and results of "AE" group which was scored with ASA and EuroSCORE II were compared. Low, medium and high risk groups predicted and observed mortality showed linear relationship.

Preoperative determination of mortality risk of a patient may give clues to the anesthesiologists, surgeons and other health care professionals, on the course of the disease for that patient. Precise scoring may decrease length of hospitalization for patients and decrease costs for institutions.

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The gender preference of the obstetricians and gynecologists for Chinese woman and their partner

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Abstract

Background: This study investigated whether Chinese woman and their partner have gender preference on the obstetricians and gynecologists.

Methods: A detailed survey was distributed to patients and their partner in obstetrics and gynecology waiting room. Using methods of qualitative and quantitative analysis, we interviewed the gender preferences of obstetrics and gynecology.

Results: 91.1% patients and 95.3% partner preferred female obstetrician-gynecologists and 8.9% patients and 4.7% partner indicated that they had no preference when only consider the gender of obstetrician-gynecologist. 98.4% patients and 96.9% partner exhibited a higher percentage of preference for a female obstetrician-gynecologist when considered intimate examinations. When considered not only the physicians' gender, 70.7% patients and 63.8% partner would like to select physicians have good skill. Physicians' experience, ability and moral character were considered important characters by the survey population. However, the physician's attitudes are no relationship with the gender of physicians. About 59.8% partners minds the selection of their wife, and 38.6% partners would interference the patients' selection. And 45.1% patients would change their selection if their partner mined their choice.

Conclusions: Our study found that although Chinese survey population did prefer a female obstetrician - gynecologist, professional skills were more important than gender when they choose a physician.

Key words: gender preference, obstetricians, gynecologists, Chinese woman.

Introduction

Many studies have investigated the gender preference on selecting obstetrician-gynecologists. Despite the widely assumption that woman prefer to see female obstetrician-gynecologists, some studies did find the majority of women prefer female providers[1,2], for survey population felt female physicians understand their problems better.

Others suggested most of women surveyed did not select their obstetrician-gynecologists based solely on physician gender. Although a small percentage of survey respondents did indicate a gender preference, it rarely influenced physician selection. When compared with other desirable physician characteristics, the gender is less important[3]. After surveying 1544 obstetric-gynecology patients in a large California military hospital, Lund's[4] found that 60% of respondents either had no gender preferences.

Do Chinese women prefer female obstetrician-gynecologists? Does their partner prefer female obstetrician-gynecologists? Few studies in the field of obstetrics and gynecology have investigated this topic. So we conducted a qualitative and quantitative study to investigate the topic of gender preference for obstetrician.

This survey was undertaken to quantify the incidence of obstetrics and gynecology provider gender preferences among our hospital and thereby provide pertinent data for clinic and hospital planning.

Materials and Methods

The Ethics Committee at the Second West China Hospital approved all aspects of the study. A self-administered, anonymous questionnaire included 45 items, which referred to the previous surveys[5, 6], written in Chinese was used to investigate the gender preference of the obstetricians and gynecologists for Chinese woman and their partner. These items include basic demographic data, preferences regarding the care patients received, gender of their

current health care provider, and other preferences they had in the obstetrics and gynecology setting. The items also constructed to try to find the reason why their preferences happened. The survey, distributed by trained research personnel, typically took minutes to complete while patients were waiting for their respective appointments. Similar questions were offered to their partner.

After acquiring oral consent from participants, we conducted the survey between June and July 2010 in obstetrics and gynecology waiting room. Patients who were at least 18 years of age were eligible. Some patients were accompanied by their partner, and interested partners completed anonymous surveys independently.

Descriptive statistics were used to describe participant characteristics in the study. Chi-square analysis and t-test were performed as needed. We chose $p \leq 0.05$ as indicating statistical significance.

Results

In obstetrics and gynecology waiting room, the 191 patients and 127 partner participated in the study. The age range of the patients and partners was from 18 to 74 years and 24 to77 years respec-

Table 1. Characteristics of patients and their partners

Characteristics	Patients		Patient partners	
	N=191	%	N=127	%
Age				
< 20	17	8.9		
20-30	96	50.3	54	42.5
30-40	53	27.8	51	40.2
40-50	19	9.9	16	12.6
> 50	6	3.1	6	4.7
Educational level				
Primary school	2	1.0		
Middle school	105	55	59	46.5
College	71	37.2	51	40.2
Graduate school	13	6.8	17	13.3
Martial status				
Unmarried	28	14.7	14	11.0
Married	155	81.2	113	89.0
Divorced	6	3.1		
Others	2	1.0		
Occupation				
Technician	26	13.6	22	17.3
worker	11	5.8	3	2.4
Peasant	13	6.8	8	6.3
Public official	29	15.2	28	22.0
Service industry	59	30.9	41	32.3
Others	53	27.7	25	19.7
Household income level				
＜￥2000/month	96	50.0	52	40.9
￥2000-4000/month	67	35.1	48	37.8
￥4000-6000/month	15	8.1	18	14.2
＞￥6000/month	13	6.8	9	7.1
Health insurance				
Yes	105	55.0	77	60.6
No	86	45.0	50	39.4

tively. 81.2% (155) of the women and 89%(113) of the men were married. In educational attainment, 37.2% of the patients and 40.2% of the partners had received education at college level or above. The patients and their partner's basement information were presented in Table 1.

From the survey study, 91.1% patients and 95.3% partner preferred female to be their obstetrician-gynecologists and 8.9% patients and 4.7% partner indicated that they had no preference when only consider the gender of obstetrician-gynecologist (P≤0.01) (Table 2). 98.4% patients and 96.9% partner exhibited a higher percentage of preference for a female obstetrician-gynecologist when considered intimate examinations include rectal, vaginal examinations and pelvic examinations (Table 3). But when considered the professional skill, 70.7% patients and 63.8% partner preferred doctors with good professional skill (Table 4).

Among all respondents, most of women patients feel no gender preference for physician attitudes (P≤0.05). 46.1% patients feel embarrassed when faced male physicians, and 38.7% patients feel embarrassed when faced male or female physicians. About one third of patients feel female physician are more comfortable, more understanding, and easier to talk. Compared with the woman physician, some patients thought that male physicians would be more gentle and respectful (Table 5).

The most important characteristic when selecting an obstetrician and gynecologist, 31.1% patients choose experience, 27.4%, ability and 24.9%, moral character. When asked about the patient's partner, 30.2% of them choose ability, 29.2%, experience, 21.7%, moral character (Table 6).

In this study, when patients were asked whether partner attitude influence their choice, there was 45.1% patients would instituted their choice, whereas 36.1% patients would change their choice. But when asked their partner, 59.8% partners mind the patients' choice, only about 38.6% of them would give some suggestion, 42.5% of them would not interference the choice of their partner (Table 7).

Table 2. Responders' gender preference for physicians

Procedure	Preference	Patients		Patients partners	
		N=191	%	N=127	%
obstetric visit and gynaecologic surgery	Physician				
	Prefer male	0	0	0	0
	Prefer female	174	91.1 *	121	95.3 *
	No preference	17	8.9	6	4.7

: P≤0.05

Table 3. Responders' gender preference for physicians on intimate examinations

	Patients		Patients partners	
	N=191	%	N=127	%
Physician				
Prefer male	0	0	0	0
Prefer female	188	98.4 *	123	96.9 *
No preference	3	1.6	4	3.1

: P≤0.05

Table 4. Responders' gender preference for physicians when considered professional skill of physicians

	Patients		Patients partners	
	N=191	%	N=127	%
Physician				
Prefer male	0	0	0	0
Prefer female	52	27.4	43	33.8
Good professional skill	135	70.7 *	81	63.8 *
No preference	4	2.1	3	2.4

: P≤0.05

Table 5. Preference for physician attitude

Procedure	Respondent preference by gender of physicians % (n)		
	Male	Female	No preference
Feel embarrassed	46.1 (88)	15.2(29)	38.7(74)
More comfortable	2.1 (4)	38.7(74)	59.2 * (113)
Easier to talk to	3.1(6)	37.7(72)	59.2 * (113)
More gender	23.0(44)	6.8(13)	70.2 * (134)
More understanding	2.1(4)	26.7(51)	71.2 * (136)
More knowledgeable	6.8(13)	6.8(13)	86.4 * (165)
More respectful	27.7(53)	6.8(13)	65.5 * (125)
Spent more time with patient	5.8(11)	8.4(16)	85.9 * (164)

: P≤0.05

Table 6. Most important physician characteristics when selecting and obstetrician-gynaecologist as ranked by patient preference

Physician characteristic	Rank				
	1	2	3	4	5
patients	Experience	Ability	Moral character	Knowledge	Reputation
	31.3%	27.4%	24.9%	11.1%	1.8%
Patients partner	Ability	Experience	Moral character	Knowledge	Reputation
	30.2%	29.2%	21.7%	7.3%	2.6%

Table 7. The influence of patients partners attitude on selecting a physician

	N	%
Patients (n=191)		
Do your partner mind your choice?		
Yes	17	8.9
No	119	62.3
Don't know	55	28.8
If they mind the choice, do you institute?		
Yes	86	45.1
No	69	36.1
Don't know	36	18.8
Partners (n=127)		
Do your mind the choice of your partner?		
Yes	76	59.8
No	35	27.6
Don't know	16	12.6
Do you give some suggestions to your partner?		
Yes	49	38.6
No	54	42.5
Don't know	24	18.9

Discussion

The role of physician gender had been examined on many areas in medicine, such as gynaecology and obstetrics, pediatrics, surgery, nursing and so on[7-11]. The study results about the physician gender had been controversial. Some studies did find the majority of women prefer female providers[1, 2], for survey population felt female physicians understand their problems better. Others suggested most of women surveyed did not select their obstetrician-gynecologists based solely on physician gender.

Our study investigated the gender preferences for obstetrician and gynecologists in Chinese patients and their partner. We interviewed patients in obstetrics and gynecology waiting room, and had a very low refusal rate. The study found that, when only considered the physicians' gender, patients and their partners had gender preferences for female obstetrician-gynecologist and especially prefer female obstetrician-gynecologist for intimate examination. We wondered why Chinese woman and their partner had a significant preference for female obstetric-gynecology providers. In our investigate, 46.1% patients felt embarrassed when faced male physicians, and 38.7% patients felt embarrassed when faced male or female physicians. About 59.8% partners minded the choice, and 36.1% partners of them would interference the patients' selection. More than one third of the patients would change their choice if their partner minded their choice. These results were very interesting. Laffa[12] stated that preference was associated with social tradition and religious beliefs. Among very religious patients, gender was the most important factor than other parameters. Similar results were reported by Lund's[4] that Asians were more likely to have a gender preference than African Americans. And Chandler's[1] results also suggested that Asian woman had a significant preference for female obstetric-gynecology providers. According to the results above, gender preference for obstetrician and gynecologists in the Chinese patients and their partner could be expected.

The further study investigated the physicians' attitudes in relationship to the gender on selecting a doctor. We found that most of patients felt no gender preference for physician attitudes. Only one third of patients felt female physician were more comfortable, more understanding, and easier to talk. And less than one-third of patients thought that male physicians would be more gentle and respectful. But other researchers had found that female physicians conducted the communication style different from the male physicians, and engaged in longer medical visits, more positive talk, question asking and more understanding[13,14].

But when considered not only the physicians' gender, 70.7% patients and their partner would like to select doctors prefer professional skills over gender. These results also supported by the rank of most important physician characteristics when selecting an obstetrician-gynecologist. According to the selection of our study population, the most important physician characteristics were experience, ability and moral character. Hadar Amir[15] also pointed that Ultra orthodox women considered professional skills more important than gender, including the ultra-orthodox responders.

In light of the above, although Asian traditions are conservative in comparison, they more want to have a good physician compare with the gender preference. This investigate would seem to have significance to clinic and hospital planners and schedules. When patients come to see a doctor, the providers should consider the patient's feeling and respect their choices. We suggested that, as far as possible, provided female obstetrician-gynecologist with good professional skills.

Acknowledgments

This work was supported by the National 973 Program of China (2010CB529905) and the National Natural Science Foundation of China (Grant no. NSFC81071861).

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Central nervous system agents to control food intake and energy balance

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Abstract

The prevalence of obesity is on a rise and is taking on pandemic proportions worldwide despite significant reductions in dietary fat consumption. The increasing incidence of obesity worldwide has renewed interest in the control of food intake and energy homeostasis. The brain–gut axis is central to the mechanism by which the signals energy status to central and peripheral nervous appetite centers. Evidence suggests that most obese individuals have an inappropriate control of their food intake rather than a metabolic defect in energy expenditure. Enhanced knowledge of the complexity of energy balance regulation and the mechanisms that sustain obesity indicate the challenge presented by management of the obesity epidemic. This article reviews that central nervous system agents to control food intake and energy balance.

Key words: Obesity, Central Nervous System, Energy Balance, Food Intake, Appetite.

Introduction

The World Health Organization (WHO) estimates that over one billion people worldwide are overweight, and will increase in 30 years [1]. Despite significant reductions in dietary fat consumption, the prevalence of obesity is on a rise and is taking on pandemic proportions. The increasing incidence of obesity worldwide has renewed interest in the control of food intake and energy homeostasis [2]. So,there has been an increasement of research about this matter over the past decade, leading to the discovery of many new hormones and neurotransmitters involved in the regulation of food intake [3]. Enhanced knowledge of the energy balance regulation and the mechanisms that sustain obesity indicate the challenge presented by management of the obesity epidemic [2]. This article reviews that central nervous system agents to control food intake and energy balance.

Central Nervous System (CNS) Mechanism

Appetite regulation is important because it modulates the energy consumption side of the equation. Appetite includes various aspects of eating patterns such as frequency and size of eating episodes, choices of high or low fat foods, energy density of foods consumed, variety of foods accepted, palatability of diet in day-today intake. Feeding behavior is controlled by a series of short-term hormonal, psychological and neural signals which act at several central nervous system (CNS) sites but the pathways converge on the hypothalamus, which contains a large number of peptides and other neurotransmitters that influence food intake [4].

Neuroanatomy and Neurophysiology of Regulating Appetite

The hypothalamus, located on the ventral aspect of the brain, is the primary brain site that integrates incoming information from internal and external sources and then propagates and transmits appropriate signals to sustain energy homeostasis [5]. The hypothalamus and dorsal vagal complex are directly involved in the CNS regulation of appetite. The arcuate nucleus (ARC) of the hypothalamus plays an integrative role, receiving both hormonal and neuronal inputs from the periphery [6]. Neurons of the ARC express receptors for gut hormones, and its anatomical proximity to the median eminence, an area lacking a blood–brain barrier, renders the ARC susceptible to influence by circulating factors. Neurons inhibiting or stimulating apetite project to the paraventricular nucleus and other hypothalamic areas important in energy balance [7,8].

Agents in Central Nervous System

Controlling energy balance demands extensive coordination from the CNS. The CNS regions that control energy homeostasis are accessible to numerous circulating hormones and other factors including information generated by the sensory

experience of eating, and from the periphery indicating the ingestion of food and the utilisation of its component nutrients [2].

Neuropeptide Y: Neuropeptide Y (NPY) contains 36 amino acid residues, including a tyrosine at each end. The appetite stimulating effects of NPY and related pancreatic polypeptides (PP) were demonstrated in 1984 [9] and NPY is one of the most potent stimulators of food intake identified to date. It is widely distributed throughout the CNS [10] with high concentrations in the hypothalamic appetite-regulating nuclei, particularly within the ARC where most NPY is derived. NPY injection into the CNS or directly into the PVN or LHA promotesmeal initation and delays the onset of satiety such that the size and duration of the first meal is increased resulting in hyperphagia [11]. The hyperphagic effects of NPY are mediated by specific NPY receptor subtypes. Of the six NPY receptor subtypes identified to date, the Y1 and Y5 receptor subtypes mediate the orexigenic effects of NPY released in the magnocellular PVN (mPVN) [12-14]. This notion that NPY-evoked appetitive drive originates from a subpopulation of Y1 and Y5 coexpressing targets in the mPVN is supported by the evidence that a subpopulation of neurons in the mPVN co-express Y1 and Y5 receptors, Y1 and Y5 receptor antagonists blocked NPY-induced c-fos activation selectively in the mPVN and Y1 and Y5 receptor antagonists individually failed to completely suppress feding [12-15].

Melanin Concentrating Hormone (MCH): Melanin-concentrating hormone (MCH) is an orexigenic cyclic 19 amino acid neuropeptide. It is cleaved from its precursorprepro-MCH (ppMCH) along with several other neuropeptides whose roles are not fully defined [16]. MCH is expressed in a discrete population of neurones in the zona incerta and LHA of the hypothalamus [17] and is often co-expressed with CART [18]. MCH containing neurones project widely throughout the CNS suggesting an involvement in numerous physiological functions. However, the most widely investigated role for MCH is in the regulation of energy homeostasis. This role was first suggested by the observation of elevated MCH mRNA and peptide levels in the hypothalamus of ob/ob mice as well as fasted animals. Additionally, repeated central injections to satiated rats produce a rapid and dosedependent increase in food intake, whilst chronic central infusion of MCH to rodents results in persistent hyperphagia and enhanced body weight and adiposity [19]. Two MCH receptors have been identified. MCH was originally associated with an orphan G-protein-coupled receptor (GPRC) termed SLC-1, although this is now referred to as MCHR1. The structure of MCHR1 is highly conserved across rodents and higher mammals [20] and receptor mRNA and protein levels are widely distributed throughout the brain in a pattern consistent with that of the terminal fields of MCH neurones. High-levels have been found in the nucleus accumbens, amygdala, hippocampus and various hypothalamic appetite-regulating nuclei [21]. With this distribution MCHR1 is likely to mediate the orexigenic effects of MCH in conjunction with other feeding-related functions such as taste, reward and olfaction. The second MCH receptor, termed MCHR2, has only a 38% homology with MCHR1. Furthermore, functional MCHR2 has not been identified in rodents, but is present in human and animals [22].

Orexins/Hypocretins: The neuropeptides orexin A and orexin B produced in hypothalamic neurons, were initially identified as endogenous ligands for two orphan G-protein-coupled receptors [23]. Orexin A is a 33-amino acid peptide of 3562 Dalton with two sets of intrachain disulfide bonds. It has an N-terminal pyroglutamyl residue and C-terminal amidation [24]. The primary structure of orexin A predicted from the cDNA sequences is completely conserved among several mammalian species. On the other hand, rat orexin B is a 28-amino acid, C-terminally amidated linear peptide of 2937 Da that is 46% identical in sequence to orexin A. The C-terminal half of orexin B is very similar to that of orexin A (73%), whereas the N-terminal half is variable. Orexin B also has a high degree of sequence similarity among species. They were recognized as regulators of feeding behavior because of their exclusive production in the lateral hypothalamic area (LHA1), a region known as the feeding center, andtheir pharmacological activity [24,25]. There appear to be two orexin receptors, OX1R and OX2R, to which orexin A binds to both with high affinity whereas orexin B binds preferentially to OX2R [24]. The distribution of the two receptors is also different. Within

the hypothalamus OX1R is most highly expressed in the ventromedial nucleus (VMH) and OX2R most highly in the PVN [25]. It has been suggested that orexins induces food intake through regulation of homeostatic pathways. Orexin neurones are stimulated by falls in plasma glucose and by fasting but are promptly inhibited by prandial satiety signals arising from the presence of food in the gut [26]**.** Such inhibitory signals could include gastric distention and a rise in portal glucose levels both of which are transmitted indirectly to the LHA via the NST as a consequence of vagal signalling [27]. These central responses along with evidence that peripheral orexin neurones located in the enteric nervous systemmay sense nutritional status in order tomodulate GI secretion andmotility to prime the digestive tract for the ingestion of food [28] suggests that the orexin system may constitute a mechanism for initiating and terminating feding episodes possibly tomodulate glucose homeostasis. Centrally, orexin neurones sense glucose availability to regulate their own activity and could interact with other neurotransmitters to control feeding including ARC, NPY and POMC neurones [29].

Agouti-related peptide (AGRP): AGRP is 132-amino acid peptide that has generated intense interest because of evidence of its role in the regulation of feeding and body weight. AGRP has sequence similarity to the product of the Agouti coat color gene in mice, a paracrine-signaling molecule produced normally in the skin that inhibits the effect of MC-1 receptor [30]. Agouti is constitutively expressed throughout the body of yellow Agouti mice, and this expression gives rise to pleiotropic effects including yellow coat color, obesity insulin resistance, hyperglycemia, and increased body length. Mice homozygous for null mutations of Agouti do not display abnormalities of weight regulation [30]. Humans also have an agouti gene that is normally expressed in adipose tissue [31]. Within the CNS, AgRP is expressed exclusively in the Arc and AgRP mRNA colocalises with NPY mRNA in 95% of NPY positive cells in this nucleus [32]. The one study has shown that like NPY, AgRP is an orexigenic peptide when injected ICV [33] or directly into the PVN or DMH [34] **.** Uniquely, AgRP acts as an endogenous antagonist at the MC3R and MC4R [35]. The MC4R is constitutively active in the absence of a ligand and AgRP functions as an inverse agonist, suppressing this constitutive activity [36]. Thus AgRP is orexigenic even in the absence of a-MSH. Overexpression of AgRP under a ubiquitous promoter leads to obesity [37].

Galanin and Galanin-Like-Peptide: Galanin is a neuropeptide which is not a member of any known family of amino-acid neuropeptides, was isolated in 1983 by Tatemoto et al [38]. It is a 29 amino acid C-terminally amidated, highly conserved but unique neuroendocrine peptide originally isolated from intestine. The first 14 aa are fully conserved in almost all species. The first 16 N-terminal amino acids appear to contain galaninagonist activity on increasing food consumption [39] . This bioactive peptide distributed widely throughout the central and peripheral nervous system and other tissues [38]. As yet, three types of galanin receptors have been identified in these tissues by molecular cloning and characterized pharmacologically in various species [40]. In brain galanin is synthesized in the dorsal raphe nucleus, locus coeruleus (LC), rostral ventrolateral medulla, central nucleus of the amygdala, the paraventricular nucleus (PVN) and the supraoptic nucleus [40]. Besides brain, galanin is also located in the spinal cord and gut. Injection of galanin into the PVN preferentially increases fat intake in comparison to NPY injection into the same area, which induces preferential carbohydrate intake [41]. In addition, a galanin receptor antagonist injected into the PVN reduced spontaneous fat intake [42] suggesting galanin has a physiological role in stimulating fat intake. However this effect appears to be strongly dependent on the test conditions [43,44] and thus a physiological role for galanin in regulating fat intake is yet to be proven.

Galanin-like peptide (GALP), while being structurally similar to galanin, and identical in the first 13 amino acid residues, is encoded by a separate gene, on a different chromosome to galanin [45]. GALP binds to the galanin receptors GalR1, GalR2 and GalR3, with the highest affinity to GalR3 [46]. The distribution of GALP mRNA is much more restricted than that of galanin. GALP expression is restricted largely to the Arc, with a few cells in the DMH [47] and that make close contacts with leutinizing hormone-releasing hor-

mone (LHRH) neurons in basal forebrain. Furthermore, GALP neurons express leptin receptors and respond to leptin treatment by increasing their expression of GALP mRNA. Centrally administered GALP activates LHRH-immunoreactive neurons and increases plasma LH levels [48].

Opoids: The opioid system is composed of three families of biologically active peptides, b-endorphin, dynorphin, enkephalins and their receptors, l-opioid receptor, j-opioid receptor, d-opioid receptor, respectively [49]. Since their discovery almost 40 years ago the evidence implicating opioid peptides in the control of food intake has expanded dramatically. The inhibitory effects of opioid antagonists, particularly naloxone, on food intake have been well documented in rodents, with evidence that m, d and k opioid receptors are all İnvolved in the short-term control of feeding [50]. Specifically the endogenous opioid system is implicated in the sensory pleasure derived from food consumption. In animals opioid antagonism seems to selectively reduce the intake of highly palatable foods, characterised by either sweetness or high fat content. Conversely, the consumption of palatable food stimulates hypothalamic b-endorphin release [51]. This has led researchers to conclude that the endogenous opioid system may stimulate food intake through palatability rather than hunger based mechanisms [52].

Endocannoboids

It has long been recognised that food intake increases following the administration of cannabis. Consequently, discovery of cannabinoid receptors and their endogenous ligands in the 1990s led to them being linked with various aspects of feeding behavior [53]. The cannabinoid system consists of two receptors whose subtypes are classified as the central CB1 receptor, which is widely distributed in the CNS and many peripheral tissues, and the 'peripheral' CB2 receptor, which is not significantly expressed in the CNS [54]. It is generally accepted that the influences of cannabinoids on feeding behaviour aremediated by the CB1 receptor. The selective CB1 antagonist, rimonabant, dose-dependently reduces food intake and body weight in rats and blocks overeating produced by the endogenous CB1 ligand anandamide. The endocannabinoids anandamide and 2-arachidonoylglycerol act on CB1 in the brain and many peripheral tissues causing a net anabolic action. This includes increasing food intake, and causing increased lipogenesis and fat storage in adipose tissue and liver [55]. It also selectively inhibits consumption of palatable food and drink, with decreased intakes of sucrose, alcohol and a sweet diet observed in rats, mice and marmosets, respectively [56]. This indicates that similar to the opioid system, the cannabinoid system may be linked to the hedonic aspect of eating. Furthermore, synergistic actions of rimonabant and the opioid receptor antagonists, naloxone and nalmefene, on food intake have been observed [57].

Cocaine and amphetamine-regulated transcript (CART): CART was initially identified as a result of its positive regulation by the psychomotor stimulants cocaine and amphetamine [58]. CART is expressed in the PVN, SON, LH, VMH, DMH and Arc in the hypothalamus [59]. In the PVN CART is colocalised with oxytocin and vasopressin and in the LH is colocalised with MCH. CART colocalises with POMC in the Arc [60] and consistent with the anorectic effect of activation of POMC neurons Additionally, CART antiserum increases food intake suggesting endogenous CART is anorectic [61]. Current rationale places CART in the role of an endogenous satiety factor which modulates the actions of NPY and leptin. Administration of CART inhibits NPY-induced feeding while central infusion of anti-CART antibodies increases food intake [62]. CART and its receptor present another potential therapeutic target to control feeding and ultimately obesity.

Melanocortins: The melanocortin system, comprising of receptors, naturally occurring agonist and inverse agonist, plays a crucial role in the regulation of energy homeostasis and food intake[63]. So, the central importance of the melanocortin system to the regulation of body weight makes it an attractive target as a therapeutic strategy for obesity. This system includes an array of melanocortin peptides that are derived from the common precursor proopiomelanocortin (POMC) and several melanocortin receptors through which they signal their effects [64]. a-Melanocyte stimulating hormone (a-MSH) is thought to be the most important melanocortin peptide in the control of food intake. The melanocortin-3 and melanocortin-4

receptors (MC3-R and MC4-R) are prominant in hypothalamic nuclei that are concerned with energy homeostasis. Both receptors probably mediate the hypophagic effects of the melanocortins, but recent studies have given MC4-R a central role. Huszar et al reported that MC4-R nullmice are hyperphagic and exhibit an obese phenotype [64]. In human studies, increasing amounts of data provide strong evidence that the MC4-R is involved in mediating the effects of melanocortin peptides on appetite and plays an important role in the development of human obesity. The activity of the melanocortin system is regulated by leptin. Approximately one third of POMC neurones express the leptin receptor and are stimulated by leptin [65]. Leptin administered to ob/ob mice in turn increases the release of a-MSH into the circulation suggesting a possible feedback loop between the sites of a-MSH release and the release of leptin from the adipose tissue. However, physiological significance of this putative feedback probably depends upon the underlying state of energy balance, since in the fasting state there is a parallel decrease in plasma leptin and plasma a-MSH [63,65].

Serotonin (5-HT): Serotonin is an important modulator of many developmental, behavioral, and physiological processes such as sleeping, temperature regulation, pain perception, and motor activity [66]. Moreover, 5-HT activation has been associated with the within-meal processes of satiation and the post-meal state of satiety approximately 30 years ago [67]. 5-HT neurones are located in the raphe nuclei of the midbrain particularly to the hypothalamic appetite-regulating nuclei. These neurones express leptin receptors and may partly mediate the effects of leptin on energy homeostasis [68]. Furthermore, activation of the 5-HT system is linked to peripheral signals triggered by fat ingestion. In addition, animals consuming a high carbohydrate diet show increased CNS levels of the 5-HT precursor tryptophan [69]. Increased CNS 5-HT functioning as a consequence of these signals reduces food intake and bodyweight in both rodents and humans; effects that are also demonstrated by serotonergic drugs [70,71]. Over fifteen 5-HT receptor, which research into potential treatments for obesity has centred upon the 5 5HT1A, 5-HT1B, 5-HT2A and 5-HT2C subtypes, have been identified [72]. Of these, the 5-HT2C subtype appears to play a particularly important role in regulating food intake. Knockout mice possessing no functional 5-HT2C receptors demonstrate marked hyperphagia and consequently obesity [73]. Furthermore, 5-HT2C specific antagonists inhibit the hypophagic actions of sibutramine and other 5-HT enhancing drugs. While most of the focus on 5-HT and weight control has been on drugs against the 5-HT2C receptor. The 5-HT6 receptor is one of the most recent additions to the 5-HT receptor family [74]. It is almost exclusively expressed within the CNS with high levels in cortical and limbic regions [71,73]. 5-HT6 selective antagonists have been reported to produce significant reductions in food intake when administered to ob/ob mice, dietary obese mice and rats with these hypophagic effects being accompanied by significant reductions in body weight and insulin levels [75-77]. As reductions in food intake are not accompanied by nausea/malaise, taste aversion or sedation [78] and antagonist administration accelerates and increases post satiety resting in the behavioural satiety sequence [79] these observed effects of 5-HT6 antagonists on food intake are consistent with enhanced satiety.

Corticotropin Relasing Factor (CRF): Corticotropin releasing factor (CRF) is a 41-amino acid mammalian neurohormone that is best known as the major physiological regulator of pituitary ACTH secretion [80]. In addition, it stimulates complimentary stress-related endocrine, autonomic, and behavioral responses [81,82]. There is considerable evidence indicating that CRF is an endogenous anorectic and thermogenic agent [83]. CRF secretion modulates food intake in the absence of stres by exerting an inhibitory tone on appetite [2]. Injections of CRH centrally decrease food intake, including NPYinduced food intake. CRH also promotes a negative energy balance by stimulating energy expenditure, by increasing thermogenesis via sympathetic pathways. CRH levels are regulated by leptin, suggesting a role for CRH in mediating the effects of leptin. Another member of the CRH family is urocortin, which is more potent at suppressing food intake than CRH. The effects of both urocortin and CRH are mediated by CRHR1 or CRHR2 receptors. However, the weights and basal food intake of both CRHR1 and CRHR2 knockout mice are normal [3].

Conclusions

Numerous mechanisms regulating energy regulation and the expression of appetite have been identified for the last fifteen years. The regulation of appetite and food intake is a complex process. It involves a coordinated response to many orexigenic and anorexigenic factors in multiple brain regions. Feeding behavior is controlled by a series of short-term hormonal, psychological and neural signals. All these signals act at several CNS sites but the pathways converge on the hypothalamus, which contains a large number of peptides and other neurotransmitters that influence food intake. Gut peptides also constitute a key means by which the periphery signals satiety and hunger to the CNS. It is this integration of the endocrine and the neurological systems which underpins much current thought on the regulation of appetite and increasing food intake concluded to obesity.

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Chlamydia trachomatis detection in urogenital specimens by the Vidas Chlamydia Test

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Abstract

Introduction: *Chlamydia trachomatis* is one of the most frequent agent of sexually transmitted diseases (STDs) which causes a wide spectrum of infections including urethritis in men and endocervicitis in women. In this study, we would like to present our findings and evaluate the Vidas Chlamydia Test in detection and verification of *C. trachomatis* in urogenital specimens among tested male and female population.

Material and methods: Thirty specimens were processed from patients attended at the STD Clinic. The diagnostic of *C. trachomatis* was done by the Vidas Chlamydia Test, an enzyme-linked fluorescent immunoassay (bioMerieux Vitek, Inc., Hazelwood, Mo.). Additionally, all positives were confirmed by the Chlamydia Direct IF (bioMerieux, France).

Results: Out of thirty tested, 1 83.33%) male and 29 (96.66%) females were analyzed. *C. trachomatis* was detected in two patients both sexes, in age category group 18-25.

Conclusions: Subsequently to our sample-size, the study has shown that the Vidas CHL has a performance which is similar to the Direct IF for male and female urogenital specimens.

Key words: *Chlamydia trachomatis,* Vidas CHL, Direct IF.

Introduction

Chlamydiae are distinquished from all other microorganisms on the basis of a unique life cycle (1). They are obligate intracellular parasites, placed in their own order and family; *Chlamydiales, Chlaymdiaceae* (2). *Chlamydia trachomatis* as a cause agent of sexually transmitted diseases (STDs) is the most frequent bacterium among youth worldwide. The Centers for Disease Control and Prevention recommends that sexually active teens and adults ≤24 years of age be routinely screened for *C. trachomatis* (3). The long-term consequences of chlamydial infections may be severe, particularly in women; including pelvic inflammatory disease (PID) in 20-40%, tubal infertility (20%), ectopic pregnancy (9%), and (18%) will develop chronic pelvic pain (4,5). Infections are predominantly asymptomatic among as many as 70 to 80% of infected women and 50% of infected men (6). The asymptomatic nature of chlamydial infection makes screening essential if control of this infection is to be achieved. Because of the severity of the complications of infection with chlamydia, several other countries including the UK, France, Holland and Finland have now taken action to reduce the prevalence ot this infection.

Nowadays, accurate laboratory tests for the diagnosis of *C. trachomatis* are available. Some of these are more sensitive than the others. Although culture is still considered to be "gold standard", antigen detection techniques are easier to perform and are used in an increasing number of laboratories. The majority of laboratories have moved away form culture, as it is expensive, time-consuming and technically difficult. Direct detection methods currently in use are direct immunofluorescence microscopy, enzyme immunoassay, and nucleic acid amplification tests (NAATs). They appear to be highly sensitive and specific and also effective for use of non-invasive specimens such as urine or vulval swabs.

In this study, we would like first time to present our findings and evaluate the Vidas Chlamydia Test in comparison to Chlaymdia Direct IF in detection and verification of *Chlamydia trachomatis* in urogenital specimens among tested male and female population.

Material and methods

This prospective study took place at the Department of Microbiology, School of Medicine, University of Sarajevo and the STD Clinic during the period of spring 2013. Thirty (30) male and female patients were included. The Vidas Chlamydia Test - CHL (bioMerieux Vitek, Inc., Hazelwood, Mo.) is an enzyme-linked fluorescent immunoassay for the detection of *C. trachomatis*. A mouse monoclonal antibody directed against the chlamydial lipopolysaccharides (LPS) is used. Positive and equivocal Vidas CHL specimens are confirmed with a blocking assay by same manufacturer. Swab specimens for the Vidas CHL assay were collected with the Vidas Specimen Collection Kit. Male urethral specimens were obtained randomly. Each dacron swab was inserted 2 to 3 cm into the urethra, rotated, and withdrawn. Female cervical specimens were collected in random order. The swab or cytobrush was rubbed against the endocervical canal and removed; contact with vaginal surfaces was avoided. Specimens were held at 4°C until were transported to the laboratory. When the Vidas CHL Assay is completed, the results are analyzed automatically by the computer, a test value is generated, and a report is printed for each sample. A test value is generated for each sample by subtracting the Relative Fluorescence Value (RFV) of a standard from that of the patient test. The test value is then compared to a set of thresholds stored in the computer and a final result is interpreted (Table 1.).

Table 1. Thresholds and Interpretation of Results

Test Value Threshold	Interpretation
< 60	Negative
≥ 60 to < 80	Equivocal
≥ 80	Positive

Additionally, we verified all positive Vidas CHL specimens by the Chlamydia Direct IF (bioMerieux, France), routinely used in *C. trachomatis* detection in our laboratory. Chlamydia Direct IF is intended for direct detection antigen of *C. trachomatis* from swab specimens in the period of acute genital chlamydial infection. It is based on the use fluorescent, labeled monoclonal antibodies directed to the Major Outer Membrane Protein (MOMP) that reacts to all *C. trachomatis* serotypes. Chlamydial cells appear like an intensive green spots called "green apple" on dark background illustrated by fluorescence microscope. Test procedures were followed according to the manufacturer′s package insert. Positive smears contained five or more Chlamydia elementary bodies (EBs).

Standard methods of descriptive and inference statistics were analyzed. All data were performed using SPSS software version 17. (SPSS, Chicago, IL) for Windows.

Results

In the study, thirty (30) male and female patients were evaluated on *C. trachomatis* detection in clinical specimens for the first time by the Vidas enzyme linked fluorescent assay. All positive specimens were finally confirmed by the Chlamydia Direct IF. Out of 30, 1 (3.33%) male and 29 (96.66%) females were tested. Out of 29 females, in three patients there were no age data. The median age of 26 tested females was 27.54 (SD= ±5.74), ranging from 21-46 years. Prevalence of *C. trachomatis* according to demographic characteristics of tested male and female population was presented in Table 2. *C. trachomatis* was detected on one male and female patient, in age group 18-25. Additionally, Graph.1. showed structure of respondents according to gender. Table 3. presented Positive Predictive Value (PPV) of the Vidas CHL in patients positive on *C. trachomatis*.

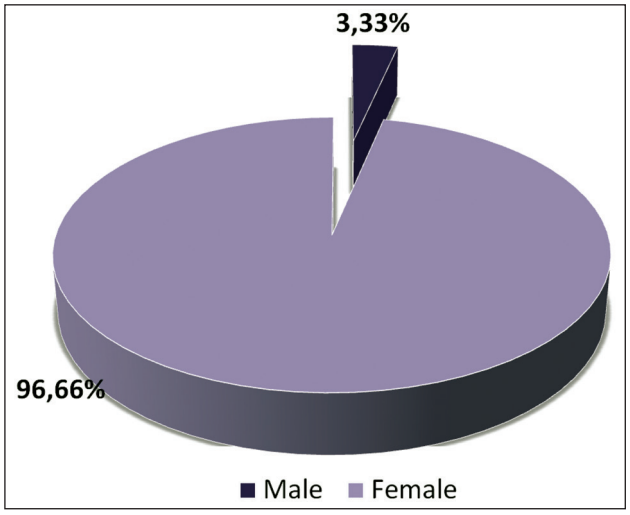
Table 2. Prevalence of C. trachomatis according to demographics characteristics of tested male and females

Variable	n	Vidas CHL test		Prevalence
		Positive	Negative	
Gender				
Females	29	1	28	3.6%
Males	1	1	0	100.0%
Total	30	2	28	6.6 %
Age group				
18-25	14	2	12	12.8%
26-34	10	0	10	0.0%
≥35	3	0	0	0.0%
Total	27	2	25	7.40%

Table 3. PPV of the Vidas CHL in patients positive on C. trachomatis

Vidas CHL		Direct IF		Total	PPV* (%)
		Positive	Negative		
	Positive	2	0	2	100.0%

**Positive Predictive Value*



Graph. 1. Structure respondents according to gender (n=30)

Discussion

In our country, there are no national or local guidelines for the screening of STDs. Besides, statistical data of STDs were unreliable having in mind the high proportion of unreported cases (7). Chlamydia causes a sexually transmitted disease that can be treated easily and its sequelae are prevented by a short course of inexpensive antibiotics.

The purpose of the study was for the first time to evaluate the Vidas Chlamydia Test based on *C. trachomatis* detection from urogenital specimens in our laboratory settings. It is proved that direct antigen detection methods have provided more rapid and less expensive alternatives. The CHL assay is widely used in Europe, as well as in the United States. Advantage of the Vidas CHL is that it allows high-volume testing on *C. trachomatis* which is very useful condition in acceptability for Chlamydia Screening Programmes. In the test procedures, blocking assay was used for confirmation of positive and equivocal specimens by same manufacturer. For the first time, we used a small-scale sample as the experimental phase (processed 30 samples) in order to obtain good quality of given results. Additionally, all positive the Vidas CHL specimens were confirmed by the Chlamydia Direct IF in detection and verification of *Chlamydia trachomatis*, permanently and routinely used in our laboratory condition. This test is also confirmed and approved by the WHO. The Direct IF is non-invasive test used in diagnostics of chlamydial infection, which enables the unique possibility of identification both asymptomatic and symptomatic patients. The advantages of the test included following characteristics: usage of the monoclonal antibodies, one of the most acceptable screening test in our condition, reference for the practical work, 100% sensitivity, 100% specificity, species specificity, and cost-effectiveness. Furthermore, the advantages of using the Chlamydia Direct IF are the faster turnaround time (approximately 30 minutes) and the visualization of Chlamydia EBs in the specimens.

The advantage of the Vidas CHL is equal or more sensitivity then other direct staining methods even culture in some conditions. The study has shown that the Vidas CHL has a performance that is similar to that of the Chlamydia Direct IF for male and female urogenital specimens. Expected values and specific performance characteristics of the Vidas CHL will vary upon patient population, specimen collection techniques, and other factors as well. There were a few studies which compared performance characteristics for the Vidas CHL and cell culture, EIA and Direct IF from different specimens (6,7). The data from manufacturer confirmed usefulness of the Direct IF test in resolving the Vidas positive/culture negative results. A specimen was considered to be positive if either the Direct IF test or blocking assay was positive. Other investigators have used Direct IF to confirm grey zone EIA and CHL specimens (8). Furthermore, the Vidas CHL is an ideal test for a screening programme and should have the capacity to be used in both sexes (9,10).

In our study, all positive specimens were tested by the Direct IF. The results were remarkable: all

two (2) CHL positive confirmed positivity by the Direct IF too. Our sample size was to small and requires further investigation, but the Vidas CHL with high throughput could compete with the more sensitive procedures (9,10).

Conclusions

Having in mind our small-size sample and the fact that the Direct IF was performed only on two positive urogenital specimens, we were not able to evaluate sensitivity, specificity and negative predictive value (NPV). We would like to mention and emphasize just for two positive patients, PPV of the Vidas CHL was 100%.

Subsequently to our sample-size, the study has shown that the Vidas CHL has a performance that is similar to the Direct IF for male and female urogenital specimens.

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The profiles of 299 patients referred to emergency department in the Van district eastern Turkey diagnosed with acute cerebrovascular disease: A 1-year retrospective study

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Abstract

This paper presents data concerning the demographical and clinical features of patients who presented to the emergency department (ED) of that presented to Yüzüncü Yıl University Medical Faculty Hospital, Van, Turkey, from October 2010 to October 2011 and were diagnosed with acute cerebrovascular disease. The related data was obtained by the review of the patient files reached using the ICD-10 diagnosis coding system. The data consists of an examination of their patient's complaints and the time they presented to the ED, the results of the neurological examination and patients score on the Glasgow coma scale, the present risk factors, the topography of any lesion with neuroimaging methods.

299 patients from the ED diagnosed with acute cerebrovascular disease were included in the study. 52.51% of the patients were male. The average age was determined as 64.04±14.29 year (18-90 year). An evaluation of the patients' history showed; hypertension in 51.81% of the cases. Ischemic stroke in 56.19%, hemorrhagic stroke in 23.41%, transient ischemic attack in 15.38% and subarachnoid hemorrhage in 5.02% of the patients.

The highest number of patients (13.71%) presented in June. No statistical difference was established between groups in terms of circannual rhythm ($p>0.05$). A lesion was determined in 68.56% of the patients via brain tomography with only 31.44 % being evaluated as normal. It was observed that only 21.40 % of patients with ischemic stroke history were admitted to ED in the first three hours after initial symptoms. After acute myocardial infarction and all forms of cancer forms Though it is the most common third cause for death in the world after acute myocardial infarction and all cancer forms, it should be kept in mind that health expenditures, mortality and morbidity rates can be reduced by risk factor controls, early emergency applications and treatments.

Key words: Cerebrovascular disease, emergency department, risk factors, mortality.

Introduction

Worldwide, cerebrovascular diseases (CVD) are the third cause of death following acute myocardial infarction and all forms of cancer (1). The neurological evaluation of 8-15% of patients admitted to the emergency department (ED) of a hospital must be carried out by a neurologist (2). Nearly half the patients presenting to ED with neurological problems had CVD, headache and epilepsy diagnoses and such diseases have the most common incidence among elderly population. CVDs lead to high mortality, morbidity and health expenditures (3). In this study, patients referred to our hospital's ED that were diagnosed with CVD after neurological consultation were examined in detail retrospectively.

Material and Method

Approval to utilize the patient data was obtained from Yüzüncü Yıl University Medicine Faculty Hospital, Van, Turkey. Of 41,298 patients presenting to the hospital between October 2010-October 2011 299 were diagnosed with acute CVD after neurology consultation and formed the study population. To access the patient files,

patient protocol figures were taken by entering various codes from ICD-10 coding system. The ED files were obtained through these protocol numbers. Demographic characteristics, known medical histories, reasons for presenting to ED, results of initial blood pressure in ED, the hour of the day, the month and season that the patient present to the ED, Glasgow coma scale scores following neurological examination, electrocardiography findings, neuroimaging methods (CBT), brain magnetic resonance imaging (MRI) and topography of lesion, the diagnoses obtained and the latest condition of patient was analyzed in detail.

Statistical Evaluation

The statistical evaluation of the data was undertaken using the SPSS11.0 program. Descriptive statistics, average deviation, standard deviation and frequency tables were used as the basic statistical analysis. Continuous variables were stated as average ± standard deviation however, categorical data were signified as a percentage. The Chi square test was utilised for the significance of the difference among the ratios in the advanced analysis. $P<0.05$ was accepted as significant.

Results

157 (52.51%) of the patients diagnosed with CVD were male, 142 (47.49%) were female. The average age was 64.04±14.29 years (ranging 18-90). When the personal medical history was examined, there was hypertension in 51.81% of the cases, heart disease in 15.18%, past medical history of stroke in 8.91%, diabetes mellitus in 13.20%, chronic obstructive lung disease in 3.96%, renal failure in 1.42%, smoking in 20.46% and alcohol in 3.63%. Ischemic stroke (IS) was diagnosed in 56.19% of the patients, hemorrhagic stroke (HS) in 23.41%, transient ischemic attack (TIA) in 15.38% and subarachnoid hemorrhage (SAH) in 5.02%.

The most frequent presentation to ED was seen in June (13.71%) and the least number of patients presented in August (3.68%). When their seasonal distribution was examined, 31.10% of the patients were diagnosed in the spring, 24.75% in winter, 24.41% in summer and 19.73% in autumn and there was no statistical difference between the groups in terms of circannual rhythm ($p>0.05$).

The average systolic arterial blood pressure of patients was 151.57± 31.64 mmHg initial blood pressure in ED whereas their diastolic arterial blood pressure was estimated as 91.30±17.87 mmHg. In the electrocardiography (ECG) of patients, normal sinus rhythm was seen in 68.90 % (n=206), atrial fibrillation in 20.40% (n=61), ventricular tachycardia in 10.70% (n=32) and also ventricular extrasystoles were observed. A lesion determined by CBT was found in 68.56% of the patients, of these lesions only in 31.44% were evaluated as normal.

Considering the emergency service application period after emergence of symptoms in ıschemic stroke (IS), it was observed that 21.40% of patients referred to ED in the first three hours, 27.09% from 3 to 6 hours and for 51.51% the period was longer than 6 hours. Clinical complaints consisted of impaired consciousness (39.46%), focal symptom (32.11%), headache (8.36%), and other complaints (20.07%) such as epileptic seizure, cranial nerve paresis and intracranial pressure.

Patients were divided into 3 subgroups according to their scores on the Glasgow coma scale; I st group: ≤8, 2nd group 9-12, 3rd group 13-15. Group 1 contained 22.74%; group 2 consisted of 21.40% and group 3 contained 55.85% of the total number of patients. Lesions were found most commonly in the left hemisphere (43.48%) and lobar damage was present in terms of topographic examination (35.45%).

69.23% (n=207) of the patients were hospitalized and followed up in the neurology department, 7.02% (n=21) were in the care of the neurosurgery service, 1.0% (n=3) were admitted to the neurology intensive care unit, 13.38% (n=40) were treated in ED and 7.36% (n=22) of them were referred to another medical institution and in addition 2.01% (n=6) of patients in the study had self-referred to another hospital. In follow-ups, it was determined that 2 patients died and mortality rate was estimated to be 0.67%.

The level of consciousness was statistically significant among cerebrovascular disease subgroups ($p<0.0001$). The consciousness level was as follows: transient ischemic attack (TIA), (IS), (HI) and SAH, from the best to the worst, respectively. Clinical complaints showed important differences between the groups ($p<0.0001$). While impaired

consciousness and direction symptom were mostly present in the HI and IS groups, focal symptoms in the TIA group, abrupt impaired consciousness and complaint of headaches were apparent in the SAH group. Hypertension was an important factor for both the IS and HI groups due to their past medical history, on the other hand, heart disease and hypertension were significant for the TIA group ($p=0.0022$). The presence of atrial fibrillation in EKG was a statistically more significant finding in the IS group ($p<0.0001$).

Discussion

The World Health Organization defines a stroke as findings relating to the impairment of focal or global cerebral functions that can develop rapidly, last 24 hours or more and sometimes result in death (4).

While annual stroke incidence is 1.3-3.6/1.000 ranging between 54-64 years, it reaches 4.9-8.9/1.000 with an apparent increase between the ages of 65-74 and it is reported that 70% of patients with a past medical history of stroke are over 65 years old (5). The average age was found to be 64.04±14.29 years in the patients with stroke history in our study and supported the fact that age is a substantial risk factor for stroke. Stroke incidence is 2-3 times more frequent in women aged 55-64 and decreases towards 85 years (6). Our cases were composed of male (52.51%) and female (47.49%). It was observed both in many studies carried out in our country (7, 8) and our study that acute stroke was more common in males in all age groups. The most frequently seen risk factors in CVD are hypertension, diabetes, heart disease and high cholesterol rate (9). We determined that the risk factors of our patients were hypertension (51.81%), smoking (20.46%), heart disease (15.18%), diabetes mellitus (13.20%) and past medical history of stroke (8.91%). While 80-85% of all stroke cases were of ischemic origin, 15-20% were of hemorrhagic origin (4). The distribution of our cases was compatible with literature.

Since many physiological events are related to the human circadian rhythm, it can be considered that diseases can show rhythmicity. The relationship between circannual rhythm and CVD has been analyzed since the 19th century. Though there is no clear unanimity, it has been established that there is high frequency in CVDs in winter and autumn months (10). In this study, it is suggested that cold air leads to stroke by increasing sympathetic activity, blood pressure, arterial spasm, the number of blood platelets and volume, blood viscosity, lipids and the activation of blood clotting activation (10, 11). We also determined two peaks in spring and winter months though they were of no importance in terms of statistics.

It was shown that elevation in blood pressure, high cholesterol level, carotid stenosis and atrial fibrillation have a causative relationship with ischemic stroke as displayed in randomized studies and if these conditions are treated, there is a decrease in the incidence of stroke (9, 12). While the average systolic arterial blood pressure of our patients' was 151.57± 31.64 mmHg, diastolic arterial blood pressure calculated as 91.30±17.87 mmHg. In their EKGs, however, there was a normal sinus rhythm at 68.90%, atrial fibrillation was 20.40%, ventricular tachycardia was 10.70% and the presence of ventricular extrasystoles was followed up. Hypertension was a significant finding for both the IS and HI group, heart disease and hypertension were important for the TIA group and the presence of atrial fibrillation was significant for the IS group. When intravenous tissue plasminogen activators and thrombolytic treatment were applied in the first three hours following the onset of the stroke, this led to a noticeable improvement in the clinical outcomes of patients with acute ıschemic stroke (13). This method of treatment shows that patients must be at the ED within the first three hours from the initial emergence of symptoms. Unfortunately, most patients could not reach the necessary medical help within this period. The percentage of patients suffering from a stroke that arrive at ED in the first three hours ranges from 21% to 48% (14,15). In the current study it was 21.40%.

Unfortunately there were limitations in terms of patient data which was obtained using ICD-10 codes. For some patients their files could not be accessed because their codes were incorrectly written or there were problems with locating the files from the archives incorrectly. Furthermore some details in the file were absent.

The results of our study show that a history of CVD is more frequently seen in males and over

65 years old. The most common risk factors are hypertension, smoking, heart disease, diabetes mellitus and a previous medical history of stroke. Access to medical services within the first three hours of the emergence which is important for urgent thrombolytic treatment was only observed for one fifth of the patients. Though the CVD is the third most common cause of death in the world, it should be taken into account that health expenditures as well as mortality and morbidity rates can be minimized by reducing risk factors and early treatment, early access to medical services, early diagnosis and accurate treatment.

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The presence of antimicrobial resistance in Gram-positive aerobic bacteria isolated from infected surgical wounds

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Abstract

Introduction: Infection of surgical wounds is a significant problem since there is surgery . Infection of surgical wounds (Eng. Surgical Site Infections, SSIS) is an infection that occurs 30 days after surgery and one year after the installation of operating a foreign body (implant), the part of the body where the surgery was performed . Infections of surgical wounds are the second most common cause of nosocomial infection, after urinary tract infections, causing 17 % of all nosocomial infections and thus contribute significantly to the deterioration of the outcomes of hospitalized patients.

Materials and Methods: The study included patients undergoing surgery and observed in surgical department and intensive care unit of the General Hospital ¨Prim. Dr. Abdulah Nakas¨ in Sarajevo. The study included a total of 76 patients. From each patient included in the study were assessed clinical parameters, laboratory parameters, was carried out by microbiological biological materials. Samples of biological material represented a surgical wound swabs, taken appropriately and in adequate numbers. Samples of the material were collected with sterile swab.

Results: Of the 76 respondents, 44 (57.9 %) were male and 32 (42.1%) females. During the study a total of microbiologically treated 76 patients and associated swabs surgical wounds. We studied the antimicrobial resistance of Gram - positive bacteria in infected wounds surgically treated patients where most fortified to Penicillin (75%), Ampicillin (55%), Azlocillin (45%), Cloxacillin (44.8 %), Kotrimoxazole (42.5 %), Oxacilin (39.4 %), Cefixime (37.5 %), Lincomycin (36.9 %), Azithromycin (30 %).

Conclusion: Infections of surgical wounds are still important problems of modern medicine. Therefore, in Bosnia and Herzegovina should introduce active monitoring of these infections, and continuous monitoring in order to prevent and combat these infections

Key words: wound infections, antimicrobial resistance, Gram-positive bacteria

Introduction

Assuming the existence of microorganisms and their role in the emerging infections presented in ancient Rome, Marcus Varro (116th to 27th BC) warned that in certain areas there are invisible animals that can cause illness by inhalation (1).

Discovery of antibiotics was a major contribution to the prevention and treatment of infections. Alexander Fleming 1928th finds Penicillin, which was first clinically applied during the Second World War. Quantitative bacteriology introduces the concept of the microorganism and the resistance of the organism, and thus results in the first information on the pathophysiology of infection (2).

Infection of surgical wounds is a significant problem since there is surgery. Infection of surgical wounds (Eng. Surgical Site Infections, SSIS) is an infection that occurs 30 days after surgery and one year after the installation of operating a foreign body (implant), the part of the body where the surgery was performed (3).

Center for Disease Control and Prevention (Center for Disease Control and Prevention, CDC) has defined three types of these infections, and has recently added a fourth type, surface, deep incisional and organic (4,5). Surgery at the normal anatomical membranes allow entry of exogenous bacteria or spread endogenous to earlier uncontaminated zones. The infection develops in 1-3 per 100 patients after surgery (6,7).

It is estimated that in the United States annually carry 27 million surgical procedures, as assessed by the CDC annually appears around 290,000 infections of surgical wounds, and about 8,000 patients have fatal outcome due to these infections (8,9).

Infections of surgical wounds are the second most common cause of nosocomial infection, after urinary tract infections, causing 17 % of all nosocomial infections and thus contribute significantly to the deterioration of the outcomes of hospitalized patients (8).

Research shows that 10% of the wound becomes infected by the air and even 90% of contact. Height incidence depends on the contamination level of the operating site. The lowest rates of incidence of surgical site infections are the pure operating center (1-3 %), slightly more in clean contaminated (3-4 %), and much more at contaminated and dirty operating center (6 %, 10% or 30 %) (10).

When selecting antibiotics in surgical prophylaxis is important to take into account certain specific circumstances . With pure selective surgical intervention, showed no damage to the tissue that contains the normal microflora, antibiotics are not indicated . In these cases, the risk of possible side effects resulting from the application of antibiotics is greater than the benefit of any prophylaxis. The exceptions are the only operations, in which the implanted bone or joint implants (11).

Antibiotic chosen for prophylaxis should act on the most common causes of surgical-site infections, but do not necessarily act on all possible causes . Selection of antimicrobial drug depends mostly on anatomical stay surgery. In addition, a drug used in prophylaxis should be different from the drugs used in the treatment of the same anatomical region, in order to prevent the emergence of bacterial resistance and preserve those drugs that are effective in the treatment of infection of each anatomical area (12).

The aim of the study

The aim of the study is to show the presence of antimicrobial resistance of Gram -positive aerobic bacteria in infected wounds surgically treated patients at the General Hospital in Sarajevo.

Patients and methods

The study was prospective - retrospective, clinical - microbiological studies, the time period 2009th-2011th years. The study included patients undergoing surgery and observed in surgical department and intensive care unit of the General Hospital ¨Prim. Dr. Abdulah Nakas¨ in Sarajevo. The study included a total of 76 patients. From each patient included in the survey was carried out by microbiological analysis of biological materials taken in the standard way and then he made a preparation that is afraid of Gram and mikroskopirao (13).

During treatment early, take the material inoculated in culture medium and incubated under appropriate conditions, the appropriate time. Isolation and identification of infectious agents, is performed by standard microbiological methods . Swabs taken from the relevant biological materials were sown on blood agar, Endo agar or McConkey agar and incubated for a period of 24-48 hours at 35-37 0C. Processing of samples taken was done in accordance primary and secondary microbial treatment. Pathogens have been identified based on the characteristic appearance of colonies, biochemical and antigenic test strains . For each type of microorganisms isolated, done by testing their antibiotic sensitivity / resistance to appropriate antimicrobial (13).

In each of the isolated pathogens tested corresponding disk - diffusion method for their sensitivity / resistance to the appropriate representatives of the group of antimicrobial drugs, using the Kirby - Bauer by NCCLS (55).

According to the collected data was performed statistical analysis of the data. For statistical data processing program was used SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Of the 76 patients participating in the study, 50 (65.8%) were in a group of abdominal surgery, 14 (18.4%), vascular surgery, 7 (9.2%), orthopedic surgery, 3 (3.9%) urologic surgery, and 2 (2.6%) patients were in the group, type of intervention in the field of plastic and reconstructive surgery.

Of the 76 respondents, 44 (57.9%) were male and 32 (42.1%) females. The average age of the respondents was 66 (55.3 to 76.0) years.

Table 1. Surgical patients by type of surgery

Type of procedure	Number	%
Abdominal	50	65,8
Vascular	14	18,4
Orthopedic	7	9,2
Urolological	3	4,0
Plastical surgery	2	2,6
Total	76	100,0

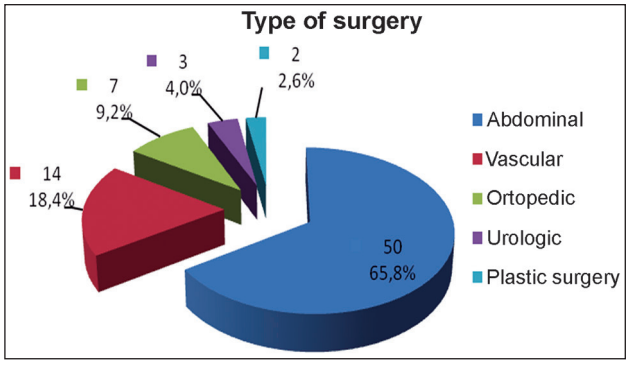

Figure 1. The ratio of representation of patients according to the type of intervention

Table 2. Gender structure of the total sample (n=76)

Gender	Number	%
Male	44	57,9
Female	32	42,1
Total	76	100,0
(medijan, IQR)	66	55,3-76,0

The total number of subjects involved in the study, divided into age groups: 54 and younger, 55-64, 65-74 years old and 75 or more years of life.

In the age group of patients up to 54 years of age, a total of 18 respondents in the age group of patients 55-64 years of age, a total of 16 subjects. Of 76 patients, 19 belonged to the age group of 65-74 years of age, and 23 patients were older than 75 years of age. (Table 3)

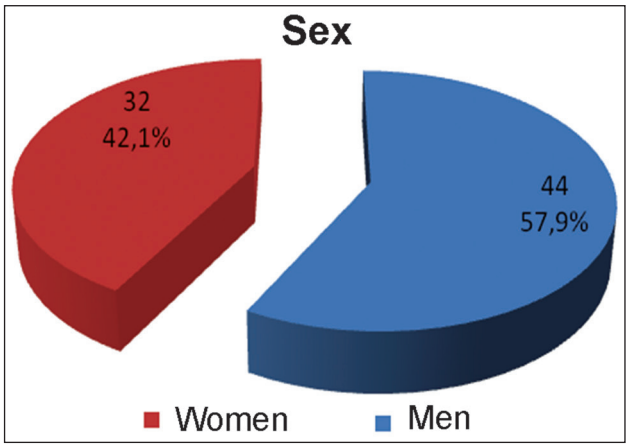

Figure 2. The ratio of representation of all respondents by gender

Table 3. Age structure of respondents

Age	Number (%)
0-54	18 (23,7%)
55-64	16 (21,1%)
65-74	19 (25,0%)
75+	23 (30,2%)
Total	76 (100 %)

In all hospitalized patients involved in the study (n=76), the average length of hospitalization in days (median, IQR) was 28 (15.0 to 49.0) days. Recovery time in days (median, IQR) was 17.5 (10.3 to 35.8) days. Duration of surgery in minutes (median, IQR) was 55 (22.5 to 86.2) minutes.

Figure 3 outlines antimicrobial susceptibility / resistance of Gram-positive bacteria, total isolated from infected wounds, surgical patients.

Table 4. The duration of hospitalization, rehabilitation and surgery

	Type of operative intervention				P	Effect
	Abdominal	Vascular	Orthopedic	Other		
Duration of surgery in minutes (medijan, IQR)	60 (30-90)	30 (4-53)	70 (60-120)	20 (1-150)	0,017*	0,89
Duration of hospitalization in days (medijan, IQR)	22 (12-33)	57 (28-73)	46 (25-62)	19 (8-64)	<0,001*	0,79
Recovery time in days (medijan, IQR)	15 (10-28)	23 (13-55)	37 (17-49)	17 (9-61)	0,026	0,90

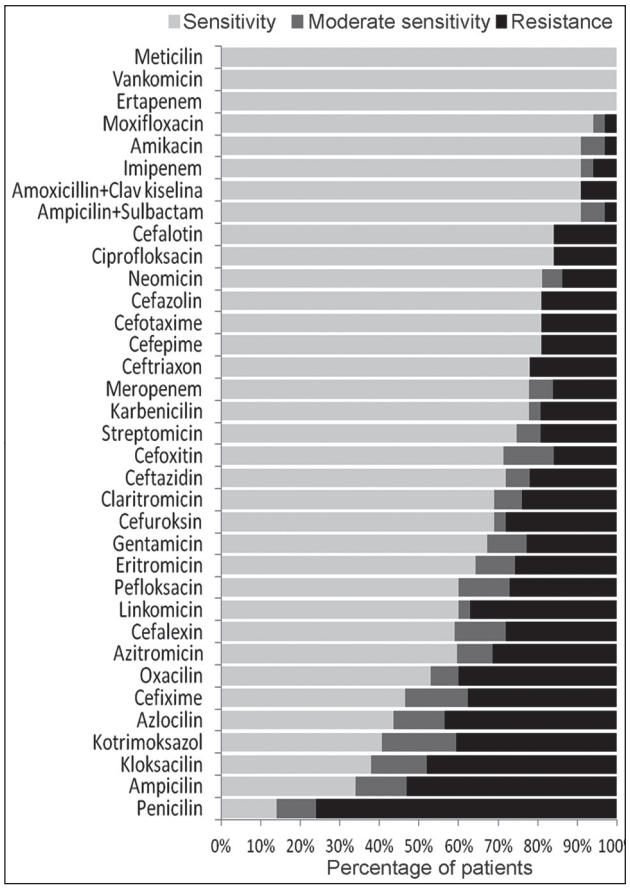

Figure 3. The relationship of the results of antimicrobial susceptibility / resistance of Gram-positive bacteria

Discussion

Of the 76 respondents, 44 (57.9 %) were male and 32 (42.1%) females. During the study a total of microbiologically treated 76 patients and their appurtenances swabs surgical wounds. Of the 76 patients participating in the study, 50 (65.8%) were in a group of abdominal surgery, 14 (18.4%), vascular surgery, 7 (9.2%), orthopedic surgery, 3 (3.9%) urologic surgery, and 2 (2.6%) patients were in the group, type of intervention in the field of plastic and reconstructive surgery.

The average age of the respondents was 66 (55.3 to 76.0) years. Similar results to more frequent infections in men 31 (64.6 %) cases, compared to women, the authors came to the study, using data protocols for Clinical Microbiology Laboratory for Sanitary and Clinical Microbiology, Cantonal Institute of Public Health, Zenica, analyzing retrospectively information on the sensitivity of pathogen infection of surgical wounds in patients in the 1st period January 2004th to 31 december 2007. years (14).

In all hospitalized patients involved in the study (n = 76), the average length of hospitalization in days (median, IQR) was 28 (15.0 to 49.0) days. Recovery time in days (median, IQR) was 17.5 (10.3 to 35.8) days. Duration of surgery in minutes (median, IQR) was 55 (22.5 to 86.2) minutes.

Our results show that the antimicrobial resistance of Gram -positive isolates were identified most in the penicillin (75%), ampicillin (55%), Azlocillin (45%), Cloxacillin (44.8 %), cotrimoxazole (42.5%), Oxacilin (39.4 %), Cefixime (37.5 %), lincomycin (36.9 %), azithromycin (30 %).

The introduction of antimicrobial treatment in everyday use, treatment of wounds is greatly simplified, and additional rigorous application of appropriate principles during surgery and post-operative care, and knowledge of risk factors of patients, it is possible to reduce their appearance (15).

Due to the large number and the risk of developing infections of surgical wounds, with the aim of prevention, CDC has developed a campaign to prevent the development of antimicrobial resistance in health care facilities, including education, both for patients and for clinicians (3,8,9)

Care of patients who develop surgical site infections after discharge, significantly higher costs than patients without infection, since patients with infection 7.5 times more visit the doctor, but also the urgent Hospital Center, but those who have the infection. It is therefore necessary to develop a strategy for the prevention of these infections, as well as all other generated in health care facilities, as part of national programs so far developed in many countries, with the aim of patient safety (16, 17, 18).

Conclusion

Our results show that the antimicrobial resistance of Gram -positive isolates determined the most Penicillin (75 %), Ampicillin (55 %), Azlocillin (45 %), Cloxacillin (44.8 %), Cotrimoxazole (42.5%), Oxacilin (39.4%), Cefixime (37.5%), lincomycin (36.9%), azithromycin (30%). Infections of surgical wounds are acute problem of modern medicine because of the frequency of complications, prolonged hospitalization, big material costs and increasing resistance to antibiotics. Continuous monitoring can be viewed global situation surgical wound infections, and epidemiological mo-

nitoring is considered the main link in the program for the prevention and control of these infections.

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Antibiotic sensitivity of isolates of *Staphylococcus epidermidis* in clinical samples

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Abstract

Among coagulase-negative staphylococci (CNS) *Staphylococcus epidermidis* is the most isolated and the predominant species in the human environment, which generally has symbiotic relationships with its host. Despite this fact, this mikroorgnism is one of the major causes of nosocomial infections (Stevens, 2003) as well as infections of implanted medical devices (Weigel *et al.* 2003; O 'Gara and Humphreys, 2001). Disturbing the integrity of the system of cutane organs by trauma, inoculation of needles, or implantation of medical devices *Staphylococcus epidermidis* enters the host and then gets the role of a pathogen. Infections caused by *Staphylococcus epidermidis* are becoming more demanding to treat because of the increasing resistance of its strains to antibiotics.

The aim of this paper is to present the antibiotic sensitivity of isolates of *Staphylococcus epidermidis*, which were isolated from 112 clinical specimens from RMC "Dr.Safet Mujić" in the period of 2009-2010 years. We analyzed 66 clinical samples of blood culture (33 sets), 19 wound smears, 18 samples of drainage facilities, 6 catheter smears and two bronchial aspirate samples. Antimicrobial susceptibility testing was performed using disk diffusion Kirby-Bauer method on Mueller-Hinton agar, a determination of sensitivity to penicillin and other beta-lactams was carried out using the oxacillin screening test.

According to the oxacillin screening test 82 isolates (73%) were resistant to meticillin (MRSE), and 30 isolates (27%) were meticillin sensitive (MSSE). The determined percentages of resistance of clinical isolates of *Staphylococcus epidermidis* to the other tested antibiotics were: penicillin and ampicillin 96.42%, amoxicillin / clavulanic acid 73.21%, cefoxitin 62.5%, eritromycin 75%, azithromycin 76.78%, clindamicin 33.92 %, trimethroprim-sulphametoxazol 63.39% garamycin 62.5% and vankomycin 0%.

Key words: *Staphylococcus epidermidis,* clinical samples, antibiotic sensitivity, C

Introduction

Coagulase-negative staphylococci (CNS) constitute the majority of the human microbiota of the skin and mucous membranes. These organisms were previously considered harmless skin commensals and in clinical samples they were considered contaminants. Today, the coagulase-negative staphylococci have become a significant, often isolated pathogen in laboratories of clinical microbiology worldwide (Kloos and Bannerman, 1994; Cerca *et al.*, 2005; Arciola *et al.* 2006; Bayram and Balci, 2006; Widerström *et al.* 2006). These microorganisms are usually isolated in clinical samples, where many species are identified as causes of nosocomial infections particularly in immunocompromised individuals, neonatuses and patients with implanted biomaterials (Jarlov, 1999 and Kleeman *et al.*, 1993). Among coagulase-negative staphylococci *Staphylococcus epidermidis* is the most frequent isolate (65% to 90% of all isolates from human samples), and it is clinically the most important species within the coagulase-negative staphylococci in humans (Kleeman *et al.* 1993; Kloos and Bannerman, 1994; Rogers *et al.*, 2009).

Coagulase-negative staphylococci (CNS) are the most common microorganisms isolated from blood culture. Until the seventies the isolation of CNS in blood culture was considered to be due to contamination or improper blood sampling and inadequate treatment of samples in the microbiological laboratory (Wilson *et al.*, 1975). According to the results of various studies in the last thirty years the prevai-

ling view is that the isolation of CNS in blood culture indicates infection of the blood in 10 to 12% of patients (Sewell *et al.*, 1982; Souvenir *et al.*, 1998). And according to more recent data from the Centers for Disease Control and Prevention's National nosocomial infection, *Staphylococcus epidermidis* was identified as a cause of nosocomial infection of the blood in 33.5% of cases (U.S. Department of Health & Human Services). These bacteremia are mostly caused by infections associated with the use of implanted medical devices (artificial heart valves, vascular grafts, central venous and peripheral catheters, prostheses of the hip, knee, etc.), as well as imunocompromised patients (Suljagic and Mirovic, 2006). The most common type of CNS isolated from blood samples of patients with signs of blood infection is *Staphylococcus epidermidis* (Souvenir *et al.*, 1998). Today hospital bacteremia caused by *Staphylococcus epidermidis* are becoming a growing problem (Kotilainen, 1990; Rupp *et al.*, 1994).

Infections caused by *Staphylococcus epidermidis* are becoming more demanding to treat because of the increasing resistance of its strains to antibiotics, and this is particularly evident in the hospital environment. *Staphylococci* in general, especially coagulase-negative staphylococci, have a documented history of the development of resistance to antimicrobials. In accordance with the present, up to 80% of isolates of *Staphylococcus epidermidis*, which causes infections associated with medical devices, are resistant to meticillin and multiple resistant to many other antibiotics. Co-resistant schemes reported in world literature are based on resistance to oxacillin.

Material and methodes

In this study we analyzed 112 clinical isolates of *Staphylococcus epidermidis* in RMC "Dr.Safet Mujić" in the period of 2009-2010. We analyzed 66 clinical samples of blood culture (33 sets), 19 wound smears, 18 samples of drainage facilities, 6 catheter smears and two bronchial aspirate samples. From individual departments only one sample of aerobic and anaerobic blood culture was submitted, while in some cases we made a series of 2 or 3 blood cultures (aerobic and anaerobic) for one patient and all samples were taken into consideration. Also in smears of wounds and drainage facilities that were submitted more samples were related to the same patient and all samples were taken into consideration.

To create the antibiograms for *Staphylococcus epidermidis* the following antibiotics from the manufacturer Liofilchem were used: penicillin, ampicillin, amoxicillin / clavulanic acid, oksacillin, cefoxitin, erithromycin, azitromycin, Bactria (co-trimoxasole, trimethroprim and sulphametoxazol), clindamycin, gentamicin (garamycin) and vankomycin. *S.epidermidis* isolates were identified based on colony morphology and other characteristics of the growth medium (blood agar and mannitol salt), Gram stain, catalase and coagulase test (coagulation of the plate and tube). In some cases the Api-Staph identification system was used according to the manufacturer's instructions (Biomerieux).

To create antibiograms the preparation of bacterial suspensions was made with sterile physiological solution and a final bacterial concentration was adjusted by comparing with 0.5 McFarland standard. Clinical isolates of *Staphylococcus epidermidis* were tested for selected antibiotics using the disc diffusion Kirby-Bauer method on Mueller-Hinton agar. Sensitivity to penicillin and other beta-lactam antibiotics was determined by the oxacillin-screeing test.

Kirby-Bauer method is a standardized system that takes into account all the variables. The recommended medium is Mueller-Hinton II agar, which has a pH value between 7.2 and 7.4

Inoculation of the medium surface with a suspension of bacteria was performed with a sterile swab. Discs with antibiotics were placed on the agar surface with sterile tweezers. After 16 to 18 hours of incubation at 37 ° C the measuring of diameter of the zone of inhibition of bacterial growth was carried out around the discs with antibiotics. The measured values were presented in millimeters.

Results

Despite the fact that *Staphylococcus epidermidis* is increasingly acknowledged as a major cause of hospital infections, so far there are only few studies concerning the question of whether pathogenic strains of *Staphylococccus epidermidis* persist in the hospital environment, and whether they colonize patients, causing infections that are extre-

mely difficult treatment. According to the oxacillin screening test 82 isolates (73%) were resistant to meticillin (MRSE), while 30 isolates (27%) were meticillin sensitive (MSSE) (Figure 1).

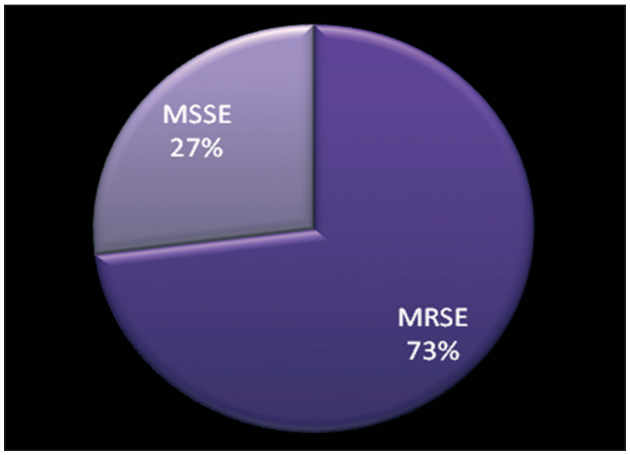


Figure 1. Percentage of clinical isolates of MRSE and MSSE

Table 1. Review of antibiotic sensitivity of isolates of S.epidermidis to the tested antibiotics

Antibiotic	R	R%	I	I%	S	S%
P	108	96,42	0	0	4	3,57
Amp	108	96,42	0	0	4	3,57
Amc	82	73,21	0	0	30	26,78
Ox	82	73,21	0	0	30	26,78
Fox	70	62,5	8	7,14	34	30,35
Er	84	75	2	1,78	26	23,21
Az	86	76,78	0	0	26	23,21
Cc	38	33,92	6	5,35	68	60,71
TSH	71	63,39	0	0	41	36,6
Ga	70	62,5	2	1,78	40	35,71
Va	0	0	0	0	112	100

The Determined percentage of resistant clinical isolates of *Staphylococcus epidermidis* to the tested antibiotics were: penicillin and ampicillin 96.42%, amoxicillin / clavulanic acid and oxacillin 73.21%, cefoxitin 62.5%, eritromycin 75%, azithromycin 76.78%, clindamicin 33,92%, trimethroprim-sulphametoxazol 63.39%, garamycin 62.5% and vankomycin 0% (Table 1). Intermediate strains were reported while testing cefoxitin, eritromycin, clindamicin and garamycin on clinical isolates of *Staphylococcus epidermidis* (Table 1).

Staphylococcus epidermidis is primarily a normal inhabitant of healthy human skin and mucosal microflora, and is thought to be a saprophytic bacterium with a low pathogenicity potential. However, recent studies indicate that *Staphylococcus epidermidis* is a common cause of many hospital infections (Ziebuhr *et al.*, 2006). Recent studies have examined the percentages of resistant strains of *Staphylococcus epidermidis* in various hospital departments (Claesson *et al.*, 2007). Table 2 gives an overview of resistance of clinical isolates of *Staphylococcus epidermidis* to the tested antibiotics listed below.

Percentages of resistant isolates of *Staphylococcus epidermidis* in various departments of the hospital RMC "Dr. Safet Mujić": pediatric services 92.85%, Surgery 56.25%, Anesthesiology 100%, Infectious disease 0%, ATD 64.21%, and the neurosurgery service as well as Orthopaedy 100% (Table 2 and figure 2).

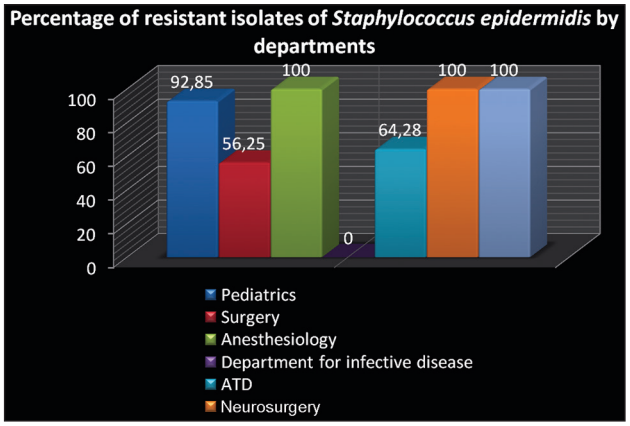


Figure 2. Percentage display of resistant isolates of S.epidermidis by services (departments)

Table 2. Samples submitted by different departments (services) and the percentages of resistance

Department	Num. S samples	Num. R samples	% S samples	% R samples	Total
Pediatrics	2	26	7,14	92,85	28
Surgery	14	18	43,75	56,25	32
Anesthesiology	0	10	0	100	10
Department for infective disease	4	0	100	0	4
ATD	10	18	35,71	64,28	28
Neurosurgery	0	6	0	100	6
Orthopaedy	0	4	0	100	4

Table 3. Antibiotic sensitivity of S.epidermidis to oxacillin by samples

Sample	Num. S samples	Num. R samples	% S samples	% R samples	Total
Blood culture	16	50	24,2424	75,7575	66
Wound smear	14	6	73,6842	31,5789	19
Drainage facilities	0	18	0	100	18
Samples of the catheter	0	6	0	100	6
Bronchial aspirate	0	2	0	100	2

Table 3 gives an overview of antibiotic resistance of clinical isolates of *Staphylococcus epidermidis* isolated from different samples (Figure 3). Clinical isolates included: 66 blood cultures, 19 wound smears, 18 drainage facilities, 6 samples of the catheters and two bronchial aspirate samples. The percentage of resistant strains of *Staphylococcus epidermidis* to oxacillin (MRSE) were: in blood cultures 75.75%, wound smears 31.57%, drainage facilities, samples of the catheter and sample of bronchial aspirate 100%.

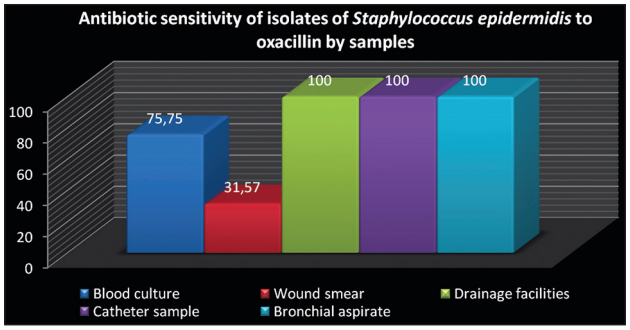
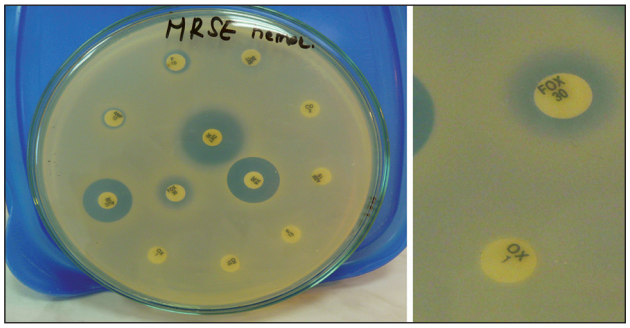
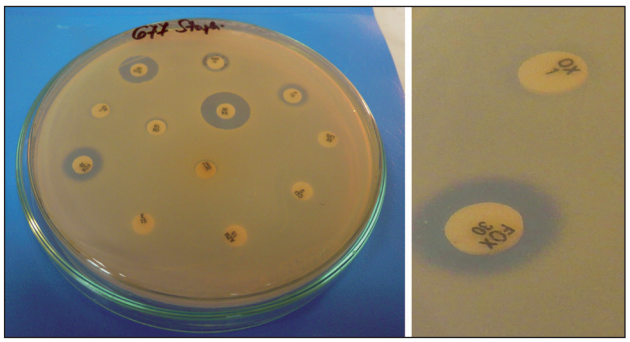


Figure 3. Antibiotic sensitivity of isolates of Staphylococcus epidermidis to oxacillin by samples



Picture 1. Submitted sample: Clood culture, Isolated: MRSE, Protocol: 598



Picture 2. Submitted sample: Drainage facilities, Isolated: MRSE, Protocol: 677

Discussion

In this study, in the period of 2009-2010 in the hospital environment RMC "Dr.Safet Mujić," we analyzed 112 clinical isolates of *Staphylococcus epidermidis*. Of this total 59% of clinical isolates of *Staphylococcus epidermidis* were related to blood culture samples. Testing a blood sample for the presence of bacteria is called a blood culture, while examining several samples is called a series of blood cultures. A set of blood cultures includes seeding a blood culture sample onto the surface of the substrate for aerobic and anaerobic bacteria. Optimal performance depends on sticking to the principles of antisepsis when taking and handling samples. It is not possible to formulate strict guidelines on the number of blood cultures in the series. It is known that one set is often or almost always insufficient. Two or three sets are necessary and sufficient to rule out or confirm a bacteremia especially if we isolate the "usual blood culture contaminants." Because of the increased incidence of infections caused by bacteria that are normal inhabitants of the skin interpretations of the findings of such bacteria in the blood culture is increasingly difficult.

To assess whether a coagulase-negative staphylococci is the cause of sepsis or is a conta-

minant it is necessary to take more than one blood culture from two different places. If contamination occurs in laboratories in over 3% of cases it is necessary to consider the ways of taking blood and the possibility of contamination. However, recently a dramatic rise in the incidence of coagulase-negative staphylococcal that cause sepsis was determined. Among coagulase-negative staphylococci the most common cause of sepsis was *Staphylococcus epidermidis*. This can be associated with increasing and ever more widespread use of intravascular devices (catheters, respiratory tract, urinary tract), on which coagulase-negative stafiloke can adherate, and this is becoming a increasingly important cause of bacteremia (Mirovic, 2002). Given that the finding of bacteria in the blood does not automatically mean an infection, the interpretation of a positive blood culture is necessary to consider each case separately, taking into account all clinical and microbiological findings. It is important to note that for proper blood culture interpretation the collaboration of clinicians, microbiologists, and staff that take the blood for blood culture is necessary.

Infections caused by *Staphylococcus epidermidis* are becoming more demanding to treat because of the increased occurrence of strains resistant to antibiotics, and this is particularly evident in the hospital environment. *S.epidermidis* strains circulating hospitals may be up to 70% resistant to meticillin (York *et al.*, 1996). For coagulase-negative staphylococci the prevalence of meticillin resistance is usually correlated with the resistance to other classes of antibiotics (Miragaia *et al.*, 2002).

CLSI has recommended the oksacillin screening test for detecting resistance to PSB (penicillinaza stable penicillin) as a phenotypic test. Various studies report oxacillin sensitivity and stability compared to other methods used to detect sensitivity to PSB. CLSI suggests the oxacillin agar screening test as an adjunct to the dilution and disk diffusion test. But, as is well known that for the detection of mecA gene the PCR method is the "gold standard" for determination of meticillin resistance, especially in isolates of *Staphylococcus aureus*. However it must be taken into consideration that the sensitive strains with mecA gene do not always express it.

A controversial issue is whether to use oxacillin or cefoxitin screening test. In some studies cefoxitin screeing test is confirmed as reliable for determining resistance to meticillin (Lee *et al.*, 2007). In contrast to that, other studies show that the results of oksacillin screening test are in high correlation with the results obtained by mecA PCR testing (Skov *et al.*, 2005).

In this study, based on the oxacillin screening test, there were 73% MRSE among clinical isolates of *S.epidermidis*. Susceptibility testing of clinical isolates of *Staphylococcus epidermidis* to ten other antibiotics found that most isolates were resistant to penicillin and ampicillin (96.42%), azithromycin (76.78%), erithromycin (75%), amoxicillin / clavulanic acid (73, 21%), TSH (63.39%), garamycin and cefoxitin (62.5%). Weaker resistance to clindamycin was reported (33,92%), while all tested isolates were sensitive to vankomycin. When these results are compared with the percentage of meticillin-resistant coagulase-negative staphylococci in some hospitals, where it approaches up to 90% (John Harvin, 2007), we can conclude that our findings are not of concern.

Conclusion

By oxacillin screening test we determined that in clinical material of patients treated at RMC "Dr.Safet Mujić" among the isolates of *Staphylococcus epidermidis* 73.21% were resistant to meticillin (MRSE), while 27.79% were meticillin sensitive (MSSE).

The testing of antibiotic sensitivity of clinical isolates of *Staphylococccus epidermidis* using disc diffusion Kirby-Bauer method according to 11 selected antibiotics are as follows: the majority of isolates were resistant to penicillin and ampicillin (96.42%), resistance to azithromycin was 76.78%, 75% erithromycin , amoxicillin / clavulanic acid and oxacillin 73.21%, TSH 63.39%, garamycin and cefoxitin 62.5%, clindamycin 33.92%. All tested isolates of *Staphylococcus epidermidis* were sensitive to vankomycin.

The percentage of MRSE isolates within different departments in the hospital RMC "Dr.Safet Mujić" were: pediatric services 92.85%, surgical services 56.25%, ATD 64.28%, Service of Anesthesiology, Neurosurgery Service and Service Orthopedics 100%. Meticillin resistant strains of *Staphylococcus epidermidis* were not isolated in the department of Infectious Diseases.

Analysis of antibiotic susceptibility of isolates of *Staphylococcus epidermidis* found that the largest number of meticillin-resistant strains was observed in samples of bronchial aspirate, drainage facilities, and catheter samples (100%), followed by blood culture specimens (75.75%), while a significantly lower percentage of resistance was recorded in samples of wound smears (32.57%).

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Effects of Naphthalan therapy in danish psoriasis patients treated in naftalan special hospital for medical rehabilitation in a 5-year period 2006-2011

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Abstract

Naphthalan has been known for over a 100 years for its medical benefits. Curable effects of the naphthalan on skin are mostly reserved for the inflammatory conditions, such as psoriasis. The aim of this study was to determine the effect of naphthalan therapy on psoriatic skin lesions in a group of Danish patients, treated from 2006 to 2011 in Naphtalan Special Hospital for Medical Rehabilitation. The mean initial PASI score was 13.8 (female 15 and male 12.5), while the mean final PASI score after 3 weeks of treatment was 3.8 (female 3.6 and male 4.0).The results of this study confirmed previously known excellent antipsoriatic effect of naphthalan.

Key words: Naphthalan, psoriasis, PASI score

1. Introduction

Medical benefit and curable properties of naphthalan have been known from the end of the 19th century. Detailed description of the effects of naphthalan oil on various organ systems of human body was published in 1898 with special accent on the skin and joint diseases (1,2). The Naftalan Special Hospital for Medical Rehabilitation uses naphthalan found at the Kriz oil field near Ivanic Grad in Croatia and it is considered to be a unique institution both in Europe and worldwide for the management of psoriasis, psoriatic arthritis, and rheumatic diseases (3).Naphthalan is obtained from naphtha, a complex mixture of various compounds, mostly hydrocarbons (4). Two forms of naphthalan are available for topical treatment of psoriasis: brown naphthalan, which has been used for the last 15 years, and yellow naphthalan, enriched with active components-steranes and also with a reduced content of polyaromatics(5). Yellow naphthalan is available in the form of oil for topical treatment and according to experimental model studies is not genotoxic (6). This study reports the results of using naphtalan as topical agent in treatment of psoriasis patients.

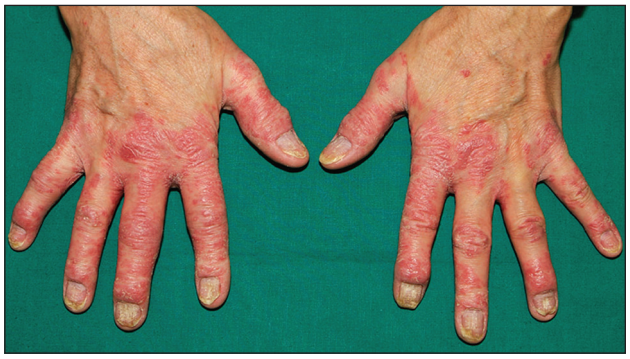
2. Patients and methods

During the 2006-2011 period, 63 psoriasis patients (25 female and 38 male) from Denmark were reffered by Denmark Insurance company for treatment at Naftalan hospital. The mean patient age was 48.3 (range 15-78) years: 47.9 (15-76) years in female and 48.8 (18-78) years in male patients. The mean length of treatment was 21 (18-28) days. During their stay at Naftalan Hospital, the patients received baths in naphthalan oil tubs for 15 minutes/day for 6 days, naphthalan oil temperature 38°C. Naphthalan cream and neutral cream (Belobaza®, Belupo) were applied once daily. During their stay at Naftalan Hospital, three female and two male patients continued taking methrotrexate and one male patient cyclosporine, initiated in Denmark. The effect of naphthalan oil therapy was monitored by PASI score and photo documentation (figures 1-3).

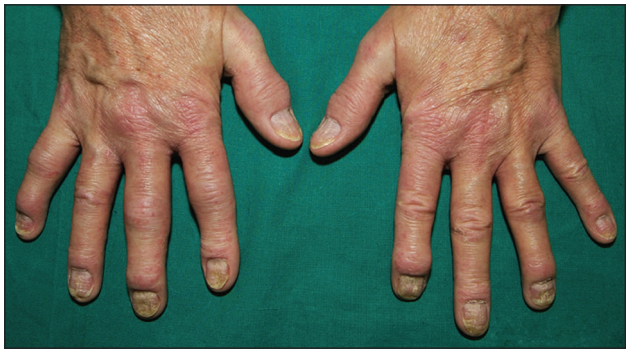
3. Results

PASI score at the beginning and at the end of treatment in 63 Danish patients (25 female and 38 male) is reported. The mean initial PASI score was 13.8 (female 15 and male 12.5), while the mean final PASI score was 3.8 (female 3.6 and male 4.0). PASI 90 was achieved in six (24%) female and three (4.9%) male patients; PASI 75 in eight (32%) female and 11 (28.9%) male patients; PASI 50 in

seven (28%) female and 18 (47.4%) male patients; and PASI <50 in four (16%) female and six (15.8%) male patients (figures 4-7). Naphthalan oil therapy was well tolerated by the patients and no side effects were observed.

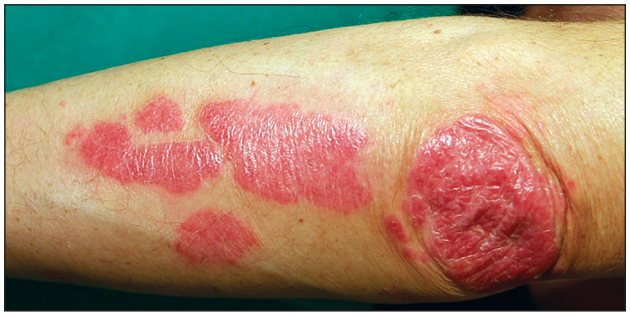


A) Before treatment

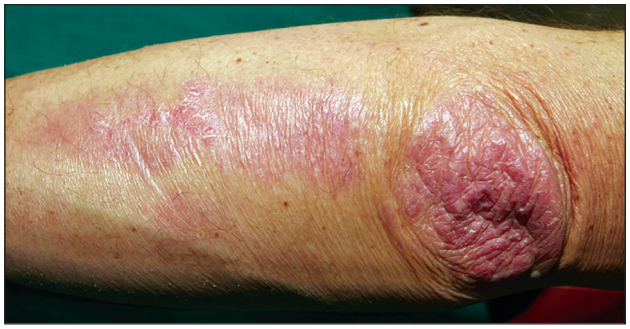


B) After treatment

Figure 1. Effect of naphthalan oil therapy

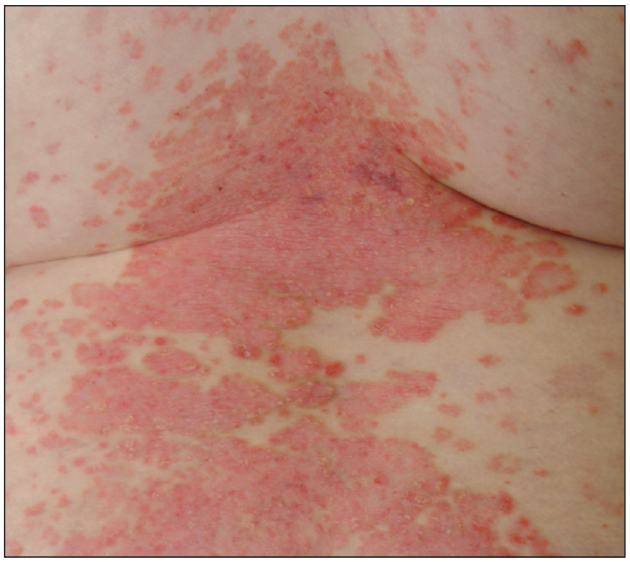


A) Before treatment

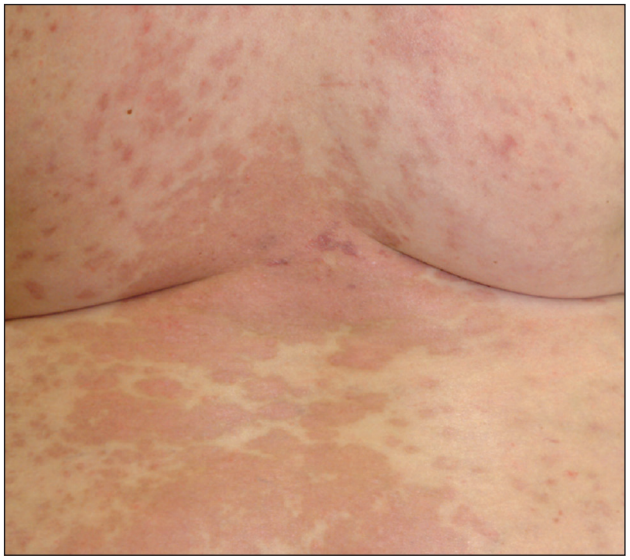


B) After treatment

Figure 2. Effect of naphthalan oil therapy



A) Before treatment



B) After treatment

Figure 3. Effect of naphthalan oil therapy

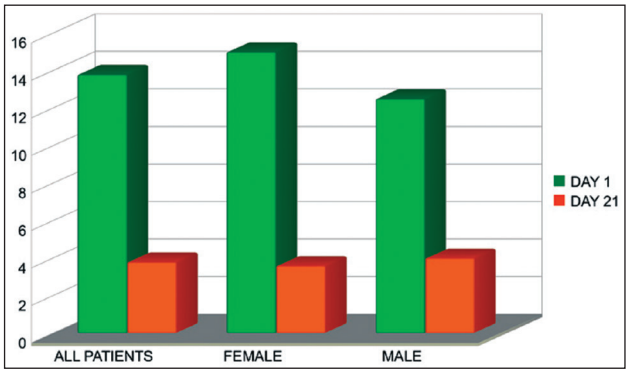


Figure 4. PASI score day 1/21

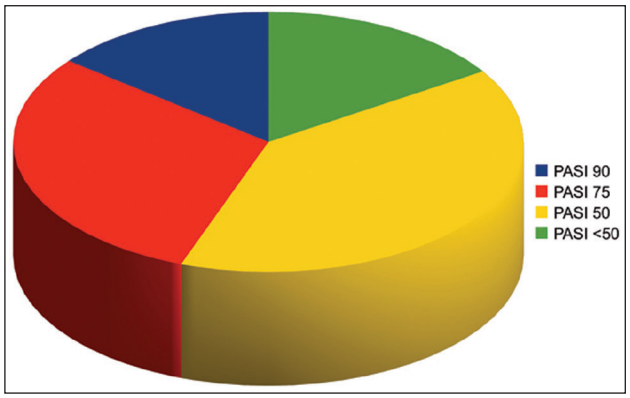


Figure 5. PASI 90, 75, 50 – all patients

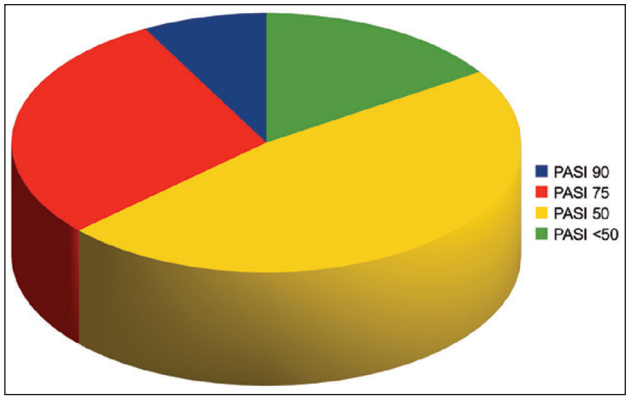


Figure 6. PASI 9, 75, 50 – male

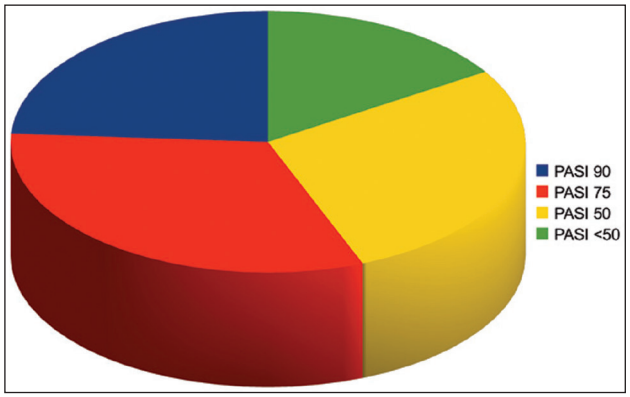


Figure 7. PASI 9, 75, 50 – female

4. Discussion

The antipsoriatic properties of heavy naphthalan oil are basis of an antipsoriatic treatment regimen that has been used over the last decade at Naftalan Special Hospital in Ivanic Grad, Croatia. Bioactive properties related to antipsoriatic effect of naphthalan oil have been an object of interest of several studies.

According to Vrgožić et al., naphthalan therapy modifies the expression of angiogenic factor in psoriatic lesions (7). Immunohistochemical results of this study point out the reduction role of naphthalan therapy in neovascularization of psoriatic lesions (7).

Vrgožić et al. were also considering antiproliferative effect of naphthalan(8).CD3, CD4, and CD8 lymphocyte count were selected point of interest and the researchers have noticed decrease of the immunocompetent cell count in psoriatic lesions which lead to conclusion about naphthalan antiproliferative activity (8,9). Antiproliferative effect of naphthalan on immortalized keratinocytes were demonstrated in vitro by Thaci et al. (10) Investigators also described an increased expression of the differentiation markers keratin 10/11 and involucrin (10).

Topical application of naphthalan is not significant just because of its plain use. In fact, classical topical therapy for psoriasis, besides it is the cornerstone of the treatment, is also considered to be extremely effective in selected patient populations even though its role can sometimes be neglected (11, 12).

Taking into consideration that all of the patients were hospitalized in specific type of institution with is well known speciality- naphthalanotherapy, there is a certain possibility of the appearance of the placebo effect. Unfortunately, because of the specific fragrance of the naphthalan, double blinding of the design would be impossible. We would like to present our excellent results in group of Danish patients treated in our institution, regarding to these limitations, and also suggest performation of another controlled studies for providing other strong evidence of benefits of the naphthalanotherapy.

5. Conclusion

Regarding on the results of previously conducted studies about inhibitory effect of naphthalan on intraepidermal proliferative activity, on intraepidermal and dermal inflammatory cells, and on neoangiogenesis, and concerning the very positive clinical results of present study, we conclude that naphthalan is a useful topical agent for the treatment of mild to moderate psoriasis.

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Clinical analysis of the role of micronutrients in combination of orthomolecular therapy for threating Osteoarthritis genus

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Abstract

Orthomolecular medicine is based on a principle :"Maintaining good health through supplementation and increasing the concentration of substances that are essential to our body and that helps our immune system." [1.2]

The study was designed to evaluate potential role of micronutrients in orthomolecular combination for threating Osteoarthritis genus. Duration of the study was 4 months.The study was conducted over five examiners on 30 patients in 3 cities of Bosnia and Herzegovina.

The criteria for inclusion of patients in the study entailed: female persons aged 45-65 years divided into two sub-groups (45-55,) and (55-65 years); clinical manifestation of osteoarthritis,radiologically confirmed changes, arthrosis II and III level by Kellgren & Lawrence and consent to the inclusion in the study. Criteria for exclusion of the patients from the study were: self-exclusion of patients from the study; acutisation of some other underlying disease that requires additional treatment or complete dietary treatment and surgical treatment. No patient was excluded from the study. The study contained several phases: Completing a questionnaire containing information on sex, occupation, risk factors; clinical examination of the patient; visual analog pain scale (VAS); enrollment of RTG verified changes of knee; presence of arthrosis II and III degree by Kellgren & Lawrence; Completing a questionnaire for accurate evaluation and grading (Oxford Knee Score). This phase is being completed threw the form, which will serve as the baseline for measuring the success of treatment.The evaulation is being done after one month and after three month of using orthomolecular combination of micronutrients.

The conclusion of the study was that group of subjects (30) with osteoarthritis of the knee (unilateral and bilateral gonarthrosis) after 3 months of using orthomolecular combination of micronutrients showed measurable and statistically significant improvement seen in VAS pain scale, improving range of motion measured by goniometer and improving scores on the Oxford Knee test.

Key words: Osteoarthritis, orthomolecular therapy, Oxford Knee Score

Introduction

Linus Pauling - founder of orthomolecular medicine (Greek: Ortho = true, good.) defined a principle on which is based this medicine:"Maintaining a good health through supplementation and increasing the concentration of substances that are essential to our body and that helps our immune system." [1.2] Under the substances that are essential to our body include: vitamins, minerals, phytonutrients, fatty acids, amino acids, probiotics, prebiotics and trace elements. In order to micronutrients could act synergistically as a unity, you must reach a certain amount, content and complexity of natural micronutrients.

Degenerative osteoarthritis implies the chronic joint disease characterized by degeneration of articular cartilage and surrounding bone.Studies show that over 70% of the population over age 60 suffer from symptoms caused by this disorder of locomotor system. Every year the number of people suffering from degenerative osteoarthritis increases by about 2% and 50% of new cases, the diagnosis is associated with symptoms. Every year, in 4% of patients there is a progression of their condition.[3]

The clinical picture is dominated by disease: pain, swelling, effusion, muscle spasm and reduced mobility.

Materials and methods

Duration of the study was 4 months.The study was conducted over five examiners on 30 patients in 3 cities of Bosnia and Herzegovina. The criterion for inclusion of patients in the study entailed: female persons aged 45-65 years divided into two sub-groups (45-55,) and (55-65 years); clinical manifestation of osteoarthritis (pain, movements tracked crepitations, deformed joints, limit movement, posture articular surfaces, island) radiologically confirmed changes (X-rays, CT or MRI); arthrosis II and III level by Kellgren & Lawrence and consent to the inclusion in the study. Criteria for exclusion of the patients from the study were: self-exclusion of patients from the study; acutisation of some other underlying disease that requires additional treatment or complete dietary treatment and surgical treatment. No patient was excluded from the study.

Phase I - Survey study

- Completing a questionnaire containing information on sex, occupation, risk factors, clinical examination of the patient (measured trophic of thigh muscles, knee circumference measurement, measuring range of mobility of the knee), visual analog pain scale (VAS); enrollment of RTG verified changes of knee, presence of arthrosis II and III degree by Kellgren & Lawrence.

- Completing a questionnaire for accurate evaluation and grading (Oxford Knee Score) *** This phase is being completed threw the form, which will serve as the baseline for measuring the success of treatment. After completing the form , patients get the precise instructions for the use of orthomolecular combination of micronutrients.

*** In order to accurately evaluate performance and efficacy of the product , patient will be able to use painkillers 1-3 tablets as needed and perform massage with ice.

Phase II - After the first month of using orthomolecular combination of micronutrients,there is an evaluation on all these parameters from the first phase (clinical examination, pain scale (VAS) and Oxford Knee Score)

* After the first evaluation , patient gets two packages of orthomolecular combination of micronutrients with precise instructions for use and the same recomendations for use of painkillers and cryo therapy for increased pain.

Phase III - After three months of using orthomolecular combination of micronutrients,there is an evaluation on all these parameteres from the first phase (clinical examination, pain scale (VAS) and Oxford Knee Score).

Phase IV - Evaluation. As part of this phase, there is a comparative evaluation of data obtained from three measurements, the performance of the treatment between the first and second stages, the second and third phases, and the success of treatment at the start and end of treatment after three months.

Orthomolecular combination of micronutrients:

Daily meal to 100g
Aminosugars
Glucosamine sulfate 1,100 mg 6.7 g
Chondroitin sulfate 400 mg 2.4 g
Hyaluronic Acid 50 mg 303 mg
Vitamins
Vitamin A 375 mg (1,250 IU *) 2.3 mg (7,566 IU *)
Vitamin C 475 mg 2.9 g
Vitamin E (TE **)
(including alpha and gamma tocopherol) 70 mg 424 mg
Vitamin B1 24 mg 4 mg
Vitamin B2 5 mg 30 mg
Nicotinamide 30 mg 182 mg
Vitamin B6 5 mg 30 mg
Vitamin B12 9 mg 55 mg
Vitamin K1 60 mg 364 mg
Vitamin D3 7.5 mg (300 IU *) 45 mg (1,816 IU *)
Folic Acid 400 mg 2.4 mg
Pantothenic acid 18 mg 109 mg
Biotin 150 mg 909 mg
Minerals and trace elements
Calcium (Ca) 200 mg 1.2 g
Zinc (Zn) 10 mg 61 mg
Selenium (Se) 50 mg 303 mg
Manganese (Mn) 2 mg 12 mg
Copper (Cu) 1.000 mg 6 mg
Molybdenum (Mo) 50 mg 303 mg
Phytonutrients
Citrus bioflavonoids 50 mg 303 mg
Mixedcarotenoid
(including beta-carotene, lutein and lycopene) 3 mg 18 mg
Amino acids
Collagen hydrolyzate 2.5 g 15.2 g
Acetylcysteine 80 mg 485 mg
Essential fatty acids

Fish oil, including: 1.1 g 6.7 g
Eicosapentaenoic acid (EPA) 500 mg 3 g
Docosahexaenoic acid (DHA) 167 mg 1 g
Energy value 259 kJ (61.6 kcal) 1,567 kJ (373 kcal)
Protein 2.8 g 17 g
Carbohydrate 8.5 g 51 g
Fats 1.1 g fat 6.9 g

Results

1. Total number of the patients: 30
1. GROUP A (WOMEN OF 45-54 YEARS): 15 women with avarage age - 48 ± 8.6 years
2. GROUP B (WOMEN OF 55-65 YEARS): 15 women with avarage age -60 ± 9.3 years

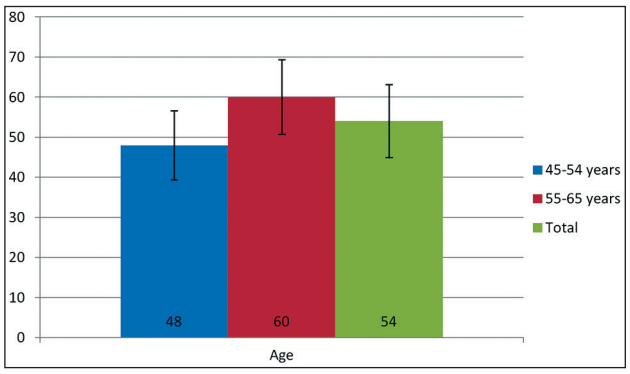

Figure 1. Average age of all respondents: 54±9.1 years

2. Occupation:
Retired: 16 respondents
Economically active: 10 respondents
Housewife: 4 respondents

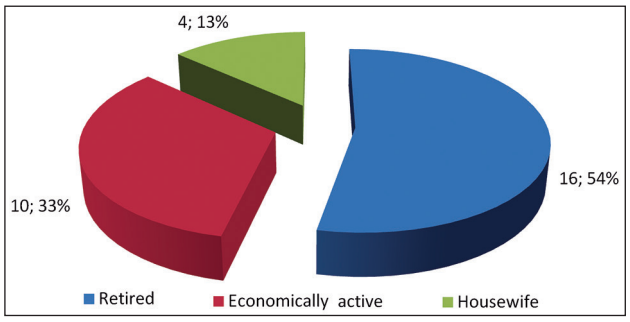

Figure 2. Occupation

3. Classification of arthrosis by Kellgren & Lawrence:
1. Grade II (change of shape of the joint space, osteophytes): 20 respondents
2. Grade III (inequality of joint space , significant osteophytes, damaged articular surface): 1O respondents

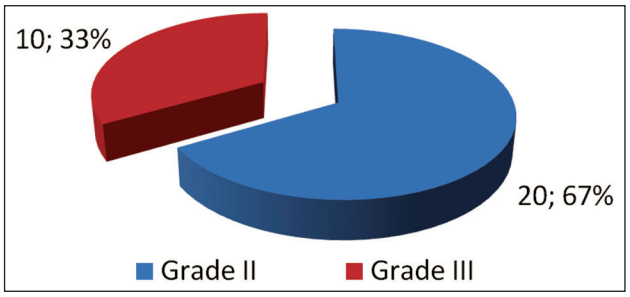

Figure 3. Classification of arthrosis by Kellgren & Lawrence

4. BODY MASS INDEX (BMI):
1. Minimum BMI: 22
2. Maximum BMI: 31

Average BMI of 30 females: 27.5 ± 4.5 – overweght (25-29.9)

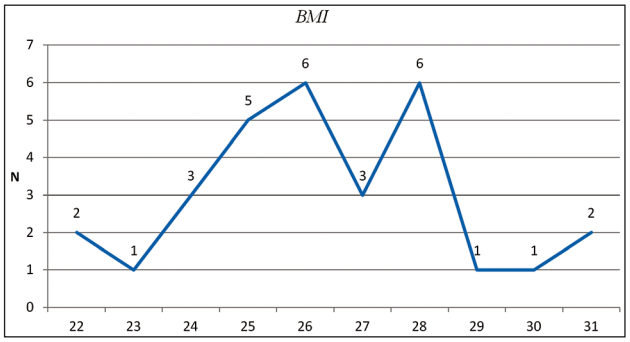

Figure 4. BMI

5. Involvement of the joint sphere:
1. Unilateral knee OA: 22
Left unilateral knee OA: 8
Right unilateral knee OA: 14

2. Bilateral gonarthrosis: 8

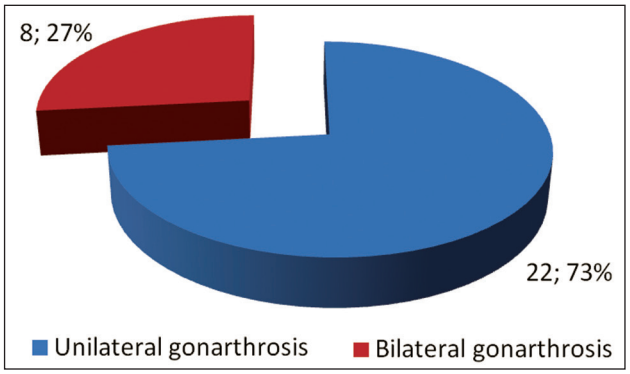

Figure 5. Involvement of the joint sphere

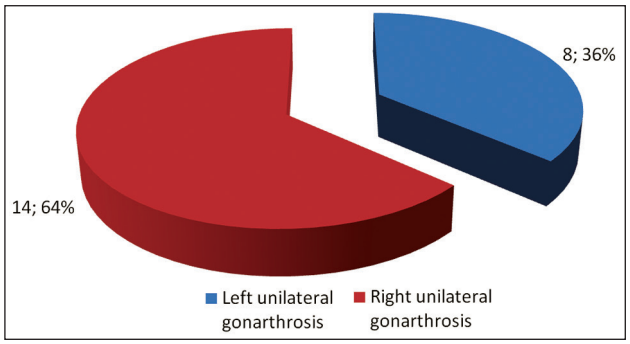

Figure 6. Unilateral gonarthrosis

First Measurement (before taking the therapy)

1. Range of motion
A) Average value of right extensions: - 15 º
B) Average value of the right flexions: -10 º
C) Average value of the left extensions:-1O º
D) Average value of the left flexions : -10 º
2. Visual Analogue Pain Scale (0-10)
First measurement: 7
3. OXFORD KNEE TEST
Lowest Value: 15

Highest value: 29
Average value of the 30 respondents: 23 which is „a moderate to severe form of osteoarthritis (20-29)“
Second mesaurement (After one month of using supportive therapy)

1. Range of motion
A) Average value of right extensions: - 10 º
B) Average value of the right flexions: -10 º
C) Average value of the left extensions:-5 º
D) Average value of the left flexions : --5 º

Difference between 1. and 2. measurement:
RIGHT:
a) The extension of the right shows an improvement for 5 º
b) The flexion of right is -10 º , the same value
LEFT:
a) The extension of the left shows an improvement for 5 º
b) The flexion of the left shows an improvement for 5 º
2.Visual Analogue Pain Scale (0-10)
Second Measurement : 6
Difference between 1. and 2. measurement in VAS is an improvement from 7 to 6 (the difference is 1)

3. OXFORD KNEE TEST
Lowest Value: 18
Highest value: 39

Average value of 30 respondents: 29
Difference between 1.and 2. measurement in relation to Oxford Knee Test is an improvement from avarage value 23 to an avarage value 29.In avarage, respondents are in the same group as in 1. measuremet (moderate to severe forms of osteoarthritis).However, improvement is being reflected in a fact that the average score of Oxford Knee Test in the 2. measurements with value 29, is being located on the border of the group “moderate to severe forms of osteoarthritis,” with a group of “mild to moderate forms of osteoarthritis.”
Third measurement (after 3 months of using supportive therapy)

1. Range of motion
A) Average value of right extensions: - 5 º
B)Average value of the right flexions: -5 º
C) Average value of the left extensions: 0 º
D)Average value of the left flexions : --5 º

Difference between 2. and 3. measurement:
RIGHT:
a) The extension of the right shows an improvement for 5 º
b) The flexion of right is shows an improvement for 5 º
LEFT:
a) The extension of the left shows an improvement for 5 º
b) The flexion of the left shows the same value as in the second measurement

Difference between 1. and 3. measurement:
RIGHT:
a) The extension of the right shows an improvement for 10 º
b) The flexion of right is shows an improvement for 5 º
LEFT:
a) The extension of the left shows an improvement for 10 º
b) The flexion of the left shows an improvement for 5 º

Between 1. and 3. measurement there is a significant difference in measurement of the range of motion.

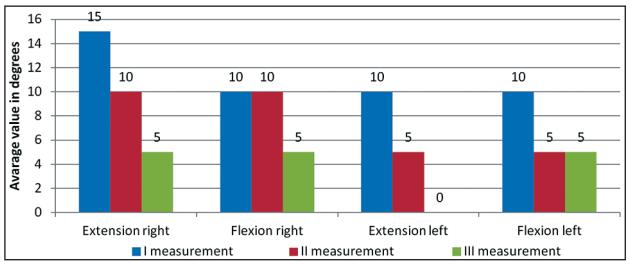

Figure 7. Range of motion

2. VAS (0-10)

Third measurement: 4

Difference between 2. and 3. measurement in VAS is an improvement from 6 to 4 (the difference is 2)

Difference between 1. and 3.measurement in VAS Pain Scale is an improvement from 7 to 4 (the difference is 3)

It was observed a significant difference in pain reduction measured on the visual analogue scale of pain between 2. and 3. measurement and even more significant difference between 1.and 3. measurement.

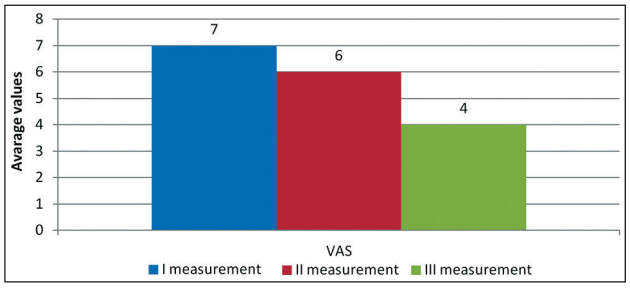

Figure 8. Visual analog scale of pain

3. OXFORD KNEE TEST

Lowest value: 20

Highest value: 40

Average values of 30 respondents: 33

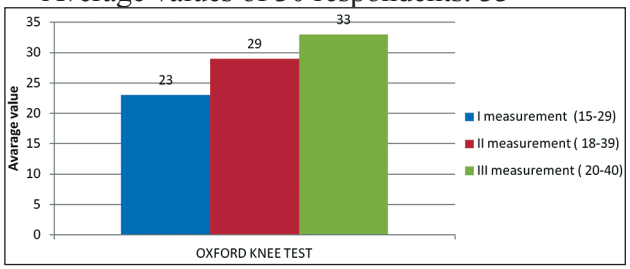

Figure 9. Oxford knee test

Difference between 2. and 3. measurement in relation to Oxford Knee Test is an improvement from an avarage valeue of 29 to an avarage value of 33.Comparison of the results shows an improvement and moving the respondents from the group "moderate to severe forms of osteoarthritis" to the group "mild to moderate forms of osteoarthritis."

Difference between 1. and 3. measurement in relation to Oxford Knee Test is an improvement from an avarage valeue of 23 to an avarage value of 33.Comparison of the results shows an improvement and moving the respondents from the group "moderate to severe forms of osteoarthritis" to the group "mild to moderate forms of osteoarthritis."

Average value of Oxford Knee test after first (23), the second (29) and third (33) measurement showed a significant improvement between the second and third measurement ,and particularly marked improvement in comparative analysis of first and third measurement.

Discussion

A progressing osteoarthritic process will lead to the increasing destruction of cartilage tissue. The associated pain and restriction of movement often cause the patient's immobilization, which will itself result in progressing symptoms in the sense of avicious circle. The primary objective of osteoarthritis therapy must there for be to alleviate pain and keep the joints functional and mobile. In addition to weight reduction, physiotherapeutic measures and supportive drug therapy, there are a number of chondroprotective cartilage nutrients that can beneficially influence the disease process on the basis of nutritional medicine. Nutritional medicine offers a number of appropriate measures with the possibility of supplementing conservative therapy in a useful and effective way.The use of chondroprotective cartilage nutrients is of particular interest in this context. Besides glucosamine sulphate, chondroitin sulphate and collagen hydrolysate,specific micronutrients, such as vitamins, trace elements and long-chain omega-3 fatty acids are of importance.If you study the effect of glucosamine and chondroitin sulfate, you can see that they: contribute to the metabolism of cartilage, stimulate chondrocytes to create elements of cartilage,they are building blocks of hyaline cartilage and synovial fluid (proteoglycans),they hav e antiinflammatory effects (in vitro) ,antioxidant

effects (in vitro), reduce the use of NSAID and helps reduce the clinical symptoms of the disease (pain, stiffness, difficulty in movement).

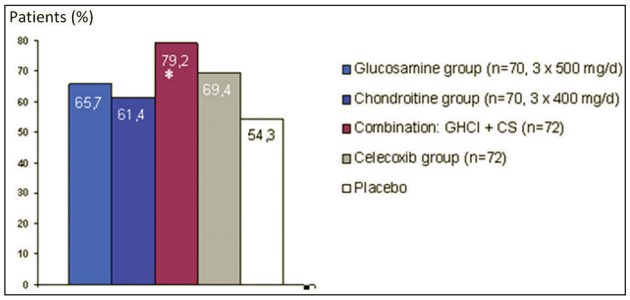

Figure 10. Subgroup of patients: Womac index of pain 301-400 mm (moderate to severe pain)

20% or more improvement in WOMAC painful scale (main scale Vs. 24 weeks) * p = 0.002 (GHCl + CS vs. Placebo) [4]

Table 5 Level of evidence based on the literature search, and strength of recommendation based on both evidence and expert opinion

Intervention	Level of evidence	Effect size Range	Strength of recommendation
Acetaminophen/paracetamol	1B		A
Opioid analgesics	1B		B
NSAIDs			
Conventional NSAID	1A	0.47–0.96	A
Coxibs	1B	0.5	A
Antidepressant	1B		B
Topical NSAID	1A	−0.05–1.03	A
Topical capsaicin	1A	0.41–0.56	A
Sex hormones	2B		C
SYSADOA			
Glucosamine	1A	0.43–1.02	A
Chondroitin	1A	1.23–1.50	A
Diacerein	1B		B
ASU	1B	0.32–1.72	B
Nutrients	1B	0.65	B
Herbal remedies	1B	0.23–1.32	B
Minerals/vitamins	1B		C
Education	1A	0.28–0.35	A
Exercise	1B	0.57–1.0	A
Telephone	1B	1.09	B
Acupuncture	1B	0.25–1.74	B
Laser	1B	0.87	B
Pulsed EMF	1B		B
Spa therapy	1B	1.0	C
TENS	1B	0.76	B
Ultrasound	1B		C
Weight loss	1B		B
Insoles	1B		B
Orthotic device (knee brace/ patella tape/elastic bandage	1B		B
IA Hyaluronic acid	1B	0.0–0.9	B
IA Corticosteroid	1B	1.27	A
Lavage/tidal irrigation	1B	0.84	B
Arthroscopy ± debridement	1B		C
Osteotomy	3		C
UCKR	3		C
TKR	3		C

Figure 11. EULAR Recommendations (2003)[5]

If you study the effect of collagen hydrolyzate you can see that it: contributs to the metabolism of cartilage, proline + glycine are important amino acids for protein building blocks of cartilage, it also reduce the use of NSAIDs and helps reducing the clinical symptoms of the disease.

When you consider the role of Omega-3 fatty acids, you can notice their anti-inflammatory effect (in our body, anti-inflammatory agents are formed from omega-3 fatty acids-Eikosanoza), they are antagonists of proinflammatory action of arachidonic acid and they inhibits the formation of signaling substances that can cause degradation of collagen (TNFa + interleukin 1a/-b).

Why antioxidants should be used in dietary management of osteoarthritis? Vitamin D should be used becouse it supports calcium absorption from the gut and helps integration of calcium in bone. Vitamin K1 should be used becouse it increases bone mineral density. Vitamin B6 is co-factor for the cross-linking of collagen .Vitamin E helps reducing use of NSAID s and acetylcysteine is cross-linking component of cartilage. The aim of study was to explore a potential role of micronutrients in orthomolecular combination for threating Osteoarthritis genus and the result was that use of orthomolecular combination of micronutrients can show clinical benefit fot the patients with Osteoarthritis genus.

Conclusion

Group of subjects (30) with osteoarthritis of the knee (unilateral and bilateral gonarthrosis) after 3 months of using orthomolecular combination of micronutrients showed measurable and statistically significant improvement seen in VAS pain scale, improving range of motion measured by goniometer and improving scores on the Oxford Knee test.

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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 keywords.

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 and 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 acknowledge-ment) 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. For the figure captions 12 pt *Italic* font should be used. (1)

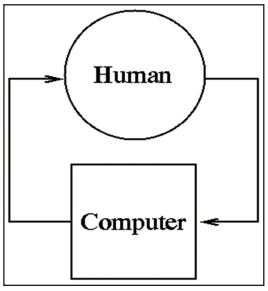

Figure 1. Text here

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.

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1. *Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. N Engl J Med 1999; 341: 1284–1291.*
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