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Medical students as healthy carriers of *Staphylococcus Aureus* and *Streptococcus Pyogenes*: its prevalence and importance

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Abstract

Aim: To assess the frequency of *Staphylococcus aureus* and *Streptococcus pyogenes* isolates from naso-pharyngeal microflora among preclinical and clinical medical students in relation to gender, age and year of the study.

Materials and methods: A cross-sectional study included 311 students who performed sanitary survey during a period from the last and the first trimester of 2008/2009 academic year. Swabs were collected and identified by conventional methods according to CLSI standards. The study was carried out at the Department of Microbiology, School of Medicine, University of Sarajevo.

Results: *Staphylococcus aureus* carriage rate was (10.6%), while *Streptococcus pyogenes* isolates was (0.6%), respectively. Regarding to all positive *S. aureus* isolates 37.1% were male, and 57.2% were female. In relation to the year of study, *S. aureus* was isolated in 11 (31.4%) students of the first year, 16 (45.8%) students of the fifth, and 6 (17.1%) of the sixth year of the study. According to age distribution, *S. aureus* was isolated in 9 (27.3%) students in group 29-38, 10 (30.3%) aged 24-28 and 14 (42.4%) aged 19-23, respectively.

Conclusion: Frequency *of Staphylococcus aureus* and *Streptococcus pyogenes* carriers among medical students in our area was median in compare with other findings. The study highlights the imoprtance of medical students as potential source of cross-infection in our hospitals.

Key words: *Staphylococcus aureus, Streptococcus pyogenes*, students

Introduction

The human body provides a very conducive environment for the growth of many microorganisms. All microorganisms that are regularly found at any anatomical area are determined as normal microflora. Bacteria are the most numerous and the most evident component of the normal microflora (1). The human body is rich in organic substances and growth factors necessary for heterotrophic microorganisms, providing a relatively constant temperature, pH and osmotic pressure.

Staphylococcus aureus is one of the most important human pathogen, causing a wide range of diseases (2). Typical forms of staphylococcal infections include local infections of the skin (blain, carbuncle, bullous impetigo, cellulitis), generalized infection of the skin (burned skin syndrome or Ritter's disease), and very frequent, and postoperative infections of surgical wounds, burns, infection of the breast after birth, pneumonia, osteomyelitis, endocarditis (1). *Streptococcus pyogenes* is β-hemolytic streptococci of serological group "A". Streptococcal disease is divided into two groups. The first group includes the following purulent inflammation of the disease, pharyngitis, scarlet fever, erysipelas, impetigo, pneumonia, necrotizing fasciitis, streptococcal bacteremia, osteomyelitis, otitis media, sinusitis, meningitis and brain abscess (1). The second group includes consequences of infection by *Streptococcus pyogenes* like acute rheumatic fever and acute glomerulonephritis as well. Pathogens can be transmitted through direct contact and fomites (3).

Colonization rate of *Staphylococcus aureus* is higher in health care workers (HCWs) and are one of the important sources of infection transmission in hospital environment (4). About 25% of the HCWs are permanent nasal carriers, and 30-50% of them also possess the bactera on their hands (5).

The study highlights the importance of medical students as potential source of cross-infection in our hospitals.

Materials and methods

Cross-sectional study included 311 students who access the sanitary inspection for a period from the last and the first trimester of 2008/2009 academic year. All tested students performed their practical training at the Gynecological and Obstetric Departments of the Clinical Centre University of Sarajevo-KCUS. Nasal and pharyngeal swabs were taken. Isolation of microorganisms was performed by standard methods at the Department of Microbiology, School of Medicine, University of Sarajevo.

Students are divided in pre-clinical and clinical groups. They were compared to age, gedner and years of the study. According to age distribution, students were ranged into three groups: 19-23, 24-28, and 29-38 years of age, respectively.

Staphylococcus aureus and *Streptococcus pyogenes* were identified according to standard microbiological methods (6). The plates were incubated in 5% CO2 atmosphere or aerobic 24-48 hrs on 35-37 °C. *Staphylococcus aureus* ATCC 25923 control strain was applied.

Statistical analysis was performed using chi-square test with Fisher correction for 2x2 tables, and the Yates correction for small samples. Statistically significant difference was considered at $p<0.05$.

Results

For the purpose of this study 311 students were selected in order to determine the prevalence of *Staphylococcus aureus* and *Streptococcus pyogenes* as well.

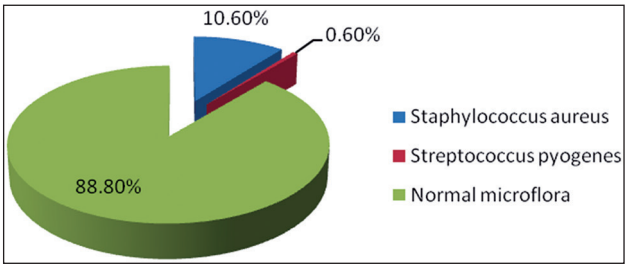
Out of 311, 101 (32%) were male, and 210 (68%) were female ($p> 0.05$). Table 1 showed gender structure of tested students of the first, fifth and the sixth year of the study. *Staphylococcus aureus* carriage rate was 33 (10.6%), while *Streptococcus pyogenes* isolates was 2 (0.6%), respectively (Figure 1). Out of 33 *S. aureus* positive isolates, 13 (37.1%) were male, while 20 (57.2%) were female (Table 2). Diferential function analysis was performed statistically to evaluate the contribution of the following criteria: students age, gender and years of study to *S. aureus* and *S. pyogenes* isolation.

The frequency of *S. aureus* isolates based on age distribution was 9 (27.3%) students in group 29-38, 10 (30.3%) aged 24-28 and 14 (42.4%) aged 19-23, respectively ($p> 0.05$) (Table 3). The greatest number of *S. aureus* was present in the fifth year of the study, 16 (45.8%), and the lowest in the sixth year 6 (17.1%), respectively. *S. aureus* was isolated in 11 (31.4%) pre-clinical students of the first year of study, while *S. pyogenes* with only 2 (0.6) as well (Table 4).

Table 1. Gender structure of students in relation to year of the study

Year of study	Gender	Number	%
I year	male	17	5
	female	45	14
	total	**62**	**19**
V year	male	49	16
	female	73	23
	total	**122**	**39**
VI year	male	35	11
	female	92	31
	total	**127**	**42**

$\chi 2=6.411$ $p=0.0468$



$\chi 2=0.133$ $p=0.7148$

Figure 1. The frequency of Staphylococcus aureus and Streptococcus pyogenes isolates among students

Table 2. The frequency of Staphylococcus aureus and Streptococcus pyogenes isolates in relation to gender

Isolates	Gender	Number	%
Staphylococcus aureus	male	13	37.1
	female	20	57.2
	total	33	100
Streptococcus pyogenes	male	0	0
	female	2	5.7
	total	2	100

Table 3. The frequency of Staphylococcus aureus in relation to students' age

Age group (years)	No of isolates	No (%) of positive isolates
19-23	70	14 (4.5)
24-28	175	14 (4.5)
29-38	66	9 (2.9)

Table 4. The frequency of Staphylococcus aureus and Streptococcus pyogenes in realtion to year of the study

Isolates	Year of the study	Number	%
Staphylococcus aureus	I year	11	33.3
	V year	16	48.5
	VI year	6	18.2
total		33	100
Streptococcus pyogenes	I year	2	100
	V year	0	0
	VI year	0	0
total		2	100

Discussion

The anterior nares have been described as primary ecologic niche for *Staphylococcus aureus.*

This study details the effect of exposure to the hospital environment, on the nasal carriage of *Staphylococcus aureus* and *Streptococcus pyogenes* among pre-clinical and clinical medical students in 2008/2009 academic year.

For the purpuse of this study, we separeted the results taken from nasal swabs and throat of 311 medical students of the first, fifth and sixth years of the study in order to determine prevalence of *Staphylococcus aureus* carraige and *Streptococcus pyogenes* as well. Students were performed their practical training at the Gynecological and Obstetric Departments at the KCUS, Sarajevo, in the academic year 2008/2009.

Nowdays, a big problem presents hospital strains of *Staphylococcus aureus* resistant to methicillin (MRSA), which usually causes severe infections in patients (7). To prevent the spread of MRSA the support of hospital administration, continuing education of staff and patients, controlled use of antibiotics, implement standard procedures for hygiene, cleaning and decontamination should be implemented. Strict observance of hand hygiene should minimize the risk of transmission of pathogens of nosocomial infections. Hands are decontaminated before and after the contact with the patient. Hand decontamination is performed by applying the basic washing and disinfectants (8).

To reduce the risk of infection in the mother and newborn at the gynecological and obstetric departments, the principles of asepsis and antisepsis sholud be implemented (9).

Our results confirmed *Staphylococcus aureus* carriage rate, it was (10.6%), while *Streptococcus pyogenes* isolates was (0.6%), respectively. No significant differences were found in the frequency of isolates in relation to age and year of study, but it was found in realtion to gender ($p<0.05$). These results were in accordance of the results of Chigbu and Ezeronye (10) and the ranges described by Adesida et al. (11).

In the study analyzed at the School of Medicine, Lagos, Nigeria (11) *S. aureus* was isolated in 14% of all isolates produced hemolysin, none of the strains were resistant to methicillin.

Cross-sectional study which included 346 patients was conducted at the University Putra Malaysia, in December 2006 (12). *Staphylococcus aures* from nasal mucosa was isolated in 23.4% of respondents. This study concluded that MRSA colonization outside of health facilities was low, in smokers and beneficiary of oral contraceptives the incidence rates of *Staphylococcus aureus* was high.

According to the published data from the Bosnia and Herzegovina, Canton Sarajevo, *S. aureus* has non-multidrug resistance profile, providing increased options for empirical and directed therapy of infections (13). However, majority of the staphylococci were multi-drug resistant and these

multi-drug resistance patterns has been documented already (14, 15).

Our results confirmed that frequency *of Staphylococcus aureus* and *Streptococcus pyogenes* carriers among medical students in our area was median in compare with other findings (10-12, 16).

Procedures for decolonization of the nasal mucosa and throat should be carried out as recommended and supervised by a team of infection control (17). Data from Shea (American Association of Health Epidemiology) recommendations state that decolonization and treatment of infected staff significantly contributed to controlling MRSA outbreaks in hospitals.

In conclusion, continuation of education among hospital staff and medical students should be implemented, including control usage of antibiotics, as well as implementation of standard hygiene procedures.

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Propionic acid derivatives synthesis as cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibitors by rheumatoid arthritis

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Abstract

Aim: Synthesis propionic acid derivatives as potential cyclooxigenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibitors by rheumatoid arthritis.

Material and methods: For determination of melting points for synthesized components apparatus Büchi Melting Point B-545 has been used. Infra-red spectrum has been determinate by instrument Perkin Elmer FT-IR Spectrum 1000. Sample preparation: Pastila KBr. For thin-layer chromatography have been used Merck HPTLC-Plates 20 X 10 cm Silica gel 60 $F_{254,}$ Statistical calculations are made on computer. All used chemicals were purity degree of proanalisy. This is an example of Friedel-Craft's reaction of *acylation*.

Results: The 2-(4-benzoylphenyl)-2-methyl propionic acid has been obtained. Synthesis itself flow in few phases until final product, therefore obtained are also: 4-methyl benzophenone (compound I), 4-bromomethyl benzophenone (compound II), 4-cyano-methyl benzophenone (compound III), 2-(4-benzoyl-phenyl)-*2-methyl-propionitril* (compound IV) and 2-(4-benzoylphenyl)-2-methyl propionic acid (compound V). The 2-(4-benzoylphenyl)-2-methyl propionic acid has been identified by thin-layer chromatography and IR method.

Discussion: An anti-inflammatory effects of non-steroidal anti-rheumatic drugs (NSAR) is based on cyclooxigenase blocking, the key enzyme in prostaglandin synthesis. There are two types of cyclooxigenase: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Cyclooxigenase-1 synthesize prostaglandins which are necessary for normal physiological functions of organism, but cyclooxigenase-2 (COX-2) isn't normally to be in organism, but appears by inflammatory processes, playing role in synthesis of pathologic prostaglandins, whose emphasize inflammation. It is oblivious that blocking COX-2 the inflammatory processes are going to seize. It is known that propionic acid derivatives as ibuprofen, ketoprofen,naproxen, fenoprofen, flurbiprofen, tiaprofenic acid, aminoprofen etc. have anti-inflammatory effects. Synthesized compound 2-(4-benzoylphenyl)-2-methyl propionic acid is methylated derivative of propionic acid, and it could be further used for examination of its anti-inflammatory properties, i.e. to examine how it has effects on inflammatory diseases as rheumatoid arthritis.

Conclusion: The most appropriate synthetic way for obtaining molecule of 2-(4-benzoylphenyl)-2-methyl propionic acid has been chosen. In first phase the most important thing is to get acyl ion ($C_6H_5C{\equiv}O^+$), because this is reaction of electrophilic substitution in aromatic chain. Homolytic bromination of methyl group in 4-methyl-benzophenone is made with N-bromosuccinimide. Especially great importance has bromination in allylic position, because double bond remained unchanged. By substitution with cyanide ion the carbon chain extends for one unit and functional group on the end of chain is going to be protected. Synthesis of nitrile is important for their easiness how functional group could be transformed in other much more important groups. As noticed there is (-CH_2CN) functional group at the end of molecule which could be eas-

ily transformed in the other important functional groups, methylation has been performed with two molecules of methyl-iodide. This functional group in hydrolysis reaction in presence of acids is converted in carboxylic group (-COOH).

Key words: propionic acid derivative, synthesis, cyclooxygenase-2 (COX-2), rheumatoid arthritis.

Introduction

Synthesis represents array of reactions in which appropriate compound, as starting material, is transformed into the molecule with desired structure. The first and most basic rule in organic synthesis is the plan. The plan of synthesis should consist of following elements: studying of desired molecule for synthesis, define key intermediary compounds in synthesis, determination of starting compounds and estimation of total yield. Studying of the molecule which is desired to synthesize especially should have consideration about carbon skeleton complexity, nature and disposition of functional groups and finally exact stereochemical relationships in the final molecule. Functional groups protection during synthesis often makes synthesis processes more simple because desired selectivity could be achieved where otherwise couldn't be. Each functional group could be protected with convenient transformation into derivative which is non-reactive under specific experimental conditions. Basic principle for good synthesis is that it could be achieved in as few phases as possible, i.e. the number of operations or reactions for carbon skeleton construction and manage functionality should be as smaller as possible. Criteria of same importance for good synthesis is that chosen reactions should be executed in highest possible yields, because total yield in synthesis reduces itself very much if low yields in particular phases of synthesis are low too. The most characteristic reactions of benzene and the others aromatic hydrocarbons are those where hydrogen atom attached on aromatic core is changed with some electrophilic group. By electrophilic substitutions, benzene acts as nucleophil giving electron couple which make new bonds between carbon atom in aromatic core and electrophilic part. Rheumatoid arthritis is chronic, systemic, progressive connective tissue disease. It is inflammatory disorder primary including synovial joint membrane, with characteristic pain in joint, stiffness, reduced mobility and fatigue. The disease is manifested in different way by particular patients, so results could vary. Rheumatoid arthritis could appear in any age and intensives itself in elderly. It is known that propionic acid derivatives have anti-inflammatory properties as ibuprofen, ketoprofen, naproxen, fenoprofen, flurbiprofen, loxoprofen, tiaprofenic acid etc. but especially methylated propionic acid derivatives have these properties. Since this molecule is methylated propionic acid derivative, it is expected that it has anti-inflammatory properties.

Material and methods

The all used chemicals were purity degree of proanalisy.

Chemicals	Conc. (%)	Density (g/cm^3)
Cyclohexane	99,5	φ=0,78
Ethyl acetate	99,5	φ=0,90
Benzene	99,7	φ=0,78
Diethyl ether	99,7	φ=0,90
Petroleum ether	95,0	φ=0,65
Toluene	99,9	φ=0,87
$AlCl_3$	98,0	φ=1,31
Benzoyl chloride	99,0	φ=1,21
Na_2SO_4-anhydrous	99,0	φ=2,70
Dibenzoyl peroxide	75,0	φ=0,53
CCl_4	99,4	φ=1,48
Ethanol	99,5	φ=0,79
Acetone	99,0	φ=0,79
Acetonitrile	99,5	φ=0,78
KCN	96,0	φ=1,55
Dioxane	99,5	φ=1,03
Thiophene	99,0	φ=1,06
KBr	99,5	φ=2,75
CH_3I	99,0	φ=2,28
NaOH	85,0	φ=2,04
H_2SO_4	96,0	φ=1,84

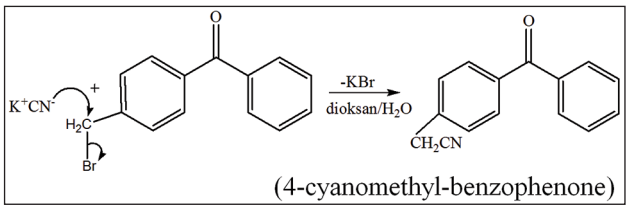
Method

Synthesis of: 2-(4-benzoylphenyl)-2-methyl propionic acid

The first phase in this synthesis is preparing of 4-methyl-benzophenone (compound I). This is an example of Friedel-Crafts reaction of acylation. As catalysts in this reaction there are aluminium trichloride and other Lewis acids. Second phase in synthesis is nucleophilic substitution of bromine ions with cyanide ion. By substitution with cyanide ion the carbon chain is getting longer for one unit and the functional group at the end of chain is protected. During homolytic bromination of methyl group with N-bromosuccinimide the 4-bromomethyl-benzophenone is obtained (compound II). Third phase is nucleophilic substitution of bromine with KCN and 4-cyanomethylbenzophenone is obtained (compound III). Synthesis of this molecule is important primary because of easiness how this functional group (-CN) could be transformed in other more important groups. Fourth phase in synthesis is methylation with methyl-iodide of 4-cyanomethyl-benzo-phenone. As solvent is used tetrahydrofurane. By this reaction,compound 2-(4-benzoyl-phenyl)-2-methylpropionitril (compound IV) is obtained.This compound 2-(4-benzoyl-phenyl)-2-methylpropionitril is purified by colon chromatography with eluent of petroleum ether-ether 6:1. Fifth phase is hydrolysis of compound IV with NaOH, then after acidification the 2-(4-benzoylphenyl)-2-methyl propanoic acid is obtained. (compound V). In this hydrolysis as solvent is used thiophene:ethanol:water (1:20:20). Excreted 2-(4-benzoylphenyl)-2-methyl propanoic acid has been extracted by ether. Ether layer is dried above anhydrous Na_2SO_4, and ether is removed by evaporation under negative pressure. Obtained compound (V) 2-(4-benzoylphenyl)-2-methyl propanoic acid is purified by colon chromatography with eluent benzene-acetone 2:1.

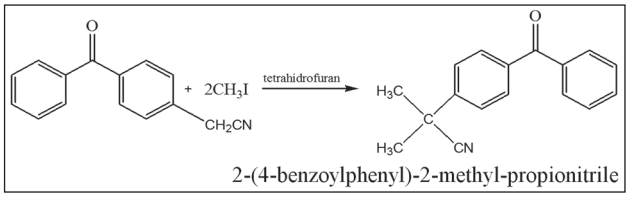
Results

Phase I: Synthesis of 4-methyl-benzophenone. It's obtained 11 grams of 4-methyl–benzophenone, compound (I), m.p. 89.2 ^{0}C. Reaction yield is 40%, calculated on benzoylchloride. Homolytic bromination of methyl group in 4-methylbenzophenone is done with *N-bromosuccinimide. Phase II: It is obtained 5,8 grams of 4-bromomethyl–benzophenone, compound (II), m.p.* 149 ^{0}C. Phase III: It's obtained 10.4 grams of 4-cyanomethyl – benzophenone, compound (III), m.p. 160 ^{0}C.



(4-cyanomethyl-benzophenone)

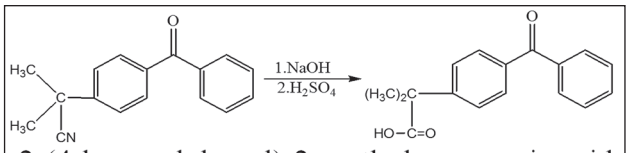
Phase IV: It is obtained 3.1 grams of 2-(4-benzoylphenyl)-2-methyl-propionitrile (IV), m.p. 190.4 ^{0}C.



2-(4-benzoylphenyl)-2-methyl-propionitrile

By hydrolysis of compound IV with NaOH, and later after acidification the 2-(4-benzoylphenyl)-2-methyl propanoic acid is obtained (compound V) with 68% yield. The solvent in this hydrolysis is thiophene:ethanol:water (1:20:20).

Phase V: It is obtained 1.8 gram of compound:



2-(4-benzoylphenyl)-2-methyl propanoic acid.

Discussion

Organic compounds syntheses today have been performed for many reasons. First, synthetic methods give new substances that doesn't found in nature, but having significant chemical, physical and biological properties. Organic syntheses also give opportunities to give new compounds having such properties that with them theoretical predictions, hypotheses and presumptions could to be checked. In the first phase of synthesis propionic acid derivatives the most important is to get an acyl ion ($C_6H_5C{\equiv}O^+$). The first phase in this synthesis is preparation of 4-meth-

ylbenzophenone. Particularly great importance has bromination in alyl position, because double bond remains untouched, thereafter compound 4-bromomethyl-benzophenone is created. By homolytic bromination of methyl group by method over N-bromosuccinimide the compound 4-bromomethyl-benzophenone is obtained. The flow of this synthesis is very difficult to follow because both starting (I) and ending (II) compounds have almost identical retention factors in every used solvents and solvent systems using thin-layer chromatography. By nucleophilic substitution of bromine with KCN compound 4-cyanomethyl-benzophenone is obtained. Nitrile is methylated with methyl-iodide resulting with 2-(4-benzoylphenyl)-2- methylpropionitrile. After hydrolysis of compound IV with NaOH, and after acidification 2-(4-benzoylphenyl)-2-methyl propanoic acid is obtained with 68% yield. Solvent in this hydrolysis is thiophene: ethanol:water (1:20:20). Excreted 2-(4-benzoylphenyl)-2-methyl propanoic acid is extracted with ether. The ether layer has been dried over anhydrous Na_2SO_4, thereafter ether is removed by evaporation under negative pressure. Created 2-(4-benzoylphenyl)-2-methyl propanoic acid is purified by colon chromatography with benzene-acetone 2:1 as eluent. Identification and isolation of molecules in this synthesis has made by thin-layer chromatography and infrared spectroscopy. After analysis of IR spectrum of synthesized molecule [2-(4-benzoylphenyl)-2-methyl propanoic acid], based on appearance of wide bands in intervals from 2500 cm^{-1} to 3600 cm^{-1}, concludes that analyzed compound has carboxylic group (COOH). Also, presence of aromatic vibrations on 692 cm^{-1} and 774 cm^{-1} confirms presence monosubstituted and disubstituted aromatic ring. The spectra have differences in comparison with ketoprofen in the area of carboxylic group absorption. The reason is that by introducing CH_3 group in synthesized compound the intramolecular hydrogen bonds occurs whose make band in mentioned area wider.

Conclusion

In the first phase of synthesis is obtained acyl ion ($C_6H_5C{\equiv}O^+$). To make reaction faster the catalyst $AlCl_3$ is added. Afterward, toluene reacts with acyl ion and as a result is obtained compound 4-methyl-benzophenone. Homolytic bromination of methyl group in 4-methylbenzophenone is done with N-bromosuccinimide. Particularly great importance has bromination in alyl position, because double bond remained unaltered. As a result, the compound 4-bromomethyl-benzophenone is created. Flow of this synthesis is very difficult to study because both starting and ending compound have almost identical retention factors by all used solvents or solvent systems in thin-layer chromatography. By substitution with cyanid ion, the carbon chain is getting longer for one unit and functional group at the end of the chain is secured. That's because the primar halides are suitable backgrounds in such syntheses. After purification of compound using colon chromatography, the yield was 54% in total 10.4 grams of 4-cyanomethyl-benzophenone is obtained. The methylation with two molecules of methyl-iodide is made because at the end of the molecule exists ($-CH_2CN$) functional group, which is easily transformable in the other important functional groups. The 4-cyanomethyl-benzophenone in substitution reaction with methyl-iodide has bonded two methyl groups instead of hydrogen atoms. By this synthesis, the compound 2-(4-benzoylphenyl)-2-methyl proprionitrile is obtained with 92% yield. It is very important to have cyanide group (-CN) at the end of the molecule. This functional group, in presence of acid, is easily transformable in reaction of hydrolysis into carboxylic (-COOH) group. By hydrolysis of compound 2-(4-benzoylphenyl)-2-methyl proprionitrile, and after acidification the 2-(4-benzoylphenyl)-2-methyl propionic acid is obtained.

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Importance of pain estimate with malignancy process in differential diagnosis of Lumbar syndrome

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Abstract

This paper points out to connection between lumbar pain and malignancy. Basic directions have been given in the sense of clinical recognition of malignancy and application of modern therapy. It is especially important to say that large number of patients with lumbar pain goes to quacks, half-educated medical workers and in such a way the malignant process gets masked, and the patient comes to a highly specialized hospital rather late, mostly with progressive metastatic processes. Untimely diagnostics decreases therapeutical success for 10 to 12%.

The research covered the territory of Montenegro. According to experience, hypothesis, aims and work methodology were determined.

The aim of this paper is clearly defined pain analysis and therapeutic possibilities with lumbar syndrome with and without malignant process.

On the basis of analysis of clinical material, the following conclusions have been made:

1. The average age of examinees with malignant illness and lumbar syndrome was 52.75±12.87, and with examinees with lumbar syndrome as a primary illness 44.23±10.67.
2. The pain therapy with lumbar syndrome with malignancy was in the highest per cent applied with the second therapy step (Tramadol, sedatives), and with lumbar syndrome without malignancy, the medicines most frequently used were NSAIL, mio-relaxants and sedatives.

Key words: malignancy process, differential diagnosis, Lumbar syndrome,

Introduction

The lumbar syndrome, as a non-typical diagnosis, has been used as a collective expression of different pathological conditions that lead up to pains in the lumbar part of the spine. Pains are with almost 99% of patients caused by degenerative processes, but also by burdening this region.

The pains caused by neoplasm, infections, trauma and other reasons are very rear. Therefore, each patient who goes to practitioners, or later to a specialist, the first clinical impression and examination of each patient who goes to practitioners or to a specialist, are directed towards degenerative changes and mechanical burdens. (1).

Cancer pain with lumbar syndrome

Main questions with painful lumbar syndrome, caused by metactatic process, are questions asking for therapeutic answers. To answer these questions, it is necessary to view present efforts in explanation of pato-physiology of pain.

Pain is unpleasant sensor or emotional sensation caused by existing or possible tissue damage or that is described with words that correspond to such damage (International Association for Study of Pain, 1979.) (2).

According to the mechanisms of pain formation, it could be:

- nociceptive-activation of nociceptors with some stimuli. It includes somatic pain (activation of nociceptors in the skin and tissues) and visceral (appeared as a consequence of infiltration, compression or extension of visceral organs).
- neuropathic, appeared because of activities in damaged sensor neurons and less modulation through low inhibitory paths.

According to time duration, pain is divided into acute and chronic pain.

Acute pain:
- cause of pain is familiar
- warns the organism of presence of harmful or potentially harmful irritation, in or out the organism
- clearly defined time of appearance
- removing the cause, pain disappears
- present symptoms and signs of increased activity of autonomous nervous system (tachycardia, tachypnoea, sweat, hypertension or hypotension).

Chronic pain:
- the pain that lasts for more than three months
- cause of pain is unfamiliar or impossible to eliminate
- the pain loses its biological warning purpose and becomes an illness by itself
- influences patient's personality, his company, disturbs his activities and stops him from living a normal life
- non-defined time of occurrence
- adjustment of autonomous nervous system and development of chronic vegetative signs, enervation, loss of appetite, insomnia, irritability
- followed by loneliness, insomnia, depression
- treatment includes elimination of pain, but also of neurosis caused by pain duration.

Before we include the pain therapy, it is necessary to have a good anamnesis related to pain, perform physical and neurological examination, and go through all documentation possessed by the patient and find out about earlier therapy for such pains. Each pain has its own dimensions. These are: quality, quantity, chronology, and circumstances of appearance, factors that increase or decrease it and joined manifestations. It is necessary to ask for localization of pain and its spreading.

Treatment of cancer pain

Cancer pain is on of the hardest pains in human organism. Therefore, the World Health Organization suggested basic rules with long-lasting cancer pains in 1986:
- By mouth,
- By the clock,
- By the ladder,
- For the individual,
- Attention to detail.

The basic recommendation of WHO is the ladder plan with cancer pain. In this concept, the pain therapy is adjusted to subjective pain intensity. The aim of such treatment is to relief the pain, so that the patient could have better everyday life. In that way the patient can independently carry out home activities, without everyday visits to his doctor. WHO suggested that with chronic pains the doctor should start with medicines such as Metamizol, Diclofenac or Ibuprofen. If the pain does not reduce with non-opiads, the doctor should take the next step, adding the weakest opiads, as Tramadol or Tilidin. If none of these drugs reduce the pain, the doctor starts with stronger opiads and application of Fenthanyl patches or long-working opioids (morfin remedies). It is especially important that the opioids have effect for longer time, so that the patient needn't take them often. An experienced therapist would start with therapy step II and III as soon as possible and alternatively change from these two groups. Decisive component in therapeutic principle is that the doctor finds a right formula for cancer pains and relieves the patient in early stage of strong pains (2).

Short resume of therapeutic steps with cancer pain (WHO, 1986)

- I step.

Non-opiate analgesics (Diclofenac, Ibuprofen, Metamizol, Acethyl acid) with adjuvant therapy.

- II step

Weak opioids (Tramadol, Talidin/Naloxon, Dihidrocodein) + non-opiads+ adjuvant therapy.

- III step

Strong opioids (Fenthanyl –plaster, Morphin, Buprenorphin) + non-opiatic analgesics + adjuvant therapy.

Adjuvant therapy implies therapy that reduces cancer pain, as opiods often do not give expected analgesic effect. With co-analgesics, corticosteroids are applied. Corticoid medicines influence pain reduction indirectly, by reduction of tumefaction. Corticosteroids are especially used with metastasis on joint structures (fasset syndrome). With metastatic processes on bones, Biphospo-

nats are applied even when the patient does not have strong pains, as prevention of development of more intensive pains. Analgesics often lead up to convulsions, even to epi-crisis, so it is necessary to include antiepileptic-anticonvulsants with their application. Anti depressants cause better mood with the patient and thus reduce pain intensity. Spasmolytics apply with pains of gall bladder or urinary paths (colic). Mio-relaxants are also used as they decrease muscle tonus that intensifies sensation of pain, caused by long lying in bed or stress condition of a patient resulting from his attitude towards final result of disease (3, 4).

With lumbar cancer or non-cancer pain, besides stated medicaments, non-medicament procedures are also used in clinical practice, such as transcutaneous electrical nerve stimulation (TENS), lymph drainage, easy massage and light therapeutic exercise, progressive relaxation by Jacobson, and also warm and cold packs can supplement therapeutic program. With some patients, autogenously training through meditation could also help. With patients who still have pains besides applied therapy, it is necessary to send them to specialized pain clinics (Pain clinic). Owing to modern medicament therapy, a small number of patients need to be treated at pain clinics. Chronic lumbar pain creates magic circle: Pains cause bad body axis, muscles become tenser, muscle hypoxia, accumulation of alopecic inflammatory substances, irritation of receptive pain system and more intensive pain. It is necessary to stop this circle in early stage, as the transfer to chronic pain means that engramic pain records have been formed (5).

It is necessary to emphasize that opioid application in long-time therapy of cancer pain can have the following side effects: Vomiting and vertigo. The doctor has to prescribe antiemetics. These effects usually disappear after two or three weeks. Many patients complain of fatigue and enervation and need for a long sleep. Also, opioids of third degree cause obstipation. The medicaments that speed up peristaltic are often applied in such cases. However, a basic rule with side effects of medicament therapy of cancer pain is that the patient should write them down in his daily notes and inform the doctor of each effect so that he could react on time (6).

Objective

1. Determine appearance of lumbar syndrome with malignancy
2. Analyze characteristics of malignant pain with lumbar syndrome
3. Therapy program of lumbar syndrome (lumbar pain) with malignancy

Method

The research involved two groups of patients:

- I group (basic group) 60 patients (30 men and 30 women), treated at Oncology ward in Podgorica, of lumbar pain.
- II group (control group) 30 patients (15 men and 15 women), treated because of lumbar pain in the Health centre in rheumatologic policlinic in Berane. All of the patients were treated with unique methodology. General characteristics of examinees-gender, occupation, residence etc were analyzed by classical research methods. Clinical analysis of lumbar syndrome was carried out through detailed neurological examination. Evaluation and graduation of pain during the therapy program was carried out by the tenths degree analogue –visual scale. (VAS scale).

Results

Between examinees with malignant illness and lumbar syndrome and examinees only with lumbar syndrome, there is a statistically significant difference in age (t-test; p=0.002). Average age of examinees with malignant illness and lumbar syndrome as supplement syndrome was 52.75$\pm$12.87 years of age and with examinees with lumbar syndrome as a basic illness 44.23$\pm$10.67 years of age.

Average duration of symptoms of lumbar syndrome with examinees with malignancy and with examinees only with lumbar syndrome has not showed a significant statistical difference (Mann Whitney U test; p=0.642). Examinees with malignancy had lumbar syndrome symptoms for 2.68$\pm$2.96 years, and examinees only with lumbar syndrome for 3.85$\pm$4.85 years.

Frequency of some occupations, between examinees with malignant illness and lumbar syn-

Table 1. General characteristics of examinees only with lumbar syndrome and in the group with malignant illness

Observed characteristics		Presence of examined characteristics at examinees with lumbar syndrome Nº (%)	
		Examinees with malignancy and lumbar syndrome	Examinees only with lumbar syndrome
Average age of examinees (X±SD)		52.75±12.87	44.23±10.67
Average duration of illness (X±SD)		2.68±2.96	3.85±4.85
Occupation of examinees Nº (%)	Workers	20 (34.5%)	24 (80%)
	Clerks	26 (44.8%)	6 (20%)
	Unemployed (housewives)	12 (20.7%)	0 (0%)
Course of illness Nº (%)	Sudden	13 (22.4%)	29 (96.7%)
	Graduate	45 (77.6%)	1 (3.3%)
Localization of original pain Nº (%)	Lumbar	13 (44.8%)	28 (96.6%)
	Leg	6 (20.7%)	0 (0%)
	Lumbar and leg	10 (34.5%)	1 (3.4%)
No of relapses Nº (%)	To five	7 (15.6%)	16 (61.5%)
	More	38 (84.4%)	10 (38.5%)
Duration of last relapse Nº (%)	With stops	5 (9.3%)	25 (89.3%)
	Constantly	49 (90.7%)	3 (10.7%)
Sphincter function Nº (%)	Incontinence	8 (13.6%)	0 (0%)
	Relapse	5 (8.5%)	0 (0%)
	Incontinence and relapse	5 (8.5%)	0 (0%)
	Normal function	41 (69.5%)	29 (100%)

drome and examinees only with lumbar syndrome had significant statistical differences (χ2-test; p=0.000). Analyses of this difference showed that:

- Examinees with malignancy were in highest per cent clerks 44.8%, 34.5% of these examinees were some kind of workers, and 20.7% were unemployed and housewives.
- 80% of examinees with lumbar syndrome were workers and 20% clerks.

The course of illness related to lumbar syndrome, between examinees in these two groups showed significant statistical difference (χ2-test; p=0.000). Intergroup analysis showed that:

- 22.4% of examinees with malignancy and lumbar syndrome as a supplement symptom had a sudden start of the illness, while 77.6% of examinees experienced gradual symptoms related to lumbar syndrome.
- Symptoms with examinees that had lumbar syndrome as basic and only illness, appeared suddenly in 96.7%, and only with 3.3% gradually.

Localization of initial pain with patients in examined groups: with malignancy and lumbar syndrome and only with lumbar syndrome showed a significant statistical difference (χ2-tes; p=0.000). Frequency of some localization in examined groups was:

- Examinees with malignancy as a basic illness and lumbar syndrome as a supplement, 44.8% had initial pain in lumbar, 20.7% in legs and 34.5% in lumbar and legs.
- 96.6% of examinees only with lumbar syndrome had initial pain in lumbar, and 3.4% in lumbar and legs.

Frequency of relapses related to symptoms of lumbar syndrome, showed significant statistical difference between examined groups (χ2-test; p=0.000). Intergroup analysis showed that:

- Examinees with malignant illness and lumbar syndrome had more relapses so that 84.4% of them had more than five relapses, and 15.6% five and less.
- With examinees only with lumbar syndrome, 61.5% of examinees had up to five relapses, and 38.5% more than five.

Duration of the last relapse between examined groups of examinees showed significant statistical difference (χ2-test; p=0.000). The analysis of this difference showed that:

- In the group of patients with malignancy, 90.7% of patients had permanently present symptoms of lumbar syndrome, while 9.3% of examinees had relapses with interruptions.
- Examinees only with lumbar syndrome had relapses with interruptions in 89.3%, while only 10.7% stated permanent symptoms of illness.

Frequency of occurrence of disturbances in sphincter function differentiated a great deal between patients with lumbar syndrome as a supplement to their basic malignant illness and patients with lumbar syndrome as a basic illness (χ2-test; p=0.000). The analysis of stated difference showed that:

- 69.5% of examinees with malignant illness had normal sphincter function, 13.6% of them had incontinence and 8.5% retention and incontinence and retention.
- Examinees only with lumbar syndrome experienced no disorders in sphincter function.

Frequency of examinees with antalgic scoliosis left and right, as well as examinees without antalgic scoliosis, showed a significant statistical difference between patients only with lumbar syn-

Table 2. Clinical symptoms of examinees only with lumbar syndrome and in the group with malignant illness

Observed characteristics		**Presence of examined characteristics at examinees with lumbar syndrome N° (%)**	
		Examinees with malignancy and lumbar syndrome	**Examinees only with lumbar syndrome**
Antalgic scoliosis N° (%)	Left	13 (22%)	13 (43.3%)
	Right	26 (44.1%)	16 (53.3%)
	Without antalgic scoliosis	20 (33.9%)	1 (3.3%)
Spinal sensibility N° (%)	Without spinal sensibility	9 (15.3%)	1 (3.3%)
	Only L5	1 (77.6%)	0 (0%)
	Only S1	4 (6.8%)	0 (0%)
	Sensibility of more vertebra	45 (76.3%)	29 (96.7%)
Limitation to flexion/ extension N° (%)	Yes	29 (49.2%)	30 (100%)
	No	30 (50.8%)	0 (0%)
Shober test N° (%)	Positive	45 (76.3%)	30 (100%)
	Negative	14 (23.7%)	0 (0%)
Shober test (X±SD)		1.44±0.65	1.93±0.65
Localization of rigidity N° (%)	Without rigidity	12 (20.7%)	0 (0%)
	Para vertebral thoracic	11 (19%)	0 (0%)
	Para vertebral lumbar	35 (60.3%)	30 (100%)
Appearance of lordosis N° (%)	Yes	14 (24.1%)	15 (51.7%)
	No	44 (75.9%)	14 (48.3%)
Appearance of kyphosis N° (%)	Yes	25 (42.4%)	1 (3.4%)
	No	34 (57.6%)	28 (96.6%)
Lazarevic's sign right N° (%)	Yes	42 (71.2%)	24 (80%)
	No	17 (28.8%)	6 (20%)
Lazarevic's sign right (X±SD)		55.71±8.31	50±10.53
Lazarevic's sign left N° (%)	Yes	42 (71.2%)	24 (80%)
	No	17 (28.8%)	6 (20%)
Lazarevic's sign left (X±SD)		55.48±8.53	50±11.52
Appearance of hypotropia N° (%)	Yes	14 (23.7%)	0 (0%)
	No	45 (76.3%)	30 (100%)

drome and malignancy and lumbar syndrome as consequence of basic illness (χ2-test; p=0.003). Intergroup analysis showed that:

- 33.9% of examinees without antalgic scoliosis were in the group of malignant and lumbar syndrome illness, while 22% of examinees had antalgic scoliosis left and 44.1% antalgic scoliosis right.
- Examinees without antalgic scoliosis in the group of patients only with lumbar syndrome had only 3.3%, while remaining 96.7% of examinees had antalgic scoliosis left in 43.3% of cases, and 53.3% right.

Occurrence of spinal sensibility of one or more vertebra on localization of L1-S1 did not make a significant statistical difference between examined groups of patients with lumbar syndrome as isolated illness and occurrence related to basic malignant illness (χ2-test; p=0.113). The following frequencies of spinal sensibilities were noted in groups:

- In the group of patients with malignancy and lumbar syndrome, 15.3% were without spinal sensibility, 1.7% of patients had spinal sensibility of L5, 6.8% had spinal sensibility only of S1, while the remaining 76.3% had spinal sensibility of more vertebras on localization L1-S1.
- With examinees only with lumbar syndrome, 96.7% of patients had spinal sensibility of more vertebra, and the remaining 3.3%

Frequency of limitations of flexion and extension between groups with malignant illness and lumbar syndrome and examinees only with lumbar syndrome, showed significant statistical difference (χ2-test; p=0.000). The analysis of this difference showed that:

- Half of the cases in the group with lumbar syndrome as supplement at examinees with malignity, or 49.2% had limitations of flexion and extension, while 50.8% did not have limitations of flexion and extension.
- In the group only with lumbar syndrome, limitation of flexion and extension was present with all examinees.

Positive and negative Shober test differed between groups with lumbar syndrome as a basic illness and malignant illness with lumbar syndrome as its consequence (χ2-test; p=0.004). Intergroup analysis found that:

- Positive Shober test in the group with malignancy and lumbar syndrome had 76.3% of patients and 23.7% had negative Shober test.
- All examinees only with lumbar syndrome had positive Shober test.

Values of Shober test between examinees in observed groups showed significant statistical difference (Mann Whitney U test; p=0.003). Higher values of this test were noted only in a group only with lumbar syndrome, the average being 1.93 ± 0.65, while the examinees with malignancy and supplement lumbar syndrome were 1.44 ± 0.65.

Localization of rigidity and its occurence showed significant statistical difference between patients in observed groups: with lumbar syndrome and malignancy and those only with lumbar syndrome (χ2-test; p=0.001). The analysis of frequency of rigidity occurrence, as well as its localization in observed groups showed that:

- Patients from the group with malignancy and lumbar syndrome did not have rigidity in 20.7% of cases, 60.3% of examinees had Para vertebral rigidity, localized in thoracic part and 60.3% in lumbar part.
- All of the patients only with lumbar syndrome and rigidity localized Para vertebral lumbar.

Percentage of examinees with lordosis between aforementioned groups showed significant statistical difference (χ2-test; p=0.01). The analysis of frequency of lordosis occurrence in groups with lumbar syndrome as separate illness and in the frame of the existing malignant illness and the group with lumbar syndrome as separate illness showed that:

- Patients with lumbar syndrome as supplement occurrence of their basic illness did not have lordosis in majority of cases (75.9%), while 24.1% of them had lordosis.
- Lordosis as a symptom of lumbar syndrome occurred more often as a symptom in the group of lumbar syndrome as isolated illness. 51.7% of patients from this group had lordosis, while 48.3% didn't.

Frequency of occurrence of kiphosis between these two analyzed groups of patients showed significant statistical difference (χ2-test; p=0.000). In comparison with occurrence of lordosis, situation related to occurrence of kiphosis showed significant statistical difference, so that frequency of this symptom occurrence was higher in the group with malignancy and lumbar syndrome. Distribution of occurrence frequency of observed syndrome was:

- 42.4% of examinees had kiphosis in the group with malignant illness and consequential lumbar syndrome, and 57.6% did not.
- At examinees with separate lumbar syndrome, kiphosis were noted only with 3.4%, while 96.7% did not have this symptom.

Examinees with positive or negative Lazarevic's sign right and left did not have significant statistical difference between observed groups of patients (χ2-test; p=0.369). Analysis of noted frequencies showed that:

- Patients with lumbar syndrome and malignant illness had positive Lazarevic's sign right or left in 71.2%, and 28.8% had negative.
- Frequency of occurrence of positive Lazarevic's sign right or left with examinees only with lumbar syndrome was somewhat higher, but not statistically significant in relation to the previous group, and it was 80%, while the remaining 20% did not have positive Lazarevic's sign.

Average values of Lazarevic's sign right in degrees between patients in these two observed groups showed significant statistical difference (Mann Whitney U test; p=0.003). Larger values of this parameter were noted at examinees with lumbar syndrome as a consequence of existing malignant illness where Lazarevic's sign was in average 55.71±8.31 degrees, while at examinees only with lumbar syndrome was 50±10.53 degrees.

Significant statistical difference was found in values of Lazarevic's sign in degrees measured left between these two groups of examinees (Mann Whitney U test; p=0.034). Average values of this parameter in the group with malignant illness and lumbar syndrome was 55.48±8.32 degrees, and at examinees only with lumbar syndrome 50±11.42 degrees.

Frequency of occurrence of muscle hypotropia between observed groups showed significant statistical difference (χ2-test; p=0.004). Intergroup analysis showed that:

Table 3. Differentiated estimate of pre-therapy pain in examined groups

Observed characteristics		Presence of observed characteristics at patients with lumbar syndrome N° (%)	
		Examinees with malignancy and lumbar syndrome	Examinees only with lumbar syndrome
Presence of initial lumbar pain N° (%)	Yes	42 (71.2%)	30 (100%)
	No	17 (28.8%)	0 (0%)
Pain intensity N° (%)	Weak pain	14 (23.7%)	0 (0%)
	Medium pain	24 (40.7%)	16 (53.3%)
	Strong pain	21 (35.6%)	14 (46.7%)
Type of pain N° (%)	Clear pain	1 (1.7%)	0 (0%)
	Dull pain	26 (44%)	2 (6.7%)
	Stabbing pain	3 (5.1%)	12 (40%)
	Aching pain	13 (22%)	1 (3.3%)
	As spasm	1 (1.7%)	14 (46.7%)
	Burning pain	23 (42.7%)	1 (3.3%)
VAS scales (X±SD)		5.93±2.61	7.23±1.25
Provoking pain N° (%)	Fatigue	18 (52.9%)	16 (53.3%)
	In rest	4 (11.8%)	0 (0%)
	Constant presence	12 (35.3%)	14 (46.7%)
Pain reduction N° (%)	Analgesics	55 (93.2%)	30 (100%)
	Spontaneously	4 (6.8%)	0 (0%)

- Patients with malignancy and lumbar syndrome had hypotropia of some muscles in 23.7%, while 76.7% was without signs of hypotropia.
- None of the patients only with lumbar syndrome had hypotropia of muscles.

Frequency of occurrence of postural disorder with patients, together with some of the forms of postural disorders, did not show significant statistical difference between observed groups of examinees (χ2-test; p=0.41). Analysis of observed frequency showed that:

- Patients with malignancy and lumbar syndrome had lordosis and scoliosis as postural disorders in 1.7% of cases, and 6.8% kyphosis, while the remaining 89.8% of examinees did not have disorders of this kind.
- 3.3% of patients only with lumbar syndrome had postural disorder – scoliosis, while 96.7% of patients had no postural disorders.

Frequency of examinees with present lumbar pain as initial symptom of existing lumbar syndrome, showed significant statistical difference between groups of patients with malignancy as a basis illness and supplementary lumbar syndrome, and the patients with lumbar syndrome as an only illness (χ2-test; p=0.001). Observed groups showed the following frequency:

- Patients with malignancy and accompanied lumbar syndrome had initial lumbar pain in 71.2% of cases, while the remaining 28.8% of patients had some other initial symptom for existing lumbar syndrome.
- In the group of patients only with lumbar syndrome, all examinees had lumbar syndrome from the very beginning of illness.

Pain intensity between patients with lumbar syndrome and malignancy and patients with lumbar syndrome as the only illness showed significant statistical difference (χ2-test; p=0.015). Intergroup analysis discovered that:

- 23.7% of patients with malignant illness as a basic illness had weak pain, 40.7% had pain of medium intensity and 35.6% of patients had strong pains.
- In the group only with lumbar syndrome, majority of patients, 53.3% stated to have had pain of medium intensity, while the remaining 40.7% described the pain as strong.

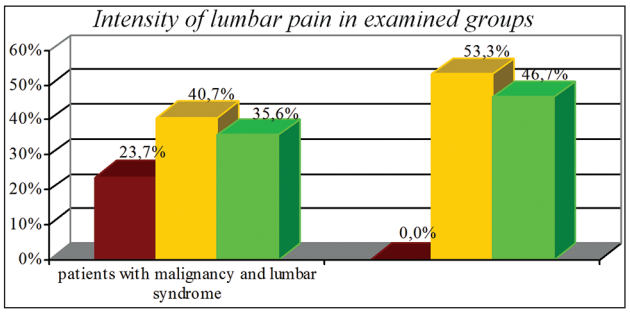

Chart 1. Intensity of lumbar pain in examined groups

Described pain character between patients with lumbar syndrome as a consequence of existing malignant illness and lumbar syndrome as the basic illness, showed significant statistical difference (χ2-test; p=0.000). Analysis discovered that

- 35.6% of patients in the group with malignant illness and lumbar syndrome, described the pain they felt as dull, 30.5% described it as burning, 15.3% as aching, 3.4% described the pain as dull and aching, dull and burning, aching and burning, while in individual cases (per 1.7% of patients) described the pain as clear pain, stabbing pain, as spasm, dull pain and stabbing pain, stabbing and burning pain.
- In the group of patients only with lumbar syndrome, 46.7% of patients described the pain as spasm, 40% of patients described it as stabbing, 6.7% as dull and 3.3% as aching and burning.

Values of VAS scales of pain between examinees with lumbar syndrome as a consequence of existing malignant illness and examinees with lumbar syndrome as basic illness did not show significant statistical difference with first measurement before any kind of therapy (Mann Whitney U test; p=0.055). Average value of this parameter in the group of patients with malignant illness and lumbar syndrome was 5.93$\pm$2.61, while higher values, but not statistically different, were measured in the group of patients only with lumbar syndrome, with average value of 7.23$\pm$1.25.

A way of provoking the pain between observed groups of patients did not show any statistical difference (χ2-test; p=0.133). Analyzing the

frequency of different ways of provoking the pain in the group of patients where lumbar pain accompanied malignant illness and the group of patients only with lumbar syndrome, it was found that:

- Examinees with lumbar syndrome and malignancy stated fatigue as provoking pain factor in majority of cases (52.9%), 35.3% complained of constant presence of pain, while 11.8% of patients had pains during the time of resting.
- 53.3% of examinees only with lumbar syndrome had pain only under strain, while 46.7% had permanent pain.

Medicament therapy that was most efficient in decrease of existing lumbar pain at patients with lumbar syndrome and malignancy and at patients only with lumbar syndrome, showed significant statistical difference (χ2-test; p=0.000). Intergroup analysis discovered that:

- Majority of patients with malignant illness and lumbar syndrome (51.8%) were most satisfied with combination of analgesics and sedatives, 16.1% the most efficient found NSAIL, analgesics and sedatives, per 5.4% of examinees found combination of corticosteroids and analgesics and combination of analgesics and miorelaxants, miorelaxants and sedatives most efficient, as well as taking only anxiolytics-sedatives. Per 3.6% of patients was taking only analgesics, combination of NSAIL and analgesics, corticosteroids, analgesics and miorelaxants, analgesics, miorelaxants and sedatives, and in 1.8% of cases the most efficient medicament therapy for pain reduction was combination of corticosteroids, analgesics and sedatives.
- Patients only with lumbar syndrome mostly used combination of NSAIL and sedatives (36.7%), 20% used only NSAIL, 16.7% used NSAIL sedatives and local infiltrations, 10% was taking NSAIL, corticosteroids, sedatives and local infiltrations, and 3.3% used: corticosteroids and analgesics, corticosteroids, sedatives and local infiltrations and NSAIL, corticosteroids, analgesics and sedatives.

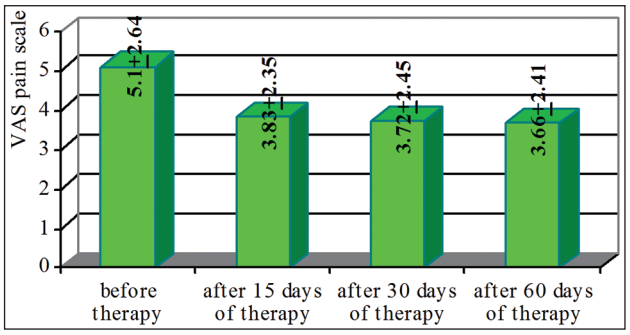

Chart 2. Average values of VAS scales in observed times of measurement in the group of patients with lumbar syndrome and malignancy

At patients with malignancy and lumbar syndrome, pain character did not show significant statistical change after the therapy, but the pain was reduced according to VAS scales (Friedman's test; p=0.000). Analysis of showed difference discovered that the pain was reduced more with longer taking of medicines, so that from the initial medium values of VAS scale of pain that was 5.1±2.64, after fifteen days was reduced to 3.83±2.35. Values of pain estimate calculated with VAS scale after thirty and sixty days remained almost the same to the previous (after fifteen days) and were in average 3.72±2.45 and 3.66±2.41. In the group of patients with lumbar syndrome and malignancy, with male examinees, the pain was not reduced after 15, or 30 or 60 days. The pain did not change intensity, or character, or values of VAS scale.

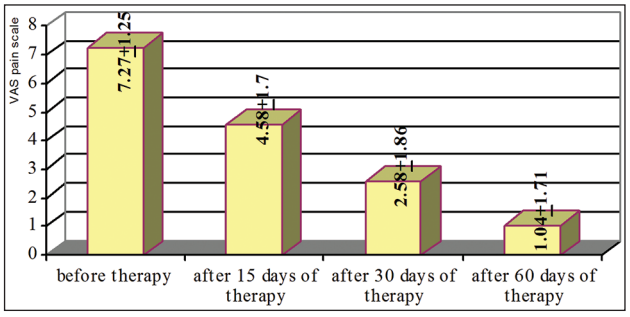

Chart 3. Average values of VAS pain scale in observed times of measurement in the group of patients only with lumbar syndrome

Values of VAS estimates observed before therapy, fifteen, thirty and sixty days after the therapy in the group of patients only with lumbar syndrome showed significant statistical difference (Friedman's test; p=0.000). Average values of VAS pain scale in observed values were: before therapy

7.27±1.25, fifteen days after therapy 4.58±1.7, thirty days after therapy 2.58±1.86 and sixty days after therapy 1.04±1.71. Intergroup analysis significant statistical difference was found in all measured times in values of VAS scale of pain.

Discussion

Between patients with malignant illness and lumbar syndrome and the patients only with lumbar syndrome, significant statistical difference in age was noted (t-test; p=0,002). Average age at patients with malignant illness and lumbar syndrome as accompanying symptom was 52.75 ± 12.87 years of age, and at patients with lumbar syndrome as a basic illness 44.23 ± 10.67 years of age. Occurrence of lumbar syndrome that was not caused by malignant process is, according to other authors, most expressed between 40 to 60 years of age (Jevtić (1984), Chollewicky and McGill, (1996), Richardson and Hamilton (1996) etc) (7, 8).

On the basis of all previous research, lumbar pain starts suddenly. Our examinations confirmed that in the group with lumbar syndrome without malignancy. Sudden start of pain with malignant processes was registered at 22.5% of patients. Group with lumbar syndrome had the sudden start in 96.7% of cases.

Significant difference between the basic and control group is pain duration. In the group of patients with malignancy, 90.7% of examinees had permanently present symptoms of lumbar syndrome. 89.3% of patients had relapses with interruptions, while only10.7% stated permanent symptoms related to illness. Explanation should be looked for in pato-physiological changes in malignant process. Namely, malignant pain is chronic pain. Lesions of tissue activate polymodal nocisensor receptors (C cords). This causes release of tachikinin at ends of peripheral nerve (Substance P and other neuropeptides). Repeated stimulations of mastocytes and other cellular elements of immune system release histamine and serotonin. Electric potentials known as axon potentials occur during time. From polymodal nocisensors through reversible collaterals, comes to spreading of blood vessels and the path that leads up to additional hypoxia and acidosis. This phenomenon has been known as antidromic vasodilatation or axon reflex. (Handwerker, 1999).

Conclusion

According to analysis of clinical sample, the following conclusions have been made:

Average age of patients with malignant illness and lumbar syndrome was 52.75±12.87 years of age, and the patients with lumbar syndrome as basic illness 44.23±10.67 years of age.

Pain therapy with lumbar syndrome with malignancy was mostly carried out with second therapeutic step (Tramadol, sedatives), and with lumbar syndrome without malignancy the most used medicaments were NSAIL, mio-relaxants and sedatives. In the group of examinees with lumbar syndrome and malignancy, with male population, there was no pain relief after therapy, either after 15, 30 or 60 days. The pain changed neither its intensity, nor character, nor values of VAS scale.

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Presence of *Streptococcus Pyogenes* in the etiology of Tonsillopharyngitis among outpatients and antimicrobial testing of the isolates

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Abstract

Tonsillopharyngitis is a common bacterial infection, which is an acute inflammation of the lining of the throat and adenoid tissue of the upper respiratory tract.

A key factor for the emergence of resistance is the inappropriate use of antibiotics.

The goal of this study is to investigate the prevalence of *Streptococcus pyogenes* in the etiology of tonsillopharyngitis and the antibiotic susceptibility/resistance of *Streptococcus pyogenes*.

The study was conducted prospectively during six-month period of 2011/2012 and included a total of 108 positive samples with isolated strains of *Streptococcus beta-haemolyticus* group A (*Streptococcus pyogenes*) tested in a microbiological laboratory of the Institute of Public Health of the Sarajevo Canton.

Most of the streptococci group A caused infections in the age group of 0-9 years (67.6%).

It is necessary to organize the supervision of antimicrobial resistance of *Streptococcus pyogenes* and ensure implementation of guidelines in order to optimize treatment.

Antibiotic resistance of *Streptococcus pyogenes* is a public health problem and a significant clinical problem.

Key words: Tonsillopharyngitis, antibiotic, resistance, Streptococcus pyogenes,.

Introduction

Tonsillopharyngitis is a common bacterial infection, which is an acute inflammation of the lining of the throat and adenoid tissue of the upper respiratory tract. This bacterial infection is caused by *Streptococcus pyogenes*, while other pathogens can be viruses (1).

The disease in case of the infection with *S. pyogenes* develops in the throat or on the skin, before than specific antibodies or competitive bacterial flora can develop. Streptococcal pharyngitis is primarily a disease of children at age from 5 to 15 year, but neonates and adults can also be affected. The bacteria are spread from person to person via droplets. To the spread of infection contributes the places where overpopulation is a big, particularly in winter. Soft tissue infections (pyoderma, erysipelas, cellulitis, fasciitis) usually develop after skin colonization with group A streptococci, after which a microorganism enters the superficial or deep skin tissues after the skin injury (2).

Numerous types of streptococci are pathogens of infections in humans. Differentiation of species within the *Streptococcus* genus is difficult due to the existence of three different schemes, which are used in the classification: based on serological properties (Lancefield groups A-W), hemolytic properties on blood agar (beta β-hemolytic completely hemolyzing, alpha- α hemolytic incompletely hemolyzing blood agar and streptococci without hemolytic features) and on the basis of biochemical (physiological) traits.

Rebecca Lancefield in 1933 established a serological classification scheme to differentiate beta-hemolytic strains. Most beta-hemolytic strains, as well as some alpha-hemolytic non hemolytic streptococci a have group specific antigens, most of which are carbohydrates. These antigens can be used for the rapid identification of streptococci by immunological tests. That is *Streptococcus pyogenes* (group A, at Lancefield scheme) is responsible for the emergence of streptococcal pharyngitis with group antigen that can be isolated directly from throat swab by rapid immunoassay. Today the Lancefield scheme is used only for few species

of streptococci (groups A, B, C, F and G). Most alpha-hemolytic and gamma-hemolytic streptococci (but not all) do not have group-specific antigens of cell walls. They are identified by biochemical tests. Classification schemes are not mutually exclusive, which further complicates the classification. Thus, members of the *Streptococcus anginosus* group can have all three hemolytic forms, can be negative in the reaction with group antisera, or may react with antisera for all groups A, C, F, or G. Similarly, *Streptococcus agalactiae* (Lancefield group B) is common beta-hemolytic but can develop without significant hemolysis (1,2,3).

Susceptibility testing of bacteria to antibiotics and chemotherapeutics is referred to as sensitivity tests. Bacterial susceptibility to antimicrobial agents can be expressed qualitatively as S-sensitive,-R - resistant and I-low sensitive or quantitative (MIC and MBC).

A key factor for the emergence of resistance is the inappropriate use of antibiotics.

An important component of any program to reduce resistance is education of health care professionals (indicated prescriptions) and patients (appropriate use of the drug). Supervision of bacterial resistance in hospitals and in the wider community is an important goal in the fight to prevent the spread of antibiotic resistance, because in addition to epidemiological monitoring it also enables effective empiric therapy.

Disk diffusion method (Kirby-Bauer principle) is a qualitative method. It is based on the principle of diffusion of antibiotics into the environment of disk or tablet and inhibition of growth of bacteria sown. The principle of the test is based on the preparation of Mueller-Hinton or other substrates in order of their seeding. The substrate is then stitched with previously prepared suspension of standard bacterial concentration (McFarland method), to which is then applied discs or tablets soaked with standardized amounts of the examined antibiotics, according to the custom and pre-agreed pattern. Then, the prepared substrate undergo incubation period of 18h at 35-37 °C and after that the reading and interpretation of the findings. The finding is interpreted for the zone of inhibition of bacterial growth within the zone of disks or tablets. Diameter of the zone of inhibition of bacterial growth is measured in millimeters and then compared with known values of standard bacterial strains. If all conditions are standardized (inoculum size, substrate, temperature, time of incubation, the pH of the substrate), the resulting diameter or zones of bacterial growth inhibition for each antibiotic is interpreted as R-resistant (not suitable for therapy), S-susceptible (suitable for therapy) and I-low sensitive (intermediate, suitable for therapy at the site of secretion). Diffusion method has its limitations and benefits. It's simple, quick and with results that are interpretable (4,5)

Goal

The goal of this study is to investigate the prevalence of *Streptococcus pyogenes* in the etiology of tonsillopharyngitis and the antibiotic susceptibility/resistance of *Streptococcus pyogenes* using a specific antimicrobial disk diffusion test.

Material and methods

The study was conducted prospectively during six-month period of 2011/2012 and included a total of 108 positive samples with isolated strains of *Streptococcus beta-haemolyticus* group A (*Streptococcus pyogenes*) tested in a microbiological laboratory of the Institute of Public Health of the Sarajevo Canton.

Isolation and identification of *Streptococcus beta-haemolyticus* group A was performed using standard microbiological methods.

Swab samples are properly seeded on blood agar and incubated for 24-48h in an atmosphere of CO_2 (the pot with a candle) at 37 ºC.

Processing of samples was made in the primary and secondary testing. *Streptococcus pyogenes* was identified based on colony morphology, beta-hemolysis, bacitracin test and commercial slide agglutination test using Slidex BioMerieux kits for determination of group A.

Data collection was performed by examining the patient's medical records.

For statistical analysis, we used the statistical software package SPSS 16.0. (Version 16.0, SPSS Inc., Chicago, Illinois, USA).

Results

The presence of *Streptococcus pyogenes* in the throat swab culture of outpatients was verified in 50.0% of males and 50.0% of female respondents.

Table 1. Gender structure of the respondents

Gender	No. of respondents	Relative frequency (%)
Female	54	50.0%
Male	54	50.0%
Total	108	100.0%

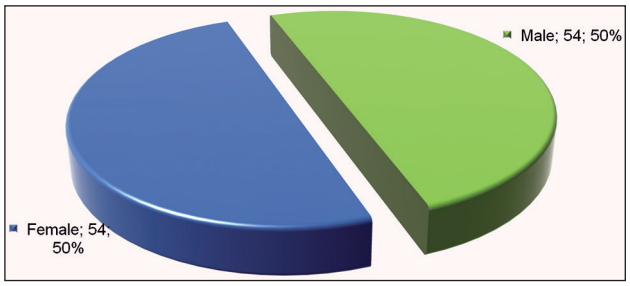

Figure 1. Gender structure of the respondents

Of the total number (n = 108), the largest number of respondents, 73 (67.6%) belonged to age group of 0-9 years. Ninety-nine patients (91.7%) were at the age up to 20 years, while the remaining nine patients (8.3%) with age between 20-60 years. Median age was 8 years and half of the patients were 8 or less than 8 years old, while the other half has 8 or more than 8 years of age. The value of the first quartile (Qil_1) is six years, respectively, quarter, or 25% of respondents have 6 or less than 6 years of age, while the value of the third quartile (Qil_3) is ten years, that is, three quarters of the respondents, or 75% of respondents have 10 or less than 10 years of age, while one fourth, or 25% of respondents have 10 or more than 10 years. Interquartile range (IQR) is four years. The youngest respondent is 3 years old, while the oldest respondent is 57 years old (6).

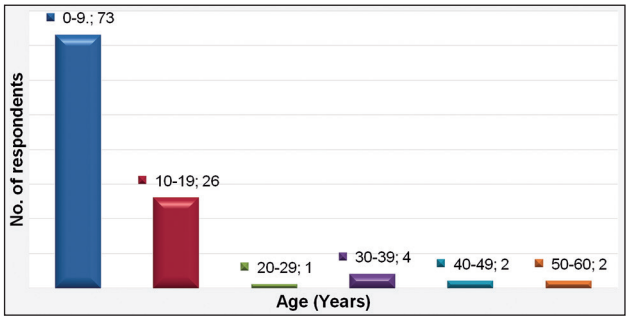

Figure 2. The age structure of the respondents

In the group of female respondents (n=54), the youngest respondent has 4.0 years, while the oldest was 57.0 years old, while in the group of 54 male participants, the youngest respondent has 3.0 years, while the oldest was 56.0 years old.

There was a statistically significant difference in median age between male and female respondents (*p=0.01*).

Of the total number of female respondents (*n=108*), isolated in clean culture from the respondents throat in 13 (12.0%) samples and in larger number in 67 (62.1%) samples, while in a smaller number in 28 (25.9%) samples.

Table 2. The age structure of the respondents

Age	No. of respondents	Relative frequency (%)	Cumulative frequency	Cumulative relative frequency
0-9	73	67.6%	73	67.6%
10-19	26	24.1%	99	91.7%
20-29	1	0.9%	100	92.6%
30-39	4	3.7%	104	96.3%
40-49	2	1.9%	106	98.2%
50-60	2	1.9%	108	100.0%
Total	108	100.0%		

Table 3. The age of respondents by gender

Gender	n	Median	Interquartile range	Z	p-value*
Female	54	8.0	4.0	- 2.584	0.01
Male	54	7.0	5.0		

**Mann-Whitney Rank Sum Test*

Table 4. Sensitivity of Streptococcus pyogenes on tested antimicrobial medications

Antibiotic	Sensitivity		Resistance		Total	
	n	%	n	%	n	%
Penicillin	108	100.0%	0	0.0%	108	100.0%
Cefuroxime	108	100.0%	0	0.0%	108	100.0%
Erythromycin	78	72.2%	30	27.8%	108	100.0%
Klindamicin	83	76,9%	23	21,3%	108	100,0%
Klaritromicin	78	72,2%	28	25,9%	108	100,0%
Ceftriaxone	108	100.0%	0	0.0%	108	100.0%

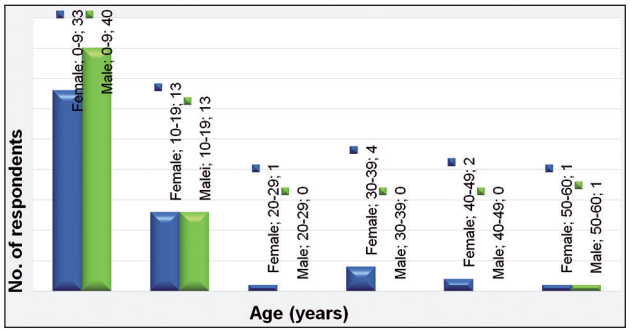

Figure 3. The age of respondents by gender

Of the total sample (n=108), in 78 (72.2%) samples with isolated *Streptococcus pyogenes* is proven antibiotic sensitivity to Erythromycin(27.8%), Klindamicin (21,3%), and Klaritromicin (25,9%) samples/cases appeared antibiotic sensitivity of Streptococcus pyogenes isolates resistance to Erythromycin, Klindamicin and Klaritromicin.

Table 5. Number of patients (Streptococcal pharyngitis) in Federation of Bosnia and Herzegovina

Year	No. of patients	Rank	Mb
2007	1054	6	45.28
2008	1129	5	48.51
2009	1220	4	52.42
2010	889	5	38.03
2011	843	7	36.05

Rank: Rank among top 10 leading contagious diseases in FB&H
Mb: Morbidity per 100000 inhabitants

Table 6. Number of patients (Streptococcal pharyngitis) in the Sarajevo Canton

Year	No. of patients	Rank	Mb
2007	426	5	101.66
2008	535	5	126.99
2009	614	4	144.93
2010	431	6	98.72
2011	499	6	113.73

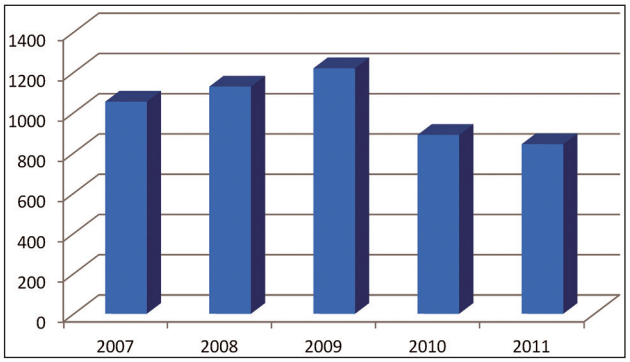

Figure 4. Number of patients (Streptococcal pharyngitis) in Federation of Bosnia and Herzegovina

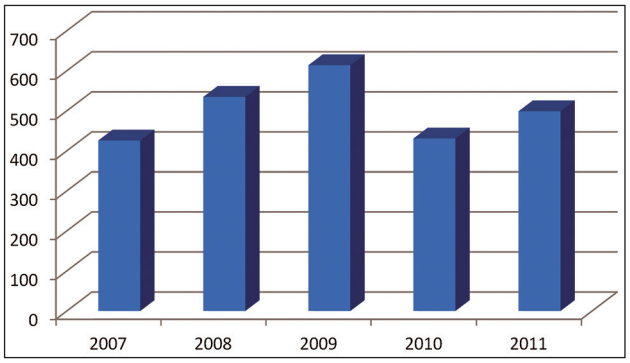

Figure 5. Number of patients (Streptococcal pharyngitis) in the Sarajevo Canton

Discussion

It is known that upper respiratory tract infections, especially in the pediatric population, represent the most common reason for visiting a doctor (1)

More and more is clearer the fact that the problem with unnecessary and inappropriate prescribing of antibiotics need to be directed towards further study of mutual relations between doctors-prescriber and patients-users, to enhance continuing education knowledge and skills on antimicrobial treatment, along with training in communication skills through specially designed programs, as

is being implemented in some countries. Therefore, it is necessary to introduce a national program for control of resistance and gaining insight into the resistance of *Streptococcus pyogenes*.

Sore throat is a disease that almost in all cases is self-healed. Symptoms disappear after 3 days in 40% of patients and after 7 days in 85% of patients. Medications of choice are penicillin antibiotics. Due to the favorable pharmacokinetics of amoxicillin the same can be used as an alternative medicine, while in case of allergy to penicillin newer macrolides can be used, noting the relatively high resistance of BHS-A to macrolides (7).

Cephalosporin's can be used in case of penicillin allergy with caution as an alternative drug, although new meta-analysis shows that cephalosporin's are superior to penicillin, experts prefer penicillin due to the rationality, as the impact of the increasing resistance and because of the cost (8).

Duration of therapy is usually 10 days for the eradication of streptococci from the pharynx and the prevention of rheumatic fever. Numerous studies show that a shorter duration of therapy is acceptable because of the good correlation between the microbiological findings and clinical recovery.

Empirical use of antibiotics is recommended in the following cases: in the presence of significant systemic symptoms associated with sore throat, unilateral peritonsillitis, information that a person has had rheumatic fever, in individuals with an increased risk of acute infection (diabetics and immunodeficient persons) (10). In streptococcal sore throat antibiotics can help prevent the spread of disease in a closed collective, but with this goal are not indicated for use in the general population. Tonsillectomy is highly recommended for recurrent tonsillitis and if it meets all of the following criteria: if a child has five or more inflamed tonsils per year, if symptoms are present for at least one year and episodes of sore throat affect the quality of life of the child (9,10).

More and more is clearer the fact that the problem with unnecessary and inappropriate prescribing of antibiotics need to be directed towards further study of mutual relations between doctors prescriber and patient users in order to enhance by continuing education the knowledge and skills on antimicrobial treatment, along with training in communication skills through specially designed programs, as is being implemented in some countries. (3,11)

Therefore, it is necessary to introduce a national program for control of resistance and gaining insight into the resistance of *Streptococcus pyogenes* (6,12).

Conclusions

The presence of *Streptococcus pyogenes* in the throat swab samples of outpatients were found in 50.0% of males and 50.0% of female respondents.

Most of the streptococci group A caused infections in the age group of 0-9 years (67.6%).

It is necessary to organize the supervision of antimicrobial resistance of *Streptococcus pyogenes* and ensure implementation of guidelines in order to optimize treatment.

Antibiotic resistance of *Streptococcus pyogenes* is a public health problem and a significant clinical problem.

The results of our study confirm the importance of knowledge of antimicrobial susceptibility in assisting clinicians in the selection of medications for empirical antimicrobial therapy.(1,6)

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Proposal for applying the optimal triangulation method in 3D medical image processing and software solution based on *Java Net Beans* environments

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Abstract

One of the most important computational geometry algorithms is a triangulation of the polygon. This algorithm is applied in computer graphics in the process of obtaining a three-dimensional view of objects. The method of 3D display objects finds its application in medicine. In order to efficiently archiving and transmission of medical images are developed special algorithms which are more suitable to the practical needs in relation to existing standards. This paper presents a method for efficiently finding optimal triangulation as well as for storing data that defines the most realistic 3D representation of objects. The main emphasis is on the speed of generating a 3D display of medical images as well as saving storage space when storing them. Storage method is based on the idea that the data in the calculation are presented in the form of the matrix. The goal of the method is that it is not calculated (and stored) data separately for each triangulation, but it works only once. The method is implemented in the *Java Net Beans* environment in the form of copyright software solutions (OT-3DMIP: *Optimal Triangulation for 3D medical image processing*) that for the storage of triangulation weights uses Java services to work with databases.

Key words: 3D medical image processing, Optimal Triangulation, Java Net Beans, Java services for database.

1. Introduction

Polygon triangulation is a process that is very important in computer graphics. Triangulation allows us that from a set of points get a display of three-dimensional objects and provides a mechanism for so-called „glazing“ of three-dimensional figures. This procedure is very important for speed, quality and resolution of the objects. Polygon triangulation has many applications in computer graphics and is used in the pre-trial phase of non-trivial operations of simple polygons. Triangulation of convex polygons is a topical issue which appears also in a two-dimensional computational geometry. Polygon triangulation is widely used in the modeling of 3D objects.

One of the good software solution for triangulation is the optional Final Surface plug-in Triangulation[1] that offers the ability to generate triangle meshes from point clouds (for example 3D scan datasets). This feature allows you to reconstruct the surfaces of scanned objects. For the 3D triangulation methods point normals are required to be present in the input dataset.

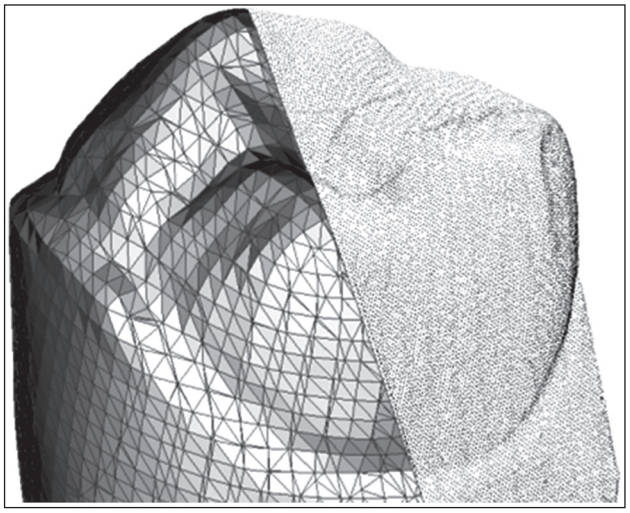

Figure 1. The optional Final Surface plug-in Triangulation

1 http://www.final-surface.com/triangulation.php

Polygon triangulation also finds its application in geo - information systems, in the process of digital terrain modeling, navigation systems and in medicine.

The image segmentation plays an important role in medical image processing. Many segmentation algorithms exist. Most of them produce raster data which is not suitable for further 3D geometrical modeling of tissues. Paper (1) presents a new approach for brain surface matching by determining the correspondence of 3D point sets between pairs of surfaces. The algorithm is based on shape using a combination of geodesic distance and surface curvature. Authors of paper presents brain surface reconstruction:

a. **First left**: initial points and triangulation (69 points and 134 triangles);
b. **First right and second left**: generated surface points and triangulation after 1st, 2nd, 3rd, and 4th iterations by their hierarchical approach (with number of points/triangles, respectively: 270/536 and 1,074 / 2,144).
c. **Second right**: the original surface (164,477 points and 329,816 triangles).

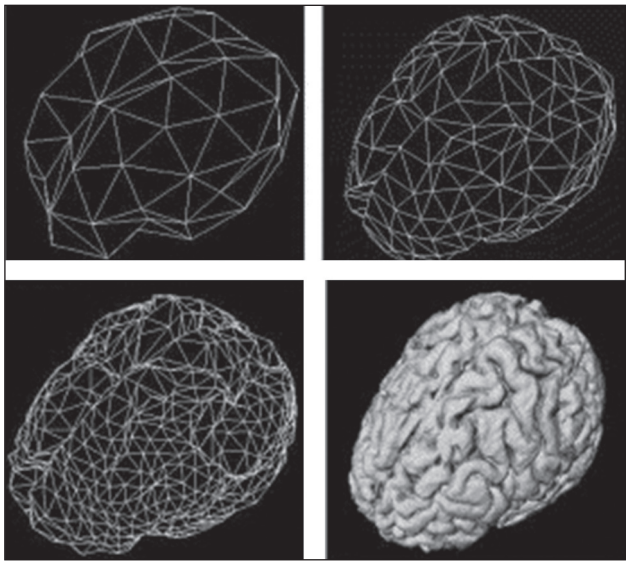

Figure 2. Brain surface reconstruction: testing results from paper (1)

A 3D scanner is a device that analyzes a real-world object or environment to collect data on its shape and possibly its appearance. The collected data can then be used to construct digital, 3D models. Figure 3 presents principle of a laser triangulation sensor.

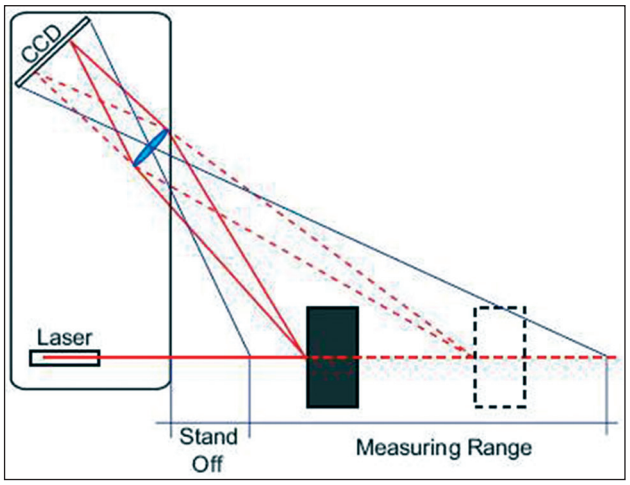

Figure 3. Laser triangulation sensor

The triangulation 3D laser scanners are also active scanner that use laser light to probe the environment (2). With respect to time-of-flight 3D laser scanner the triangulation laser shines a laser on the subject and exploits a camera to look for the location of the laser dot. Depending on how far away the laser strikes a surface, the laser dot appears at different places in the camera's field of view. This technique is called triangulation because the laser dot, the camera and the laser emitter form a triangle (3).

2. Related works

In the following are some similar works that deal with the application of triangulation in the field of 3D medical image processing.

Authors of the paper (4) presents a triangulation method which has been applied in the duplex ultrasound scanner to quantify blood flow velocities in two dimensions. Paper (5) presents numerous techniques for obtaining surface triangulations from 3D image data. Some applications require efficient triangulations, i.e. the source data should be represented as accurately as possible with a minimum number of triangles. Presented method is based on an existing algorithm, that adapts triangulation density to local surface curvature, which is useful in clinical practice.

Authors of the paper (6) presents a vector segmentation algorithm based on an adaptive Delaunay triangulation. Triangular meshes are used to divide an image into several non-overlapping regions whose characteristics are similar. Novel methods for improving quality of the mesh and its adapta-

tion to the image structure are also presented. The Delaunay triangulation is a triangulation which is equivalent to the nerve of the cells in a Voronoi diagram, i.e., that triangulation of the convex hull of the points in the diagram in which every circumcircle of a triangle is an empty circle (7).

3. The method for finding and storing optimal triangulation

In this part of the paper we present a method for finding optimal triangulations of polygons. The method is based on the storage of vertices (V) and their cost (C) based on the formula (3.2) in the matrix *M*. For triangle $\Delta v_i v_j v_k$ cost (or weight) is calculated as follows:

$$w(\Delta v_i v_j v_k) = |v_i v_j| + |v_j v_k| + |v_k v_i| \quad \text{..... (3.1)}$$

Each side of the polygon (i, j) in a given triangulation of convex polygons, with *k*-vertices can form triangles, and for them the cost are calculated (8):

$$m[i, j] = m[i, k] + m[k, j] + w(\Delta v_i v_j v_k) \quad \text{..... (3.2)}$$

At the beginning, the matrix *M* is formed, which is always quadratic, with dimensions:

$$i \times j \; = \; n \times n$$

where *i* presents rows, *j* columns and *n* is number of vertices of the polygon.

Matrix M is filled on the basis of the Algorithm 1. The Algorithm 1 for finding the optimal convex polygon triangulation consists of three steps.

Algorithm 1. Optimal triangulation

INPUT: *n - number of vertices (coordinates of the vertices are created using the click of mouse)*

Step 1: Completing the matrix

C- cost, V-vertex, i-rows, j-columns, k- vertices between

```
for (i=1; i<=n; i++)
 for (j=1; j<=n; j++)
     j:= i+l
         for (k=i+1; i<=j-1; k++)
          V[i,j]:= k
           calculated cost C[j,i,k]
```

Step 2: Selection in matrix (n-2)-cost, in generating a triangulation

t- triangles that the polygon is divided

```
for (t=1; t<=(n-2); t++)
        Triangle[t]=TriangulationPol[i,j,k]
   Triangle[t]=V[i,j,k]
    Get C[j,i] := cost[t]
```

Step 3: Sum the (n-2)-cost and storage in *Buffer*

sumAllCost[T]+=cost[t];

Storage in Buffer *sumAllCost* for current Triangulation[T]

Find OptTriang= min{sumAllcost[T_1],..,sumAllcost[$T_{c(n-2)}$]}

OUTPUT: *Optimal Triangulation*

For implementation of algorithm we used Java *Net Beans* environment. The main class in our Java software solution `(TriangPolygonOptimal)`, which corresponds to the Algorithm 1 is executed before the class that is responsible to draw all the polygon triangulation. The number of all triangulations of a convex polygon with *n* vertices is closely related with the concept of *Catalan numbers* (9). More precisely, the number of triangulations is equal to:

$$T_n = \frac{1}{n-1}\binom{2n-4}{n-2} = \frac{(2n-4)!}{(n-1)!(n-2)!} \quad \text{..... (3.3)}$$

Fields of the matrix [i,j] where *i=j* determine bisector of the matrix, which diagonally divides matrix into two segments. In one segment are recorded vertices (V) while on the other side of the bisector line for the vertices are recorded costs (C):

$$V[\mathrm{i}\,,\mathrm{j}] = C[\mathrm{j}\,,\mathrm{i}]$$

With the filling vertices it starts from the top to down, from the first row to the *n*-th row. First, at each position [i,j] marks the vertex:

$$k\,(V_1, V_2, ..., V_{n-2}),$$

which is located between these two values.

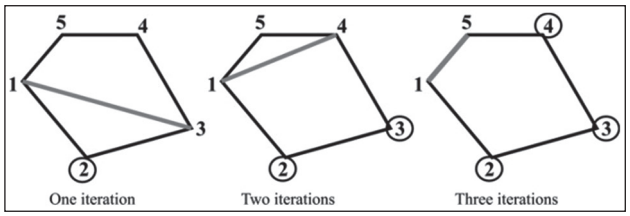

Figure 4. Graphic representation of V_1, V_2 and V_3

From Figure 4., we can see that in the first cycle (V_1), the number of vertices that are located between 1 and 3 is one and that is vertex 2 (Figure 4, left). Then in the next cycle (V_2) vertices that are located between 1 and 4 are 2 and 3 (Figure 4, central), and between 1 and 5 are (V_3) vertices 2, 3 and 4 (Figure 4, right). Number of vertices in the field of the matrix increases until the last row which marks *(n-2)*-values. That number represents the number of vertices which are located between the first and the last vertices.

The number of combinations of values of one field in the matrix depends on the polygon vertices [i,j], and that number is moving in the range of:

$$1 \leq k \leq (n-2)$$

For each obtained combination of the vertices [i,j,k] is recorded the costs on the other side of the bisector based on the formula (3.2) (segments in the matrix are marked with C_1, C_2, .. , C_{n-2}, order of their filling is from left to right, see Figure 6).

The Algorithm 1 for finding an optimal triangulation is used in combination with the Algorithm 2 for finding the internal diagonal of the polygon that is used for drawing the triangulation.

Algorithm 2. Finding all the possible diagonals
- Step 1: Set the counter $i = 1$,
- Step 2: *i-th* point connect with (*i*+2)-*nd* point,
- Step 3: Is the new diagonal internal?
  * Yes: Add it in the list and eliminate (*i*+1) *th* point
  * No: *i*=i+1
- Step 4: If *(i=n)* goto step 5
  * else Return to step 2
- Step 5: Call Algorithm 1

Algorithm 2 in the fifth step calls for Algorithm 1. This algorithm is stated from paper (10) for the sake of completeness, since it is exploited in our implementation. When drawing the triangulation, the combination of internal diagonals which determine *(n-2)*-triangles in the polygon are taken, and it defines one possible triangulation. For each triangulation they are taking a combination of the vertices [i, j, k] which correspond to a given triangle in the observed triangulation. For each triangulation in the internal buffer the (n-2)-costs are summarized.

Example 1: In the following example we demonstrate how the method works on the example of an irregular convex pentagon. Based on formula (3.3), the number of different triangulation of a convex pentagon is 5 (Figure 5).

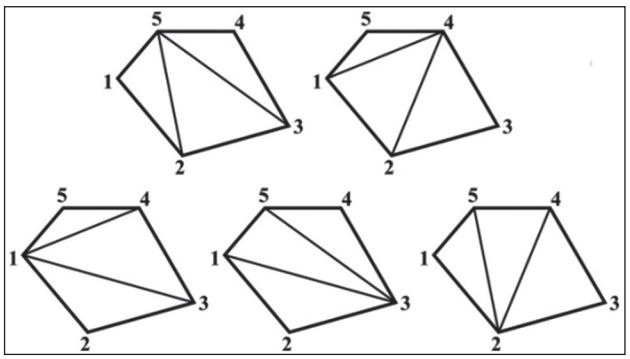

Figure 5. Five triangulation of a pentagon

Through the three steps we will explain how to reach to the optimal triangulation for above stated pentagon.

Step 1: The matrix is filled with the concrete values of *V* and *C* (Algorithm 1, step 1), as shown in Figure 6.

	1	2	3	4	5
1		N	35	42 40	31 43 25
2	N		N	35	38 41
3	2	N		N	36
4	2 3	3	N		N
5	2 3 4	3 4	4	N	

Figure 6. Completing the matrix M (general case)

Mark N presents adjacent vertices which do not have value k (diagonal, which is closest to the bisector S). In the matrix M, first the vertices are recorded (V). In the first column the matrix vertex between 1 and 3 is recorded, then between 1 and 4, and at the end between 1 and 5.

In these cases the number of vertex *k* which is recorded as a temporary value increases (firstly one

vertex {2}, then two {2,3} and at the end three vertices {2,3,4}). That means the number of iterations in the last row of the matrix is always *(n-2)*. Then are recorded the vertices in second column and so on.

For the mentioned values of vertices [i,j,k] the cost values are calculated based on formula (3.2) and its formulation in the Java source code is:

Cost=distance[i,k]+distance[k,j]+Area(i,j,k)

Step 2: The next step is taking a concrete cost from the matrix *M*. In this step the selection of those vertices are performed and *(n-2)*-costs that correspond to the current triangulation for which the cost is calculated. The selection is performed for each triangulation of the pentagon (Algorithm 1, step 2).

In the following tables, the selection procedure is presented for the first two triangulations of a pentagon.

Case 1: The selection in the matrix M for Triangulation T[1]={{1,2,5},{2,3,5},{3,4,5}}

	1	2	3	4	5
1		N	35	42 40	31 43 25
2	N		N	35	38 41
3	2	N		N	36
4	2 3	3	N		N
5	2 3 4	3 4	4	N	

Case 2: The selection in the matrix M for Triangulation T[2]={{1,4,5},{1,2,4},{2,3,4}}

	1	2	3	4	5
1		N	35	42 40	31 43 25
2	N		N	35	38 41
3	2	N		N	36
4	2 3	3	N		N
5	2 3 4	3 4	4	N	

Step 3: For each triangulation *(n-2)*-value of cost is taken from the matrix M, and in that way it obtains its cost:

$$SumAllCost[T_i]=cost_1+cost_2+...+cost_{(Tn)} \quad (3.4)$$

where *i* takes the value from the set $\{1,...,T_n\}$.

In the case of the pentagon, there are 5 such sum for costs. Algorithm 1 works in combination with a class that draws the individual triangulation. For each combination in an internal buffer of application it calculates the total sum of costs based on formula (3.4). After comparing the sum of the costs for all triangulation has taken the smallest one as optimal (Alg. 1, step 3).

$$OptT=\min\{sumAllCost[T_1],...,sumAllCost[T_n]\}$$

In the following example we obtain the optimal triangulation for the given pentagon. That is a triangulation with triangles {1,4,5}, {1,2,4} and {2,3,4}.

For the given values of vertices we get costs that correspond to them 25, 40 and 35. In Figure 7, these values are rounded on the other side of the bisector with combinations of vertices that suit them.

	1	2	3	4	5
1		N	35	(40)	(25)
2	N		N	(35)	38
3	2	N		N	36
4	2	3	N		N
5	4	4	4	N	

Figure 7. Summation of (n-2) values for optimal triangulation

The last step is the formation and drawing the optimal triangulation based on the identified triangles (Figure 8).

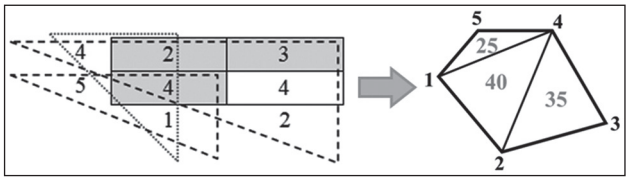

Figure 8. Generation of optimal triangulation

4. Implementation method for optimal triangulation in Java NetBeans environment

Java NetBeans environment refers to the platform for developing applications for the Internet as well as the integrated development environment (IDE) based on the NetBeans platform. *Java* as a programming language can be characterized as: simple, high-performance, object-oriented, multithreading, dynamic, distributed, secure, portable and etc. The advantage of Java is that the most programming languages interpret or compile to run on the computer, while Java itself compiles

and interprets. Java in combination with other tools for modeling (eg UML) gives excellent results when it comes to implementation of computer graphics algorithms (11). Here are some papers about the significance of applying of these advanced environments (and programming languages) in medical imaging and visualization.

Authors of the paper (12) present their experience in developing a Medical Imaging and Visualization Toolkit (implemented in the Java language) that is a set of comprehensive libraries as well as a number of interactive tools. Paper (13) describes the implementation of an interface, which can be used to plug-in and then apply a segmentation method to a medical image series. The design is based on handling each segmentation procedure as an object where all parameters of each object can be specified individually. Authors of the paper (14) deal with image processing and visualization of medical data which has become an essential support for clinical diagnosis and treatment planning. The available open source libraries such as the Insight Registration and Segmentation Toolkit (ITK) and the Visualization Toolkit (VTK) with custom designed user interface can help rapid development of medical imaging applications.

In our case we use NetBeans environment which is composed of modules that are parts of a development environment that can be added. Some of the standard modules of NetBeans environment are:

- *NetBeansProfiler* which allows the optimization of Java programs and faster execution of programs (15),
- NetBeans *JavaScript EdiorModul* provides support for JavaScript, Java Applets and CSS. *GUI design tool* allows the creation of Java graphical applications.

The application **OT-3DMIP** (*Optimal Triangulation for 3D medical image processing*) is developed in Java programming language. Computational geometry algorithms range from simple tests of co-linearity of points, their mutual positions, up to the very complex finding of the smallest convex envelope set of points. To make these algorithms work efficiently, a suitable data structure for the representation of points, segments, set of points and other geometric objects is required (16).

In this section we will mention only some parts of the source code that are responsible for drawing the triangulations of polygon (17). The method `createTriangulation` generates triangulations via 4 nested loops (corresponds to the first step in the Algorithm 1). These loops provide iterations in which all combinations are drawn in the specified order.

```
for(n = 1; n <= order ; n++ ){
for(i = 0; i < n ; i++ ) {
for(j = 0; j < pObject[i].size(); j++ ){
for(k = 0; k<pObject[n-i-1].size(); k++
){
pObject[n].addElement(
newNodes((KListD)pObject[i].
elementAt(j),(KListD)pObject[n-i-1].
elementAt(k) ));
      }
   }
}
```

That means, for generating and drawing the triangulation, are taken the values [i,j,k] and for a given combination it finds the cost in the matrix *M*. This procedure is repeated for each triangle in a given polygon. The number of elements in the instance is equal to the value of the integer *(n-2),* which represents the number of generated triangles for the given *n*-gon:

```
for(i=0; i<=(n-2); i++)
pObject[i]= new Vector();
pObject[0].addElement(new LNodes());
pGraphic = new TriangPolygonOptimal();
```

The object `pGraphic` represents an instance of the class `TriangPolygonOptimal`, the vector `pObject` is the vector that determines the number of instances. `LNodes` indicates the terminal (leaf) node. The aim of this loop is to connect the ending points of generated diagonals and thus to form the corresponding convex polygon. The procedure is repeated until all possible triangulations are drawn on the Panel of the application.

Method *cost()* is responsible for calculation using formula (3.2) for the optimality:

```
double cost(Node t){
double d,cost;
d1=Math.pow(x1-k.x1,2)+Math.pow(y1-k.
y1,2);
Math.sqrt(d1);
```

```
d2=Math.pow(x2-k.x2,2)+Math.pow(y2-k.
y2,2);
Math.sqrt(d2);
cost:=d1+d2+Area(a,b,k);
return(cost);
}
```

5. Experimental results

The application **OT-3DMIP** contains the Toolbar and central panel, which is the place for the graphical representation of the convex polygon triangulations.

The *Toolbar* containing functional buttons that appears on the application's panel immediately after its starting. Toolbar contains the following options:

- Field for selection of number of vertices (n),
- Button for drawing all triangulation,
- Button to display the optimal triangulation.

In the application on the *central panel* first will be drawn all triangulation of convex polygon (Figure 9).

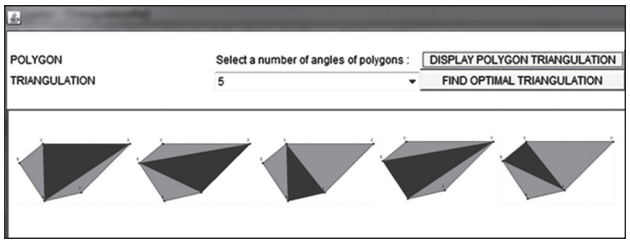

Figure 9. All triangulations of a pentagon in Java

Then clicking on the button "*Find Optimal Triangulation*" in a separate *JPanel*, optimal triangulation is drawn based on recorded values in the matrix (Figure 10).

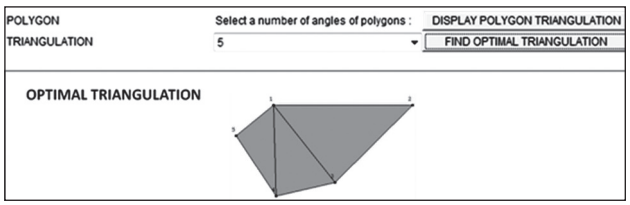

Figure 10. Optimal triangulation of a pentagon

Table 1 presents the testing results of applications for the values n={5,6,...,14}. Time is expressed in seconds and includes time of completing matrix (step 1 in Algorithm 1) and total time of drawing of all triangulation for a given value *n* and finding among them the optimal triangulation (step 2 i 3 in Alg. 1).

Table 1. Execution time for the application

Number of vertices (n)	Number of triangulations	Completing matrix	Total Execution time
5	5	0.6	1.7
6	14	0.9	2.4
7	42	1.1	2.8
8	132	1.6	3.7
9	429	2.3	5.2
10	1430	6.7	14.8
11	4,862	12.8	27.4
12	16,796	21.4	48.7
13	58,786	30.7	64.6
14	208,012	41.5	85.5

** PC performance for testing results: CPU - Intel(R)Core (TM) 2Duo CPU, T7700 ,2.40 GHz, L2 Cache 4 MB, RAM Memory-2 Gb, Graphic - NVIDIA GeForce 8600M GS.*

6. Possibilities of applying the optimal triangulation in medical diagnostic imaging

A key problem in medical computation is reconstruction of shapes (of organs, bones, tumors etc) from lower dimensional information such as CAT scans up to three-dimensional shapes. The CAT scan information is originally one-dimensional, but is transformed into two-dimensional slices by signal processing techniques. However the reconstruction of three-dimensional shapes from slices becomes a more geometric problem, which can be abstracted as that of finding a surface connecting a collection of contour lines or data points. Application of optimal triangulations is presented in project „ *Anatomical modeling research*"[2] (Rick Miranda, Colorado State), where authors give study of finding optimal triangulations for reconstruct surfaces by joining parallel contours.

In our case is presented method for optimal triangulation which can be applied in the process of 3D medical imaging processing. Primarily, this method is effective when it comes to the division of a polygon on a series of triangles, but on the other hand shows good results when it comes to storing data that define the optimal triangulation.

2 http://www.colostate.edu/Depts/Mathematics/profiles/miranda/research_CVA.html

Conclusion

We have presented an algorithm for performing optimal triangulation on three-dimensional image data. In the process of 3D display of medical images are to be achieved the most realistic display of medical images in order to better diagnose the problem. In this case it is achieved by using an optimal triangulation. The importance of present methods can be considered from two aspects. The first aspect is reflected in the speed of finding optimal triangulation, which is based on the advanced capabilities of the Java programming language. The second aspect is reflected in the storage of all possible triangulation of one polygon and savings memory space because it uses only one matrix (or table in the case when working with databases). The significance of the presented method is reflected in the fact that using the recorded values can be a very effective way to find the optimal triangulation, which enables a better reflection of medical images. Given software solution provides good results in terms of execution speed and savings of memory space.

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Determination of caffeine, theophylline and theobromine content in energy drinks from Bosnian markets

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Abstract

Objective: Methylxantines like caffeine (C), theophylline and theobromine that act simultaneously as cardiac stimulants, diuretics and smooth muscle relaxants are incorporated into many cocoa beverages and non-alcoholic energy drinks (ED). Consumption of high levels of C, theophylline and theobromine poses potential health hazards.

Material and Methods: This work has been aimed to assess C, theophylline and theobromine content in 13 different ED samples commercially available from the local market and to modify and use the HPLC with UV/VIS detection, method proposed by Sharma et al., 2005., for the determination of C in ED. HPLC was performed with a gradient mobile phase composed of acetronitrile and 0.1% ortho-phosphoric acid (w/v) in water, and peaks were detected at 210 nm. Degassed and diluted samples were analysed on Lichrospher 100 RP 18e column (250 X 4.0mm, 5μm), at 30^{o}C and 1.0 mLmin^{-1} flow rate.

Results: The C contents in ED varies according to the type of the brand, from 0.093 mg/mL to 0.304 mg/mL. Theophylline and theobromine content were detected in some energy drinks.

Conclusion: Used HPLC method is simple, sensitive and accurate and can be applied for the determination of C, theophylline and theobromine content in all kinds of ED and for fast routine analysis. Results suggest caution in the use of high amounts of ED.

Key words: Caffeine,theophylline,theobromi, energy drinks.

Introduction

Methylxantines like caffeine (C), theophylline and theobromine that act simultaneously as cardiac stimulants, diuretics and smooth muscle relaxants are incorporated into many cocoa beverages and non-alcoholic energy drinks(ED). Caffeine (C), 1,3,7-trimethyl-xanthine is a naturally occuring substance found in the leaves, seeds or fruits of different plants species worldwide (Galasko et al., 1989; Ashihara and Crozier, 2001). Most ED contain also natural products such as guarana, ginseng, and taurine. No reports were identified of negative effects associated with taurine, ginseng, and guarana used in the amounts found in most ED. Commonly reported adverse effects seen with C in the quantities present in most ED are insomnia, nervousness, headache, and tachycardia (Clauson et al., 2003: Iyadurai and Chung, 2007), but consumption of more than 1 g of C can lead to death (Rieselmann et al., 1999). The absence of regulatory oversights has resulted in aggressive marketing toward young people for psychoactive, performance - enhancing and stimulant drug effects. There are increasing reports of C intoxication from ED and it seems likely that problems with C dependence and withdrawal will also increase (Reissig et al., 2009). In a view of the possible potential adverse effects of C, theophylline and theobromine it is important that people are informed about which of the commonly consumed ED contain this substance. The purpose of this work was to assess C, theobromine and theophylline content in 13 different ED samples commercially available from the Bosnian market and to modify and use the HPLC with UV/VIS detection, method proposed by Sharma et al., (2005) for the determination of mentioned substances in ED.

Material and Methods

Energy drinks commercially available from Bosnian marketplaces:

Turbo Forte (DOO FRUCTA TRADE Derventa Bosnia and Herzegovina), Guarana (Knjaz Milos Arandjelovac Serbia), Sinalco Energi's (Sinalco International GmbH Duisburg-Walsum Germany), Burn intense energy (Coca Cola Beverages Austria GmbH Wien Austria), Booster (Nectar Backa Palanka Serbia), Effect® (MBG international premium brands GmbH Paderborn Germany), Red Bull (Red Bull GmbH Fuschl am See Austria),Red Bull-sugarferee (Red Bull GmbH Fuschl am See Austria), Fast energy (Park sistem doo Veternik Serbia), JOOP energy (Turkmali Ankara Turkey), Golden Eagle (Frutex Suva Reka Kosovo), FG Future Generation (Idol Icecek San LTD Istanbul Turkey), Big Bill (NENIKO Gostivar Macedonia).

Reagents and chemicals:

All standards C, theobromine, gallic acid, catechin and theophylline were obtained from Sigma Aldrich, Germany. Acetonitrile, methanol (both HPLC grade) and ortho-phosphoric acid were purchased from Sigma Aldrich, Germany and Carlo Erba Reagents, Italy, respectively. All solvents are degassed and filtered through a 0.45μm Sartorius Minisart® RC25 Regenerated Cellulose (RC) filters (Sartorius GmbH, Germany). Standard stock solutions of C, theobromine, gallic acid, catechin and theophylline were prepared in 70% aqueous methanol and were passed through 0.45μm filter before injecting into the HPLC. Standard curves for all standards were plotted by injecting of standard mixture and peak area responses were obtained. A standard graph was prepared by plotting concentration versus area. All peaks were plotted and integrated using sofware. The area and the retention time of the analyte peaks were compared with those of respective standards.

Sample preparation:

All samples of ED are treated during the 60 seconds in Sonorex ultrasonic bath (Bandelin electronic GmbH, Germany) and diluted. Prepared samples are filtered through 0.45μm Sartorius Minisart® RC25 Regenerated Cellulose (RC) filters (Sartorius GmbH, Germany).

High performance liquid chromatography: instrument and conditions:

A Shimadzu LC10-Avp system, equipped with a dual wavelength UV/VIS detector (Shimadzu, Kyoto, Japan) was used in this study. All the modules were controlled by PC with interface and HPLC System Manager Window based software. HPLC was performed with a gradient mobile phase composed of acetronitrile and 0.1% ortho-phosphoric acid (w/v) in water, and peaks were detected at 210 nm. Used water was prepared by Milli-Q® Ultra-Pure Water System (Millipore, Bedford, MA, USA). Degassed and diluted samples were analysed on Lichrospher 100 RP 18e column (250 X 4.0 mm, 5μm), at 30 ^{O}C column compartment temperature and 1.0 mL min^{-1} flow rate.

Results

The results obtained for caffeine concentration in the different ED are showed in Table 1.

The C content per brand differs than data indicated by the producer.

The results obtained for theophylline and theobromine concentration in the different ED are showed in Table 2.

The theophylline and theobromine content were detected in some ED brand in others were under the detection limit.

Discussion

Routine quality control methods have recently become very important for the determination of beverage quality due to its application in the food industry (Sharma et al., 2005). It is very well known that tea, coffe and different beverages contain C and other methylxantines. It is probably not widely known that allowed concentrations of C realy are the same as on the producers label and is consumption of high amounts of ED safe because of the significant C content, a drug that is not desirable in many health conditions. The C content of a number of beverages was measured using HPLC. Although several methods (Hartley et al., 1984; Muhtadi et al., 1990; Horle et al., 2002; Yus-

Table 1. Concentrations of caffeine in the different ED

Sample	Caffeine concentration (µg/mL)	Caffeine concentration Producer label (µg/mL)
Turbo forte	303.58	250
Guarana	203.15	250
Sinalco energi's	234.22	250
Burn energy	286.60	320
Booster	289.33	320
Effect®	271.11	320
Red Bull	286.43	300
Red Bull sugarfree	277.09	300
Fast energy	199.94	250
Joop energy	117.81	150
Golden Eagle	267.11	320
Future generation energy drink	128.38	150
Big Bill	93.47	120

Table 2. Concentration of theophylline and theobromine in the different ED.

Sample	Theophylline content (µg/mL)	Theobromine content (µg/mL)	Sample	Theophylline content (µg/mL)	Theobromine content (µg/mL)
Turbo forte	4,93	1,85	Red Bull sugarfree	0,90	-
Guarana	-	-	Fast energy	-	-
Sinalco energi's	1,03	-	Joop energy	-	-
Burn energy	-	-	Golden Eagle	-	-
Booster	74,99	-	Future generation energy drink	-	-
Effect®	-	-	Big Bill	-	-
Red Bull	3,38	-			

has, 2002; Mamina and Pershin, 2002; Mumin et al., 2006) have been described, a slightly different method (Sharma et al., 2005) was adopted and used in this study for a fast and simple screening of C in ED. HPLC method for the determination of C, theophylline and theobromine content was chosen because HPLC is the most oftenly used qualitative and quantitative determination and separation method and makes it a valuable separation tool in many scientific fields. HPLC screening of ED and other beverages for C, theophylline and theobromine content and within the framework of forensic examination or chemico-toxicologic investigations could be performed in a short time.

Conclusions

This HPLC method proved to be appropriate for the determination of C, theophylline and theobromine content in energy drinks. Furthermore, this method is simple, sensitive and accurate for the determination of C, theophylline and theobromine and other similar substances in all kinds of energy drinks and for a fast routine analysis. Results suggest caution in the use of high amounts of different ED.

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The reco-mmended, 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, ksdh.

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.

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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)

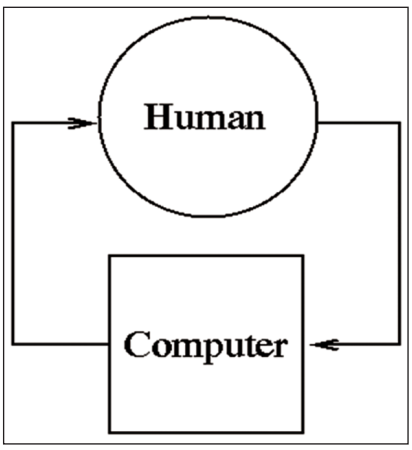

Figure 1. Text here

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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