

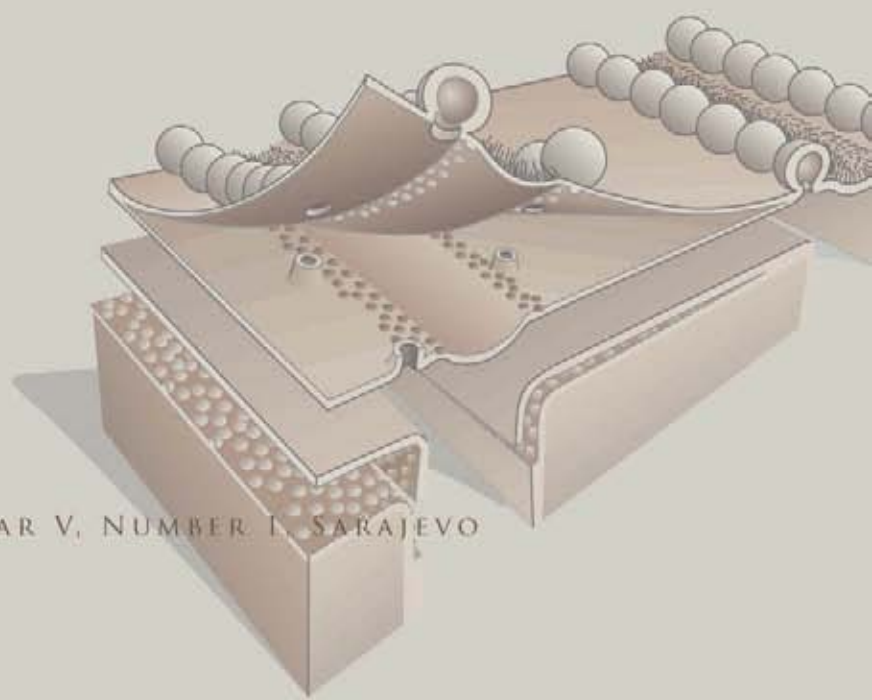
UDRUŽENJE BAZIČNIH MEDICINSKIH ZNANOSTI  
ASSOCIATION OF BASIC MEDICAL SCIENCES



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## EDITORIAL

We gladly inform our readers and collaborators that interest in publication in Bosnian Journal of Basic Medical Sciences permanently grows. Numerous letters and other communication that we receive from our scientists working abroad testify to it. This communication resulted in an idea that our scientists working in the USA, edit one issue of the Journal. The issue would bring the latest achievements in an area interesting for our medical experts.

In this issue, in the usual place in the back of the journal, we bring amended and modified "Instructions for preparation of manuscripts to be published *in extenso* in Bosnian Journal of Basic Medical Sciences". Modifications were made in almost all of the sections of the Instructions. We try to assist the authors in manuscript preparation according to the Instructions. We kindly invite the readers to support our work with their suggestions and recommendations and help us achieve further improvements.


We have already informed You that our colleagues, Diaspora scientists, have expressed interest and responded to our invitation to publish in "Bosnian Journal of Basic Medical Sciences". Considering that a meeting of our Diaspora colleagues is scheduled for the end of summer 2005 the Editorial Board will follow further arrangements and provide one copy of the last issue of Journal, free of charge for all the registered participants. To those willing to explore other means of cooperation we will provide help in exchange of knowledge with our and world scientists. Also, we are willing to organize thematic sessions, round tables and lectures on modern scientific achievements in the world. As we have great confidence in our young researchers who are successful at highly competitive international knowledge market we are prepared to dedicate special section for their individual presentation. The presentation would be made upon Your recommendation and submission of adequate documentation. We assure You that we are proud of all of them. We hope that they will serve as a model and inspiration to the future scientists for whom the knowledge of the world became much more accessible, owing to the development of informatics.

It can be arranged that we introduce a "readers section", should there be interest, where the readers would bring information on the latest achievements in medical science and reports on our countrymen in Diaspora.

Sarajevo, February, 2005

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# CORRELATION OF THE HER-2 PROTEIN EXPRESSION AND OTHER CLINICOPATHOLOGICAL FEATURES OF DUCTAL INFILTRATIVE BREAST CANCER

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## ABSTRACT

The aim of the study was to investigate the relationship between the expression of the HER-2 membrane protein and other clinical-pathological parameters such as: histological size of the tumor; degree of the tumor's differentiation, presence of vascular invasion and presence of metastases in regional lymph nodes, in cases of ductal infiltrative breast cancer. We have investigated 56 cases of ductal infiltrative breast cancer. In all patients a mastectomy with a dissection of axillary lymph nodes has been performed. All tissue samples, taken by biopsy, were embedded in the paraffin, stained by hematoxylin-eosin technique and screened, and evaluation was performed by using a semiquantitative method of the immunohistochemical expression of the HER-2 protein. A decrease of the protein HER-2 expression was noticed in cases of an increase of the tumor's diameter above 50 mm. Increased expression of the HER-2 protein was noticed in cases of moderate (grade II) and poor (grade III) differentiation of carcinoma, as well as in cases where there was no metastases in the regional lymph nodes. No relationship has been observed between the expression of HER-2 and occurrence of vascular invasion. In cases of ductal infiltrative breast cancer the expression of HER-2 protein is in correlation with the size and degree of tumor's differentiation, as well as with the presence of metastases in regional lymph nodes.

**KEY WORDS:** HER-2, immunohistochemistry, breast cancer

## INTRODUCTION

A receptor for the human epidermal growth factor HER-2 is the cell membrane 185-kd oncoprotein coded by the *c-erb-B2* gene located on the 17q12-21.32 chromosome (1). This receptor shows the activity of the tyrosine kinase and has a key role in the regulation of growth, survival and differentiation of lobular epithelial cells (1, 2, 3). Mutation (amplification) of the HER-2 gene leads to an increased expression of this protein (4). Excessive expression of the HER-2 protein causes an increased cell growth and their reproduction, whose end result is the development of cancer. Excessive expression of the HER2 protein on tumor cells' membrane is part of the process of malignant transformation and tumor's progression (5, 6). Activation of this receptor stimulates the path of signal transduction which leads to elevated concentration level of intracellular Calcium and increased potential of plasma membrane, having a mitogenic effect (7). Beside classical clinical-pathological parameters (size of tumor, histological type, a degree of tumor's differentiation, presence of local nodular and distant metastases), an independent prognostic factors such as the status of estrogen and progesterone receptors and expression of HER-2 protein are very important for the prognosis of the breast cancer. Excessive expression of the HER-2 is present in 40-60% of ductal carcinoma in situ cases, 25-30% cases of invasive breast cancer, when its level can be 10-100 times higher compared to the surrounding normal breast tissue (8, 9, 10, 11). Increased HER-2 expression is in the correlation with an increased aggressiveness of the tumor and poorer prognosis (12, 13). In the research of ductal infiltrative breast cancer, beside standard clinical-pathological (TNM) parameters, an immunohistochemical status of the HER-2 protein and its correlation with the above mentioned parameters have been investigated.

## MATERIAL AND METHODS

The study included 56 patients whose age was ranging from 20 to 80 years (mean age 57 years). Due to breast cancer, in all patients a mastectomy with axillary dissection has been performed. The size of the tumor was measured in fresh surgical biopsies. Tissue samples were fixated in 10% neutral formalin, embedded in paraffin and, afterwards, sectioned in 3-5 microns slices, stained with standard hematoxylin-eosin (HE) method and immunohistochemically with Hercep Test (DAKO, K 52049). Histological classification of the tumor was performed according to WHO criteria.

Microscopic study of the tumor included a determination of the following characteristics: size of the tumor (measuring in millimeters two biggest diameters), number of tubular formations (>75%, 10-75%, <10%), number of mitoses in 10 high magnification microscopic fields (<10/hpf, 11-20/hpf, >20/hpf), nuclear pleomorphism (slight, moderate, and severe). Ductal infiltrative breast cancers are graded according to the Bloom-Richardson scheme which, afterwards, has been modified by Elston and Ellis (well differentiated or grade I; moderately differentiated or grade II; poorly differentiated or grade III) (14); presence of vascular or lymphatic invasion and the analysis of the total number of axillary lymph nodes. Tumor's status was determined according to the criteria for determination of breast cancer's status given by the American Joint Committee (AJC) and International Union Against Cancer (IUCC) (15). In each axilla, a minimum of 14 lymph nodes was examined. Immunohistochemical evaluation of the presence and intensity of staining of the HER-2 protein was determined on the base of the score system 0-3+ (0, +, ++, +++). The result was negative (0 or 1+) if there was no staining of the cell membranes at all, if the staining was of poor intensity and/or if the staining was incomplete in less than 10% of tumor's cells. Slightly positive staining (2+) considered slight or moderate staining of the whole cell membrane in more than 10% of tumor's cells. Strongly positive (3+) staining was considered when the whole cell membrane was intensively stained in more than 10% of tumor's cells. Statistical analysis was performed by using a chi-square test. Statistical significance was defined for  $p < 0.005$ .

## RESULTS

Clinical-pathological characteristics of the ductal infiltrative breast cancer in 56 studied cases are shown in Table 1. The majority of patients (96, 4% of cases) were above 41-years-of-age. In the majority of cases (44,6%), cancers were moderately differentiated (grade II), and the majority of these tumors had 20-50 mm (pT2) in diameter with no signs of vascular invasion (60, 7% of cases) and no metastases in axillary lymph nodes (67, 8% of cases). The frequency of appearance of the HER-2 protein in 56 cases of ductal infiltrative breast cancer is presented in Table 2. In 39 cases, HER-2 protein was negative (35.7% = 0 and 33.9% = 1+). HER-2 protein was slightly positive (2+) (Figure 1.) in 8 patients (14.2%) and in 9 patients (16%) was strongly positive (3+) (Figure 2.). Results of the statistical analysis of the relation between



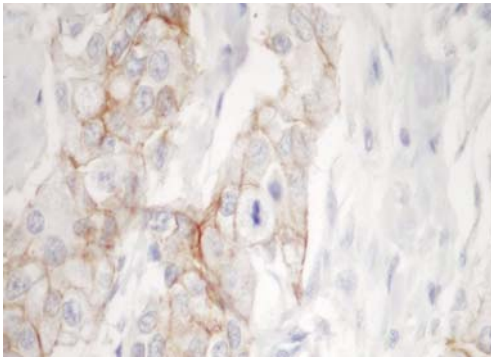


FIGURE 1. More than 90% of the ductal carcinoma cells are stained for HER2, but the staining intensity is moderate. The Hercept Test™ score is 2+. (250X)

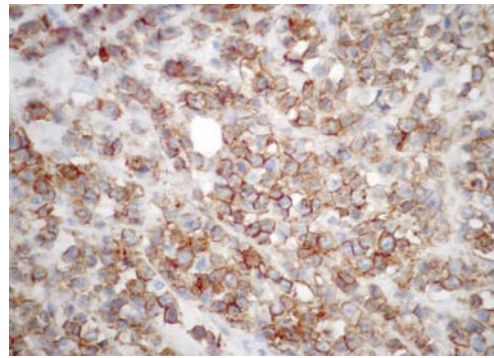


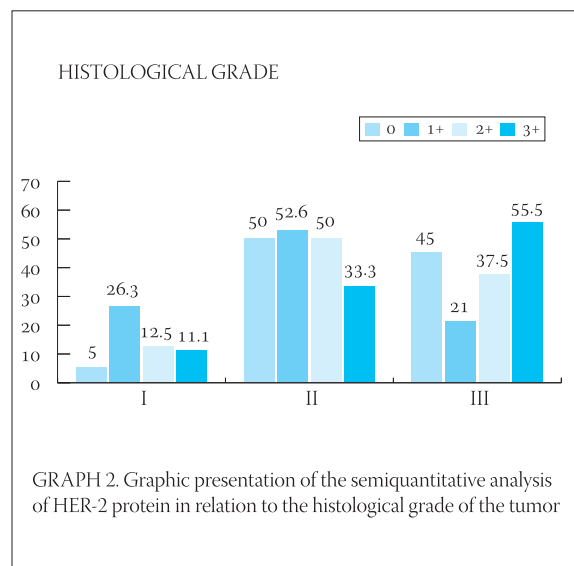
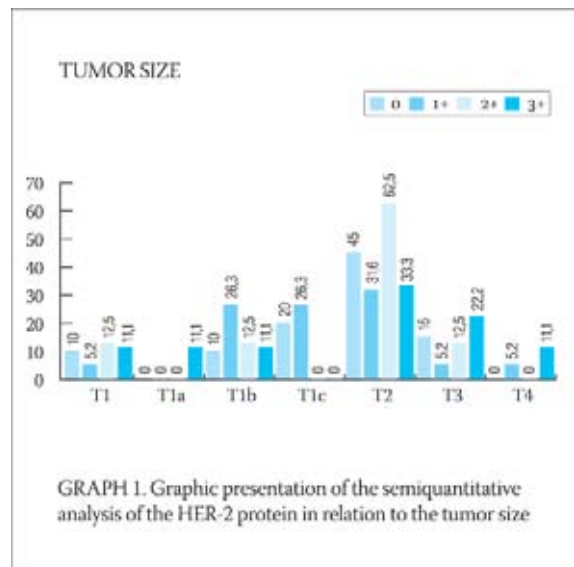
FIGURE 2. More than 90% of the ductal carcinoma cells are strongly stained for HER2 in the entire membrane. The Hercept Test™ score is 3+. (250X)

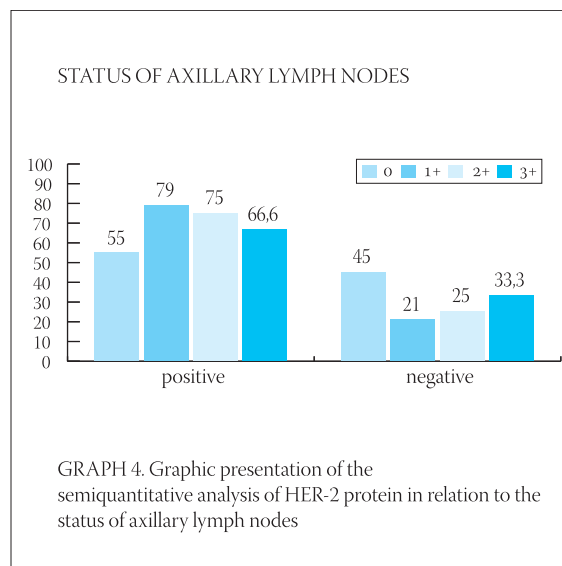
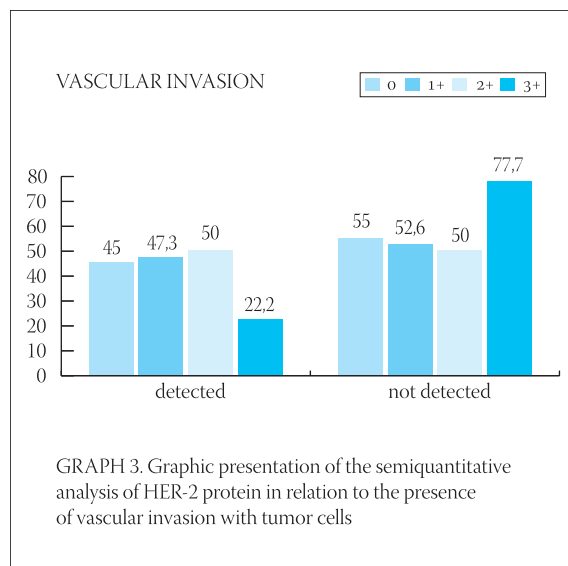
INDEPENDENT FEATURE		No	%
AGE	≤40	2	4
	≥41	54	96
HISTOLOGICAL GRADE OF TUMOR	I	10	17.8
	II	25	44.6
	III	21	37.5
TUMOR SIZE (PT)	pT1	3	45
	pT1a	1	2
	pT1b	8	14
	pT1c	9	16
	pT2	25	45
	pT3	8	14
	pT4	2	4
VASCULAR INVASION	Present	22	39.3
	Not Present	34	60.7
LYMPH NODE STATUS	No metastases	38	67.8
	Metastases	18	32.2

TABLE 1. Frequency of appearance of the HER-2 expression in 56 cases of ductal infiltrative breast cancer

INTENSITY OF THE HER-2 STAINING	No	%
0	20	35.7
1+	19	33.9
2+	8	14.2
3+	9	16

TABLE 2. Frequency of appearance of the HER-2 expression in 56 cases of ductal infiltrative breast cancer





the expression of HER-2 protein and individual characteristics of the tumor are presented in Graphs 1-4. Staining intensity of the HER-2 protein in 63.5% of cancers whose diameter was 2-5 cm (pT2) was of medium intensity (score 2+) and in 33.3% of cases of strong intensity (score 3+). With an increase of tumor's size (>50 mm), a drop in the HER-2 expression was significant ( $p < 0.05$ ). With an increase of the degree of tumor's differentiation (grade II and III), an increase of the HER-2 expression was significant ( $p < 0.05$ ). There was no statistically significant difference in the expression of HER-2 protein in cases with and without signs of vascular invasion. In cases with no metastases in axillary lymph nodes, expression of the HER-2 protein was significantly ( $p < 0.05$ ) higher in comparison to cases with no metastases.

## DISCUSSION

During the investigation of the frequency of the occurrence of an independent prognostic factor HER-2 protein and its relation to the classical clinical-pathological parameters in 56 cases of ductal infiltrative breast cancer, it was noticed that the protein expression is in the correlation with the size and degree of tumor's differentiation, as well as the absence of metastases in regional lymph nodes. Clinical behavior and metastatic potential, even in the cases of same pathological status of the breast cancer, are often very different (16). That is why this search for parameters that would point out more precisely the biological potential of the cancer continues. Some researchers believe that the HER-2 status, beside the nodal and menopausal, is the only significant and independent prognostic parameter of the breast cancer's behavior. Protooncogene HER-2/new (erB-2 or p185 HER-2) which codes the same protein is located on the surface

of the cell membrane and which acts as a receptor for the growth factor, is involved in the malignant transformation of many tissue types – breast, ovaries, stomach, lungs (5, 17, 18, 19). In breast cancer, HER-2 expression is a marker of poor prognosis (4). It is generally accepted that the expression of this oncogene on tumor cells is related to a short-time survival, low levels of estrogen and higher histological grade of tumor (4, 5, 20). It was not found that the increased expression of the c-erb-2 oncogene is related to patient's age, size of tumor, occurrence of positive lymph nodes or histological type of tumor (21). Prognostic influence of the HER-2 presence is smaller in cases of negative axillary lymph nodes, compared to the cases with positive lymph nodes (4). In our research, we noticed that the increased expression of the HER-2 protein is in correlation with the size of the tumor, which is in accordance with researches performed by other authors (22, 23). Statistically significant decrease of the expression of HER-2 protein was noticed when tumor's size exceeded 50 mm and it was most intensive in tumors whose diameter ranged from 20 to 50 mm. Since it is the correlation occurring in early phase of the disease, it is possible that the expression of HER-2 protein represents a step in the process of tumor's evolution. It is possible that the mutation of the HER-2 gene in cases of ductal infiltrative breast cancer is responsible for the increased proliferation of the cell mass only up to a certain point, when other things happen on some other genes. The fall of the expression can be a final act in the process of tumor's progression, when tumor cells cease to be dependent of this protein. It was noticed that, with an increase of cell differentiation, there is a significant increase in the amount of protein on cell's membrane which, too, is in accordance with results of some other researchers


(4). This would present another confirmation of the HER-2's true relation with early genesis of the tumor. Contrary to some quotations from literature which speak about non-existence of the correlation between the occurrence of metastases in axillary lymph nodes and expression of the HER-2 (21), in our study, the occurrence of positive nodes was followed by a decrease in the expression of HER-2 protein, which can be explained by the involvement of some other genetic anomalies that are far more potent in causing the spreading of the cancer. The occurrence of HER-2 in tumors which did not metastasize in the nodes, according to some researches, increases the risk of recurrent disease, compared to cases which showed normal HER-2 expression (6, 24). Abner (25) believes that the significance of positive HER-2 in cases with no nodal metastases is not clear, i.e. it is

controversial. Future investigations will, for sure, put some light on the relation between the HER-2 expression and occurrence, i.e. absence, of nodal metastases. There was no statistically significant relation between the occurrence of vascular invasion and expression of the HER-2 protein. Regulation of the HER-2 expression would be independent from the occurrence of vascular invasion. It can be concluded that, in ductal infiltrative breast cancer, the oncogenic effects of the HER-2 protein are potent and associated with some pathological parameters of poor prognosis. It is possible that there are multiple levels of the control and regulation factors which dictate the occurrence and intensity of the expression of HER-2 protein, which would be the goal for some researches to come since all aspects of progression and spreading of cancer are not completely explained still.

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# HISTOLOGICAL CHANGES OF THE SCIATIC NERVE IN DOGS AFTER INTRANEURAL APPLICATION OF LIDOCAINE – RELATION TO THE ESTABLISHED APPLICATION PRESSURE

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## ABSTRACT

Histological changes of sciatic nerve in adult dogs 7 days after single application of 2% lidocaine (4 ml dose, speed of injection 3 ml/min) and measurement of the application pressure was studied, with a goal to investigate structural changes of the nerve in relation to the established pressure values. The application pressure was determined by using Bio Bench software. In intrafascicular puncture an average application pressure of  $198.23 \pm 52$  kPa was found, and in interfascicular puncture its average value was  $53.3 \pm 17.9$  kPa, with a note that individual differences are regularly present. Seven days after the injection, a nerve dissection was performed and serial sections covering the region of injection's puncture and bordering proximal and distal zones, in the total length of 3 cm, were prepared. The found changes show the presence of nerves' fibers lesions with a strong reactivity of Schwann's cell, as well as the change of interstitial structure concerning hypercellularity and occurrence of cellular extravasation. The covering system of the nerve in the zone of epineurium manifests changes of inflammatory process and in perineurium a decomposition of lamella layers and the alteration of their tinctorial properties were noticed. A comparison of the found nerve reactivities in intra- and interfascicular application showed their one-way alteration, although the lesions were more noticeable in the conditions of intrafascicular application. The damages were mostly expressed in the zone of local application of anesthetic, than distally from it, while the damage to the structure in the proximal part is of the smallest degree.

**KEY WORDS:** intraneural application of local anesthetic, application pressure, histology, dog

## INTRODUCTION

Peripheral nerves are supplied with blood through an internal network of endoneurium blood vessels and an external network of epineurium blood vessels. These two networks anastomose via transperineurial blood vessels. An intact blood-nerve barrier is important for the preservation of internal nerve milieu. Accumulation of the liquid within endoneural space in different pathological conditions results in an increased endoneural pressure, reduction in the blood-nerve flow and increase in the permeability for osmotically active macromolecules. Regional anesthesia represents a significant problem from the point of nerve damage, especially the damage to the blood-nerve barrier. Carriers of the negative effect are: anesthetic itself, needle's diameter, speed of injection, volume of the injected substance and region of application (1, 2, 3, 4, 5). A strong pain that patient feels is a sign that the nerve was targeted with the needle during the performance of regional anesthesia. That is why it is suggested that the nerve blockage is not performed in anesthetized or deeply sedated patients, because they cannot feel the pain during intraneural application after which nerve damage occurs. High pressure during the application of local anesthetic, as a sign that the needle was introduced intraneurally, can have applicable clinical value which could reduce the risk of neurological injuries (6). In spite of their wide use, there are a surprisingly small number of studies dealing with neural toxicity of locally applied anesthetics (7).

## MATERIAL AND METHODS

For this research, we used 9 sexually mature male dogs, of mixed breed, with average body mass of 15 kg and average age 2-3 years. The dogs were kept in a standardized laboratory conditions. Before the experiment was performed, a premedication of animals with acepromazine (0.5 mg/kg, intravenously), atropine (0.04 mg/kg, subcutaneously) and ketamine (5 mg/kg, intramuscularly) was done. In general endotracheal anesthesia (halothane) and aseptic conditions, we surgically approached the nerve between biceps femoris muscle and semitendinosus muscle. We bluntly dissected fascias of those muscles, separated the connections with a retractor and found sciatic nerve. At an angle of 45 degrees, we intraneurally placed a 25-gauge needle in the region of nerve, under direct control of optical instrument. By using an automatic infusion pump (PHD 2000; Harvard Apparatus, Holliston, MA) we applied 4 ml of 2% lidocaine (Bosnalijek, Sarajevo) with the

speed of 3 ml per minute. The achieved pressure during the application was registered by an in-line manometer (PG 5000; PSI – Tronics Technologies Inc, Tulare, CA) connected to a computer by an analogue-digital converter (DAQ 6023; National Instruments, Austin, TX). The manometer was placed proximally from the needle with which it was connected by a non-distensible tubes (high durometer polyvinyl chloride injection tubing; 84" arterial pressure tubing; Abbot Critical Care System, Abbot Laboratories, North Chicago, IL). The data about the pressure were analyzed by using a software package (BioBench version 1.2; National Instruments, Austin, TX). After the application is done, the skin wound was sutured with an "x" stitch of non-absorbable suture. Seven days after the injection, an intravital excision of the nerve from the area of puncture and bordering proximal and distal zones, 3 cm in length, was performed. Nerve samples were fixated in 10% formalin, embedded in paraffin, sectioned in series and stained with HE method. Prepared histological sections were analyzed under the light microscope with installed digital camera. For the evaluation of the results, standard statistical methods were used. Computer based evaluation of the mean value of pressures is presented as the mean value (X), standard deviation (SD) and standard error of the mean value (SEM). For investigation of significant differences between intrafascicular and extrafascicular group we used the t-test. *P-value* ( $p < 0.05$ ) was significant.

## RESULTS

Calculated mean values of the application pressure in *intrafascicular* application were ( $198.23 \pm 52$  kPa) and in extrafascicular application ( $53.3 \pm 17.9$  kPa). At the time of lidocaine application in all nerve samples spindle-shape edemas were noticed in the puncture area and in neighboring proximal and distal zones. In the area of application in intrafascicular injections, changes in the histological structure were noticed, including: *Perineurium* showed division of lamellas with its significant disintegration at the place of puncture and the loss of demarcation toward the surrounding perifascicular connective tissue and closest nerve fibers. Blood vessels incorporated in its structure and its surroundings are more noticeable but there are no signs of hyperemia and extravasation (Figure 1). Nerve fibers of the fasciculus into which the anesthetic was directly injected showed damages in general, while subperineural ones had more intensive changes of wide range

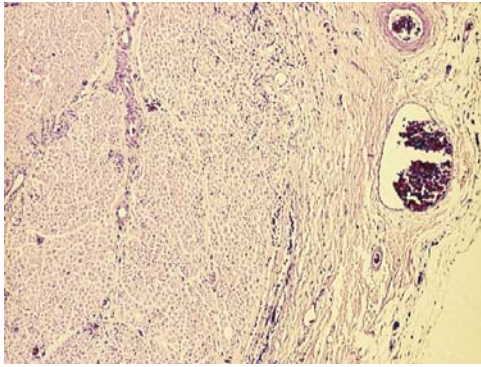


FIGURE 1. Intrafascicular application. HE, x 40.

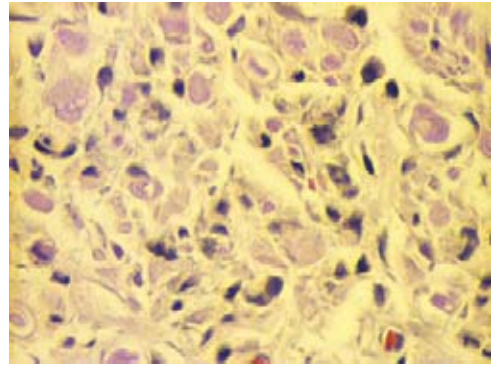


FIGURE 2. Intrafascicular application. HE, x 400.

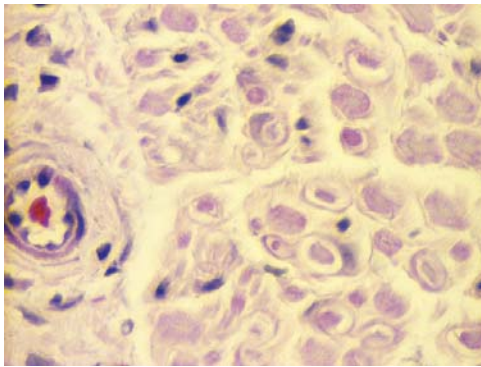


FIGURE 3. Intrafascicular application. HE, x 400.

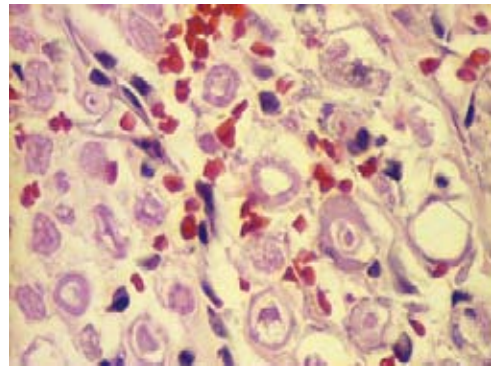


FIGURE 4. Intrafascicular application. HE, x 400.

compared to the central ones. Myelin fibers were disarranged in the space and of increased volume. Some of the axons of these fibers were dislocated and hyperacidophile. In some of them, an advanced axolysis up to the degree of complete disintegration was noticed. In those cases, residual structureless masses appear in fiber's structure. In some areas, myelin sheath showed increased acidophilia and, occasionally, loss of normal structure and transformation into a thin, hyperacidophile, structureless ring. *Amyelin* fibers were disarranged and filled with hyperacidophilic axoplasm (Figure 2, 3, 4). *Schwann's* cells (Figure 2, 3) in the structure of myelin fibers are hypertrophic and some of them noticeably stick out into the interstitium. Consequently, one gets the impression of their separation from the fiber structure. At the same time, their cytoplasm and enlarged nuclei have an extraordinary affinity for the stain. Schwann's cells in the structure of amyelin fibers have

less hyperchromatic nuclei. Apart from the above described changes within the fasciculus, a *hypercellularity* can be noticed (Figure 1, 2), contributed by both increased number of Schwann's cells and the mobile cells of the connective tissue, especially macrophages. Intrafascicular blood vessels show hypertrophic endothelium (Figure 3). Individual or grouped extravasally located erythrocytes can be seen in some areas (Figure 4). *Epineurium* (Figure 1, 5) shows hypercellularity of the mononuclear inflammatory process type, with an increased number of macrophages, lymphocytes, plasma cells, hyperemic blood vessels and bundles of collagen fibers with altered, i.e. uneven tinctorial attributes. Collagen fibers are more compactly arranged in the perifascicular zone and strongly acidophilic. In the nerve zone which is located distally from the puncture area as well as in the area proximally from the puncture site, histological changes are of the same



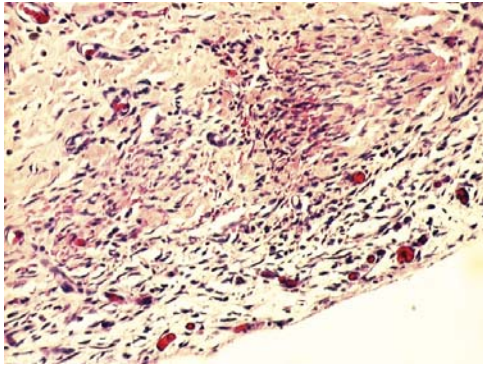


FIGURE 5. Intrafascicular application. HE, x 100

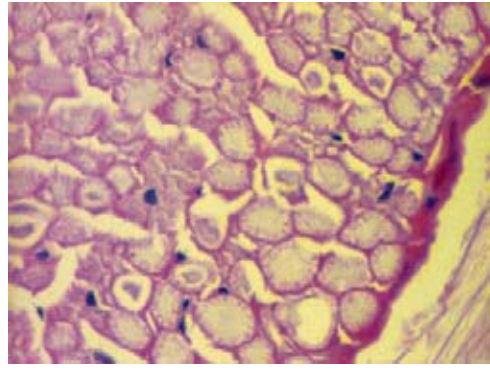


FIGURE 6. Intrafascicular application (proximal segment).  
HE, x 400.

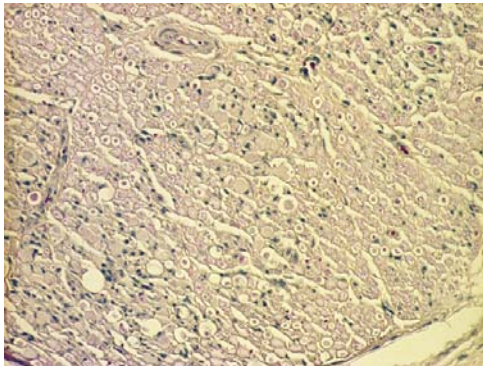


FIGURE 7. Intrafascicular application (distal segment).  
HE, x 100.

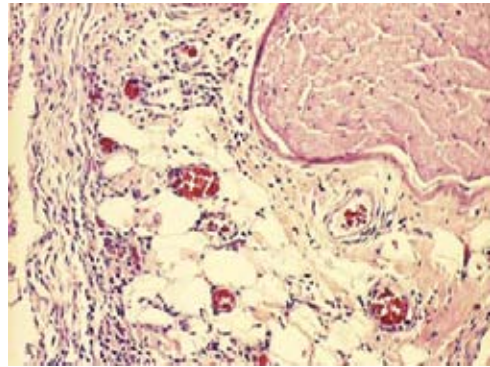


FIGURE 8. Extrafascicular application. HE, x 100.

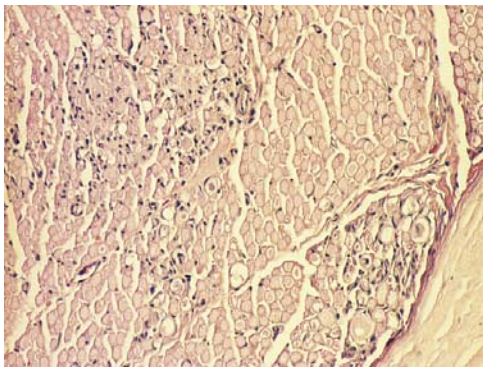


FIGURE 9. Extrafascicular application. HE, x 100.

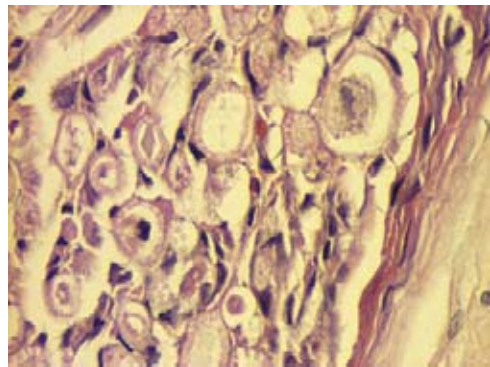


FIGURE 10. Extrafascicular application. HE, x 400.

type but of lower intensity compared to the puncture area (1 cm), especially in the proximal part (Figure 6, 7) In the nerves in which lidocaine was injected *intraneurally and extrafascicularly*, connective tissue in the structure of epineurium and perifascicular region show hypercellularity of the mononuclear inflammatory process type, so an increased number of macrophages, lymphocytes and plasma cells are registered. Groups of adipocytes with hyperemic blood vessels and bundles of collagen fibers with uneven tinctorial attributes are seen. Intrafascicularly, *subperineural* nerve fibers are more voluminous and Schwann's cells are large with hyperchromatic nuclei. Occasionally, zones of *hypercellularity*, which are the result of the outstanding of Schwann's cells and especially macrophages, are evident (Figure 8, 9, 10).

## DISCUSSION

In rare cases, clinical use of local anesthetics is related to neurological morbidity (2). The mechanism of the occurrence of neurological sequelae after intraneural application of local anesthetic is not completely clarified. Some authors state that the degree of nerve damage after intraneural application of various agents depends on the type and dose of the agent (4, 5, 8). Nerve damages during intrafascicular application of local anesthetic are results of direct trauma during application (7) or of ischemia which leads to the damage of blood-nerve barrier and endoneural edema (1). The pressure, as a factor that contributes to the damage, is mentioned, whereby intraneural application is in relation with different levels of application pressure (6). Other authors were mainly dealing with the difference in nerve damage during paraneural and intraneural application or with the differences in damages during paraneural application of different substances (3, 9). We have observed pressure differences in intrafascicular and extrafascicular application of local anesthetic, keeping in mind the different structure within the nerve itself. Our data show that the degree of nerve damage is higher in intrafascicular application which is in relation to high application pressure, with mean values found by us were  $198.23 \pm 52$  kPa, while in cases of extrafascicular application those mean values were  $53.3 \pm 17.9$  kPa, with parallel smaller damages of histological

structure. Our previous researches point out the difference in pressure as well (6). Under the conditions of paraneural and extrafascicular application, local anesthetic dilutes quickly into the surrounding tissue and its concentration falls with systemic absorption as well, which results in changes that are of significantly weaker intensity when compared to intrafascicular application; changes are dominantly present in epineurium and in subperineural region of the fasciculus and intensity of the changes depend on the dose and type of local anesthetic (4, 5, 9, 12). Our research shows this distribution of changes during extrafascicular application as well. Opposite of the topical application, the consequences of the elevation of endoneural pressure during intrafascicular application are edema and ischemia, which results in weakened flow in the fasciculus, difficult dilution and absorption of local anesthetic and extension of its acting. In cases of intrafascicular application of 2% lidocaine, we found damages at the level of nerve fibers, Schwann's cells, intrafascicular blood vessels, interstitium, perineurium and epineurium in all nerve samples. Those changes were present in the neighboring proximal and distal regions from the application site, but they were of weaker intensity. Other researchers mention similar changes during intraneural application as well, but not in all cases (6, 8, 10 and 11) because they didn't distinguish between intra- and extrafascicular application, based on the histological nerve structure and the differences in achieved application pressures that are in connection with it.


## CONCLUSIONS

1. In intrafascicular application of lidocaine, significantly higher application pressure was found compared to the one in extrafascicular application.
2. Intrafascicularly injected lidocaine results in significant lesions in the puncture area, where subperineural changes are more intensive than central ones. Segments that are distal and proximal to the puncture site show one-way changes of weaker intensity, especially proximally.
3. In extrafascicular application of lidocaine, changes in epineurium and subperineurally located fibers are dominant.



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# SATURATION WITH OXYGEN FOR DUCTAL DEPENDENT CONGENITAL HEART DISEASES BEFORE AND AFTER THE PROSTAGLANDIN THERAPY

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## ABSTRACT

Ductal dependent congenital heart diseases represent 14-20 % of all congenital heart diseases. A primary goal of the treatment of these diseases is to retain ductus open until the final cardiosurgical treatment. Prostaglandins are presently the only medicaments, which have a capability to keep ductus open. By means of a retrospective study in a period from January, 2000 until December, 2002 at the Paediatric clinic of the Clinical centre of the University in Sarajevo, 14 patients (treated with prostaglandins) diagnosed with ductal dependent congenital heart diseases were analyzed. In our sample, there are 9/14 male patients (64.3 %), 11/14 (78.6%) were full-term newborns, while 10/14 (71.4 %) were eutrophic at birth. An average saturation increase, after the prostaglandin therapy, measured in blood from the capillaries is 29, and measured transcutanly is 32 units. Duration of prostaglandin therapy in our study was on average 17.2 days. The most common cause of death was insufficiencia cardiorespiratoria (4 out of 11), but sepsis/infection (3 out of 11) and insufficiencia renalis were also common. 78.6 % (11 out of 14) patients died partly because of the complexity of these diseases, but also because a cardiosurgical treatment is delayed. A goal of this study is evaluation of saturation with oxygen before and after the prostaglandin therapy.

**KEY WORDS:** saturation, duct dependent, congenital heart disease, prostaglandin

## INTRODUCTION

Congenital cardiac diseases have an incidence of 0.8 % (1) and ductal dependent congenital heart diseases are represented with 14-20 % (2). Ductus arteriosus in a foetal period exists as a large vascular structure, with a diameter equal to lung truncus and descending aorta. About 55-60 % of foetus systemic circulation flows from the right to the left side of circulation through ductus. Lung resistance decreases with a first breath intake, ductus constriction starts and it is functionally closed 10-18 hours after the birth. Structural closure of ductus usually ends between the 15 th and the 21. day (3). Newborns with ductal dependent congenital heart diseases can be symptomatic until the moment ductus closes, so a number of children are sent home as healthy. Decrease of blood flow through ductus causes dangerous hypoxia or cardiogenic shock, metabolic acidosis, multisystemic failure and death. Ductal dependent congenital heart diseases consist of the following congenital heart disease groups:

- A. **Ductal dependent pulmonary circulation:** pulmonary atresia with intact intraventricular septum, tetralogia Fallot (more serious forms), tricuspid atresia, univentricular heart with critical stenosis or with pulmonary artery atresia;
- B. **Ductal dependent systemic circulation:** hypoplastic left heart syndrome, critical aortic stenosis, critical aortic coarctation, aortic arch obstruction;
- C. **Ductal dependent congenital heart diseases without an adequate blood mixing:** transposition of great arteries.

A common order of therapeutic measures for the above stated heart diseases is prostaglandin therapy, intubation and sedation, oxygen therapy, correction of acidosis, hypoperfusion and hypotension (4,5,6) A primary goal for the patients with ductal dependent congenital heart diseases is to medicamentally retain their ductus open. Prostaglandins are presently the only medicaments, which keep ductus open. The usual starting dose is 0.05 to 0.1 mcg/kg/BW\*/min, and a maintenance dose is lower and can be as low as 0.01 mcg/kg/BW/min in a continuous infusion. The drug side-effects are numerous, and some of them are: apnoea, convulsions, vasodilatation, arrhythmia, hypotension, fever, infection, hypoglycaemia, hypocalcaemia, bleeding, thrombocytopenia and diarrhoea (7,8).

## PATIENTS AND METHODS

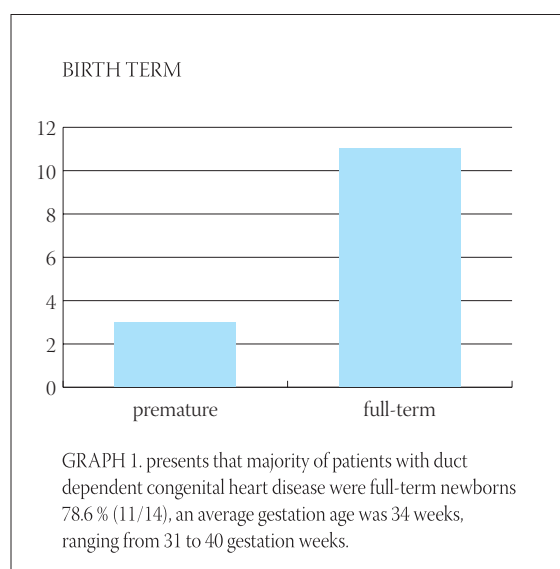
During the period from January, 2000 until December, 2002, 14 patients who were treated with prostaglandins at the Paediatric clinic of the Clinical centre of the University in Sarajevo, neonatology unit, with ductal dependent congenital heart diseases have been analyzed by a retrospective study. A source of information were patient records (disease histories) and computer information bases. Diagnosis for heart disease in all cases was obtained with an echocardiography, after the history, physical findings, ECG, lung and heart X-rays, and a complete blood analysis with the acidobasic status (ABS). The group was analyzed according to sex, birth weight, weeks of gestation, time duration of prostaglandin therapy, final outcome and a death cause. All the patients received Alprostadil (prostaglandin E<sub>2</sub>) intravenously in a continuous infusion. Saturation with oxygen before and after the prostaglandin therapy was recorded transcutanly and from capillary blood.

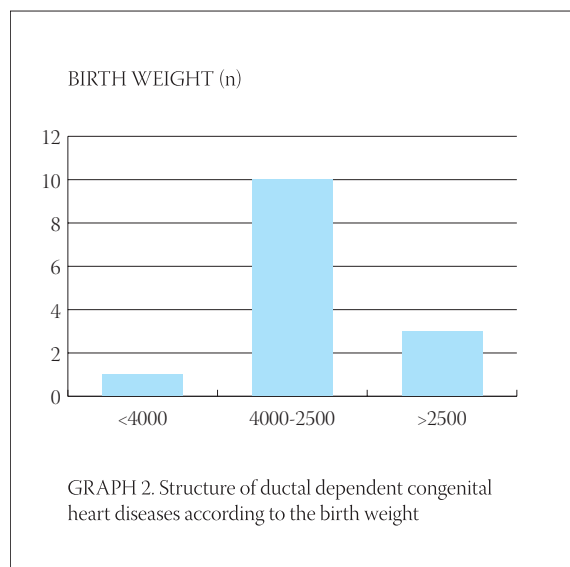
## RESULTS

A total of 14 patients formed this study. Males were presented with 64% (9/14) which has been shown in Table 1. Out of a total number of newborns with ductal dependent

SEX	NUMBER (n)	PERCENT (%)
MALE	9	64
FEMALE	5	36

TABLE 1. Sex structure of ductal dependent congenital heart diseases (n=14).





dent congenital heart diseases - 71.4 % (10/14) were eutrophic (Graph 2). Considering that oxygen exchange until birth is conducted through mother's placenta (as most important role), it is not a surprise that all the patients were well developed for their age at birth. An average increase of saturation after the prostaglandin therapy followed via gases analyses of capillary blood was 29 units, ranging from 18 to 45 (Table 2). From the same Table it is visible that increase of transcutan

saturation was 32 units, ranging from 14 to 52 units as well as an average maintenance dose of prostaglandins: 0.033 mcg/kg/BW/min (from 0.01 to 0.09). Table 3 represents data on an average duration of prostaglandin therapy was 17.2 days ranging from 3 to 41 days. Out of 14 patients, 11 of them died (78.6%), and the three who survived were transferred to the cardiosurgical centres outside of Bosnia and Herzegovina. In Table 4: causes of death: four patients (36.3 %) died due to cardiac insufficiency, three died due to systemic infection and three because of renal insufficiency. In one newborn cause of death was massive intracranial haemorrhage.

## DISCUSSION

Usage of prostaglandins goes back to the 70's. The Nobel prize for a research on prostaglandins in 1970 won Von Euler, and in 1982 Suneu K. Bergstrom, John R.Vane. The drug Alprostadil - prostaglandin E1 is in use in neonatal cardiology, but also Dinoprost - prostaglandin E2. All the patients in our study received Alprostadil by infusion. However, use of prostaglandin E2 is recommended by Silove, because he found that they have fewer side-effects (4). Unwanted effects are in a close correlation with a dose. The most common complications are apnoea, febrile states and infection. Silove

AGE	WEIGHT WHEN ADMITTED (G)*	MAINTENANCE DOSE PGE1 (MCG/KG/MIN)	INCREASE OF SAT.(CAPILLARY BLOOD-ABS)	INCREASE OF SATURATION IN UNITS (ABS)	INCREASE OF SATURATION (TRANSCUT.)	INCREASE OF SATURATION IN UNITS (TRANS.)
5 h	1300	0.05	from 0.51 to 0.90	0.39	from 62 % to 92 %	30
1 day	2590	0.07	from 0.24 to 0.42	0.18	from 67 % to 87 %	20
1 day	3890	0.01	from 0.50 to 0.89	0.39	from 60 % to 85 %	25
8 days	3500	0.025	from 0.71 to 0.91	0.20	from 82 % to 99 %	17
7 h	3900	0.03	from 0.26 to 0.71	0.45	from 84 % to 98 %	14
1 day	2090	0.05	from 0.41 to 0.76	0.35	from 35 % to 67 %	32
2 days	3600	0.05	from 0.61 to 1.00	0.39	from 70 % to 100 %	30
1 day	3280	0.01	from 0.47 to 0.65	0.18	from 47 % to 61 %	14
1 day	3100	0.03	from 0.58 to 0.99	0.41	from 52% to 92 %	40
1 day	3820	0.01	from 0.58 to 0.96	0.38	from 54 % to 74 %	20
1 day	2700	0.01	from 0.56 to 0.90	0.34	from 47 % to 99 %	52
3 days	3650	0.01	from 0.70 to 0.94	0.24	from 67 % to 82 %	15
6 days	3200	0.09	from 0.63 to 0.73	0.20	from 57 % to 80 %	23
3 days	3540	0.02	from 0.58 to 0.90	0.32	from 66 % to 83 %	21

TABLE 2. Saturation with oxygen before and after the prostaglandin therapy recorded transcutanly and from capillary blood.

g<sup>\*</sup>=grams

recommends 0.03 mcg/kg/BW/min, while larger doses are given for a few hours at the beginning. An average maintenance dose in our study is 0.03 mcg/kg/BW/min. Prostaglandins directly effect smooth muscles of ductus arteriosus and dilate it. They cause vasodilatation of all the arterioles. They inhibit aggregation of platelets. A maximum effect of the drug occurs after 30 minutes from the start of infusion for cyanotic, and after a few hours for the obstructive lesions. They have to be given before ductus closes completely, because this drug cannot open it. An effectiveness of the drug depends on the starting values of pO<sub>2</sub>; the more serious hypoxia is, the more pO<sub>2</sub> increases. Thus, we can say that age of a child and starting values of pO<sub>2</sub> have a key role in effectiveness of the drug. A way of drug intake (venous or arterial), sex or pCO<sub>2</sub> do not affect the drug effectiveness (6,10,12). In our study, 50 % (7/14) of the children started the prostaglandin therapy on the first day of life. An average increase of saturation measured in capillary blood was 29 units, ranging from 18 to 45 units. An average increase of saturation measured transcutanly was 32 units, ranging from 14 to 52. Prostaglandins are given for a relatively short period of time, a few hours to a few days, longest for a month. During that time, a child is cardiosurgically cared for. If a duration of the therapy is prolonged, side-effects are more common and more serious, and ductus becomes very brittle, so that during

a surgical intervention it can potentially burst (7,8,11). In our study, 78.6 % (11/14) of the patients died. 8/14 had a complex congenital heart disease, (Transposition of Great Arteries - TGA), which needs to be corrected within the first 2-3 weeks after the birth, and that is currently not available in Sarajevo, because the cardiosurgical treatment of paediatric patients is done 3-4 times a year during the visits of the cardiosurgeons from Vienna. The local team has not yet been trained for surgical interventions of complex congenital heart diseases. High mortality is a consequence of a discontinued cardiosurgical treatment, prolonged prostaglandin therapy, as well as a long duration of mechanical ventilation with infections.

DEATH CAUSE	DEATHS (N)	PERCENT %
INSUFF. CARDIORESPIRATORIA	4	36.3
SEPSIS	3	27.3
INSUFF. RENALIS	3	27.3
HEMORAGIA INTRACRANIALIS	1	9.1

TABLE 4. Death causes for ductal dependent congenital heart diseases (n=11).

PATIENT	DIAGNOSIS	OUTCOME AT RELEASE	NUMBER OF DAYS ON PROSTAGLANDIN THERAPY
1	TGA, ASD	Died	41
2	ASD, DAP	Died	39
3	ASD	Died	23
4	TGA	Died	3
5	VSD	Died	5
6	TGA	Died	3
7	TGA, VSD	Died	23
8	TGA, VSD	Died	8
9	ASD, VSD	Died	16
10	TGA, DAP	Died	6
11	VSD, ASD	Survived	8
12	VSD, DAP, ASD	Survived	26
13	TGA, VSD	Survived	26
14	TGA, ASD, VSD	Died	14

TABLE 3. Diagnosis for ductal dependent congenital heart ; Diseases, mortality and time duration of prostaglandin therapy.




## CONCLUSION

This randomized, clinically selected study confirms a justification for the use of prostaglandins in neonatal cardiology, and their useful effect. However, in order to increase a survival rate and life quality of these patients, it is necessary to improve and to establish a proper paediatric cardiosurgery in Bosnia and Herzegovina. Cardiosurgery in Bosnia has been improved during the past four years, which can be seen in a total number (160 patients) and outcomes (figures in co-

relation with European centres) of the treated patients by the local team and the cardio surgeons from Austria. However, improvement concerns primarily a continuous prostaglandin supply, education in the foetal echocardiology (which would significantly decrease a number of complex congenital heart diseases) and a paediatric cardiosurgery, so that the patients with congenital heart diseases can have an adequate treatment on time.

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# THYROID VOLUME MEASUREMENT BY ULTRASOUND IN SCHOOLCHILDREN FROM MILDLY IODINE-DEFICIENT AREA

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## ABSTRACT

Thyroid size was estimated by ultrasound and physical examination in 480 schoolchildren (238 boys and 242 girls), 7-14 years old, living in Tuzla Canton. By physical examination goiter was found in 13.5% (n=65) of subjects. When compared with the upper limits of the reference thyroid volumes reported by WHO and ICCIDD, goiter by ultrasonography was found in 12.9% (n=62) of all subjects. All goitrous children had a diffuse goiter. The differences in mean thyroid volumes between groups with and without goiter detected by physical examination were significant in all age groups ( $p < 0.05$ ). The results of ultrasound examinations correlate well with palpatory findings and show higher values for the thyroid volume in children with goiter. It generally confirms the values of the findings by palpation, even in areas with mild iodine deficiency.

**KEY WORDS:** thyroid volume, iodine deficiency

## INTRODUCTION

Determination of goiter prevalence and urinary iodine excretion in schoolchildren is recommended for assessing the extent and severity of iodine deficiency disorders in a region (1). For years, palpation was the single method available for defining thyroid volume. Although in moderately or severely iodine-deficient areas, thyroid palpation provides careful estimation of thyroid size, serious problems are encountered in areas with mild iodine deficiency, where most goiters are small. Thyroid ultrasound came to be considered the most reliable method for the estimation of thyroid volume (2,3). In epidemiological surveys, the use of this technique is strongly recommended to define the goiter endemic in areas of mild iodine deficiency. In the past, severe iodine deficiency, endemic goiter and cretinism were documented in restricted areas of Bosnia and Herzegovina. Salt production is present in Tuzla area since the medieval times and salt iodination was initiated in salt plant in Tuzla in 1934. Epidemiological surveys on goiter in Bosnia and Herzegovina were started systematically in the early 1950s. An investigation in 1953 has documented high goiter prevalence in endemic areas, varying from 34% to 60%. A law proscribing obligatory iodination of all salt for human and animal consumption, with 10 mg KI/kg of salt was proclaimed in 1953. On the basis of clinical observation of rather high prevalence of goiter among schoolchildren, the study, with the aim to evaluate the efficacy of compulsory iodine prophylaxis more than 40 years after its introduction, was carried out in all cantons in Bosnia and Herzegovina Federation in 1999(4). The results of the study show the persistence of mild iodine deficiency in Tuzla Canton on the basis of median urinary iodine excretion of 79.0 µg/L and goiter prevalence of 20.1% in schoolchildren. Thyroid size was estimated by physical examination. The aim of the present study was to measure thyroid volume and evaluate the prevalence of endemic goiter by ultrasound criteria in the schoolchildren population in mildly iodine-deficient area of Tuzla Canton.

## SUBJECTS AND METHODS

Thyroid size was assessed by ultrasound and by physical examination in 480 schoolchildren (238 boys and 242 girls) 7-14 years old, randomly selected, in Tuzla Canton. Tuzla Canton is one of the ten administrative territorial units of the Federation of Bosnia and Herzegovina, situated in North-Eastern Bosnia. Thyroid size was estimated by neck palpation, by the same examiner,

and graded according to the World Health Organization (WHO)/International Council for the Control of Iodine Deficiency Disorders (ICCIDD) criteria (1) as follows: grade 0 - no palpable or visible goiter; grade 1 - a mass in the neck that is consistent with an enlarged thyroid that is palpable but not visible when the neck is in the normal position, it moves upward in the neck as the subject swallows; grade 2: a swelling in the neck that is visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated. Thyroid ultrasound was performed by the same examiner. Subjects were examined in supine position with the neck hyperextended. Thyroid volume was estimated using real-time sonography according to Brunn et al. (5) with Toshiba SSA-220A, using a 7.5 MHz linear transducer. Longitudinal and transverse scans were performed allowing the measurement of the depth (d), the width (w) and the length (l) of each lobe. The volume of the lobe was calculated using formula:  $V(\text{ml})=0.479 \times d \times w \times l$  (cm). The thyroid volume was the sum of the volumes of both lobes. The volume of the isthmus was not included. The criteria used for defining the upper limit of normal for thyroid volume (percentile 97) in children with a normal iodine intake were those proposed by WHO/ICCIDD (6).

## RESULTS

The mean thyroid volume as determined by ultrasonography was  $5.5 \pm 2.8$  ml. Although the mean thyroid volume was greater in girls ( $5.6 \pm 3.0$ ) than in boys ( $5.3 \pm 2.5$ ) the difference was not statistically significant ( $p > 0.05$ ). The results of the ultrasound examinations confirmed the fact that the right lobe ( $3.2 \pm 1.7$ ) of the thyroid gland is larger, compared to the left lobe ( $2.2 \pm 1.2$ ), and the difference is significant ( $p < 0.0001$ ). Mean and median thyroid volumes of all subjects according to age are shown in Table 1. The median thyroid volume increases from 2.7 ml at 7 years to 8.4 ml at 14 years. Significant positive correlation was observed between the thyroid volume and age ( $r=0.97$ ,  $p < 0.0001$ ). Also, significant correlation was found between thyroid volume and body weight of children ( $r=0.98$ ,  $p < 0.0001$ ), height ( $r=0.98$ ,  $p=0.0003$ ) and body surface ( $r=0.99$ ,  $p < 0.0001$ ). When compared with the upper limits of the reference thyroid volumes, goiter by ultrasonography was found in 12.9% ( $n=62$ ) of all subjects ( $n=480$ ). All goitrous children had a diffuse goiter. By physical examination goiter was found in 13.5% ( $n=65$ ) of subjects. Mean and median

AGE (YEARS)	NO. EXAMINED	THYROID VOLUMES (ML)		
		MEAN±SD	MEDIAN	RANGE
7	54	2.8 ± 0.9	2.7	1.4-5.1
8	65	3.8 ± 1.1	3.6	1.3-6.5
9	58	4.5 ± 2.9	4.1	2.2-17.5
10	55	4.5 ± 1.4	4.3	2.7-9.6
11	66	5.5 ± 2.0	5.0	3-13.4
12	60	6.2 ± 2.2	6.3	3-12.5
13	65	7.5 ± 2.6	6.8	4.6-15.3
14	57	8.6 ± 3.1	8.4	3.3-15.4

TABLE 1. Mean and median thyroid volumes of all age groups

thyroid volumes according to age in subjects with and without goiter by physical examination are shown in Table 2. The differences of mean thyroid volumes between groups with and without goiter detected by physical examination were significant in all age groups ( $p < 0.05$ ).

## DISCUSSION

Thyroid ultrasound is reliable, practical and objective method of determining thyroid size. The results of the ultrasound examinations confirmed the fact that the right lobe of the thyroid gland is larger, compared to the left lobe (7). Thyroid volume is well correlated with age and growth parameters, what was reported also in this survey (8,9). When the upper limits of reference thyroid volumes were taken into account (6), we found goiter prevalence of 12.9% in the studied population. This finding was in accordance with the prevalence of goiter (13.5%) ob-

tained by physical examination. The volumes of thyroid gland in children where goiter was found by palpation and the volumes in children without goiter are compared and presented separately. The results of the ultrasound examinations correlate well with the palpatory findings and show higher values for the thyroid volume in children with goiter, which generally confirms the values of the findings by palpation. The recent studies (2,3) indicate that thyroid ultrasonography is more accurate than palpation in the assessment of goiter prevalence in the schoolchildren population living in mildly iodine-deficient areas. They have shown that palpation overestimates the actual size of thyroid, particularly in children. On the contrary, the data reported in this study indicate that physical examination is as precise as ultrasonography in the assessment of goiter prevalence in mildly iodine-deficient areas. This may be explained by the fact that this study was performed by the same investigator. Our findings for the volume of thyroid gland were

AGE (YEARS)	THYROID VOLUMES (ML)			
	GOITER (+)		GOITER (-)	
	MEAN±SD*	MEDIAN	MEAN±SD*	MEDIAN
7	4.2 ± 0.6	4.5	2.8 ± 0.9	2.7
8	5.3 ± 1.0	5.3	3.6 ± 1.1	3.5
9	9.4 ± 5.9	7.6	3.9 ± 1.5	3.9
10	6.3 ± 1.8	5.8	4.2 ± 1.1	3.9
11	7.1 ± 2.8	6.1	5.1 ± 1.6	4.7
12	8.4 ± 1.8	8.1	5.7 ± 1.8	5.7
13	11.5 ± 4.6	14.6	6.9 ± 1.5	6.7
14	12.4 ± 3.1	12.5	7.8 ± 2.7	7.2

TABLE 2. Thyroid volumes according to age in subjects with and without goiter by physical examination

\* $P < 0.05$

compared to the values of other authors. Our values are higher than the values of Gutekunst et Martin-Teichert (2) for Sweden, where there is no deficiency, are lower than the values of Semiz et al. (10) for goitrous area of mild to moderate degree from Turkey and are similar to the values of Aghini-Lombardi et al. (11) that refer to goitrous region in Italy


## CONCLUSION

In conclusion, the ultrasound data indicate mild degree of iodine deficiency in Tuzla Canton. Although ultrasonography provides more precise measurement of thyroid volume compared with palpation, clinical examination in the assessment of goiter prevalence in mildly iodine-deficient areas should not be abandoned.

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# ROLE OF BONE SCINTIGRAPHY AND TUMOR MARKER-CA 15-3 IN DETECTION OF BONE METASTASES IN PATIENTS WITH BREAST CANCER

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## ABSTRACT

Breast cancer is one of the most frequent types of cancer affecting women. After hematogenous spreading of cancer, axial skeleton is most frequently involved. Bone scintigraphy is commonly performed in detection and evaluation of bone metastases. In breast cancer, marker Ca 15-3 is widely accepted in follow-up and detection of disease recurrence. Aim of the study was to correlate levels of tumor marker Ca 15-3 and presence of bone metastases detected by bone scintigraphy. Study included 25 patients with breast cancer, previously surgically treated. All patients underwent total body scintigraphy. Ca 15-3 was measured by radioimmunoassay. Presence, number and location of bone metastases were correlated with Ca 15-3 levels. Bone scintigraphy revealed bone metastases in 16 (64%) patients. 11 (44%) patients with metastases and 1 patient (4%) without scintigraphically visible metastases had elevated Ca 15-3 levels. Significant difference in distribution of metastases was found for spine ( $t=3.930$ ,  $p=0.008$ ). Correlation between intensity of radiopharmaceutical uptake and level of Ca 15-3 in patients was positive ( $r=0.405$ ). A weak correlation was found between number of metastases and level of Ca 15-3 ( $r=0.139$ ). Significant differences in Ca 15-3 level was found in patients with metastases compared to patients without metastases ( $\chi^2=0$ ,  $p=1.0$ ). Since no significant correlation was found between level of Ca 15-3 and number of metastases, we consider scintigraphy an appropriate method for assessment of bone metastases in breast cancer.

**KEY WORDS:** breast cancer; bone metastases, bone scintigraphy, Ca 15-3

## INTRODUCTION

Breast cancer is one of the most frequent types of cancer affecting women with incidence peak between age 40-60 (1,2). The most common site of distant metastases is skeleton, which is affected in about 8% of all patients.

After hematogenous spreading of cancer, axial skeleton is most frequently involved with pattern that resembles red bone marrow distribution.

Complications associated with skeletal metastases including pain, pathological fractures, hypercalcemia, myelosupresion, spinal and cord compresion, lessions of nerve roots, are demanding for patients and health care resources in general (3,4) Early detection of bone metastases is mandatory in the evaluation and managment of these patients.

In diagnosis of breast cancer metastases several procedures and tests are available such as: tumor markers (CEA, Ca 15-3), x-rays, CT scan, magnetic resonant imaging, bone scintigraphy. These investigations aim to establish location of dissemination, make a specific diagnosis, prevent complications by early diagnosis and finally to assess patients response to treatment (5).

Although not the latest method, bone scintigraphy is still often requested and commonly performed in detection and evaluation of bone metastases. This investigation allows highly sensitive detection of osteoblastic activity.

Tumor markers are present in healthy individuals as well as in patients with malignant diseases but in different concentrations. In breast cancer, breast-associated mucin marker Ca 15-3 is widely accepted as a serum tumor marker in follow-up and detection of disease recurrence (5,6). This study was undertaken in order to correlate levels of tumor marker Ca 15-3 and presence of bone metastases detected by bone scintigraphy.

## PATIENTS AND METHODS

25 patients with pathologically verified and previously surgically treated breast cancer were included in study.

All patients underwent total body scintigraphy using double-head gamma camera equipped with low energy high resolution collimator 3 hours after I.V. injection of 740MBq Tc-99m MDP (methylen diphosponate).

Ca 15-3 was measured by radioimmunoassay using microparticle Enzyme-Immunoassay technology (MEIA, Abbott AXSYM system). Presence and number of bone metastases together with their location were correlated with levels of Ca 15-3 tumor marker. Statistical analyses were performed with chi-square test, Student t-test, standard deviation. Study was approved by Ethical Committee and informed consent in written was obtained from all subjects in advance.

## RESULTS

Median age of patients included in study was 50 varying from 30 to 67. 15 patients (60%) had pathologically verified cancer on the left breast, the rest of 10 patients (40%) had the right breast cancer. Bone scintigraphy revealed bone metastases in 16 (64%) patients. Scintigraphy was negative for metastases in 9 (36%) patients. 11 patients (44%) with scintigraphically proven metastases and 1 patient (4%) without scintigraphically visible metastases had elevated Ca 15-3 marker. In 9 patients with normal scintigraphy (36%) only 1 patient (4%) had elevated Ca 15-3 marker, (Table 1.).

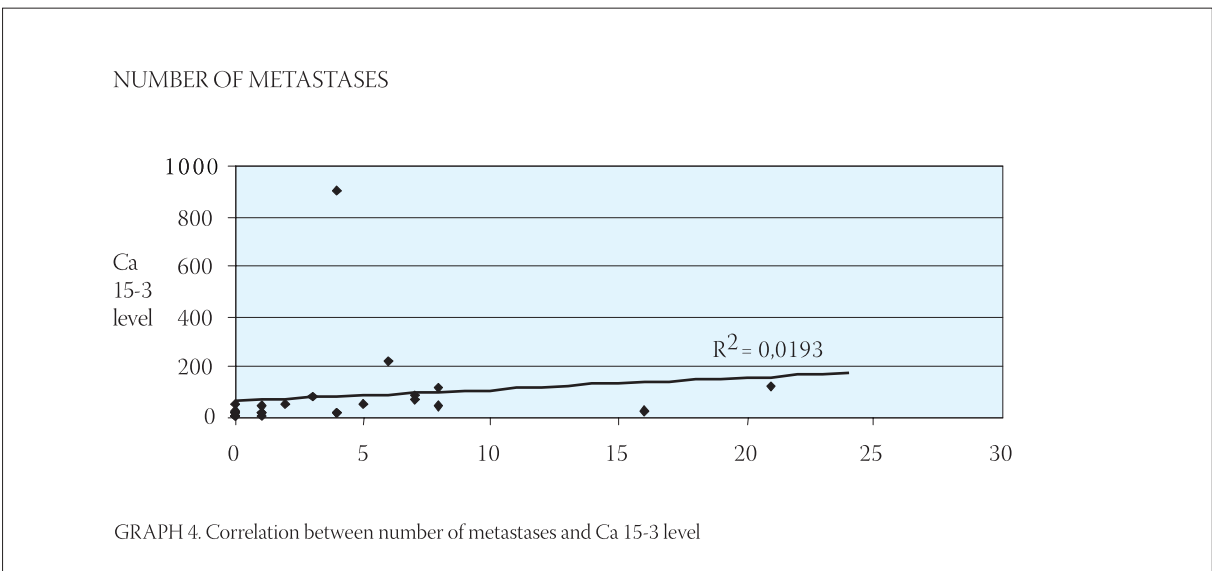
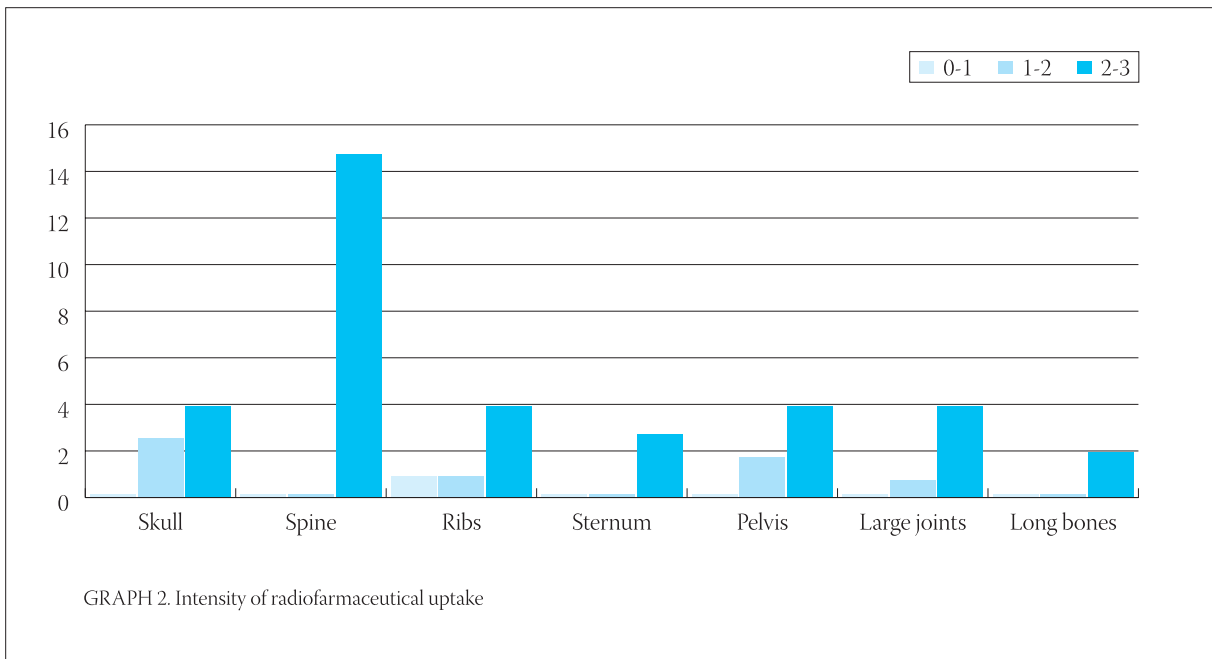
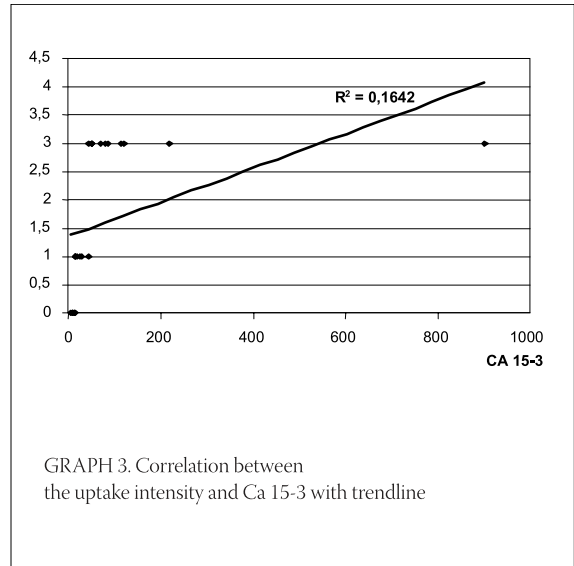
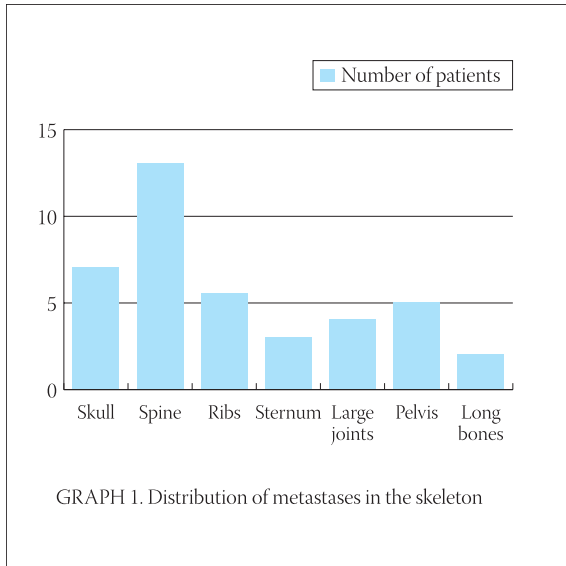
Multiple bone metastases were found in 12 (75%) patients. Four patients (25%) had solitary bone metastases. Analyzing the distribution of bone metastases the highest frequency was found for spine (34.09% of all metastases).

The difference is significantly higher compared to other bones, ( $t=3.930$ ,  $p=0.008$ ), Graph 1. The most intensive radiopharmaceutical uptake was found in spine (score 2-3).

Difference was significant ( $t=3.083$ ,  $p=0.022$ ), Graph 2.

Correlation between the intensity of radiopharmaceutical uptake and level of Ca 15-3 in patients with breast cancer was positive ( $r=0.405$ ). Graph 3.

A weak correlation was found between number of metastases and level of Ca 15-3 ( $r=0.139$ )- Graph 4. However, statistically significant correlation in level of Ca 15-3 was found in patients with bone metastases revealed scintigraphically in relation to patients without bone metastases (chi square 0,  $p=1.0$ )




## DISCUSSION

Breast cancer is the leading cause of death in women aged 35-54 (7). Although bone scan is frequently performed in evaluation of breast cancer metastases, precise role of bone scanning at different stages of the disease is still contentious. Clinical areas in which the bone scan has been evaluated include staging, systematic follow-up of asymptomatic patients, and assessment of response to therapy. In breast cancer, metastases are most often distributed in the axial skeleton, reflecting the distribution of red bone marrow, with the vertebrae being the most commonly involved site (4). In our study, most common site of bone metastases was spine.

Tumor markers sensitivity is also related to the site of recurrence, with the lowest sensitivity for locoregional relapse and highest for liver metastases (8). The disease extent of patients with bone metastases correlated significantly with elevated Ca 15-3 levels. Higher Ca 15-3 levels were found for larger disease extent. Consequently, longer survival was observed in patients with limited disease extent (8). In our study we found significantly higher levels of Ca 15-3 in patients with bone metastases, however, we found no significant correlation between level of Ca 15-3 and number of bone metastases.

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# BODY DISSATISFACTION: HOW IS IT RELATED TO STRESS AND ONE'S PERCEPTION OF INDIVIDUAL AND CULTURAL IDEAL BODY? A COMPARISON OF BOSNIAN AND AMERICAN UNIVERSITY STUDENTS

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## ABSTRACT

Cultural differences in body dissatisfaction, the relationship of stress to body dissatisfaction and individual and cultural body ideals were investigated. Forty-eight United States women and 48 Bosnian women completed the Body Shape Questionnaire (BSQ), the Impact of Event Scale (IES), and a Body Figure Rating Scale. When Body Mass Index was controlled, United States women demonstrated more body dissatisfaction and chose smaller cultural ideal body sizes than Bosnian women, but did not differ on personal ideal body size ratings. Furthermore, stress was only related to body dissatisfaction for Bosnian women. Additional research is needed to further elaborate the body dissatisfaction differences as they relate to cultural values.

**KEY WORDS:** body dissatisfaction, ideal body shape,  
women from BiH and USA, body image, stress



## INTRODUCTION

In the last few decades, body dissatisfaction has been studied as an integral component of eating disorders. Although body dissatisfaction is often associated with eating disorders (1,2), there has been an increase in body dissatisfaction among non-eating disordered women in the United States (3,4). Body image is defined as an evaluation of one's size, weight, or any other aspect of the body that determines physical experience (5). Body image is typically divided into three areas: perceptual, subjective, and behavioral. The perceptual component, also known as body image disturbance, is defined as an overestimation of the size of body parts (6). The subjective component focuses on the level of satisfaction, concern, cognitive evaluation, and anxiety about one's body. The behavioral component involves avoidance of the situation that causes the individual to experience discomfort due to physical appearance (5). In the present study, we will focus on the perceptual and subjective components of body image. Perceptual and subjective components of women's body images are a function of the standards that women are striving to achieve. These standards of beauty change as a function of time (7) as well as culture (8,9,10,11). Seid has posited that the shapes of bodies that we see today have been prevalent throughout history. What has changed is the societal or cultural ideal body shape as well as the means by which one achieves this body shape (7). Although the standards may have changed in the United States, the desire to match the societal or cultural ideal has not ceased (4). However, the standards of the ideal body shape have become more difficult, if not virtually impossible, to attain (12). Many women struggle with diets, expose themselves to extreme exercise regimes, and even undergo plastic surgery in an effort to attain the unrealistic ideal body shape (13). In examining non-Western cultures, women are not less likely to desire the ideal bodies, but instead the ideal bodies are more realistically defined (8,14,15). More specifically, women in non-Western cultures showed less body dissatisfaction, higher acceptance of their own bodies, choose body ideals that were larger than those considered ideal in Western cultures. In addition, women in non-Western cultures, although on average thinner, often selected a wider range of female body shapes as attractive than women in Western cultures. Interestingly, when women from non-Western cultures were exposed to Western culture over a length of time, either by immigrating or by attending a Western university, they adopted the ideals of beauty and body shape more similar to their Western counterparts

(9,14). In particular, Franko and Herrera suggested that women's level of acculturation to the Western culture is related to their individual level of body dissatisfaction. In addition, women's level of acculturation affects women's standards of the ideal body shape (9). In addition to the effect of culture on women's perceptions of their bodies, women's perceived level of stress has been shown to be related to the way they perceive and feel about their bodies (16). Koff and Sangani studied coping strategies in order to explain the relationship between body shape dissatisfaction and coping with stressful events. They found that women who used emotional coping as a way to deal with stress reported higher levels of body dissatisfaction. Slade might explain the relationship between coping with stress and body dissatisfaction in terms of control (17). Stress often leads to situations where women do not feel in control of the situation. Slade suggested that when women feel dissatisfied with their lives due to lack of control in their environment, they attempt to exert control over the one thing that they are able to control—their body shapes. When exploring the relationships between women's general perceptions and feelings about their bodies and stress, we became interested in how American women compared to Bosnian women on body dissatisfaction. Traditionally, Bosnian women's roles in society are moral provider and caretaker. Women's values and their identities are tied to their homes; they are often equated to the well-being of their family members and the organization of their homes. Because women's roles are defined by their domestic ability, the value of thinness and achieving particular body shape may not be as important as in Western cultures. However, these values are traditional and, although they are still widespread in villages and smaller communities, they are somewhat different in the cities. In the cities, women are expected to attend universities, to work, and to financially contribute to their households. Thus, women's roles in the city are not limited to the role of a provider. In addition, women in the cities are exposed to Western culture through the Western media and Western companies that are currently operating in Bosnia. Irrespective of whether they live in the rural areas or the cities, women in Bosnia have experienced the stress associated with war and recovery from war. Since stress has been shown to affect women's body shape dissatisfaction in the United States (16), it is possible that women in Bosnia may be equally affected. The purpose of the present study was to investigate the relationship between body dissatisfaction and stress in university women from Bosnia and from United States and to compare body dissatisfaction as well as judg-

ments of their personal and cultural ideal body shape. We predicted that American women would show higher levels of body dissatisfaction, select a thinner body size as their ideal, and select a thinner cultural ideal body size than Bosnian women. In addition, we predicted that for both Bosnian and American participants, stress would be positively correlated with body dissatisfaction.

## METHODS

### PARTICIPANTS

A total of 96 participants from the University of Tuzla (48 participants) in Tuzla, Bosnia and from Western Washington University (48 participants) in Bellingham, Washington volunteered to participate in the present study. American participants received extra credit in their undergraduate psychology classes while Bosnian participants received extra credit in their general studies classes as a result of completing the study. All of the participants were female students between the ages of 18 and 26. It should be noted that there was a statistically significant difference in the average age of the American participants ( $19.19 \pm 1.16$ ) and the Bosnian participants ( $22.02 \pm 1.96$ ) ( $p < 0.01$ ).

### MATERIALS

Participants completed the following measures: Body Shape Questionnaire (BSQ)(18), Impact of Event Scale (IES)(19), Body Figure Rating Scale, and a demographic survey. The BSQ is a 34-item questionnaire constructed to measure body shape satisfaction. Participants are asked to respond to items using a 6-point scale (1=*never*, 6=*always*) with higher score representing dissatisfaction with one's body. The BSQ includes questions that assess important body image symptoms such as marked preoccupation with one's weight and shape, embarrassment in public due to body shape, and excessive feelings of fatness after eating. A sample item from the BSQ is *Have you felt ashamed of your body?* The BSQ has a test-retest reliability of 0.88 for a two-month delay. In addition, the BSQ has a high degree of construct validity ( $r=0.77$ ). The IES is a 15-item scale that measures the impact of a stressful event. The IES contains two subscales that indicate the continued intrusiveness (I) of an event into consciousness, and the level of psychological avoidance (A) in dismissing the event. For the present study, the total of the two subscales is used to measure subjective stress (20). Participants are asked to name a stressful event and to respond to questions about how distressed they are about the event. A sample item from the IES is *I had troubles falling asleep*

*or staying asleep*. Each item is rated on a 5-point scale (0 = *not at all*, 4 = *extremely*) with higher scores indicating more stress. IES was found to have high reliability ( $\alpha = 0.86$  to  $0.90$ ) with pooled patients and non-patients samples over three time periods. The validity of the IES subscales was supported by confirmatory factor analysis (19). The Body Figure Rating Scale is a three-item questionnaire based on nine figure drawings that were designed and illustrated by Stunkard, Sorenson, and Schulsinger (21). It consists of the various female body types that range from very thin (skeletal body shape) to very heavy (obese body shape). Each figure corresponds to a number from 1 to 9 (where 1 = skeletal body shape and 9 = obese body shape). The figures and questions assess participants' individual ideal and their perception of culturally ideal body type. The participants are asked the following questions: What is your current body shape? What is your ideal body shape? What do you see as an ideal body shape in your country? The demographic survey includes height, weight, and age. Each measure was constructed by translating each item into Bosnian, then back-translating the items into English. Two translators constructed the translated version and back translated version independently. The two versions were then compared to check for any meaningful differences. All differences were resolved through compromise.

### PROCEDURE

Participants were tested at the University of Tuzla and Western Washington University during a lecture hour. Participants were informed about the confidentiality of responses and their right to discontinue at any time. Participants were also given an informed consent form. They were asked to read and sign the consent form. During this time, participants had an opportunity to ask questions about the study. When all questions were answered, participants received the packets including the Body Shape Questionnaire, Impact of Event Scale, Body Figure Rating Scale, and the demographic survey. Packets also included the information about the Counseling Center on campus for the American participants if they had concerns regarding body image issues. Because the University of Tuzla does not have a counseling center, participants were informed about the psychological resources that were available in Tuzla. Participants were told that the instructions for the individual tests were included at the top of each page. Participants were allocated 45 minutes in which to complete the packet. (*All matters regarding human subjects and their participation complied with the ethical principals outlined in World Medical Association Declaration of Helsinki*).

## RESULTS

### DEMOGRAPHIC INFORMATION

American and Bosnian participants were compared on their average height, weight and the Body Mass Index. For the women from USA, their self-reported heights were converted to centimeters and their self-reported weights were converted to kilograms. There was no significant difference in the average weight of the American ( $59.59 \pm 7.77$ ) and Bosnian participants ( $58.54 \pm 7.29$ ) ( $p > 0.05$ ). However, an independent samples t-test revealed a significant difference in the average height between American participants ( $166.37 \pm 6.35$ ) and Bosnian participants ( $164.42 \pm 4.45$ ) ( $p < 0.01$ ). The Body Mass Index (BMI) was calculated for all participants. An independent samples t-test yielded a significant difference between American participants ( $21.51 \pm 1.22$ ) and Bosnian participants ( $20.25 \pm 1.64$ ) ( $p < 0.05$ ).

### BODY SHAPE QUESTIONNAIRE

The total BSQ scores were compared for participants from Bosnia and participants from the United States. An independent samples t-test yielded a significant difference in total BSQ scores such that American participants ( $93.73 \pm 27.09$ ) scored higher than Bosnian participants ( $72.21 \pm 19.72$ ) ( $p < 0.01$ ). Based on these findings, American participants seemed to have higher body dissatisfaction than Bosnian participants. Since the BMI was higher for American women than Bosnian women and Bosnian women were older than American, the difference in BSQ scores could merely be a function of these factors. Thus, we reanalyzed the data using the BMI and age as covariates. An ANCOVA yielded a significant difference in total BSQ scores with American women expressing more body shape dissatisfaction than Bosnian even when BMI and age were taken into account ( $p < 0.05$ ).

### IMPACT OF EVENT SCALE

The total IES scores were also compared for participants from Bosnia and the United States. An independent samples t-test revealed that there was no significant difference in stress scores between American women ( $37.64 \pm 9.99$ ) and Bosnian women ( $36.94 \pm 10.76$ ) ( $p > 0.05$ ). These results suggest that the current level of stress as perceived by the participants may not be any different for the two groups of women and thus cannot account for the difference in body shape dissatisfaction.

### WOMEN'S RATINGS OF THEIR BODIES

The selections were made from the Stunkard's (21) Body figure rating scale. This scale consists of body fig-

ures ranging from skeletal (1) to extremely obese (9). The ratings were compared for the participants from United States and Bosnia using a chi-square test of independence since the data were ordinal measures. The responses of women from both countries were between ratings of 2 to 6. The chi-square test of independence revealed that there was no significant difference between the ratings of the current body shape of American and Bosnian participants ( $p > 0.05$ ). The frequency of current body shape ratings by country can be seen in Table 1. Using same scale, participants from both countries were asked to rate their personal ideal body shape. The responses of women from both countries were restricted to ratings of 2, 3, or 4. The chi-square test of independence revealed that there was no significant difference between the ratings of American and Bosnian participants' ideal body shapes ( $p > 0.05$ ). The frequency of personal ideal body shape ratings by country are presented in Table 1. Irrespective of country, all of the participants chose the ideal personal body shape that is smaller than the average body shape (5) on the Stunkard 9- point scale. Finally, the participants from Bosnia and United States were asked to choose a body shape that they considered the ideal in their country. The ratings of women from Bosnia ranged from 2 to 5 whereas the ratings of women from the United States ranged from 1 to 4. The chi-square test of independence revealed that there was a significant difference between the ratings of American and Bosnian participants' perceptions of the ideal body shape in their country ( $p < 0.01$ ). The frequency of ratings of perceptions of the ideal body shape in each country are in Table 1 and indicate that women from Bosnia believe that their cultural ideal body shape is larger than the women from the United States.

RATING	2	3	4	5	6
CURRENT BODY FIGURE RATINGS					
BOSNIA	9	15	18	3	3
UNITED STATES	3	16	21	7	1
PERSONAL IDEAL BODY FIGURE RATINGS					
BOSNIA	12	30	6	--	--
UNITED STATES	12	33	3	--	--
CULTURAL IDEAL BODY FIGURE RATINGS					
BOSNIA	0	6	32	7	1
UNITED STATES	7	23	14	3	0

*Note.* Only ratings of 2-6 are reported since none of the participants used ratings of 1, 7, 8, or 9.

TABLE 1. Frequency of Current, Personal Ideal, and Cultural Ideal Body Figure Ratings as a Function of Culture

## IES AND BSQ

The relationships between BSQ and IES were analyzed for American and Bosnian participants separately as well as for the two groups combined. Pearson correlation revealed significant positive correlation between IES and BSQ ( $r = 0.40$ ,  $p < 0.01$ ). These results indicate that for the overall group higher levels of stress were associated with more body dissatisfaction. For Bosnian participants only, there was also a significant positive correlation between BSQ and IES ( $r = 0.64$ ,  $p < 0.01$ ), demonstrating for Bosnian participants that as stress increased body dissatisfaction also increased. American participants also showed positive correlation of IES and BSQ. However, this correlation was not significant ( $r = 0.24$ ,  $p > 0.05$ ). BSQ and the Perception of the Country's Ideal Body Shape. The relationship between body shape concerns and country's ideal body shape as perceived by the participants was analyzed for American and Bosnian participants. For Bosnian participants, there was significant negative correlation between BSQ and perception of their country's ideal shape ( $r = -0.19$ ,  $p = 0.05$ ) as well as for American participants ( $r = -0.21$ ,  $p = 0.05$ ). These findings for both groups suggest that the perception of a culture's endorsement of a smaller ideal body shape is associated with higher levels of body dissatisfaction for the participants from both Bosnia and United States.

## DISCUSSION

In conducting this study, we were interested in the cultural differences between Bosnian and American women in their perceptions of bodies, body dissatisfaction, and the relationship between perceived stress and the level of body dissatisfaction. The way women view their bodies and their choices of the ideal body shapes are influenced by their culture (8,15). Women from Western cultures tend to show more body dissatisfaction (22), choose thinner ideal body shapes, and view heavier bodies more negatively than women from non-Western cultures (8,15). In exploring the difference in the ideal bodies of American and Bosnian women, we were interested in both individual and cultural ideal bodies. The findings of our study revealed a difference in cultural ideal body size. In particular, Bosnian women reported their country's ideal body size as bigger than the perceived country's ideal body size of American participants. However, we found no difference between the two groups of women in their choice of individual ideal body size. Both American and Bosnian women chose an ideal that is smaller than the average body size on

the Stunkard Body Figure Scale. Interestingly, Bosnian women reported a smaller body type as their personal ideal than as their country's ideal. It is possible that on an individual basis Bosnian women, especially women who live in major cities and who are attending a university, are more likely to accept Western standards of thinness. This may occur because they are exposed to Western influences more than women from rural areas. However, when these women are asked to consider their country's ideal, they may be able to move beyond their personal biases and consider the body size that is acceptable in the country as a whole. Given this difference between the personal and cultural ideal body shape in Bosnia, we may see a change in the Bosnian culture's ideal body shape in the future. As more Bosnian women are exposed to the sources of Western influence, they may adopt ideal body standards more similar to those of Western women, ignoring the cultural ideal as they perceive it presently exists. As Western influences become more commonplace, individual or personal ideal body shapes may become the cultural ideal as well. Thus, accepting thinner bodies as ideal may affect Bosnian women's level of body dissatisfaction as the gap between the current and the ideal body becomes wider. We also predicted that women from United States would show more body dissatisfaction than women from Bosnia. Consistent with the previous studies (8,9,15), American women demonstrated higher levels of body dissatisfaction. In order to control for the possible weight difference between the two groups, we calculated body mass index and found that the American women on average had a higher BMI than Bosnian women. It is possible that the difference in body dissatisfaction is simply a reflection of the weight difference between the two groups. In other words, American women may have demonstrated higher levels of body dissatisfaction based on their desire to lose extra weight. However, when we controlled for both differences in age and body mass, American women still expressed more body dissatisfaction than Bosnian women. These body dissatisfaction differences may be related to the different body ideals within the two cultures. That is, Bosnian women's lower level of body dissatisfaction may be a reflection of achieving the cultural ideal body shape more frequently than their American counterparts since the cultural ideal body shape may be more realistically defined and easier to obtain. Furthermore, we predicted that the levels of stress in both American and Bosnian participants would be positively correlated with their levels of body dissatisfaction. This prediction was based on Slade's (17) theory that stress influences the way women view and treat their bodies. Slade stated



that when women are under stress they might feel they have no control over events in their lives. Since they are unable to control these events, they may turn to something over which they perceive they have absolute control, their bodies. When this attempt is unsuccessful, it leaves women feeling dissatisfied with their bodies. It is also possible that women who experience higher levels of stress than normal may perceive their bodies more negatively as a result of their negative feelings about the stressful situation. Consistent with our prediction, Bosnian participants showed a tendency for higher body dissatisfaction when their level of stress was higher. Although American participants showed similar trend, the stress and body dissatisfaction were not significantly correlated for the American participants. Contrary to our predictions, Bosnian and American participants reported similar levels of stress. We initially expected Bosnian women would report higher levels of stress since they have survived the Bosnian war and are still coping with the effects of the war. It is possible that Bosnian participants' perceptions of stress were based more on their personal experiences than uncontrollable events of war and the aftermath of war. Furthermore, it is possible that Bosnian women felt more stability in other areas of their lives such as their relationships and jobs, which compensated for the stress of the war. In addition, there are some limitations of the scale we used in this study to measure stress. To complete the Impact of Event Scale, participants are asked to choose an event from the past that has been affecting their level of functioning in the last week. It is possible that Bosnian women did not choose the war as that event. We do not have access to this information since most women chose not to write down the name of the event when completing the IES. In future research, this information may be obtained by asking Bosnian participants to what degree the war and the aftermath of war still affects them today.

## CONCLUSION


In addition to the limitations that result from the measure of stress that we employed, there are limitations due to sample size and generalizability. The number of participants was limited in both Bosnian and Ameri-

can samples. Although the number of participants was large enough for statistical analysis, it may be difficult to generate conclusions about either Bosnian or American culture based on these limited samples. In addition, the Bosnian sample mainly consisted of young middle class women who live in the city. Their perception of body issues in Bosnia as well, as their level of stress and body dissatisfaction, may be very different than those of young women who live in villages or are displaced due to the war. Except for the age of participants, the American sample was matched with Bosnian sample very well. However, it is possible that the variations are larger within Bosnian community. A more accurate picture of body issues in Bosnia may be obtained by sampling the population in such way that it includes women from both villages and cities and those who are not as involved in the educational system, especially at the university level. In order to curb the behavioral and perceptual changes that have occurred in other non-Western countries as a result of acculturation to Western culture and its beauty ideals, a few steps could be taken in the future. Social influence, such as the media (23,24), seems to impact women's formation of their self-images. Perhaps, these social influences could be used in a positive way. For example, Bosnian women could be encouraged through sources of the local media to maintain positive outlook on their current bodies by emphasizing the health and emotional problems that may come as a result of trying to reduce one's body size to fit Western's ideals. The media could also help by using models and actresses on the television programs that are representative of the average Bosnian women. In addition, social influence derived from the involvement in school and extra curricular activities could be used to help young women form more holistic views of themselves. Young women could be encouraged to excel in their school, sport, and music work. This could be accomplished by organizing activities in which young women would be awarded for their intellect, physical abilities, communication skills, etc. By being exposed to a complex, supportive environment, young women may realize that their worth is much more than their physical appearance.



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# CAUSAL FACTORS OF ACUTE GASTROENTERITIS IN INFANTS AND YOUNG CHILDREN

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## ABSTRACT

Respiratory, gastrointestinal and skin diseases represent the most common diseases in infants and young children. Causal factors of these diseases are important infectious agents and causes of pathological conditions in children, but they are also very important for their parents, as well as for people in their close environment. Greater incidence of infections in infants and young children can be explained in different ways. A cause can be insufficient maturity of their immune system, but also their exposure to infections within collective accommodations (cribs, nurseries, pre-school institutions), where they are, at the same time, exposed to a number of unknown agents. Today, a great emphasis is devoted to the ways and kinds of children's nutrition. The problem of relation between infected young organism and infectious agent itself, is also reflected in a long resistance and excretion of microorganisms in their exterior environment. It is well-known that microorganisms resist and excrete much longer in younger organisms, compared to adults, where their resistance and excretion is much shorter or very rare. Actually, adults have already formed protective immunity against particular infectious agents. It doesn't prevent infections in adults, colonization of pathogens, nor eventual development of disease. Established immunity can shorten the time necessary for excretion of microorganisms in their exterior environment and, if disease gets developed, it is of shorter duration and slower progress.

**KEY WORDS:** acute gastroenteritis, infants and young children, viruses, bacteria, parasites.

## INTRODUCTION

The number of infections of digestive and respiratory system in children within collective accommodations is important, and can have the influence on their total health system. It is well-known that, during the first months of every new-born child, the possibility of their contamination and disease development is greater, compared to the older age. Their contamination and disease development can be caused even by a small number of microorganisms within inoculum, because of the fact that immune system in infants and young children is underdeveloped, or has a slower progress. Likewise, decision of parents to put their new-born child in the cribs, results in distraction of the rhythm, or absolute discontinuation of nursing. This results in the direct influence on possible genesis and development of children's first infections, which is demonstrated by rotavirus or salmonella infections.

## CLASIFICATION OF MICROORGANISMS

### VIRUSES

**Hepatitis A** represents a well-known disease caused by hepatitis A virus. In developed European countries, the number of seropositive individuals is gradually decreasing; examinations of 18-years-old individuals, during 1977, showed their seropositivity in 50% of cases and, during 1997, in only 11.5% (1). This demonstrates significantly reduced presence and circulation of hepatitis A virus in examined areas, as well as the possibility for developing more severe forms of epidemics, due to daily increasing of population at risk, which is susceptible to this virus. In FB&H, during 1999, 421 cases of hepatitis A were registered, while during 2003, only 53 individuals suffered from this disease. In FB&H, during 2000, 12 hepatitis A epidemics were registered, with 919 ill individuals. It is well-known that, in younger individuals, hepatitis A develops in much greater number as an asymptomatic form, and that the number of contaminated individuals is always greater than officially reported number. According to the CDC's reports, more than 25% of children younger than 6, who were in contact with contaminated adults, suffer from asymptomatic infection forms (2). Some authors have estimated that hepatitis A infection ends as asymptomatic form in 60-80% of cases of very young individuals. *Hepatitis A virus (HAV)* is classified within the genus *Hepatovirus*, family *Picornaviridae*. There is only one known serotype of this virus, but its various genotypes are being discovered in different parts of the world.

The virus has a spherical shape, icosahedral symmetry and the size of 27 to 32 nm. The outer lipoprotein envelope is absent. The viral capsid is composed of 32 capsomers. The virus genome is made of a single stranded RNA with the size of 7.8 kb and with other components. Hepatocytes are primarily infected with hepatitis A viruses; complete cycle of their multiplication is running through hepatocyte's cytoplasm. The virus doesn't express significant cytotoxicity towards infected hepatocytes. Actually, in hepatitis A pathogenesis, immune reactions within infected organism are of predominant importance, on particular cellular level. Hepatitis A virus, as well as hepatitis E virus, only causes acute forms of hepatitis. The virus is resistant in exterior environment, it persists for 1 hour on 60°C, reserves its infectiveness for years on -20°C, and resists for only 5 minutes on 100°C. It is susceptible to UV rays influence, formaline, chlorine compounds and oxidative medium. Dry sterilization and autoclaving deactivate and destroy the virus. In exterior environment, it persists in contaminated water, feces, shells and food. The disease is diagnosed epidemiologically, clinically, according to the results of laboratory analyses (increased values of serum AST, ALT, alkaline phosphatase) and viral-serology examinations. Discovery of specific IgM anti-HAV antibodies indicates the acute phase of viral infection. After treatment, the patient has protective, practically lifelong immunity. The treatment is symptomatic and one of recommendations is resting and diet nutrition. Human immunoglobulin can be used in infected areas. In specific prevention, appropriate vaccine is available. Vaccine is being used for risk groups of populations, populations from highly-endemic areas, as well as individuals who were in contact with contaminated people, or travel to high-risk areas. **Rotaviruses** (lat. Rota – wheel) are classified within the family *Reoviridae*, genus *Rotavirus*. Those are ubiquitous viruses in human environment. They can contaminate water-pump systems, sewages, food or nearest environment. During the period of baby-nursing, over 80% of cases get contaminated with rotaviruses, which means that they are the most frequent causal factors of acute gastroenteritis in this, as well as in infant population of pre-school age. Initial virus infection resigns as diarrhea, the next contact is the cause of mild distraction, while the next, third infection, resigns unknowingly (3). In the life of young population, important source of rotaviruses are malnourished or immunosuppressed children who are, after completed treatment, asymptomatic agents of the virus, for one to three weeks. Asymptomatic virus

carriers can also include inapparent infected children or adults who were in contact with contaminated children. Along with the human representatives, those of animal rotaviruses are also well-known. They all belong to the group of RNA viruses, whose genome is made of double stranded RNA with 11 segments. RNA encodes synthesis of 6 structural and 5 non-structural proteins. VP6 determines classification of human rotaviruses to A, B and C groups. Most of the human rotaviruses species belong to the group A, while, in China, group B is being determined in the cases of epidemiologic disease forms, and group C in sporadic infections of different parts of the world. VP7 glycoprotein determines the serotype (G) rotavirus classification, while glycoprotein VP4 is responsible for their P serotype classification. Group A human rotaviruses are divided in the subgroup I and II. They are consisted of more than 10 serotypes G. Human serotypes 1, 2 and 4 are causes of infections in more than 95% of cases, while serotypes 3, 8 and 9 are rarely found (4). Infected organism synthesizes neutralizing antibodies against viral antigens VP4 and VP7. Along with the human rotavirus representatives, monkey and veal rotaviruses can also be pathogenic for people. Size of the rotavirus is approximately 70 nm. The inner capsid lamina is made of 32 capsomers. Viruses have characteristic shape which can be immediately noticed on the electronic microscope image. Viral infections are possible during the whole year, but they are the most frequent during cold seasons. Hospital infections are registered in new-born children's departments (5). Nosocomial infections, caused by rotaviruses, have one of the most important positions in total number of these infections, since their number is increasing every day. Important source of viruses within hospital departments are asymptomatic carriers; in hospital departments for children's bronchiolitis treatment, rotaviruses are found in 5%, by accidentally analysing feces (1). The same possibilities of rotavirus transmission to the healthy population of children can occur within the institutions for their collective custody, cribs or other interior environments. In the case of every smaller or greater acute gastroenteritis epidemic, it is very important to determinate a casual factor, so that appropriate sanitary and other precautions, as well as preventing the casual factors from transmission from children to adults and in the opposite way, can be performed on time. Today, the values of Rhesus rotavirus tetravalent active vaccine (RRV-TV) (RotaShield, Wyerst-Ayerst Pharmaceuticals, Wayne, Pa), which is composed of RRV mixture (Rhesus rotavirus, serotype G3) and three types of genetically modified monkey-hu-

man virus types (reassortant), type G1, G2 and G4, is being intensively clinically examined. The vaccine would be ordinated per os, in the new-born children aged 6 weeks, in three doses and with the distance of 3 weeks. It would also protect infected individual from development of severe gastroenteritis forms. **Reoviruses** are classified within the family *Reoviridae*, genus *Reovirus*. There are three known antigen types of this virus, type 1, 2 and 3. Virus types have common, as well as different antigen characteristics, which can be demonstrated by reactions of neutralization or inhibition hemagglutination. All reovirus serotypes agglutinate human "O" blood group-erythrocytes, and type 3 agglutinates beef and sheep erythrocytes. The viruses have a rounded shape, the size of 80 nm, and virus genome which is composed of 10 double stranded RNA-segments. They have double-laminated capsid, but the outer lipoprotein envelope is absent. In infected cells of digestive system, infection development is slow, the time of virus multiplication is long, and multiplied viruses leave the cell after the cellular lysis and go to intercellular area. In intestinal cells, reovirus serotype 1 is the best multiplied. Multiplied virus' capability of spreading into Peyer's plates and mesenteric lymph nodes, depends on activity of their hemagglutinins (6). Reovirus infections in children have been found in different parts of the world, but they can also be discovered in water-pump systems and canalization water. They are transmitted to humans by the faecal-oral route. It is demonstrated, by epidemiological studies, that these viral infections occur in childhood, and that examined individuals, aged to 3 years, show seropositivity in approximately 70% of cases. Viral infections can resign inapparently, or manifest through the symptoms of digestive system infections. Digestive problems are caused by serotype 1 and sometimes serotype 2. Diarrhea is mild, lasting 2-3 days, often ending without any therapy. If the treatment is necessary, virus etiology methods are used, as in cases of other diarrheal conditions. Reovirus serotype 3 infects upper parts of the respiratory system. There is no specific prevention from these viral infection; general sanitary precautions are available. **Coronaviruses** are classified within the family *Coronaviridae*. They are named by their morphology, lat. corona = crown. They belong to RNA virus group. RNA is single stranded and non-segmented. Their size is approximately 160 nm. On the outer viral lipoprotein envelope, there are glycoprotein grafts with antigen and hemagglutinating characteristics. The grafts look like flower lobes, through which viruses communicate or adsorb to receptors of sensitive cells. They multiply in the cy-

toplasm of infected cells, and mature budding on the Golgi device and endoplasmic reticulum. This group of viruses doesn't have the possibility of budding on the cellular membrane itself. New-multiplied viruses are self-protecting from cytoplasmic enzymes, by agglomerating within cellular vesicula, which they leave after their fusion with the inner side of cellular membrane. Coronaviruses transmit to the susceptible organism by respiratory, faecal-oral or mechanical route, and infect susceptible cells of respiratory, digestive or nerve systems. They are prevalent in all parts of the world, with possibility of infection in all age- and sex-groups of population. In pre-school aged children, infections of gastrointestinal system are the most frequent and discovered during the whole year. The infection is manifested by diarrhea and, in new-born children, necrotizing enterocolitis can also develop. In feces samples, viruses are demonstrated by EM or immunoelectronic microscopy (IEM). The treatment is symptomatic. There is no specific prophylaxis; general sanitary precautions are available. Representatives of human calciviruses, virus **Norwalk** and morphologically similar viruses (**Norwalk-like, Hawaii, Snow Mountain, Tounton Cokle, Paramatta**) are classified within the family Calciviridae. All these viruses can infect susceptible organism and cause acute gastroenteritis in young children and infants. Calciviruses belong to the group of RNA viruses. Viral capsid is composed of 32 capsomers which are concave and cup-shaped (lat. Calix-cup). This is the group of viruses without lypoprotein envelope. On the EM image, they have characteristic morphology and the size of 35 to 40 nm. They multiply in the cytoplasm of infected cells and leave them after cellular lysis. They infect new-born children, aged from one month to one year. Disease has a short incubation period (48-72 hours), with diarrhea, lasting from 1 to 11 days. Infections occur during the whole year, but they are more frequent in winter. Viruses are transmitted within the population by the faecal-oral route, with more frequent occurring disease within internal collectives, institutions, colleges, schools and hospitals. In feces samples, the viruses can be demonstrated by EM, IEM or ELISA method. Viruses excreted through feces can contaminate water, food or objects of general use. The treatment is not specific, there is no specific prophylaxis. General principles are used in treatment, and general sanitary precautions in prevention from infection are recommended. Norwalk and similar viruses are classified within the family Calciviridae. Norwalk virus is discovered in Norwalk, Ohio, SAD, as well as in other parts of the

world. Similar particles are discovered later. Norwalk virus' size is 27 nm. It has single stranded RNA. Based on his morfology, antigen and serology characteristics, it is different from other members within the family. Also, individual members of this group of viruses can be differentiated by serology methods. Infection in young and non-immune organism leads to development of acute gastroenteritis with short incubation period (around 24 hours). The disease occurs suddenly and shows general infective symptoms. Viruses are excreted from infected organism through feces, and transmitted to susceptible organism by the faecal-oral route. Along with infected and ill individuals, contaminated water and food can also be the source of these viruses. After treatment, short protective serotype immunity remains. General-accepted principles are recommended in treatment. There is no specific prophylaxis. Norwalk-like, Hawaii, Snow Mountain, Taunton Cokle, Paramatta and other Norwalk-like particles can cause disease with similar clinical and epidemiological characteristics. They can be differentiated and identified by IEM. **Adenoviruses type 40 and 41** belong to the genus **Mastadenovirus**, within a large family **Adenoviridae**. All members of this genus primarily infect humans, playing important role in their total health. There are a few groups and 50 known adenovirus serotypes. They all have double stranded DNA, whose genome is capable for encoding proteins, thanks to 30 and 40 included genes. Their size is from 70 to 90 nm, they have icosahedral symmetry, with the capsid composed of 252 capsomers. They don't have lypoprotein envelope. They have penton, hexon and fibrous antigens (with hemagglutinational capabilities). Viruses are connected to specific receptors of sensitive cells via fibrous antigens. They infect epithelial cells of susceptible organism, multiplying in their nucleus and cytoplasm. New-multiplied viruses leave the infected cells after cellular lysis. Human adenovirus types can infect susceptible organism, causing various clinical entities. Diseases can be caused by one or more different adenovirus serotypes, but different diseases can also be caused by the same virus types. Some adenovirus types (type 40 and 41, 31 rare) infect epithelial intestinal cells, where they multiply and excrete to exterior environment through feces. 40 and 41 adenovirus types are causes of acute gastroenteritis in infants and young children, with similar clinical and epidemiological characteristics, as in the case of rotavirus infections. In the therapy and prophylactic sense, there is no difference compared to other viral infections. **Astroviruses** are classified within the family **Astroviridae**. They are the causes of easier infection forms and



diseases in pre-school aged children. Except human, there are also animal infections (calves, lambs, poultry, dogs). Viral particle has the size of 27 to 30 nm, single stranded RNA is placed within nucleus. Viruses have four structural proteins. Based on antigen morphology, there are eight discovered human astrovirus serotypes. Acute gastroenteritis in infants and young children is characterized by occurrence of abdominal cramps, nausea, high temperature and diarrhea. Diarrhea lasts between 3 and 14 days, feces is liquid and mucous. Easier form of dehydration is present. Viruses are excreted in exterior environment through feces, and transmitted to a susceptible organism by the faecal-oral route. Intrafamilial infections are recorded in the case of these viruses. In feces samples, viruses can be demonstrated by IEM and ELISA method. In the treatment of ill individuals and prevention from infections, generally-accepted principles are in use, since there is still no specific medicines nor preventive precautions. **Echo viruses** (32 serological types) represent potential infective agents in infants and young children. They are classified within the family Picornaviridae, genus Enterovirus. This is a group of RNA viruses, sized from 24 to 25 nm, with icosahedral symmetry. They multiply in the cytoplasm of infected cells, which they leave after cellular lysis. They express significant selectivity and tropism. Since they are resistant viruses, they persist very long outside of infected organism. They are excreted through feces, and transmitted by the faecal-oral route. During the infection of susceptible cells in upper part of respiratory system, they spread towards the cells of digestive system, from which they can get to target cells through blood, causing easier forms of serous meningitis (6). Infections in young individuals can be manifested by febrile rash condition, as well as by development of respiratory and digestive problems. Isolation and identification of Echo viruses is performed in GMK cells, which is exception compared to above described viruses. After treatment, type specific, opposite and short immunity is developed. There is still no specific medicine, nor vaccine for these viral agents.

## BACTERIA

**Salmonellae** are the group of bacteria which can cause infections and diseases in children older than 5. In examined areas of the world, the most frequent isolated salmonellae from examined group belong to particular serotypes (*S. enteritidis*), which depends on epidemiological situation within examined region. Salmonella infections in infants younger than 5, result in development of bacteria-carrier occurrence, which can last longer than

three months (1). This date is important in the case of accommodating asymptomatic salmonella-carriers in institutions of collective accommodation, since these infectious agents transmit to susceptible individuals by the alimentary route, via contaminated hands or objects from their nearest environment. This is the reason why various salmonella types become important causal factors of epidemic acute gastroenteritis type, within the institutions for retenting children aged to one year, or within the institutions for accommodating individuals with decreased resistance towards infectious agents. With the purpose of prevention from occurring particular infections and diseases, continuous systematic studies and discovering asymptomatic carriers of particular kinds of microorganisms are necessary, as well as performing particular contraepidemic precautions within the institutions for accommodating pre-school aged children. Bacteria within the genus *Salmonellae* belong to the family **Enterobacteriaceae**, as well as the genus *Shigellae*. Salmonellae are Gram-negative, mostly mobile bacilli, which have somatic-O, flagellar-H and envelope-Vi antigens. They don't have a capsule and do not form spores. H-flagellar antigen occurs in two forms, as phase 1 and phase 2. Salmonellae which have a phase 1 antigens are marked as monophasant, compared to biphasant salmonellae, with phase 1 and 2 antigens. Phase 1 and 2 H-antigens enable division of salmonellae to particular serotypes. Today, there is around 2300 known salmonella serotypes which can infect humans and various animal species. Several various salmonella serotypes can have some of the common flagellar antigens. O or somatic antigens are composed of lipopolysaccharides of the cellular wall. They have complex constitution, in one of their parts they have components with characteristics of bacterial endotoxins. Based on O antigen, all known salmonella serotypes are divided into particular groups. It is well-known that, within the first five groups, there are representatives of salmonellae pathogenic for humans. Vi or envelope-antigen represents the surface antigen, which can cover O antigen. Vi antigen has limited number of salmonellae. All salmonellae are cultivated on Salmonella-Shigella (SS), Wilson-Blair, deoxycholate-cytrate agar or other media. Human infections with particular salmonella species can cause development of general cyclic infectious diseases (abdominal typhoid and paratyphoid A and B), alimentary toxic-infections or enteritis. As causal factors of diseases in infants and young children, salmonellae are also important as infective agents of salmonellosis, as well as of alimentary toxic-infections. In ill children, diarrhea can endanger their general health condition, with pos-

sible development of septicaemia. It is necessary to pay a special attention to asymptomatic carriers as the source of infectious agents in children' and adults' population, as well as to their relation towards this infection (1, 7). In FB&H, during 2002, 4195 individuals were registered with clinical diagnosis of enterocolitis, as well as 351 cases of salmonellosis and 19 salmonellosis carriers. From the data source, information about the age of ill individuals or carriers is not available, nor about their geographic distribution. Also, during 2002, 1021 cases of alimentary toxic-infection were registered. In FB&H, during 2003, 4199 individuals were registered with clinical diagnosis of enterocolitis, as well as 419 cases of salmonellosis and 29 carriers. During the same year, 1024 alimentary toxic-infections were registered, but without indicated age of infected and ill individuals.

**Shigellae.** Infections by this bacteria species in pre-school children, can end with development of severe infection form and with damaging colonic mucous cells, with possible complications and particular number of deaths (1). Compared to salmonella infections, shigella infections are limited to epithelial cells of colonic mucous and the surface side of lamina propria. Practically, there is no penetrating of these bacteria into the blood (8). Infections by this bacteria group are possible within internal children' collectives. Around 50% of infected and ill children gets hospitalized. It is important to emphasize that all infection cases should be evidenced and reported to appropriate institution. Occurrence of only one shigellosis case requires systematic research, which includes discovering asymptomatic or mild-infected individuals. In particular cases of children' infections, the disease can develop a form of acute dysentery syndrome, which is manifested through hemorrhagic inflammation of the colon mucous, with occurring mucous-bloody feces, tenesmus and cramps. Shigellae are transmitted by the faecal-oral route, via contaminated hands and objects, or through contaminated excrements of ill individuals. Ill individuals and carriers are important source of infective agent, and flies play an important role as the way of their spreading, through contaminated food and water. Carrying is rare, of short duration, for only few weeks, with rare exceptions for more than a year. Developed disease form, along with the hospitalization, also requires rehydration and diet nutrition, as well as antibiotic therapy when necessary. It is important to emphasize that, in children' institutions, rigorous sanitary precautions should be performed, and in the case of disease occurrence, particular objects and institutions should be closed. Bacteria from *Shigella* genus are Gram-negative and immobile bacilli, which do not form any spores, nor

synthesize the capsule. They have fimbriae. Based on their biochemical and antigen characteristics, they are classified in four subgroups (A, B, C, D). The subgroup A (*Sh. dysenteriae*) includes 12 serotypes, subgroup B (*Sh. flexneri*) 6 serotypes and 2 varieties, while there are 18 serotypes in the subgroup C (*Sh. boydii*). Only one serotype with two phases of occurrence belong to the subgroup D (*Sh. sonnei*). All mentioned bacterial serotypes can cause acute disease or bacillary dysentery. The disease is manifested as a local illness with intracellular multiplication of bacteria, development of inflammatory changes, cellular necrosis, occurrence of ulceration and hemorrhage. *Sh. dysenteriae* type 1 excretes Shiga toxin, enterotoxin and cytotoxin. Disease diagnosis is determined based on the clinical findings, epidemiological data and laboratory examination of feces samples (coproculture). Except microscopic analysing, feces is cultivated on the blood, endo and Salmonella-Shigella agar. Media are incubated on 37°C for 24 hours. Grown bacterial colonies undergo biochemical and serology typing, to the subgroup or serotype level. In FB&H, during 2002, 20 cases of bacillary dysentery were registered, without available data about the age of ill individuals, type of isolated bacteria, or their regional distribution. During 2003, only 17 cases of infection with these bacteria were registered in FB&H.

**Compylobacter jejuni.** In the countries with the low, as well as the high sanitary standard, compylobacteria are quite frequent causal factors of diarrheal diseases in infants and young children. Infections can resign as asymptomatic or severe disease forms. Enteritis caused by these bacteria are included in the group of zoonosis, since animals (poultry, cows, dogs) and their products, milk and meat, represent the source of infection. Inter-human transmission of these bacteria is possible within departments for new-born or young children, or within families. The disease occurs during warmer months. Morphologically, there is no difference between *C. jejuni* and *C. coli*, *C. fetus* and other species, which are one of 15 known species and subspecies within the genus (9). They are Gram-negative, bent and thin little rods. They have flagella, do not form any spores. They are cultivated on special selective media (Skirrow) in microaerophil conditions and 42°C. Grown colonies express the poor biochemical activity. For humans, pathogenic compylobacter species, oxidase and catalase are positive. Some *C. jejuni* species excrete cytotoxin, and all species have endotoxin and excrete enterotoxin. Infection in new-born and young children can be manifested as acute enterocolitis. In the cases of severe disease forms, development of dehydration is present, which requires

hospitalization. Caring is possible, of short duration, for only several weeks. The therapy is symptomatic, compensation of lost liquid and electrolytes is recommended, as well as diet nutrition. Severe cases require hospitalization, as well as antibiotic treatment (erythromycin). Except *C. jejuni*, it is also possible to isolate *C. coli* from feces samples. Its presence in causing the disease varies between 5 and 10%, although there are reports about its more frequent isolation. According to the reports from our neighborhood about acute enterocolitis causal species, *Compylobacter* is found in 3.86%. *C. jejuni* is isolated in 75% of cases (9). There is no possibility for specific disease prevention; general sanitary precautions are available, along with sanitary controlling the water and food product quality. In our region, **Escherichia coli** still participates as an important etiological factor, causing diarrheal diseases in pre-school aged children. In developed countries, the importance of *E. coli* in causing acute gastroenteritis in new-born children is minimal, but it is significant in infant's infections with enterohemorrhagic *E. coli*. In the case of diarrhea, caused by enterohemorrhagic *E. coli* (O157), it is necessary to perform all required examinations, to analyse available feces samples from environment and perform appropriate precautions. This infection is important especially within nurseries or internal children' collectives. The members of the genus are Gram-negative and mobile bacilli which have O, H i K antigens. Most of the *E. coli* species isolated from feces samples, are included in facultative anaerobic flora of the human digestive system, but only small number of isolates can be associated to the human infections. All types of these bacteria have fimbriae or pili, which provide their adhesional capability. Most of the isolated species are successfully cultivated on in vitro media (blood and endo agar), they are biochemically active and belong to the group of lactose-positive bacteria. Most of them ferment carbohydrates to the acid and gas. *E. coli* isolates can have characteristics of enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC) and enteroaggregative *E. coli* (EAEC). Enterotoxigenic *E. coli*, with the enterotoxin, causes diarrhea in new-born and young children in developing countries. Enterohemorrhagic *E. coli* causes sporadic and epidemic forms of hemorrhagic colitis, through the local excretion of Shiga like toxin 1 and toxin 2. A special attention should be paid on the patients from which *E. coli* O157:H7 is isolated. Actually, this bacterial infection in these patients can develop hemolytic uremic syndrome.

## PARASITES

**Giardia intestinalis** represents one of the causal factors of acute gastroenteritis in pre-school aged children. After the first contact between a non-immune person and this parasite, short diarrhea can develop, which can be treated and properly cured. Lamblyase is cosmopolitan disease, whose causal factor is isolated from analysed feces samples in 2-7% of examined individuals from Northern and Central Europe, and in 10% from Southern Europe (8, 10). Vegetative or, more frequent, cystic parasite forms can be found in feces. *L. intestinalis* cysts are transmitted by the faecal-oral route, contaminated water or food. Hydric epidemics are also discovered. In infants and young children, cysts can also be transmitted via contaminated hands. If disease is suspected, diagnosis is based on laboratory analysis of cysts or vegetative parasite forms from feces samples or from duodenum contents. There is no specific prevention, general precautions are available, as well as recommendations about the use of microbiologically clean water and personal sanitary precautions, such as washing hands or fruits and vegetables.

## CONCLUSION

Causal factors of acute gastroenteritis in infants and young children are viruses, bacteria and parasites. Acute gastroenteritis of viral etiology in infants and young children is caused by hepatitis A viruses, rotaviruses, caliciviruses, *Norwalk* and *Norwalk-like* viruses, adenoviruses, astroviruses, coronaviruses, as well as Echo viruses. Acute gastroenteritis of bacterial etiology is caused by several salmonella and shigella serotypes, but also by *Compylobacter jejuni* and different types of *Escherichia coli* and, when we talk about parasites, by *Giardia intestinalis*. Etiologic role of these causal factors in causing diseases must be laboratory-demonstrated, since this is not possible to perform based on clinical image of disease or epidemiological data. This requires knowledge and the use of available laboratory diagnostic methods in virology, bacteriology, parasitology and immunology domain. Each year, a certain number of infected and ill individuals that suffer from hepatitis A is registered in Federation of Bosnia and Herzegovina and, during 2000, 12 hepatitis A epidemics with 919 ill individuals were also registered. Rotavirus infections are not being officially registered, as well as other virus infections, but, according to the reports from several hospital centres, they are being discovered and described in their reports and published works. In the Clinic for Infective Diseases in Tuzla, between 1999-2000, rotaviruses are demonstrated as etiology agents in 23.9%, enteric adenoviruses in 1.5%,

and mixed infection in 1% of individuals infected by rota and *Shigella sonnei*, and 0.5% by rota and EPEC (enteropathogenic *Escherichia coli*) (11). In FB&H, during 2002, 4195 individuals with clinical diagnosis of enterocolitis, 351 cases of salmonellosis and 19 salmonella carriers were registered. Information about the age of ill individuals or carriers is not available from the source. Also, during 2002, 1021 cases of alimentary toxicoinfection were registered. In FB&H, during 2003, 4199 individuals with clinical diagnosis of enterocolitis were registered, as well as 419 cases of salmonellosis and 29 carriers. During the same year, 1024 alimentary toxicoinfections were registered, but without indicated age of infected and ill individuals. In FB&H, during 2002, 20 cases of bacillar dysentery were registered, without available data about the age of ill individuals, the type of isolated *Shigella* or their regional distribution. During 2003, 17 cases of infections by these bacteria were registered in


FB&H. We do not have any data about other bacterial or parasite causal factors of these diseases. In specific prophylaxis of diseases, appropriate vaccine is developed for several causal factors. For other causal factors, specific vaccines are being investigated, but they all should:

- give specific protection to new-born children against infective agent, even in the first weeks of their life,
- decrease pathogenic influence of microorganisms, prevent development of severe disease forms,
- provide development of local protective immunity,
- provide specific protection towards all serotypes of specific infective agents.

Along with specific prophylaxis, supervisions of collective accommodation objects for young children are also important, as well as performing rigorous sanitary procedures, individual or collective.

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# EFFECTS OF NEBIVOLOL ON ARTERY HYPERTENSION- MULTICENTRE STUDY BOSNIA AND HERZEGOVINA

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## ABSTRACT

Hypertension is a major risk factor for cardiovascular diseases; drugs that reduce blood pressure and simultaneously improve or reverse endothelial dysfunction, as nebivolol, may be advantageous in terms of cardiovascular protection. The objective of this study is to show the anti-hypertensive efficacy and safety of nebivolol (5 mg once a day) given to patients with arterial hypertension for 3 months. It should also provide information about drug's influence on laboratory tests - fasting blood glucose and serum cholesterol, triglyceride and creatinine concentrations. Six centers - Tuzla, Sarajevo, Mostar, Bihać, Zenica and Banja Luka participated in this prospective study with follow-up period of 3 months that included 3 visits. The study group consisted of 328 hypertensive patients. Results showed a significant decrease in both systolic and diastolic blood pressure and heart rate at the end of the study. Fasting blood glucose level and serum cholesterol, triglyceride and creatinine changed significantly during the study, with lower levels of all the tests. Nebivolol seems to be free from some of the problems that generally accompany not only the classical beta-blockers but sometimes also newer classes of antihypertensive drugs. With its high anti-hypertensive efficiency and safety, and presence of statically significant difference in laboratory tests and beneficial effects, absence of adverse interaction with glucose and lipid metabolism, patients treated with Nebivolol may show an optimal adherence to therapy.

**KEY WORDS:** arterial hypertension, endothelial dysfunction, nebivolol



## INTRODUCTION

Arterial hypertension is a major modifiable risk factor for cardiovascular diseases that can, if untreated, result in serious morbidity and mortality from cardiac, cerebrovascular, vascular and renal diseases (1). The treatment of hypertension has evolved over the last years as we accumulated knowledge of the natural history, pathophysiology, and risk factors for hypertension as well as the effects of therapy and interactions of this factors. The goal of treating high blood pressure is to reduce blood pressure and prevent or reverse end-organ damage. Beta-adrenoreceptor blockers have long been established for the treatment of hypertension and much of the evidence that they reduce the risk of developing serious cardiovascular complications is based on clinical trials that used this class of drugs (2,3). They were recommended as one of the initial medications for the treatment of hypertension by the fifth and sixth National Committees on Detection, Evaluation and Treatment of High Blood Pressure and the World Health Organization-International Society of Hypertension (4-7). Effective antihypertensive therapy should reduce vascular resistance without impairment of cardiac output, a measure of both systolic and diastolic function. Nebivolol, a highly selective  $\beta_1$ -adrenoreceptors blocker, actually decreases arterial blood pressure by reducing systemic vascular resistance without depressing left ventricular function (8,9), inducing an endothelium-dependent vasodilatation, which arises from the release of nitric oxide (NO). In hypertensive patients the basal and stimulated production of nitric oxide is reduced and the normal balance between vasodilating and vasoconstricting factors is modified with a decrease in vasodilation and an increase in vasoconstriction, so endothelial dysfunction may be considered as a target for the treatment of hypertension. The most common cause of death in patients with high blood pressure are complications from atherosclerosis. Nitric oxide plays a protective role as it prevents

monocyte adhesion, platelet aggregation, vascular smooth-cell proliferation and migration, events known to be associated with arteriosclerosis and thrombosis development. Beta adrenergic blockers have been shown to reduce hypertension-related cardiovascular and cerebrovascular morbidity and mortality in long-term clinical trials (10,11). Antihypertensive therapy should be directed toward controlling all the patient's cardiovascular risk factors. Fasting blood glucose, serum cholesterol and triglycerides and serum creatinine provide information on potential cardiovascular risk factors and also establish a baseline for the effects of drug therapy.

## MATERIALS AND METHODS

The study was designed as a prospective study with follow-up period of 3 months including 3 visits for each patient. The study population consisted of 328 patients, 177 female and 151 male, mean age  $56,78 \pm 9,47$ , mean weight  $80,59 \pm 14,29$  and mean body mass index (BMI)  $27,38 \pm 4,26$ . We included patients with blood pressure  $> 120/80$  who were recruited from centers in Tuzla, Sarajevo, Mostar, Bihać, Zenica and Banja Luka. Patients were classified according to JNC classification in three groups: (Table 1-3; Figure 1,2)

1. Group with pre-hypertension (blood pressure level- 120-139/80-89 mm Hg),
2. Group with the first-degree hypertension (blood pressure level- 140-159/90-99 mm Hg) and
3. Group with the second-degree hypertension (blood pressure level 160-230/100-140 mm Hg).

At every follow-up visit a thorough clinical was performed (including measurements of systolic blood pressure, diastolic blood pressure and heart rate, which were

CATEGORY	SBP (MM HG)	DBP (MM HG)
NORMAL	<120	<80
PREHYPERTENSION	120-129	80-84
	130-139	85-89
STAGE I	140-159	90-99
STAGE II	160-179	100-109
	$\geq 180$	$\geq 110$

When a patient's systolic (sbp) and diastolic (dbp) blood pressures fall into different categories, the higher category should apply.

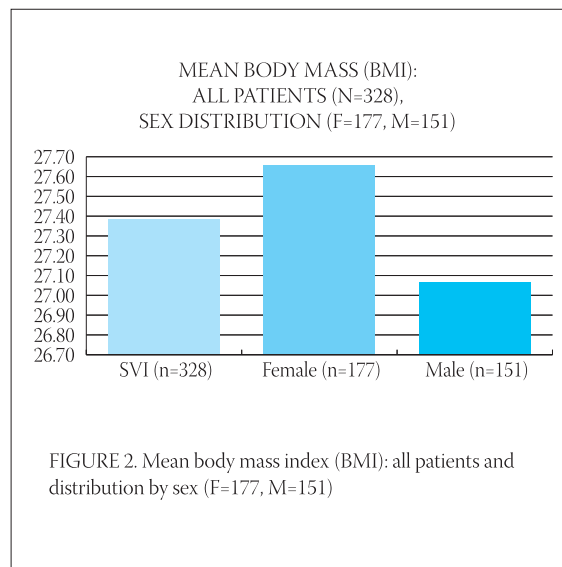
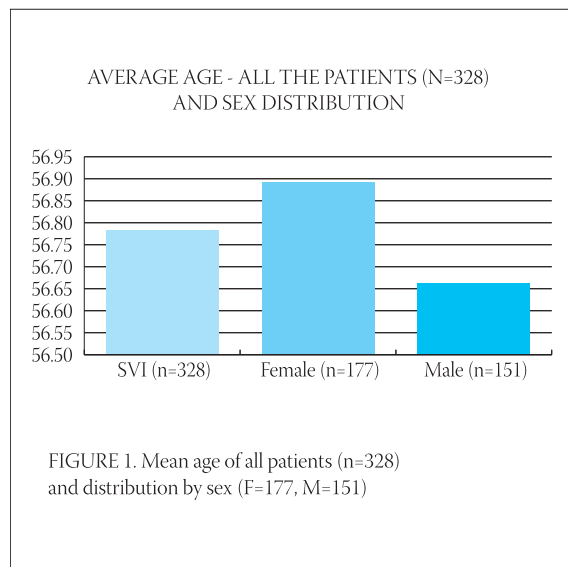
TABLE 1. Blood Pressure Classification by JNC 7.



AGE	ALL (N=328)	FEMALE (N=177)	MALE (N=151)
AV	56,78	56,89	56,66
SD	9,47	9,13	9,88
MAX	80,00	80,00	78,00
MIN	30,00	35,00	30,00
MED	56,00	56,00	56,00
COUNT	328,00	177,00	151,00

Note: AV – average value, SD – standard deviation, MAX – largest value, MIN – smallest value, COUNT – counts of numbers.

TABLE 2. Age of patients, all patients and distribution by sex



BMI	All (n=328)	FEMALE (n=177)	MALE (n=151)
AV	27,38	27,65	27,06
SD	4,26	4,72	3,65
MAX	49,59	41,82	49,59
MIN	17,86	17,86	19,33
MED	27,16	27,47	26,86
COUNT	328,00	177,00	151,00

Note: AV – average value, SD – standard deviation, MAX – largest value, MIN – smallest value, COUNT – counts of numbers.

TABLE 3. Body mass index (BMI), all patients and distribution by sex

evaluated prior to the drug intake using mercury sphygmomanometer) and blood samples drawn for blood chemistry (fasting blood glucose and serum cholesterol, triglyceride and creatinine). After initial evaluation patients were prescribed nebivolol in daily dose of 5 mg, and monitored during period of 3 months. We calculated basic descriptive statistical parameters (mean value- x, standard deviation- SD, largest value, smallest value, median) and used Student t-test, Wilcoxon Signed Rank Test and Kruskal-Wallis One Way Analysis of Variance on Ranks to determine statistical differences.

## RESULTS

The study was completed by 328 patients (95,5%), while 18 patients (4,5%) withdrew.

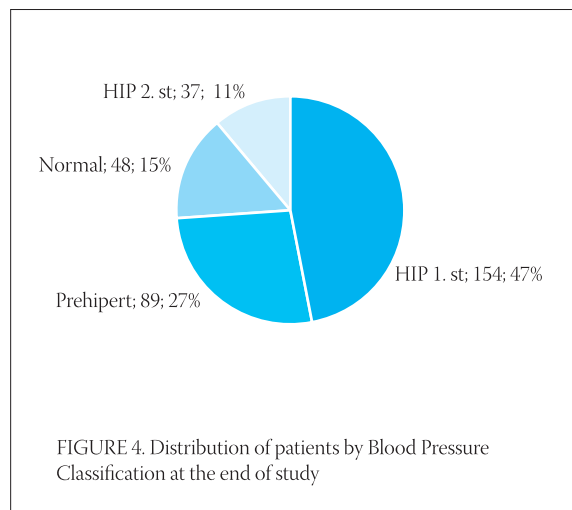
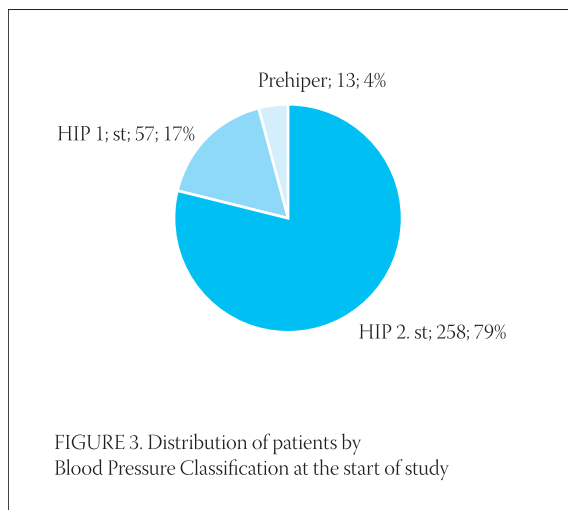
Table 4. and Table 5. show results of descriptive statistic- mean and standard deviation for systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate at the start and at the end of study.

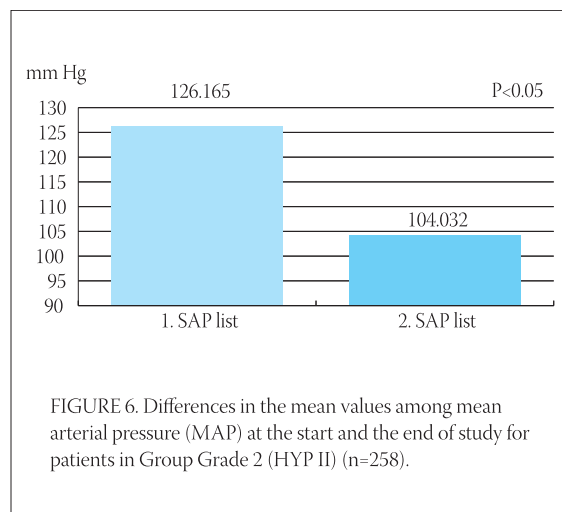
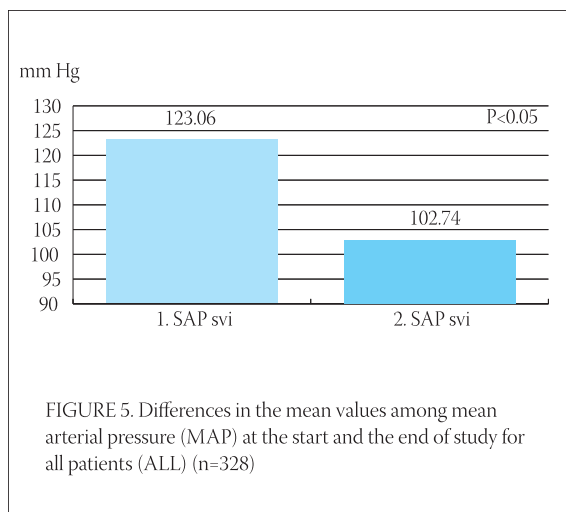
BLOOD PRESSURE CLASSIFICATION	n	SYSTOLIC BLOOD PRESSURE (SYS) MM HG		DIASTOLIC BLOOD PRESSURE (DIA) MM HG		MEAN ARTERIAL PRESSURE (MAP)	
		1. measurement ±SD	2. measurement ±SD	1. measurement ±SD	2. measurement ±SD	1. measurement ±SD	2. measurement ±SD
Prehypertension	13	130±0	122,3±4,29	89,23±2,77	80,77±4,94	102,82±1,85	94,62±4,42
		W= -55,000 T+ = 0,000 T- = -55,000 P(est.)= 0,002 P(exact)= 0,002		W= 81,000 T+ = 86,000 T- = -5,000 P(est.)= 0,004 P(exact)= 0,002		W= -66,000 T+ = 0,000 T- = -66,000 P(est.)= 0,003 P(exact)= <0,001	
Stage I	57	147,86±5,07	131,3±7,96	97,28±6,93	82,67±6,68	114,14±4,84	98,88±5,94
		W= -1431,000 T+ = 0,000 T- = -1431,000 (P = <0,001)		W= -1485,000 T+ = 0,000 T- = -1485,000 (P = <0,001)		W= -1596,000 T+ = 0,000 T- = -1596,000 (P = <0,001)	
Stage II	258	175,58±14,18	142,12±13,37	101,46±9,16	84,99±7,42	126,17±8,87	104,03±8,37
		W= -32880,000 T+ = 8,000 T- = -32888,000 (P = <0,001)		W= -32725,000 T+ = 85,500 T- = -32810,500 (P = <0,001)		W= -32896,000 T+ = 0,000 T- = -32896,000 (P = <0,001)	
All	328	168,8±18,47	139,4±13,44	100,19±9,1	84,4±7,28	123,06±10,3	102,74±8,26
		W= -51309,000 T+ = 25,500 T- = -51334,500 (P = <0,001)		W= -51146,000 T+ = 107,000 T- = -51253,000 (P = <0,001)		W= -52648,000 T+ = 1,000 T- = -52649,000 (P = <0,001)	

TABLE 4. Mean and standard deviation for systolic blood pressure, diastolic blood pressure and mean arterial pressure for all groups of patients, at the start and the end of study.

BLOOD PRESSURE CLASSIFICATION	1. MEASUREMENT ±SD	2. MEASUREMENT ±SD
PREHYPERTENSION	72,83±4,04	71,33±2,43
	t = 2,691 with 11 degrees of freedom. (P = 0,021)	
STAGE I	79,2±10,87	69,54±6,12
	W= -879,000 T+ = 33,500 T- = -912,500 (P = <0,001)	
STAGE II	85,23±15,17	72,63±8,23
	W= -24763,000 T+ = 1839,000 T- = -26602,000 (P = <0,001)	
ALL	83,74±14,54	72,1±7,87
	W= -37046,000 T+ = 2574,500 T- = -39620,500 (P = <0,001)	

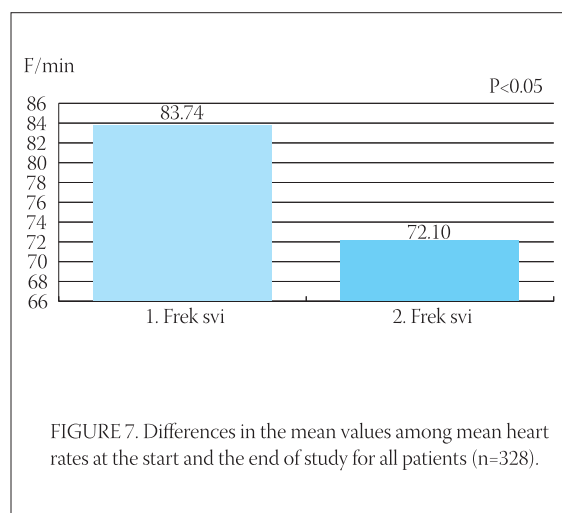
TABLE 5. Mean and standard deviation for heart rate (HR) at the start and the end of study.





KRUSKAL-WALLIS ONE WAY ANALYSIS OF VARIANCE ON RANKS

We used ANOVA test – Kruskal Wallis to analyze one group of results, there we repeat measurement in some patients- four measurements of blood pressure and heart rate and table 6 shows descriptive statistic for subgroup of patients called GroupHYP 2, n=146. Table 12. show results of t-test and significance level for fasting blood glucose, serum creatinine, cholesterol and triglyceride at the start and the end of the study. There was a significant difference ( $p< 0,001$ ) in fasting blood glucose, serum creatinine and triglyceride at the end of the study, with significant decrease, and statistical significant difference ( $p< 0,045$ ) in serum cholesterol level at the end of the study, which is shown in Table 11. and 12.



COLUMN	SIZE	MEAN	STD DEV	STD. ERROR	C.I. OF MEAN
1.Sys II st	146	178,19	13,82	1,14	2,26
2.Sys II st	146	159,77	14,75	1,22	2,41
3.Sys II st	146	150,53	14,75	1,22	2,41
4.Sys II st	146	144,16	14,42	1,19	2,36
1.Dia II st	146	103,60	9,25	0,77	1,51
2.Dia II st	146	94,78	9,34	0,77	1,53
3.Dia II st	146	89,90	9,11	0,75	1,49
4.Dia II st	146	85,87	8,12	0,67	1,33
1.Map II st	146	128,46	8,56	0,71	1,40
2.Map II st	146	116,44	9,78	0,81	1,60
3.Map II st	146	110,11	9,85	0,82	1,61
4.Map II st	146	105,30	9,11	0,75	1,49
1.HR II st	146	84,63	14,12	1,17	2,31
2.HR II st	146	77,51	10,59	0,88	1,73
3.HR II st	146	74,64	9,17	0,76	1,50
4.HR II st	146	72,30	9,29	0,77	1,52

Note: Mean – average value, Std. Dev – standard deviation, Std. Error – standard error, CI – confidence of interval, Size – counts of numbers

TABLE 6. Descriptive statistic for Group HYP 2, n=146.

GROUP	N	MEDIAN	25%	75%
1.Map II st	146	126,667	122,667	132
2.Map II st	146	116,667	110	123,333
3.Map II st	146	110	103,333	116,333
4.Map II st	146	103,333	98,333	110

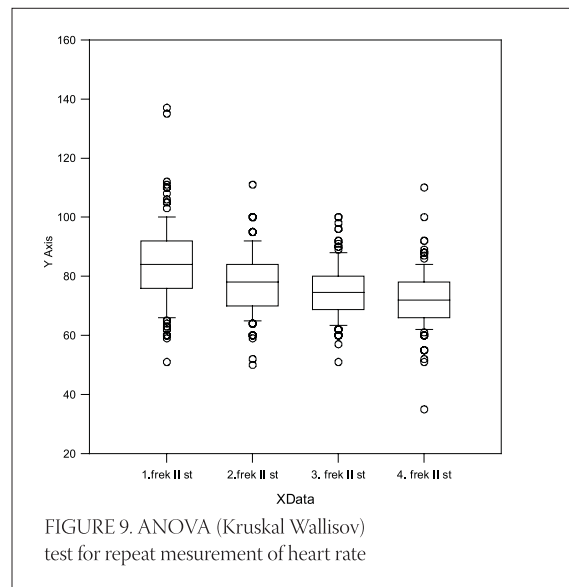
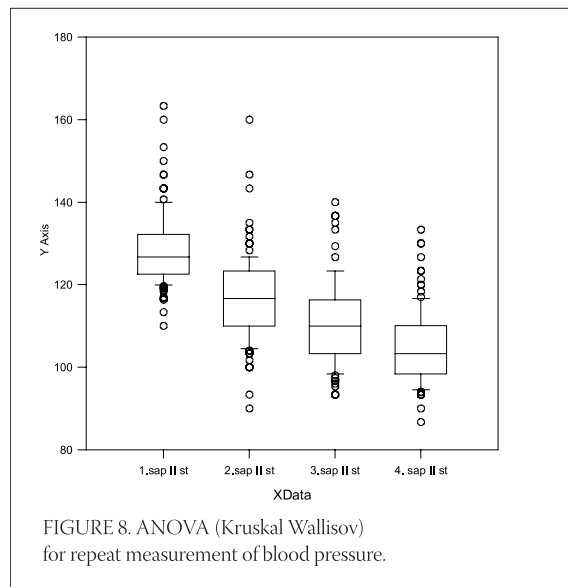
H = 287,306 with 3 degrees of freedom. (P = <0,001)

TABLE 7. Kruskal-Wallis One Way Analysis of Variance on Ranks

COMPARISON	DIFF OF RANKS	Q	P<0,05
4.Map II st vs 1.Map II st	314,77	15,94	Yes
3.Map II st vs 1.Map II st	249,72	12,65	Yes
2.Map II st vs 1.Map II st	157,39	7,97	Yes

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0,001)

TABLE 8. Multiple Comparisons versus Control Group (Dunn's Method)



Heart Rate (F/min) ANOVA test for Group HYP 2, n=146.

KRUSKAL-WALLIS ONE WAY ANALYSIS OF VARIANCE ON RANKS

GROUP	N	MISSING	MEDIAN	25%	75%
1.HR II st	146	0	84	76	92
2.HR II st	146	0	78	70	84
3.HR II st	146	0	74,5	69	80
4.HR II st	146	0	72	66	78

H = 80,120 with 3 degrees of freedom. (P = <0,001)

MULTIPLE COMPARISONS VERSUS CONTROL GROUP (DUNN'S METHOD) :

COMPARISON	DIFF OF RANKS	Q	P<0,05
4.HR II st vs 1.HR II st	166,404	8,426	Yes
3.HR II st vs 1.HR II st	131,065	6,637	Yes
2.HR II st vs 1.HR II st	83,051	4,205	Yes

COLUMN	SIZE	MEAN	STD DEV	STD. ERROR	C.I. OF MEAN
1. Sys I st	42	152,93	6,75	1,04	2,10
2. Sys I st	42	141,55	10,90	1,68	3,40
3. Sys I st	42	135,95	8,21	1,27	2,56
4. Sys I st	42	134,52	8,25	1,27	2,57
1. Dia I st	42	95,41	6,48	1,00	2,02
2. Dia I st	42	87,00	8,09	1,25	2,52
3. Dia I st	42	84,12	7,99	1,23	2,49
4. Dia I st	42	81,67	7,62	1,18	2,38
1. Map I st	42	114,58	4,08	0,63	1,27
2. Map I st	42	105,18	7,87	1,21	2,45
3. Map I st	42	101,40	6,78	1,05	2,11
4. Map I st	42	99,29	6,72	1,04	2,09
1. HR I st	42	83,62	11,45	1,77	3,57
2. HR I st	42	74,19	9,50	1,47	2,96
3. HR I st	42	70,60	7,63	1,18	2,38
4. HR I st	42	67,67	5,86	0,91	1,83

Note: Mean – average value, Std. Dev – standard deviation, Std. Error – standard error, CI – confidence of interval, Size – counts of numbers

TABLE 9: Descriptive statistic for Group HYP 1, n=42

ANOVA test for MAP Group HYP 1.

KRUSKAL-WALLIS ONE WAY ANALYSIS OF VARIANCE ON RANKS

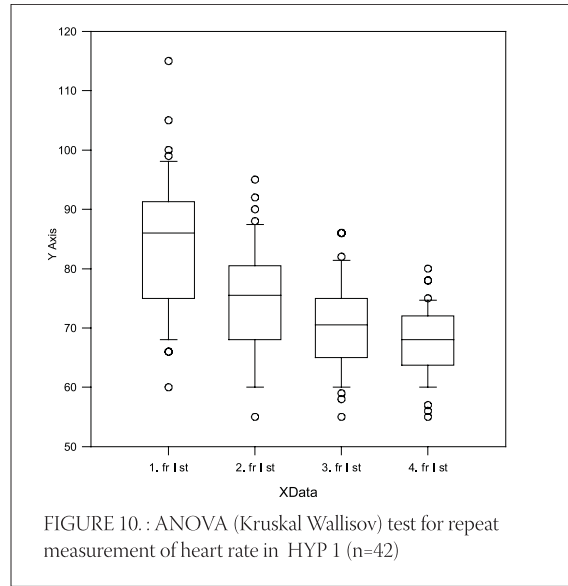
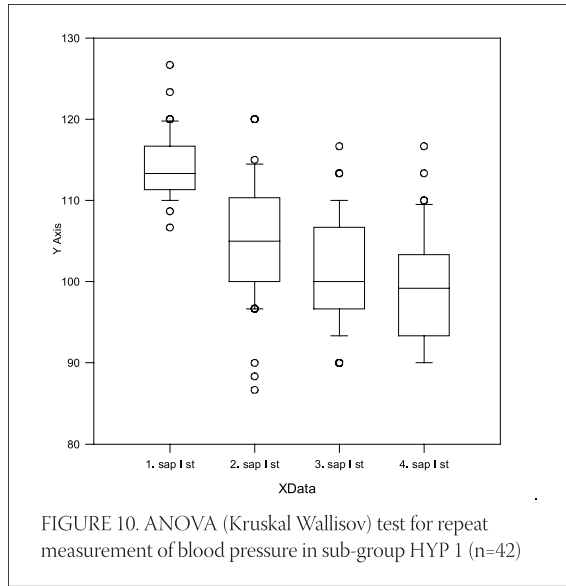
GROUP	N	MEDIAN	25%	75%
1. Map I st	42	113,333	111,333	116,667
2. Map I st	42	105	100	110
3. Map I st	42	100	96,667	106,667
4. Map I st	42	99,167	93,333	103,333

H = 78,795 with 3 degrees of freedom. (P = <0,001)

MULTIPLE COMPARISONS VERSUS CONTROL GROUP (DUNN'S METHOD) :

COMPARISON	DIFF OF RANKS	Q	P<0,05
4. Map I st vs 1. Map I st	86,429	8,143	Yes
3. Map I st vs 1. Map I st	75,024	7,068	Yes
2. Map I st vs 1. Map I st	52,595	4,955	Yes





Heart Rate (F/min) ANOVA test for Group HYP 1, n=42

KRUSKAL-WALLIS ONE WAY ANALYSIS OF VARIANCE ON RANKS

GROUP	N	MEDIAN	25%	75%
1. HR I st	42	86	75	91
2. HR I st	42	75,5	68	80
3. HR I st	42	70,5	65	75
4. HR I st	42	68	64	72

H = 48,531 with 3 degrees of freedom. (P = <0,001)

MULTIPLE COMPARISONS VERSUS CONTROL GROUP (DUNN'S METHOD) :

COMPARISON	DIFF OF RANKS	Q	P<0,05
4. HR I st vs 1. HR I st	70,119	6,606	Yes
3. HR I st vs 1. HR I st	54,083	5,095	Yes
2. HR I st vs 1. HR I st	35,655	3,359	Yes

	N	MINIMUM	MAXIMUM	MEAN	STD. DEVIATION
FASTING BLOOD GLUCOSE	328	2,80	14,00	6,05	1,70
CREATININE	328	56,20	226,00	85,00	20,67
CHOLESTEROL	328	3,20	18,00	6,13	3,93
TRIGLYCERIDE	328	0,84	12,80	2,18	1,11

TABLE 10. Mean and standard deviation of fasting blood glucose, serum creatinine, cholesterol and triglycerides at the start of the study

	N	MINIMUM	MAXIMUM	MEAN	STD. DEVIATION
FASTING BLOOD GLUCOSE	328	2,30	12,00	5,37	1,16
CREATININE	328	40,00	230,00	78,71	19,65
CHOLESTEROL	328	2,60	15,00	5,68	3,64
TRIGLYCERIDE	328	0,70	11,00	1,90	1,02

TABLE 11. Mean and standard deviation of fasting blood glucose, serum creatinine, cholesterol and triglycerides at the end of the study

	t-test	p
FASTING BLOOD GLUCOSE	7,45	0,001
CREATININE	5,56	0,001
CHOLESTEROL	2,01	0,045
TRIGLYCERIDES	5,11	0,001

TABLE 12. Results of t-test with significance level for fasting blood glucose, serum creatinine, cholesterol and triglyceride

## DISCUSSION

Results of our study shows that the study was completed by 328 patients (95,5%), while 18 patients (4,5%) were withdrawn (Figure 3,4) Blood pressure improvement was registered in 314 (95,73%) patients while in 14 (4,27%) patients blood pressure did not change significantly for 10/5 mm Hg (sys/dia), and in 48 (14,63%) patients blood pressure is fully normalized with level <120/80 mm Hg. (Table 4, 5; Figure 5-7) We used (Kruskal Wallis One Way ANOVA on Ranks) for 2 sub- groups of patients, where we used repeated measurement. The results showed statistically significant difference in blood pressure level on the first control ( $P<0,05$ ) in 188 patients (57,32%). (Table 6,9; Figure 8-10) Results of our study show improvement of serum parameters- fasting blood glucose, creatinine, cholesterol and triglyceride, with statistical significant difference ( $p<0,05$ ) at the end of study. (Table 10-12) Considering these results, in this study nebivolol is demonstrated to be suitable for therapy in arterial hypertension. The results provide evidence of good tolerability profile and lower incidence of adverse effects on glucose and lipids metabolism. Different studies compared the efficacy of Nebivolol with other antihypertensive drugs. The percentage of patients with fully normalized blood pressure was significantly higher with nebivolol than with nifedipine (54% vs 42%) (12). In the studies comparing nebivolol with nifedipine or amlodipine heart rate was decreased by nebivolol and slightly increased with the two dihydropyridines: the lower heart rate is a potential advantage of nebivolol, due to the epidemiological relation between heart rate and cardiovascular morbidity (13,14). Nebivolol was also compared with other beta-blockers, such as atenolol (15,16) and metoprolol (17). Therefore, the antihypertensive efficacy of nebivolol was superior or similar to that of other beta-blocking drugs. Antihypertensive drug must have not only blood pressure – lowering properties, but also influence other

critical cardiovascular, metabolic, and renal end points. The impairment of nitric oxide (NO) bioactivity, the main feature of endothelial dysfunction, is a key factor in the pathogenesis of many common cardiovascular diseases. High levels of blood glucose can impair not only vascular tone but also blood flow, both depending on NO controlled endothelial function (18). High blood glucose level or dislipidaemia can affect by several mechanisms- such as oxidative stress- endothelial function thus leading to vascular damage, so opportunities of treatment can arise from nebivolol, some effects of which were shown to be just mediated by NO. In most of the studies nebivolol did not significantly alter blood glucose or plasma lipid levels (19). Nebivolol seems to be free from some of the problems that generally affect not only the classical beta- blockers but sometimes also the newer classes of antihypertensive drugs. With its high anti-hypertensive efficiency and safety, and finding of statically significant difference in laboratory tests and beneficial effects, without adverse interaction with glucose and lipids metabolism, patients treated with Nebivolol may show an optimal adherence to therapy.

## CONCLUSIONS

Nebivolol administered in a single daily dose of 5 mg lowers both systolic and diastolic blood pressure in patients with arterial hypertension. It is simple one daily dosage, that can be given to patients of all groups. The major objective of our study is to provide an adequate cardiovascular protection by appropriate reduction of blood pressure values. Results data of our study provide evidence of antihypertensive effects, good tolerability profile and lower incidence of adverse effects on glucose and lipids metabolism of Nebivolol, which is in relationship with that obtained by other authors.

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# DENTAL TREATMENT OF PATIENTS WITH KIDNEY DISEASES-REVIEW

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## ABSTRACT

In their practice every dentist is brought into a situation to treat patients with grossly impaired kidney function. Kidney diseases, whether acute or acquired, imply a number of body dysfunctions such as prolonged bleeding, high blood pressure, infection tendency etc. which, in turn, pose a threat involving serious complications in cases of dental interventions in these patients. The aim of this article is to provide a review of current dental practice in patients with kidney disease. This implies dental intervention and preparations of patients with chronic renal disease, nephritic syndrome, patients on dialysis, and patients with kidney transplants. Certainly, cooperation between the dentist and nephrologist is an imperative for the appropriate dental treatment of patients with grossly impaired renal function.

**KEY WORDS:** kidney disease, dialysis, transplantation, dental treatment

## INTRODUCTION

Kidney is an organ responsible for a set of complex functions in the body and they are as follows:

- excretion of metabolic waste products,
- regulation of the salt and water in the body,
- preservation of acid balance,
- excretion of different hormones and organic substances.

Kidney diseases are as complicated as the organ itself. They can be divided into developmental anomalies and inherited diseases or acquired diseases. With respect to the further course of illness they can be divided into acute and chronic kidney diseases. In the practice every dentist is placed into a situation to treat patients with a grossly impaired renal function. Familiarity with the nature of the disease in question and possible complications that may develop in these patients is indispensable prior to any dental treatment, particularly of invasive nature as it is the case in oral surgical interventions (1). Also, we should note that dental infections may cause acute glomerulonephritis or progression of this disease into chronic failure. Experiments have shown that pyelitis or pyelonephritis can be caused by a bacterial infection which is inoculated into the kidney from any chronic dental infection. Experimental evidence support a connection between kidney calculus and oral infection. The aim of this article is to present current dental practice in patients with kidney diseases. This implies the dental treatment and preparations of patients who suffer from chronic kidney disease, nephrotic syndrome but also the patients who use dialysis and those with kidney transplants.

## NEPHROTIC SYNDROME

Nephrotic syndrome (syndroma nephroticum) is a clinical disease which comprises of the following stages:

- proteinuria (3g per 24 hours)
- hypoproteinemia
- hyperlipidemia
- appearance of characteristic edemas in face (lids) and lower extremities (knees)

The most common causes of nephritic syndrome are SLE, diabetes and amyloidosis while among the primary kidney diseases the most important are the diseases involving immune deficiency, i. e. glomerulonephritis. The nephrotic syndrome commonly appears in children 2-6 years

old. They show characteristic tendency to infection (1). So-called **Epstein's syndrome** is well described in literature. It is an idiopathic nephritic syndrome with typical changes in oral mucus of newborns in the form of salty, pseudo-diphtheric layers stretching over the soft palate in the shape of butterfly. We can also detect wounds in mucus in the form of Bednar's acnes (1). With respect to dental treatment we should bear in mind that patients with necrotic syndrome are prone to infection and therefore, the endodontic treatment of deciduous teeth and multi-root teeth can show counter-indications. In any case, prophylactic antibiotics protection is a must prior to invasive dental treatment (1). Therefore, we should always bear in mind that dental intervention is not to be undertaken in patients with renal insufficiency unless in urgent cases, and this should be done only after consulting either the nephrologist or urologist.

## PATIENTS WITH CHRONIC KIDNEY DISEASES

Chronic kidney diseases are results of progressive deterioration of kidney nephrons and dysfunction of glomerular filtration. As a result, the kidney function is impaired followed by high loss of fluids from the body due to the increased excretion of urine (polyuria). Besides, in patients who have not been treated properly, the concomitant effects are also polydipsia, tremor and hematuria. In a more severe form of the disease we can see edemas in the face, particularly on lids as a result of fluid retention and the impaired balance of electrolytes. With chronic renal deficiency we should pay attention to the following:

1. The immune system of patients is grossly weakened, and consequently, there is greater tendency to infection. Candidiasis and ulcers are common in the oral cavity. Soft tissues in the oral cavity are pale due to anemia. As a result, excretion of saliva is reduced, food retention in the mouth is increased and halitosis is an ultimate outcome. In extreme cases stomatitis uremica may develop. In fact, uremia is invariably followed by stomatitis. This stomatitis is characterized by thickening and redness of the buccal mucus and the presence of pseudo-membranes that cover oral mucus, gingiva, soft palate and pharynx. We can rarely encounter surface and deep ulcerations smaller than 1 cm in radius without a specific localization (2). The bottom of these ulcerations does not bleed easily. The histological lesions indicate inflammatory process accompanied by necrosis. A similar



form of stomatitis can appear with nephritis without azotemia. Vincent's microorganisms are commonly the cause of secondary infection of uremic stomatitis.

2. Absorption of medications administered per os is reduced due to reduced absorption capacity of the gastrointestinal tract.
3. Forms of B and C hepatitis are frequent, and as a result, there is a tendency to bleeding.
4. Anemia is a result of the reduced erythropoietin production. As yet, the standard for assessing the value of hematokryte in patients with renal dysfunction who have to undergo the operation has not been ascertained. A study has shown an increase in intra-operative complications for hematokryte values of 20-26%(3). The acceptable value of hematokryte is 36 % which can be achieved by administering erythropoietin for several weeks prior to the operative procedure. Fresh blood transfusion should be avoided whenever possible, primarily because it reduces the chances of a successful transplantation in case of need. In other words, every transfusion is the introduction of new antigens into the body, and the latter can, in turn, react by producing anti-bodies (3,4).
5. Tendency to bleeding is increased because of platelets dysfunction. Consequently, APTT and INR have to be monitored very carefully(3)
6. There is also a tendency to hypertension and hypotension. Pre-operative and intra-operative tension is quite common in patients with chronic renal disease. This is attributed to fear, increased katecholamine secretion and hypertension caused by renal dysfunction (5).
7. In patients with more severe renal disease there changes appear on paradontium. Thus, in patients with uremic dystrophy a loss of lamina dura and trabecular build of jaw bones occurs (5).
8. Secondary hyperparatireoidism is also very common. It is a result of phosphate retention and their influence on hyper production of paratireoid hormone resulting in the increased loss of calcium in bones. In children with more severe chronic disease retardation in teeth development and jaw malformation may occur, but also changes in the tooth structure and porcelain abnormalities, precocious loss of teeth etc (6).
9. Acid- base disorders. Acidosis in patients with chronic renal disease may reduce the effectiveness of local anesthetics (3,7).
10. Hypercalcemia. General anaesthetic is to be avoided in patients with chronic renal disease whose potassium level is over 5.5 mmol/l. Otherwise, there is an increased risk of aritmia (3).

## DENTAL TREATMENT

Since kidney diseases may be more or less severe, there is no uniform dental treatment. Nevertheless, an invasive dental treatment requires consultation with the nephrologist who administers prophylactic antibiotic therapy (8). Penicillin or cephalosporin are usually administered. Tetracycline and streptomycin should be avoided because they are nephro-toxic. Patients should be asked if they suffer from allergies because allergy to penicillin is quite common. Due to poor gastrointestinal resorption, antibiotics should be administered i.v. I.m. administration may show counter-indications because of kreatinine increase. In patients with chronic kidney disease the endodontic treatment of the deciduous and multi-root teeth should be avoided at all costs because of increased infection risk. We should also avoid treatments of gangrenous teeth and those with apical parodontitis because they can later develop into an inflammatory focus. Extractions should be done with local anesthetic. As for anesthetics, anesthetics of amidic type should be applied such as lydocain, xylocain because of their reabsorption potential in the liver. Analgetics of almost any kind may be administered and also codeine-based medicaments that are also metabolized in the liver. Since these patients often suffer from hepatitis B or C a dentist must undertake all the precautionary measures (protective glasses, mask, cap, gloves, and inoculation against B hepatitis). If the dental intervention must be done with a total anesthetic consultations with the nephrologist or anesthetist prior to the intervention are obligatory (7).

## PATIENTS WHO USE DIALYSIS

There are two possible therapies for patients with kidney dysfunction:

1. dialysis (hemodialysis, peritoneal dialysis)
2. kidney transplants

The level of kreatinine in serum should be 600-800  $\mu\text{mol/l}$  if a patient is to use dialysis. Dialysis represents the perfusion of the patient's blood and the dialysis solution on either side of the membrane. At this, it is necessary to note that in the course of dialysis the patient is given heparin in order to prevent blood coagulation outside the body. The majority of patients undergo dialysis three times a week in the duration of 4 hours. Heparin is an anti-coagulant agent for parenteral administration and its effect is prevention of activated coagulation factors Xa and trombone, and thus prevention of coagula-

tion. It is retained in the circulatory system 4-6 hours upon administration. This fact is important because of proper timing of dental intervention. Accordingly, since heparin prolongs the bleeding time, the tooth extraction should be done a day after dialysis when the anti-coagulant agent's presence is reduced to the minimum while the dialysis effect is maximal. APTT and INR should be checked prior to the surgical intervention. Heparin can also bring about mild trombocitopeny (4).

Prevention and therapy against bleeding should include the following:

1. K vit amp. i.v.
2. Etamsylatum amp. i.v.
3. Protamine sulphate (1:5)

The nephrologist should undertake all the preparatory measures prior to sending the patient to his/her dentist. In addition to bleeding, the patients who undergo dialysis are also very sensitive to infection. Because of possible bacterial infection the prophylactic administration of antibiotics of broad specter is strongly recommended. We can administer cefalosporines. Penicillin should be administered in the dosage of 2 mg after dialysis. Surgical interventions should be made with local anesthetic. The total anesthesia is to be avoided because of concomitant hypertension, arteriosclerosis and anaemia (7).

## PATIENTS WITH KIDNEY TRANSPLANTS

In general, kidney transplants involve the risk of transplanted organ rejection. In order to prevent this, patients who have undergone an organ transplant operation, are given huge doses of immunosuppressants such as corticosteroids, azatioprin, cyclosporine A and anti-lymhocyte globulin (8,9). These patients are extremely sensitive to infection. After tooth extraction the wound healing is significantly impaired. The immunosuppressant therapy may involve many side effects that, in turn, may largely affect oral surgical intervention. The side effects are hypertension, increased bleeding, diabetes (5,8). Accordingly, in patients who underwent kidney transplant operations prophylactic antibiotics should be administered in consultation with the patient's physician. Because of potential adrenal crisis risk it is necessary to alter steroid therapy. If the stress suffered during the oral surgical intervention is minimal, the therapy should not be altered. If the stress is insignifi-

cant, it is recommended to increase the steroid dosage twice a day two days prior and following the oral surgical intervention. If the stress is great, 100g of hydrocortisone should be administered i.m. prior to the operation, gradually reducing dosage by 50 % on a daily basis for three days after the intervention until the dosage of 20mg which should be administered twice a day for the subsequent 7 days. In any case, steroid dosage is administered by the expert physician after consultations with the dentist and the expected stress assessment (9). The patients who are getting prepared for kidney transplantation should be treated with regard to the following:


1. Comprehensive treatment of the oral cavity should be done (including prophylactic measures, treating the teeth with caries, doing the necessary extractions, particularly of the gangrenous, pulpal and parodontopathic teeth and the remaining roots) (1,10).
2. Advise the patient on the importance of oral hygiene since oral infection may bring about the rejection of the organ transplant.
3. Mouth rinsing with hlorhexidine solution (0.2%) is recommended one day prior to the organ transplant operation in order to prevent candidiasis and bacterial infection.
4. The effect of immunosuppressant therapy (cyclosporin A) may induce gingival hyperplasia similar to dilantine gingivitis in epileptic patients (11).  
Prior to gingivectomy it is necessary to clean the salivary calculus and remove any signs of infection (rinsing with hlorhexidine solution) but also to motivate the patient to maintain a regular oral hygiene. It is indispensable to administer antibiotics with these patients (1,8).

## CONCLUSIONS

1. Patients with kidney diseases are an extremely delicate group of patients.
2. They have tendency to infection and therefore, prophylactic antibiotics treatment is a must prior to surgical interventions.
3. They are also prone to bleeding and therefore surgical interventions should be undertaken in the days when the patient does not use dialysis.
4. We should always bear in mind that patients with kidney transplants are prescribed immunosuppressant therapy.
5. Dental treatment of such patients implies close cooperation between the dentist and the nephrologist.

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# THE EFFICACY OF SUPPLEMENTAL INTRAOSSEOUS ANESTHESIA AFTER INSUFFICIENT MANDIBULAR BLOCK

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## ABSTRACT

It is a well-known scientific fact that only a small percentage of infiltration of inferior alveolar nerve is clinically proven to be efficient. The objective of this study was to determine the anesthetic efficacy of supplemental intraosseous injection, used after the insufficient classical mandibular block that didn't provide deep pulp anesthesia of mandibular molar planned for extraction. The experimental teeth consisted of 98 mandibular molars with clinical indication for extraction. Based on the history of disease, we indicated the extraction of the tooth. After that each tooth was tested with a electric pulp tester P1. We tested the pulp vitality and precisely determined the level of vitality. After that, each patient received classical mandibular block, and the pulp vitality was tested again. If the pulp tester indicated negative vitality for the certain mandibular molar, and the patient didn't complain about pain or discomfort during the extraction, the molar was extracted and the result was added to anesthetic success rate for the classical mandibular block. If, five minutes after receiving the mandibular block, the pulp tester indicated positive vitality (parameters of vitality) or the patient complained about pain or discomfort (parameters of pain and discomfort), we used the Stabident® intraosseous anesthesia system. Three minutes after the application of supplemental intraosseous injection the molar was tested with the pulp tester again. The anesthetic solution used in both anesthetic techniques is lidocaine with 1:100.000 epinephrine. The results of this study indicate that the anesthetic efficacy of the mandibular block is 74.5%, and that supplemental intraosseous anesthesia, applied after the insufficient mandibular block, provides pulpal anesthesia in 94.9% of mandibular molars. The difference between anesthetic efficacy of the classical mandibular block and anesthetic efficacy of the supplemental intraosseous anesthesia, applied after the insufficient mandibular block, is obvious.

**KEY WORDS:** intraosseous, anesthesia, supplemental

## INTRODUCTION

Retrospective analysis shows that need for supplement to inefficiency of mandibular block was noticed 1968, when Magnes and co-workers(1) published their first study, which at the same time promoted intraosseous anesthesia. Technique described in this study was very popular during early seventies. The first study, which elaborated scientifically in overwhelming manner all properties of system of intraosseous anesthesia was published by Leonard (2) this study brought data of workability of this method and described proper technique of application of Stabident system. Coggins and co-workers(3) published 1996 efficiency of supplemental intraosseous anesthesia in maxillary and mandibular molars and announced to the scientific public success of supplemental intraosseous anesthesia, by measuring vitality of the first lower molars and brought results of success of 93%. In December 1997 Reisman and Reader(4) investigated effects of supplemental intraosseous anesthesia in vital tooth, which require endodontic treatment. They have published that 75% of patients requested additional anesthesia after mandibular block because of subjective feeling of pain in attempt of endodontic treatment. The first intraosseous anesthesia showed success of 82% and the second was successful in 98% cases. In January 2000 Gallatin and co-workers,(5) published the study, which apart from efficiency of intraosseous anesthesia, cleared up impact of intraosseous anesthesia on heartbeat, what was for the certain period of time subject of scientific discussions. Analyzing relevant studies related to intraosseous local anesthesia, which was published recently, it is concluded that there is not published study, which would bring results on efficiency of additional intraosseous anesthesia when extraction of vital teeth with clinical indication for extraction.

## MATERIAL AND METHODS

Teeth sample are 98 mandibular molars, which show signs of vitality and clinical indication for extraction was established. Operative procedure was done with respect to ethical standards regulated by Helsinki Declaration. After history procedure and establishment of indication for extraction, standard apparatuses for testing of tooth pulp P1 (Jugodent) is to be tested vitality of tooth in subject, and precisely determined level of vitality on the scale 1 to 10. The first to patient is to be applied classical mandibular block. Five minutes after application of mandibular block again is to be tested vitality of tooth pulp in the same way and subjective feeling of numbness of

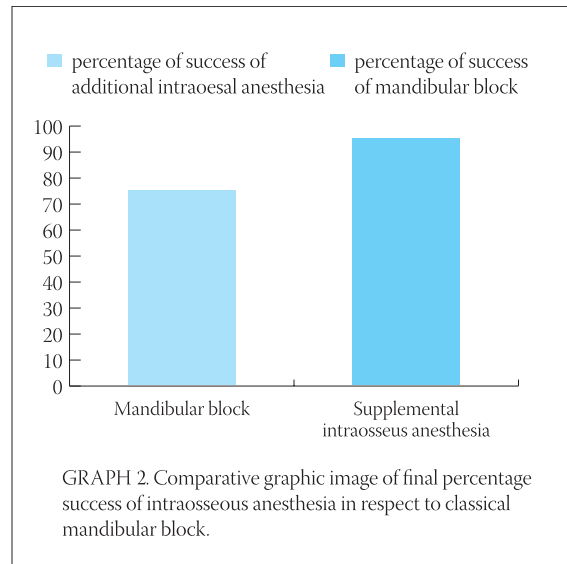
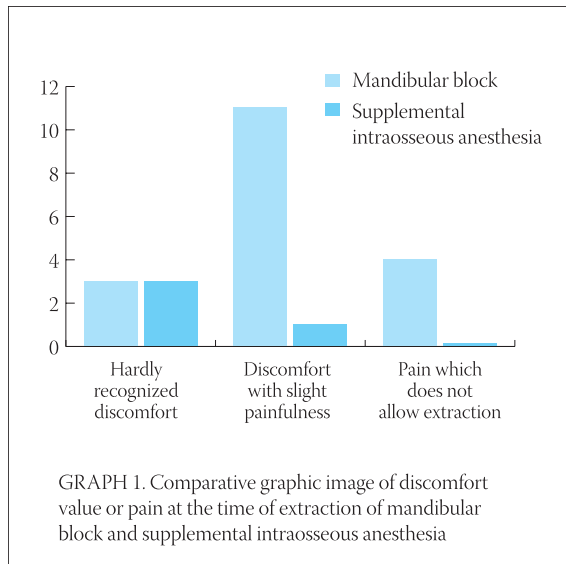
lower lip and tongue. Patients to whom apparatus for vitality shows that tooth in subject is entirely under anesthesia (0 on the vitality scale), and do not complain about discomfort or pain at the time of work, are to be treated by extraction of tooth in the standard manner, their results are registered into the research chart in order to enter into final percentage relation on success of mandibular block. Patients whose tooth show signs of vitality on apparatus for measure of vitality of tooth pulp (1 to 10 on the vitality scale) 5 minutes after application of mandibular block or if they complain about discomfort and pain at the time of extraction attempt, what would understand absence of full affect of conventional block, to them are applied intraosseous Stabident anesthesia, following the rules of application recommended by manufacturer, as described above. Three minutes after application of intraosseous anesthesia the following parameters are followed up and registered in the research chart:

1. Vitality test graded on the scale from 1 to 10
2. Subjective feeling of discomfort at the time of extraction graded from 0 to 3.

## RESULTS

After application of conventional mandibular block, out of total number of mandibular block under anesthesia – 98 teeth, 73 of them have positive signs of numbness of lower lip and tongue of the side in subject, negative vitality test and show absolute absence of pain during the work. Out of this come out that percentage of success of classical mandibular block is 74.5%. Tooth extraction was not made in those cases where vitality signs are positive in order to avoid pain and discomfort in patients at the time of extraction. Number of mandibular molars with negative test of vitality and show absence of pain during the work (73 molars) is statistically significantly higher than number of teeth which show either positive test of vitality or some pain and discomfort at the time of attempt of extraction (25 molars). Value  $\chi^2$  of test is  $\chi^2 = 23.515$ ; d.f.= 1, and level of significance  $p < 0.0001$ . Total number of mandibular molars, which 5 minutes after application of classical mandibular block show either positive vitality test (1 to 10 on the vitality scale) or some pain and discomfort at the time of extraction attempt (1 to 3 on discomfort scale) is 25 teeth. Out of total number of mandibular molars under anesthesia 5 minutes after application of classical mandibular block, seven of them show vitality signs (1 to 10 on the vital-





ity scale) and eighteen mandibular molars show apart from negative test of vitality. Out of total twenty five molars, which showed either positive vitality test or some painfulness and discomfort at the time of extraction attempt, statistically significant were less molars (7) which show vitality signs than number of molars, which show apart from negative vitality test painfulness at the time of extraction attempt (eighteen molars). Value  $\chi^2$  test is  $\chi^2 = 4.84$ , d.f.=1, and level of significance  $p < 0.005$ . Out of total 18 molars with negative vitality test, significantly the highest number of molars (11 molars) were with discomfort and slight pain (2 on discomfort scale) in respect to number of molars (3 molars) with hardly recognized discomfort (1 on discomfort scale) and number of molars (4 molars) with extremely strong pain at the time of extraction attempt (3 on discomfort scale). Value of  $\chi^2$  test is  $\chi^2 = 6.33$ ; d.f.=2, level of significance  $p < 0.005$ . Out of seven tested teeth three minutes after application of intraosseous anesthesia 1 mandibular molar show vitality sign (1 to 10 on the vitality scale). Out of total number of teeth which 3 minutes after application of intraosseous anesthesia fail to show vitality signs by apparatus for testing of vitality of teeth pulp (0 on the vitality scale) 6 molars, none show any painfulness or discomfort at the time of extraction (0 on discomfort scale). Patients to whom apparatus for vitality of teeth pulp 5 minutes after application of classical mandibular block show negative test of vitality (0 on vitality scale) and they felt discomfort or pain at the time of extraction attempt (1 to 3 on discomfort scale) intraosseous anesthesia was applied too. Total number of such mandibular molars is 18 teeth. Out of eighteen mandibular molars 3 minutes after application of intraosseous anesthesia 14 fail to show any discomfort or pain at the time of extraction attempt (0 on discomfort scale).

Remaining 4 teeth show some discomfort as follows:

- 3 mandibular molars with hardly recognized discomfort (1 on discomfort scale)
- 1 mandibular molar with discomfort and slight pain (2 on discomfort scale)
- None mandibular molar with pain which does not allow extraction (3 on discomfort scale)

Difference in number of teeth which show negative vitality test (5 molars) after application of intraosseous anesthesia in respect of number of teeth which show positive signs of vitality (1 molar) is differ significantly on the lower level of significance. Value of  $\chi^2$  test is  $\chi^2 = 3.57$  and level of significance is  $p < 0.10$ . Sum of all stated statistic data brings and percentage of success of intraosseous anesthesia which we supplement to insufficient mandibular block in our research. As result we get total percentage of success of supplemental intraosseous anesthesia of 94.9%.

## DISCUSSION

Scientific interest for percent of success of mandibular block presented during last decades, when were published number of studies dealing with those issues. Kaufman and co-workers(6), (1987) Reisman and co-workers (4) published scientific studies where was used apparatus for testing of vitality of tooth pulp as the basic parameter of proving of efficiency of mandibular block. The first study which brings results on efficiency of additional intraosseous anesthesia published Leonard (5) In clinical study author published success of intraosseous anesthesia of 88%. Coginns and co-workers(3) published efficiency of primary intraosseous anesthesia in mandibu-

lar molars and published percentage of success of 93%. The same year Dunbar et al(7). published success of additional intraosseal anesthesia by measuring of vitality of the first lower molars and brought results on success of 98%. Reisman and co-workers.(4) researched efficiency of additional intraosseal anesthesia in vital teeth which require endodontic treatment. They published extremely low percentage of success of conventional block with data that 75% of teeth after classical block required additional anesthesia because of subjective feeling of pain at the time of attempt of entering of endodont instrument into root channel of vital tooth. Quoted publications represent scientific base for further research. Taking sample of 98 mandibular molars we got percentage success of classical mandibular block of 74%. Percentage of success responding to up to now studies, which light up problem of effect of mandibular block. Out of total number of teeth 25 require additional intraosseal anesthesia. From this group of teeth, which show insufficient effect of classical mandibular block 7 show positive vitality signs and after application of intraosseal anesthesia positive vitality sign is registered on one sample only. Difference in number of teeth which show negative vitality test of tooth pulp after application of additional intraosseal anesthesia (6 molars) in respect to those which show vitality signs (1 molar) differs significantly on lower level of significance  $p < 0,10$ . Further more out of total sample of mandibular molars 18 have signs of painfulness or discomfort at the time of extraction attempt after classical mandibular block. It is specially significant data that out of total number of those teeth 11 have discomfort and pain feeling (2 on discomfort scale) which is statistically significant in respect of number of teeth with

1 and 3 level of discomfort with level of significance of  $p < 0,005$ . On the other side discomfort after application of additional intraosseal anesthesia was registered in four samples only: 1 and 2 on the discomfort scale, but none sample had pain which does not allow extraction (3 on the discomfort scale). As result we got total percentage of success of additional intraosseal anesthesia, which we applied on insufficient classical mandibular block, which is in our research 94.9%. Out of all stated is notable that results of our research are in immediate relation with results of already published studies, which treat the same issues, but using different methodology principles, what prove the hypothesis set for such researches.

## CONCLUSION

- Conductive anesthesia on alveolaris inferior is clinically successful in some percentage only, what is the conclusion of achieved percentage of success of 74.5%. Achieved percentage of success is in close relation with up to now published researches.
- Additional intraosseous anesthesia as supplement to insufficient mandibular block shows success of 94.9%, which is in any case in close relation with achieved results of up to now researches with different methodology approach to this scientific problem.
- Positive signs of numbness of lower lip and tongue after application of classical mandibular block does not always mean and clinically successful anesthesia, which would show negative vitality and painless work on mandibular molars with clinical indication for extraction.

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# COMBINED APPLICATION OF AMOXICILLIN AND CLAVULANIC ACID AFTER ORAL SURGICAL INTERVENTIONS

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## ABSTRACT

Antibiotics represent a powerful weapon against infections. As dentists we are faced almost on a daily basis with the need to prescribe antibiotics. At the same time, we can often see that the antibiotics use tends to get out of control or that they are used indiscriminately with no real need. The aim of this case study is to investigate the effectiveness of amoxicillin and clavulanic acid combination in various dental ailments but also to demonstrate possible difference in the severity of symptoms after the use of amoxicillin and antibiotic combination of amoxicillin and clavulanic acid after surgical and oral interventions. The investigation involved 102 patients who were divided into two groups (the first group consisting of 59 and the second one of 43 patients). Following surgical treatment the first group of patients was proscribed antibiotic combination of amoxicillin and clavulanic acid in the dosage of 625 mg, 3 times per day. The second group of 43 patients was proscribed amoxicillin in the dosage of 500 mg, 4 times per day. The recommended therapy for antibiotic combination of amoxicillin and clavulanic acid was 5 to 10 days after the operation and 8 to 10 days for amoxicillin. In other words, both groups of patients started to use antibiotics after the surgical or oral intervention such as operative removal of impacted wisdom teeth, apicotomy or complicated extractions, and also after the treatment of odontogenic abscesses etc. The same parameters were measured prior to the surgical intervention in cases when patients demonstrated the symptoms before the operational treatment while in all other cases the parameters were measured 48 hours and seven days following the operation. The measured parameters were: pain, swelling, body temperature, dysfunction such as dysphagus trismus, chewing disorder and possible allergic or gastrointestinal reactions. All parameters observed were precisely set in order to harmonize the investigation criteria and facilitate statistical data processing. With respect to pain before the operation there was no substantial statistical difference,  $p > 0.05$  ( $t = 0.56$ ;  $t = 0.69$ ). With respect to the onset of pain and the use of antibiotics after 48 hours there is a significant difference in favor of antibiotic combination of amoxicillin and clavulanic acid ( $\chi = 14.83$ ,  $p = 0.002$ ;  $p < 0.01$ ). Thus, pain is less acute if antibiotic combination of amoxicillin and clavulanic acid is administered. With respect to swelling and administration of antibiotics 48 hours after the operation there is no significant difference between the use of the two antibiotic therapies ( $\chi = 4.89$ ;  $p = 0.18$ ;  $p > 0.05$ ). The investigation conducted seven days after the operation with regard to pain and the use of either antibiotic therapies demonstrated significant statistical difference ( $\chi = 9.35$ ,  $p < 0.01$ ) in favor of antibiotic combination of amoxicillin and clavulanic acid. In other words, patients who used amoxicillin and clavulanic acid felt significantly less intense pain. With respect to swelling, significant statistical difference between the two groups of patients was established in favor of antibiotic combination of amoxicillin and clavulanic acid, i.e.  $p < 0.05$  ( $\chi = 6.45$ ,  $p = 0.03$ ). The combination of amoxicillin and clavulanic acid has proven to be significantly more effective in comparison with the use of amoxicillin after oral - surgical interventions, and therefore antibiotic combination of amoxicillin and clavulanic acid is recommended for use in further practice.

**KEY WORDS:** antibiotics, oral-surgical interventions

## INTRODUCTION

Antibiotics are still considered to be powerful agents against infections. As dentists we find ourselves almost on a daily basis in situations when we have to proscribe antibiotics. At the same time, we are aware of the uncontrolled and indiscriminate use of antibiotics in dental practice. The questions being asked at this point are: "What are the indications for antibiotics application in dental practice? What kind of antibiotics should we opt for?" Hooley and Whitecare (1) deal with the question of indications for the use of antibiotics in dental practice. Together with their associates they addressed this issue as early as 1984. According to these authors antibiotics can serve as a useful tool in the treatment of infection. On the other hand, random and inappropriate use of antibiotics can prove detrimental for a patient suffering from dental infection but also for the treatment of other infections. The antibiotics abuse in the treatment of infections is of minor therapeutic value and only incurs unnecessary costs. In addition, it can complicate clinical picture and compromise further treatment. Broad abuse of antibiotics, according to the same authors, has led to an increase of resistance in bacteria, widespread allergies in patients so that the long-term effectiveness of antibiotics is reduced. Based on the above the same authors refer to indications and counter indications in the use of antibiotics in dental practice:

### INDICATIONS

For treatment of acute infection

If the immune system of the host is seriously threatened because of primary disease or use of drugs.

As a prophylaxis from infection in patients with artificial valves, hip transplant etc.

When infection is the cause of systemic lymphadenopathy.

With facial and cervical cellulitis

With acute pericoronitis

With osteomyelitis

With fungal infection

With acute periapical and periodontal abscess

When appropriate therapy cannot be applied and/or surgical intervention (extraction, incision)

### COUNTER INDICATIONS

With minor chronic, well localized infections (chronic periodontal abscess).

For improved wound healing

As a prophylaxis in minor surgical and dental interventions

For sterilization of the root canal

For treatment of chronic pericoronitis and chronic gingivitis

For treatment of localized osteitis, acute osteitis and acute alveolar osteitis

As a prophylaxis in oroantral communications smaller than 2 mm

After minor surgical interventions such as: tooth extraction, small excisions on the palate, cheek, alveolar mucosa etc.

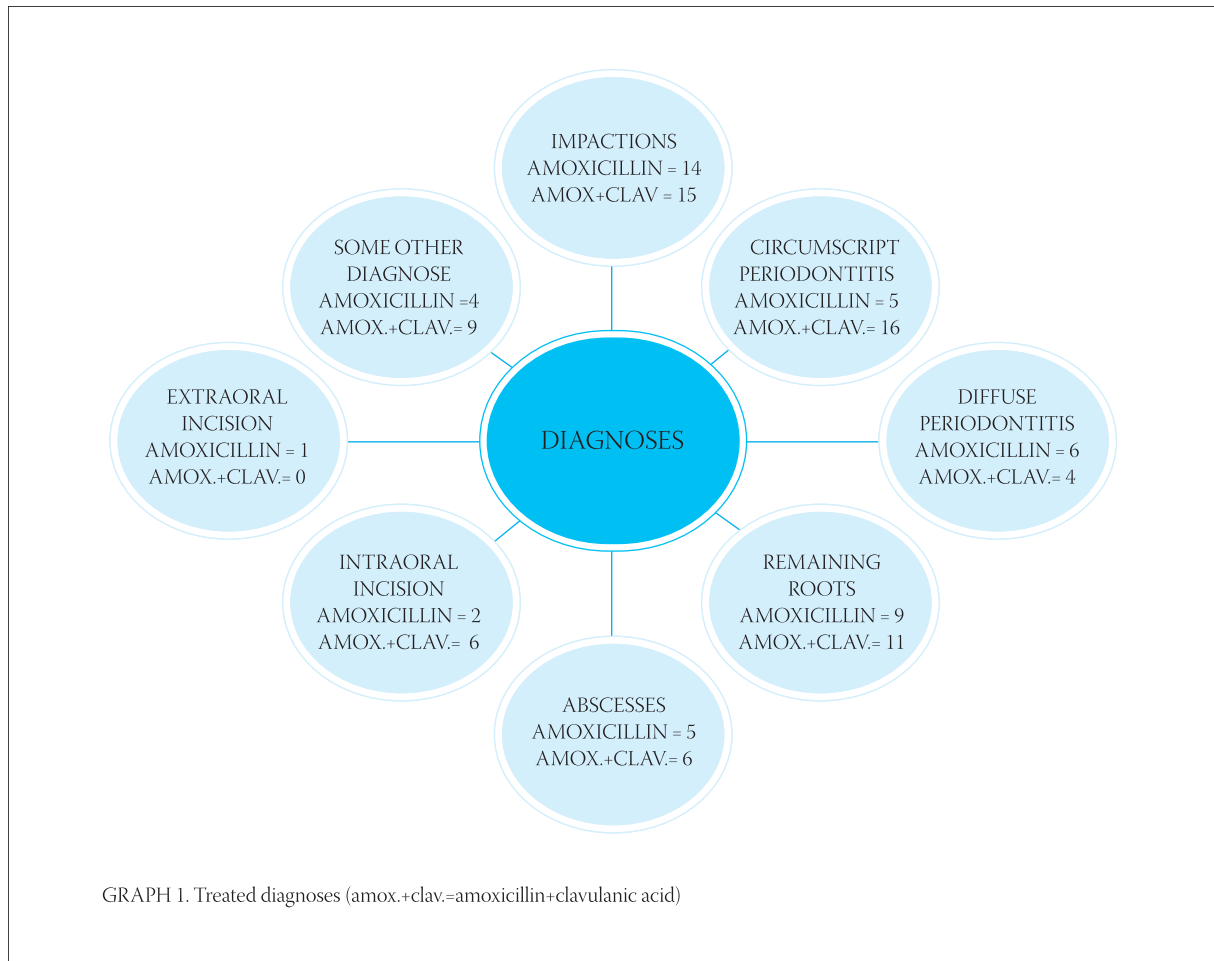
As a component of periodontal packaging

As packaging is applied in surgical wound

In dental medicine the most commonly proscribed antibiotics are broad-specter penicillin drugs which are used either orally or parenterally. Pain and swelling in the area of oral cavity or face, the impaired function and increased body temperature are the most common symptoms that bring patient to the dentist. The combat against these symptoms implies the use of antibiotics. The same symptoms appear after oral and surgical interventions such as:

- Surgical removal of wisdom teeth
- Apicotomy
- Surgical removal of the remaining roots
- Abscess incisions
- Surgical closing of the maxillary sinus
- Treatment of osteomyelitis etc.

In order to reduce the symptoms and to make the post-operative period of recovery more comfortable for the patient we have opted to proscribe the penicillin antibiotic Amoxicillin\*. Amoxicillin is polysynthetic penicillin of a broad specter similar to ampicillin. Its range of action is based on inhibiting transpeptidasis, the enzyme which prevents the synthesis of mucopeptides in the bacterial membrane and the former prevents its building. Amoxicillin belongs to  $\beta$  group of lactam antimicrobes and is sensitive to  $\beta$  lactamasis. It acts against gram-positive and gram-negative microorganisms (bacteria). The dosage for the adult patients is 500 mg or 4 times per day. At this, one capsule contains 500 mg of amoxicillin in the form of amoxicillin trihydrate. Its good property lies in a wide range between its therapeutic and toxic dosage. Similar to this antibiotic is amoxicillin+clavulanic acid antibiotic, which is in fact the combination between amoxicillin and clavulanic acid. Clavulanic acid is irreversible inhibitor of  $\beta$  lactamasis which forms stable and inactive compounds together with enzymes, and in this way, prevent further resorption of amoxicillin increasing at the same time its ef-



fect on poorly sensitive and penicillin-resistant bacteria. Anti-bacterial range of effect of this compound involves:  
 Aerobe gram-positive bacteria  
 Aerobe gram-negative bacteria  
 Anaerobe bacteria

Unlike Amoxicillin which is manufactured in capsules and forte suspensions, antibiotic combination of amoxicillin and clavulanic acid is manufactured in film tablets, suspension, forte suspension and injections. The aim of this case study is to investigate the effectiveness of antibiotic combination of amoxicillin and clavulanic acid in various dental ailments and to find evidence that supports the existence of differences in the symptom intensity after the application of amoxicillin in comparison with antibiotic combination of amoxicillin and clavulanic acid after the oral - surgical interventions.

## SUBJECTS AND METHODS USED IN THE INVESTIGATION

This investigation was done respecting ethical standards stipulated in Helsinki Declaration. The total of 102 patients were included in the investigation. They

were divided into two groups: (one group consisted of 59 patients and the other of 43 patients). The first group (59 patients) were proscribed antibiotic combination of amoxicillin and clavulanic acid after the operation in the dosage of 625 mg, 3 times per day. The second group (43 patients) was proscribed amoxicillin after the operation in the dosage of 500 mg, 4 times per day. The recommended therapy duration for antibiotic combination of amoxicillin and clavulanic acid was 5 to 10 days and 8 to 10 days for amoxicillin. Thus, in both groups the patients started using antibiotic after an oral-surgical intervention such as

- Operative removal of wisdom tooth
- Apicotomy in diffuse and circumscribed periodontitis
- Operative removal of the remaining roots
- Treatment of dental abscesses etc. (Graph. 1.)

The same parameters were measured before the surgical intervention in cases when the patients had the symptoms before the operation and in all cases after 48 hours and 7 days following the operation. The parameters measured were pain, swelling, body temperature, dysfunction such as dysphagia trismus, chew-

ing dysfunction. All the parameters were strictly defined in order to harmonize the investigation criteria and facilitate statistical data processing. The parameters follow: Pain 0- the patient does not feel any pain  
 Pain 1- insignificant pain that does not impair function  
 Pain 3- a considerable pain with the impaired function  
 Pain 4- an intense pain which prevents peaceful sleeping

Swelling 0 - it is not shown visually  
 Swelling 1 - insignificant swelling detected by palpation  
 Swelling 2 - swelling is visually noticed, ex. the erased nasolabial furrow.  
 Swelling 3 - significant swelling which affected one area  
 Swelling 4 - exceptionally visible swelling which has spread to other areas

## RESULTS

	PAIN 0	PAIN 1	PAIN 2	PAIN 3
AMOXICILLIN	1	7	10	8
AMOXICILLIN+CLAV.ACID	4	16	9	6

TABLE 1. Pre-operative symptoms: Pain

	SWELLING 0	SWELLING 1	SWELLING 2	SWELLING 3	SWELLING 4
AMOXICILLIN	7	7	4	6	2
AMOXICILLIN+CLAV.ACID	13	16	4	2	0

TABLE 2. Pre-operative symptoms: Swelling

With respect to the pain symptom before the operation there was no significant statistical difference,  $p > 0.05$  ( $t = 0.56$ ;  $t = 0.69$ ). With respect to the swelling symptom before the operation there was no significant statistical difference,  $p > 0.05$ ;  $t = 0.1$ ;  $p = 0.48$ ). The results of the above tables indicate that the sample was exceptionally homogenous and therefore, valid for the above-indicated calculations.

	PAIN 0	PAIN 1	PAIN 2	PAIN 3
AMOXICILLIN	19	16	5	2
AMOXICILLIN AND CLAV.ACID	27	22	8	0

TABLE 3. The first check-up after 48 hours: Pain symptom

Between the onset of pain and the use of two antibiotics there is significant statistical difference in favor of antibiotic combination of amoxicillin and clavulanic acid ( $\chi = 14.83$ ,  $p = 0.002$ ;  $p < 0.01$ ).

	SWELLING 0	SWELLING 1	SWELLING 2	SWELLING 3	SWELLING 3
AMOXICILLIN	12	13	13	4	0
AMOXICILLIN AND CLAV.ACID	10	30	12	5	0

TABLE 4. The first check up after 48 hours: Swelling symptom

Between the onset of swelling and the use of two antibiotics there is not a significant statistical difference ( $\chi = 4.89$ ;  $P = 0.18$ ;  $P > 0.05$ ).

	PAIN 0	PAIN 1	PAIN 2	PAIN 3
AMOXICILLIN	32	7	1	0
AMOXICILLIN AND CLAV.ACID	56	1	0	0

TABLE 5. The second check-up after 7 days: 'Pain' symptom

After 7 days there is a significant statistical difference in relation to 'pain' symptom and the use of two antibiotics:  $H_2 = 9.35$ ,  $p < 0.01$ , and it tilts significantly in favor of antibiotic combination of amoxicillin and clavulanic acid use. In other words, the pain is significantly smaller after the administration of antibiotic combination of amoxicillin and clavulanic acid.



	SWELLING 0	SWELLING 1	SWELLING 2	SWELLING 3	SWELLING 4
AMOXICILLIN	32	6	2	0	0
AMOXICILLIN AND CLAV.ACID	55	21	0	0	0

TABLE 6. The second check-up after 7 days: 'Swelling' symptom

After 7 days, with respect to swelling there is a significant statistical difference between the patients regarding the treatment with the two antibiotics,  $p < 0.05$  ( $H_2 = 6.45$ ,  $p = 0.03$ ) in favor of antibiotic combination of amoxicillin and clavulanic acid.

	EXCELLENT THERAPY	GOOD THERAPY	UNSATISFACTORY THERAPY
AMOXICILLIN	31	10	2
AMOXICILLIN AND CLAV.ACID	50	7	2

TABLE 7. Effectiveness of therapy

With regard to the therapy outcome between the users of the two antibiotics there is a significant statistical difference in favor of antibiotic combination of amoxicillin and clavulanic acid ( $\chi^2 = 15.00$ ;  $p = 0.001$ ;  $p < 0.001$ ).

We have also monitored the emergence of potential allergic reactions and gastrointestinal disorders in course of the antibiotic administration:

GRADE*	NUMBER OF PATIENTS
ALLERGIC REACTIONS 0	42
ALLERGIC REACTIONS 1	0
ALLERGIC REACTIONS 2	0
ALLERGIC REACTIONS 3	3
GASTROINTESTINAL REACTIONS 0	41
GASTROINTESTINAL REACTIONS 1	0
GASTROINTESTINAL REACTIONS 2	1
GASTROINTESTINAL REACTIONS 3	1
OTHER UNDESIRABLE REACTIONS	0

\*Grade: 0 = no reaction; 1 = mild reaction; 2 = moderate reaction; 3 = severe reaction

TABLE 8. Undesirable reactions to amoxicillin

GRADE*	NUMBER OF PATIENTS
ALLERGIC REACTIONS 0	57
ALLERGIC REACTIONS 1	0
ALLERGIC REACTIONS 2	2
ALLERGIC REACTIONS 3	0
GASTROINTESTINAL REACTIONS 0	48
GASTROINTESTINAL REACTIONS 1	6
GASTROINTESTINAL REACTIONS 2	3
GASTROINTESTINAL REACTIONS 3	2
OTHER UNDESIRABLE REACTIONS	3

\*Grade: 0 = no reaction; 1 = mild reaction; 2 = moderate reaction; 3 = severe reaction

TABLE 9. Undesirable reactions to antibiotic combination of amoxicillin and clavulanic acid

## DISCUSSION

As it was stressed at the beginning of this case study report antibiotics play an exceptionally important role in medicine in general, and also in dental medicine. General dental practitioner and especially oral surgeon are often brought into a situation when he/she faces a dilemma regarding antibiotic prescribing. Uncritical and careless antibiotic prescribing brings more harm than benefit. This problem is still tackled by many authors. Thus, Haas and his associates (2) deal with the problem of resistance to antimicrobes in their study conducted in 1998. They emphasize that the resistance of bacteria, fungi and viruses to antimicrobes is steadily increasing with detrimental effects. The role of dentistry in this respect is still unclear. Nevertheless, the dentists need to know how to proscribe antibiotics properly, treat infections effectively and minimize likely development of bacterial resistance to antimicrobes. The aim of the above authors study was to raise the awareness of dentists of the dangers involved in treating antimicrobial resistance and to the ways of avoiding the same (2). Furthermore, Epstein and his associates (3) assert that antibiotics play an important role in the treatment and prophylaxis of infections. Because of the resistance to antibiotics the authors have conducted their research with the aim of benefiting assessment of particular antibiotics in the dental practice. The data were gathered on the basis of the sample filled in by trained dentists in British Columbia in Canada. The total of 2.542 sample forms were sent to them, out of which 19.9 % were returned either by fax or mail. The collected data were analyzed by applying chi test. It was proven that penicillin and its derivatives were the most commonly proscribed antibiotics following a dental treatment. An average duration of antibiotic therapy was 6.9 days. On average, dentists proscribed antibiotics 1.15 times per week as prophylaxis against bacterial endocarditis, 17.5 % of physicians proscribed antibiotics as prophylactic protection postoperatively in the duration of one to seven days. Antibiotics were proscribed preoperatively to patients who suffered from rheumatic fever, heart murmur or those with an artificial hip. Antibiotics were commonly proscribed in surgical interventions to patients who suffer from the immune-deficiency syndrome. In their conclusion, the above authors have stressed that the appropriate and timely antibiotic administration is a way to ensure an effective treatment and avoid bacterial resistance to antibiotics. In order to raise the standards, dentists are advised to get to know pharmacology and be committed to lifelong learning (3).

Beckford – Ball (4) states that since the discovery of penicillin antibiotics have had a significant impact on health maintenance. However, practitioners are faced with the common proscribing dilemma. How can we continue with the practice of appropriate antibiotic prescribing with the minimum risks of bacterial resistance to antibiotics (4)? Our view is that an appropriate antibiotic prescribing is a very topical issue. In our environment beset with very low standards regarding oral hygiene and health, the dentists are often placed into a situation to proscribe antibiotics. In order to ensure full effectiveness of the proscribed drug it is important to know the appropriate dosage and duration of its administration. In our study report in view of the severity of the clinical picture we proscribed antibiotic combination of amoxicillin and clavulanic acid for 5 to 10 days in the dosage of 625 mg 3 times per day, and amoxicillin for 8 to 10 days in the dosage of 500 mg 4 times per day. We had to stop administering antibiotic combination of amoxicillin and clavulanic acid in two patients after the expiration of two days because of side effects. In other cases when we proscribed antibiotic combination of amoxicillin and clavulanic acid the patients used it arbitrarily for 2, 3, 6 or 7 days in accordance with their personal feelings. In cases of amoxicillin prescription some patients have also arbitrarily reduced the duration of the therapy to 3 or 5 days depending on their individual state. Some patients took the medication only for 2 days because of allergic reaction. Therefore, the patients themselves are often responsible for the bacterial resistance to antibiotics because of their failure to follow the proscription instructions. Pallasch (5) stresses that due to the antibiotic abuse people have brought about the situation wherein all microorganisms acquired resistance to some antibiotics while other microorganisms became resistant to all antibiotics. The greatest benefit of the antibiotic use is assistance to the host in controlling and defending against infection. On the other hand, antibiotics may cause toxic and allergic reactions, super-infections followed by resistant bacteria, chromosome mutations that lead to microbe resistance and also they can instigate eruption of dormant, resistant genes (5). Palmer with his associates (6) deal with the same problem. In their work (published in 2001) they point to the problem of unselective and inappropriate antibiotic prescribing in the form of solutions that are given to children. This can contribute to the development of bacterial resistance. The way to prevent such a situation is to follow clear instructions about the kind of antibiotic that is to be proscribed, its

dosage and the duration (6). The aim of the study conducted by Anderson and his associates (7) in 2000 was to compare the level of proscripting and the kind of antibiotic proscribed by general practitioners (GPs) and dentists for various dental ailments. In their conclusion they state that GPs tend to proscribe antibiotics for dental problems more often than dentists. There are also differences in proscripting broad-specter antibiotics. According to these authors GPs and dentists as well should rationalize the administration of antibiotics (7). In our investigation we came across problems when GPs did not obey the dentist's proscriptioin of antibiotic combination of amoxicillin and clavulanic acid but instead proscribed some other antibiotic, mostly cephalosporins. Unfortunately, this is the case of lack of cooperation between the GP and the dentist. Oral cephalosporins are not effective against many anaerobic gram-positive cocci such as peptococcus and peptostreptococcus as well as against many anaerobic gram-negative bacteria such as *Bacteroides species*, i.e. *Bacteroides fragilis* which is often the cause of odontogenic infection. Thus, the use of oral cephalosporin for the treatment of odontogenic infection can result in super-infection (1). The issue of rational antibiotic administration is also topical. In this respect, the aim of the study conducted by Palmer and his associates (6) was to investigate whether the clinical revision may improve antibiotic proscripting by general dentists. The investigation was conducted in general dental surgeries in the north-west of England involving 175 general dentists. The records were kept about the kind of antibiotic proscribed, its dosage and the duration of therapy, clinical indicators and the health state of patients, but also about other reasons which led to the antibiotic proscriptioin. The comparison was made between the antibiotic proscripting before and after the clinical revision. The study results have shown that antibiotics proscripting has decreased by 42.5% after the revision (or following the instructions). In all situations the most commonly proscribed antibiotics were amoxicillin (57.6%), metronidazol (23.8%), penicillin (9.3%), erythromycin (4.8%) and the combination of amoxicillin and metronidazol (1.7%). In the aftermath of issuing the instructions with regard to the antibiotic proscriptioin a significant decrease in proscriptioins followed ( $p < 0.05$ ). The results of this study lead to the conclusion that the clinical revision in view of antibiotic proscripting and the publication of written instructions and the educational training may rationalize the use of antibiotics in general dental surgeries (8). In this way, in order to avoid indiscriminate and scientifically unfounded use of antibiotics, it is an imperative

to follow rigorously strict indications for the use of antibiotics. It is also a way to decrease costs and increase good results of the treatment. We believe that it is by and large the responsibility of oral surgeons to proscribe antibiotics for specific dental ailments. This was confirmed by the author Preus and his associates (9) in their work conducted in 1992. They investigated the practice of antibiotic proscripting by Norwegian dentists. The results showed that antibiotics were most commonly proscribed by oral surgeons and paradontologists in comparison with general dentists (9). A particular segment of antibiotic use consists of antibiotic prophylaxis. Nevertheless, not all cardiological states demand antibiotic prophylaxis. Indiscriminate use of antibiotics for these purposes can also bring more harm than benefit. Tong and his associates dealt with this problem in 2000. According to their review article the latest recommendations of AHA association were that only in few situations was the antibiotic prophylaxis necessary prior to the dental intervention. The same authors believe that, at the present moment, the risks of inappropriate use of antibiotics and the development of bacterial resistance outweigh the possible benefits. In their conclusion they recommend antibiotics use only in strictly defined situations that are scientifically grounded (10). Antibiotic combination of amoxicillin and clavulanic acid is not suitable for a single-day antibiotic prophylaxis because the dosage of 2 grams of this antibiotic would amount to the overdose with clavulanic acid, and that, in turn, could bring about the undesirable reaction such as diarrhea. One of the reasons for the use of antibiotics is the protection against the infection after the surgical intervention. Thomas and his associates (11) indicate to the fact that antibiotic use as a prophylactic measure against the infection after the removal of impacted third molars is widespread. It seems that the benefits of resorting to this kind of prophylaxis are marginal. Also, according to the same authors there is little evidence to support the use of the second and third generation of antibiotics in routine prophylaxis. In their study the above authors indicated the need to rationalize the use of antibiotics which would also be cost-effective (11). Classen and his associates tackled the same problem (12). They point to the fact that randomized control studies have shown the efficacy of antibiotic prophylaxis in the prevention of infection of surgical wounds (12). In our study we have attempted to investigate the intensity of postoperative symptoms, especially of pain and swelling after the use of antibiotic combination of amoxicillin and clavulanic acid and amoxicillin antibiotics. The idea to undertake this investigation was born after we noticed that the

administration of antibiotic combination of amoxicillin and clavulanic acid contributed to faster recovery of patients in the postoperative period. Our aim was to

find evidence to support that this was not a mere accident and we have conducted our investigation on the sample that is sufficiently large for statistical analysis.

## CONCLUSION

The combination of amoxicillin and clavulanic acid has proven to be significantly more effective after the oral-surgical interventions in relation to the effect of amoxicillin antibiotic. Therefore, we recommend the prescription of antibiotic combination of amoxicillin and clavulanic acid in the future dental practice.

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# GENETIC EXAMINATION OF CHILDREN SUFFERING FROM CYSTIC FIBROSIS

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## ABSTRACT

CFTR protein (cystic fibrosis trans membrane conductance regulator) is expressed in multiple epithelial tissues, including upper and lower respiratory tracts, pancreas, sweat glands and gastrointestinal tract. More than 800 mutations and 100 polymorphic variants of DNA sequences were identified in patients with CF (Cystic fibrosis) and CFTR- diseases. In this study, genetic CFTR analysis of the children suffering from chronic lung disease (cystic fibrosis) is presented. They are treated and regularly controlled at the Pediatric hospital Sarajevo. CFTR analysis was done in 9 cases, 4 boys (44.4%) and 5 girls (55.55%). There are 3 children (33.3%) in the age group 1 to 3 years, 1 child (11.1%) in the age group 3 to 6 years, 3 children (33.3%) in the age group 6 to 9 years and 2 children (22.2%) in the age group 9 to 12 years. Genetic analysis was conducted at the Medical center for molecular biology, School of Medicine, Ljubljana. PCR method with PAGE and direct sequestration on ABI PRISM 31 was applied. The majority of children (7 children, i.e. 77.77%) had CFTR mutation  $\Delta F 508$  whilst one child had G542X mutation and one child R 1174 mutation. The purpose of this study is to emphasize the need for CFTR gene identification in the institutes of our country.

**KEY WORDS:** cystic fibrosis, CFTR gene, children who suffer from cystic fibrosis

## INTRODUCTION

Cystic fibrosis (CF) is a complex gene syndrome revealed by dysfunction of all exocrine glands. It is defined by mutations of CFTR gene (cystic fibrosis transmembrane conductance regulator), which result in the production of hyper-viscous mucus and chloride malabsorption in the sweat glands' ducts (1, 2, 3). It is inherited as autosomal recessive trait, with gene locus for cystic fibrosis mapped at 7q31. The most frequent mutation is  $\Delta F 508$  that is found in approximately 70% of affected children. The mutation of CF gene is connected to deletion of three pairs of bases, which results in shift of phenylalanine residue to the position 508 of the mature protein (4, 5). The diagnosis of cystic fibrosis is established according to the criteria that can be divided into 2 groups (6). Group A - clinical criteria: typical pulmonary clinical features, typical gastrointestinal clinical features and positive family history of cystic fibrosis in close relatives. Group B- laboratory criteria: Chloride concentration in the sweat and CFTR mutation. At least one criterion from each group is necessary for conclusive diagnosis. The treatment is symptomatic and supportive, until the introduction of gene therapy which means replacement of mutated CFTR gene with functional one.

## SUBJECTS AND METHODS

In this study we present the results of genetic analysis of 9 children, in the age between 1 and 12 years, who suffer from cystic fibrosis. They are treated and monitored at the Pulmological department of the Pediatric Hospital Sarajevo. The diagnosis was established by repeated broncho-obstructions, X-ray examinations of the lungs, measurements of the enzyme activities in the duodenal secretions and measurement of chloride content in the sweat, which was above 100 mmol/l in

all cases. The genetic analysis was realized using PCR based method, with PAGE and direct sequestration on ABI PRISM 31 Genetic Analyzer at the Medical center for molecular biology, School of Medicine, Ljubljana.

## RESULTS

In the Table 1. the results are presented for nine children, 4 girls and 5 boys 1-12 years of age, who suffer from cystic fibrosis. In 7 cases (77.77%)  $\Delta F 508$  mutation of CFTR gene was identified. In the age group 3-6 years one G542X mutation was identified, while one R1174 mutation was identified in a girl from the age group 9-12 years.

## DISCUSSION

Cystic fibrosis is a chronic, multisystem disorder, most frequently characterized by pulmonary manifestations. Due to the modern supportive care, about half of the patients live to the age of 20 and about 25% of the patients survive until 30 years of age (6). In our patients, the diagnosis of cystic fibrosis established based on clinical features and sweat test. The sweat test is the most important and standard diagnostic procedure (7).

## CONCLUSION

1. The diagnosis of cystic fibrosis was confirmed by genetic analysis, which is especially important in the case of atypical and mild forms of cystic fibrosis.
2. In the future, cystic fibrosis will be cured when an adequate way of replacement of the defective CFTR gene with intact gene is found and an ideal vector for safe and efficient entrance of CFTR gene into the cell is discovered.

AGE	No.	MALE	FEMALE	CFTR GENE
1-3 YEARS	3 (33.3 %)	1	2	$\Delta F 508$
3-6 YEARS	1 (11.1 %)	1	0	G542X
6-9 YEARS	3 (33.3 %)	2	1	$\Delta F 508$
9-12 YEARS	2 (22.2 %)	1	1	$\Delta F 508$ R 1174
TOTAL	9 (100 %)	5	4	$\Delta F 508$ (77.77 %)

TABLE 1. CFTR mutations in children with cystic fibrosis



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# PSYCHO-SOCIAL CHARACTERISTICS OF CANNABIS ABUSING YOUTH

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## ABSTRACT

It is a well known fact that drug abuse is most common in early adolescence. The most popular substances among youth are cannabis products (made from *Cannabis sativa* L., Cannabaceae). The majority of heroin and cocaine addicts have started with marijuana. The aim of this study is to show some psycho-social characteristics of adolescents who abuse cannabis. Research conducted during the year 2001 was epidemiological and prospective. The study group included 600 adolescents of equal gender and age distribution. Q 2000 questionnaire was used, as a comprehensive tool for all aspects of adolescent life. The results show strong peer impact on one's behavior. Youth who use cannabis had 2-3 friends of the same behavior, compared to others who had none. We found positive correlation between life stressful events and cannabis abuse. We also noticed tendency to delinquent behavior related to cannabis abuse (35%).

**KEY WORDS:** adolescence, cannabis, abuse, psychosocial, peer, delinquency

## INTRODUCTION

For approximately 4.500 years, *Cannabis sativa* L. plant is grown all over the world for different purposes (production of fabric, oil, drugs). The kinds of cannabis, which are used as drugs prosper in tropical areas; and, the ones from which oil and fabric are produced prosper in subtropical areas and mild climates. Since very old times, cannabis has been used for many purposes during various rituals (similar to the use of coffee, tea or tobacco) in numerous tribal communities native to North Africa, Middle East, territories that are within the state borders of present Pakistan, India, Nepal, Afghanistan. According to the UN Convention definition, the following terms are used for cannabis:

- Cannabis plant- any plant from genus *Cannabis*
- Cannabis- represents the flowering tops with intact resin
- Cannabis resin- raw or purified resin separated from the cannabis plant
- Cannabis oil- concentrate extracted from cannabis resin or cannabis
- Cannabis - similar to tobacco, green or brown leaves and the flowering tops of the cannabis plant.

### COMMON NAMES:

marijuana grass

hemp - It is commonly used by smoking (0.5- 1 g)

ganja

bongo

Cannabis resin- black or dark brown resin-like matter processed from cannabis plant. It exists in the following forms: fine powder, tiles, unleavened bread. It can be smoked alone or mixed with tobacco (1/10 g) or used in food as infusion, cookies. Some common names in narco-slang are: hashish, kif, shit. Cannabis oil can be extracted from the resin as tar, red or dark green viscous liquid. Very potent, 1-2 drops are placed on a piece of paper or tobacco. Names: honey oil, red oil. Through detailed chemical analysis over 427 substances were found in cannabis. Some of those are psychoactive substances. It was determined that cannabis contains approximately 60 molecules of cannabinoid type. The best known cannabinoids are: tetrahydrocannabinol (THC), cannabinol, canabidiol, canabigerol, canabicyclic acid, Cannabis acid. Familiar kind of cannabis is "Sinsemilla" which produces no seeds. Such plant is produced by removing "male flowers" during their blooming, so that insemination cannot occur and the plant is persuaded to bloom further and thus produce greater amount of resin on the flowers. Sinsemilla contains enor-

mous amount of a psychoactive cannabinoid – THC. Unique way of growing cannabis is known in the countries with continental or colder climate. No matter what the outside conditions are, cannabis is grown in the closed spaces (usually a basement), which are specifically arranged for this purpose. Beside the necessary fertile soil, plastic sheets need to be installed on the walls and water and light constantly provided. Favorable conditions (fertile soil, a lot of humidity, heat and all-day light) result in very successful cultivation of cannabis. This way to grow it is called "hydroponing". Cannabis produced in this way is called "skunk". Otherwise, for "harvesting" – removing the resin from the flowering tops, various techniques are used, for example, rubbing with the hands, shaking against the wall, walking through a plantation in a special suit. "Sinsemilla" – contains 5–14% of the THC. Pharmacological effects mainly depend on the experience but also on the expectations of a user. The following experiences were described by the largest number of the interviewed cannabis users – good mood "high", euphoria, a nice relaxed state, an increased activity of sensors (receptors): vision, smell, taste, hearing. Substance abusers who used cannabis for a short period usually experience increased appetite, increased heart rate, eye redness. While the drug is still in effect, the mental and physical abilities are reduced. Increase in dosage causes distortion and confusion of vision, hearing and other receptors. Large doses cause hallucinations. Substance abusers who have used cannabis for a prolonged time usually experience loss of drive and interest in activity. One may develop moderate tolerance (psychological dependence is possible), Cannabis smoke contains 50% more tar than the worse cigarettes so there is also a risk of lung cancer, chronic bronchitis and other respiratory system diseases. Besides certain ritual values, people attribute cannabis some medicinal value, that is, ability to heal certain diseases. Modern medicine did not find any justifiable medical reason for use of the cannabinoids in treatment of diseases. Therefore, cannabis is placed on the List I in accordance with the UN Convention on narcotic drugs, 1961. The most recent research show that the synthetic THC—dronabinol (Marinol) may be effective in treatment of glaucoma, decrease muscle contractions in case of epilepsy, or as analgetic in patients on chemotherapy (used as antiemetic—a substance which decreases vomiting) and perhaps for appetite stimulation in patients with AIDS. WHO recommendation regarding the medical use: Although there has been a significant development during the past few years, there is still a need for scientific research, especially in the area of clinical and epide-

miological research about the influence of cannabis on human health and pharmacology, but also further research in the area of using cannabis as medicament (1). Although it has not been found that cannabis causes physical addiction but only a moderate form of psychological addiction, scientific studies show that many abusers of cannabis have continued—deepened their experience—with a “stronger” experience by moving onto heroin. Problems connected with abuse of psychoactive substances in Bosnia and Herzegovina was registered for the first time in early eighties of the twentieth century. According to the most recent data, the most commonly abused substances are cannabis, anti Parkinson’s disease drugs, glues and solvents, heroin, ecstasy, sedatives and hypnotizers, alcohol, tobacco, and very rarely hallucinogens, cocaine and others. Although drug abuse occurs at any stage of life, its occurrence is the most significant in adolescence. Thus, this paper focuses on that population with an aim to analyze some aspects of different behaviors of youth who use cannabis.

## MATERIAL AND METHODS

### THE KIND OF RESEARCH

The conducted study is of an epidemiological and prospective type.

### STUDY GROUP

This research includes total of 600 adolescents, 200 in Tuzla Canton (100 primary school pupils and 100 high school students) and 400 in Sarajevo Canton

(200 primary school pupils and 200 high school students). Average age of subjects was 12-17 years with equal gender representation as well as equal representation according to living conditions (rural-urban).

### RESEARCH INSTRUMENTS

In order to obtain the needed information, we used Q 2000 test, an instrument which was created during the period 1990-1991. This test is scientifically valid and widely accepted in the world, and it is adequate for the topic under consideration. It contains a wide range of questions about the relevant factors, which may influence use and abuse of drugs from all aspects of adolescent life; health culture, life style etc. In all schools the conducted survey was anonymous and voluntary. The research preparations as well as the survey were conducted simultaneously in Sarajevo and Tuzla cantons during the year 2001.

## RESULTS

The research results were subjected to standard statistical analysis using software EPI-INFO. Data are presented in table and graph formats (2). Relevant data gathered from our subjects in connection with their age and sex are presented in the following two tables and one graph, followed by the comments. Risky behavior is more often found in the young people who are susceptible to use of narcotics: in a car driven by a drunk friend 10.0%: drunk grown-up 5.0%, seat belt always used by 15.0%; and in a group which does not use narcotics against the same parameters: 0.9%, 0.7%

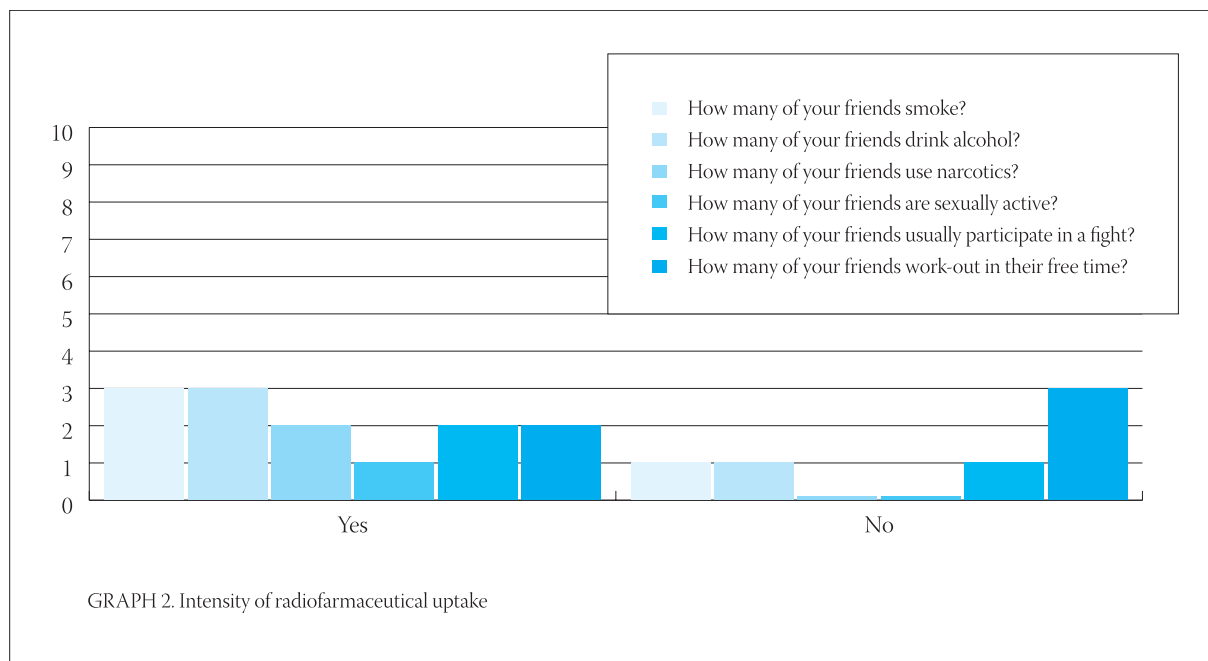
NORMAL CULTIVATION OF CANNABIS	% THC	SKUNK*
CANNABIS PLANT (MARIJUANA)	0.5 – 5	(25)
CANNABIS RESIN (HASHISH)	2 – 10	(40)
CANNABIS OIL	10 – 30	(80)

\* By selection and cultivation- hydroponing- one gets "skunk" with 25% of THC and resin with 40% of THC.

### CONTENTS OF TETRAHYDROCANABINOL

		HAVE YOU EVER TRIED TO SMOKE "GRASS"			
		YES		NO	
		N	%	N	%
HOW OFTEN ARE YOU IN A CAR DRIVEN BY A DRUNK FRIEND?	OFTEN	2	10.0	5	0.9
HOW OFTEN DO YOU TRAVEL IN A CAR DRIVEN BY A DRUNK GROWN-UP?	OFTEN	1	5.0	4	0.7
DO YOU USE A SEAT BELT, DURING A CAR RIDE ( $X^2=7.136$ P=0.00755)	ALWAYS	3	15.0	240	41.7
	NEVER	6	30.0	86	14.9

TABLE 1. Risk behavior of young people who use cannabis



		HAVE YOU EVER TRIED TO SMOKE "GRASS"			
		YES		NO	
		N	%	N	%
HAVE YOU EVER TAKEN SOME THINGS FROM A STORE WITHOUT PAYING FOR IT	IT HAPPENED MORE THAN ONCE	7	35.0	39	6.9
HAVE YOU DESTROYED SOMETHING THAT DOESN'T BELONG TO YOU ON PURPOSE OR BEEN AN ACCOMPLICE IN DOING IT?	IT HAPPENED MORE THAN ONCE	3	15.0	41	7.3
HAS SOMEONE ABUSED YOU IN ANY WAY AND MADE YOU GIVE HIM/HER MONEY,...	IT HAPPENED MORE THAN ONCE	2	10.0	18	3.2
HAVE YOU EVER ABUSED SOMEONE AND MADE THEM GIVE YOU MONEY,...	IT HAPPENED MORE THAN ONCE	2	10.0	2	0.4
HAVE YOU COLD-BLOODEDLY TAKEN MONEY OR SOMETHING ELSE KNOWING IT BELONGS TO SOMEONE ELSE	IT HAPPENED MORE THAN ONCE	5	26.3	11	2.0

TABLE 1. Risk behavior of young people who use cannabis

and seat belt is always used by 41.7%, which is shown in Table 1. Data presented by the graph show that group influence on an individual is significant in relation to the abuse of harmful and dangerous substances. Table 2. present that users of narcotics have a higher degree of inclination towards delinquent behavior. The value of  $X^2$  shows significant difference between the two groups of participants when delinquent behavior of youngsters is concerned.

## DISCUSSION

Considering a significance of the adolescent period in one's lifetime and serious disorders that can oc-

cur during that period, we conducted this study in order to determine a level of abuse of cannabis by the adolescents. We approached this problem from the aspect of behavior of youth, trying to present sociological factors, which could potentially cause an increase in cannabis abuse. Identification of these factors may help in formulation of a preventive strategy that could give positive results in wider area. Our research showed that from a total of 598 adolescents, 3.34 % abuse cannabis. As one can see from the results of our material, different forms of risk behavior (riding with a drunk friend, drunk grown-up, not using a seat belt, using narcotics, etc.) are more frequently exercised by the young people who use any of the psychoac-

tive substances compared to their peers who do not. For example, young people who consume cannabis would more likely ride in a car which is driven by a drunk friend 10.0 %: drunk grown-up 5.0 %, seat belt is always used by 15.0 % ; and in a group that does not use narcotics against the same parameters; 0.9 %, 0.7 %, and seat belt is always used by 41.7 %, which is shown in Table 1. Literature is abundant in data which suggest similar variables and results. The conclusion is that persons in that age group manifest multiple risk behavior more often (3).

Our study considered peer influence as well. It was found that youngsters who abuse cannabis, alcohol and other narcotics have, on average, two or three friends with the same habits, compared to the other group of young people who do not have any or have just one such friend. A peer influence proved to be very significant in abuse of harmful and dangerous substances. This observation is in agreement with the works of authors from around the world and many studies, which have shown that the characteristics of a group an adolescent spends time with and its attitude toward drug abuse are very relevant. Results from the literature illustrate the same conclusions: risky behavior of youngsters in comparison with the use of psychoactive substances could be a prediction for future behavior (4, 5, 6, 7, 8). A focus of our research was also delinquent, destructive behavior of young people. It is important to stress that smoking,

alcohol and drug consumption are in a direct, high correlation with destructive behavior of youngsters. Some authors have shown that easier access to weapons, drugs and alcohol increases the risk of delinquent behavior (9, 10). Young people who participate in fights assert in 20% cases that they will continue with such behavior (8). At the end of this fragmented elaboration, we can come to a general conclusion that our research is, for the most part, in agreement with the studies of some world authors, what can be seen through this discussion.

## CONCLUSIONS


In the end we can conclude that during our research we have registered risk factors for abuse of the psychoactive substances by the young people. The most important ones are:

1. Young people who use cannabis show high degree of inclination towards different kinds of risky behavior (riding with a drunk friend or drunk grown-up, non-usage of safety belt etc.).
2. There is a great probability that an individual will take on the characteristics of a group (he/she will start to consume one or more of the harmful substances if this is present in his/her immediate surroundings).
3. The consumers of cannabis are more inclined towards delinquency (theft in 35.0 %).

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# EVOLUTION OF QUIT & WIN PROGRAM CARRIED THROUGH 2002 YEAR IN FB&H

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## ABSTRACT

World Health Organization (WHO) in cooperation with National Public Health Institute of Finland carried through Quit & Win program 2002. People from all over the world try to abstain from smoking or using tobacco products for a four-week period, from 2-29 May. FB&H took part in this campaign. Quit & Win-2002 program was an optional contest for health professionals. The purpose of this paper is to present the participant's characteristic towards age, gender, education, tobacco consumption and previous attempts to quit. Results: the research encompassed 81 health professionals. Among them 71% were women. The highest participation rates on average were found in the 45-54 year group (29.7%). The most of participants were health professionals with middle school education (81%). Only 5% of the participants were physicians. The great number of health professionals (47.5%) smoked between 1-9 years. Research shows that 61% of participants were passionate smokers who smoked more than 14 cigarettes a day. Out of 81 participants 42 medical workers has never tried to stop smoking. Research shows that smoking habit is very spread among medical workers and it follows the general state in population. It's surprising because we expect that health worker must be role model of the healthy life.

**KEY WORDS:** smoking cessation, Quit&Win, health professionals

## INTRODUCTION

Quit &Win is smoking cessation contest for adults. Contest for promoting quitting were originally used in some US health campaigns in the early 1980s. The National Public Health Institute of Finland (KTL) applied Quit &Win as a cessation method for the first time in Finland in 1985 and organized the first international contest in 1994. Quit &Win has rapidly grown in popularity as a practical international smoking cessation action. This is likely to be due to Quit &Win unique, positive approach to a problem that is receiving increasing attention world wide as the truly major health threat.(1) Quit &Win 2002 competition was an optional contest for health professionals. This program encourages health professionals to act as role models in relation to smoking cessation. FB&H joined the international Quit &Win 2002 network. FB&H has made its own competition including: recruiting smokers, information activities, obtain prizes and other tasks.(2) The international core components of the program include the campaign timetable, common rules, international promotional materials and standardized follow-up procedures. The common rules were: The competition take place in May 2002 with the actual quit date on 2 May 2002. The requested abstinence period lasts four weeks. The criteria for participant were: At least 18 years age, a current daily smoker and history of daily smoking of at least one year before the contest. The participant must fill in the entry form no later than the quit day. The national/regional winners will be drawn after the abstinence period of four weeks.

The abstinence is verified by biochemical test. After the national winners have been chosen, an international super prize of US 10 000 and six regional prizes of US 2500 will be drawn among the winners of each country.

## METHODS

Quit &Win 2002 competition took place in May 2002 with actual quit date on 2 May 2002. Participants were selected among voluntary applied medical workers. Each participant must fill in the entry form (model WHO). Scientific analysis was prepared on the base of entry form for participant.

Observing variables were:

Gender

Age group: 18-24, 25-34, 35-44, 45-54, 55-65 years

Tobacco consumption (present smoking-times per day):

1-14, more than 15

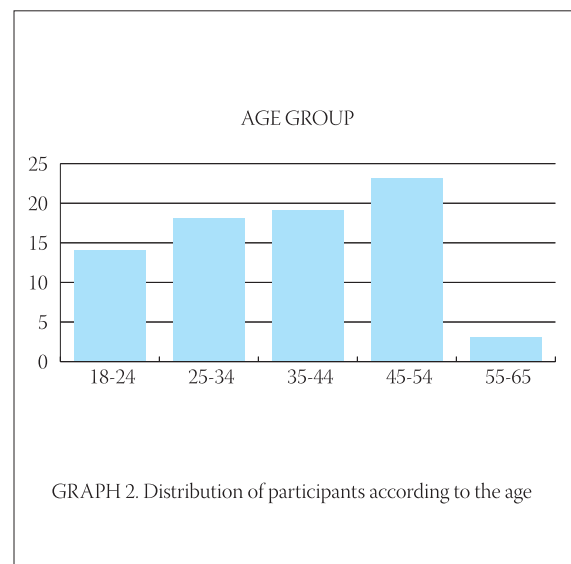
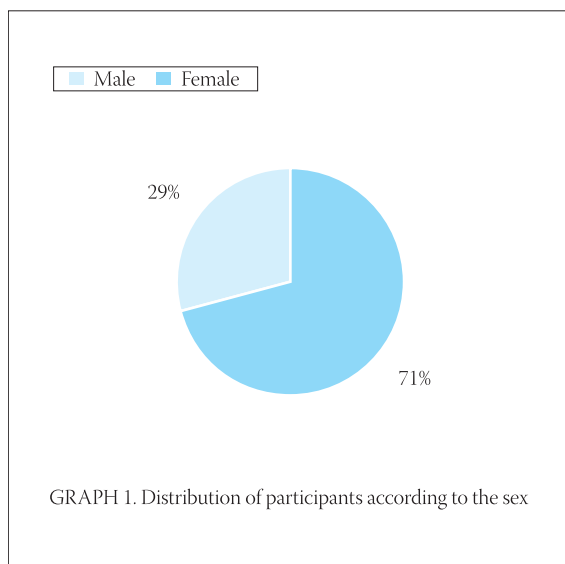
Previous attempts to quit: none; 1-2 times; 3 or more

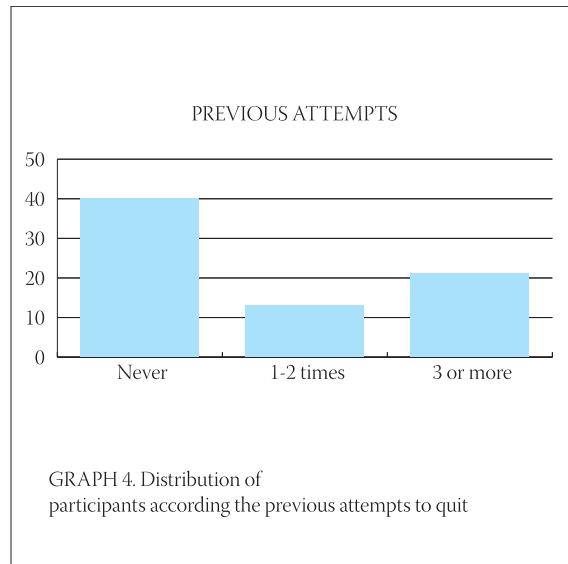
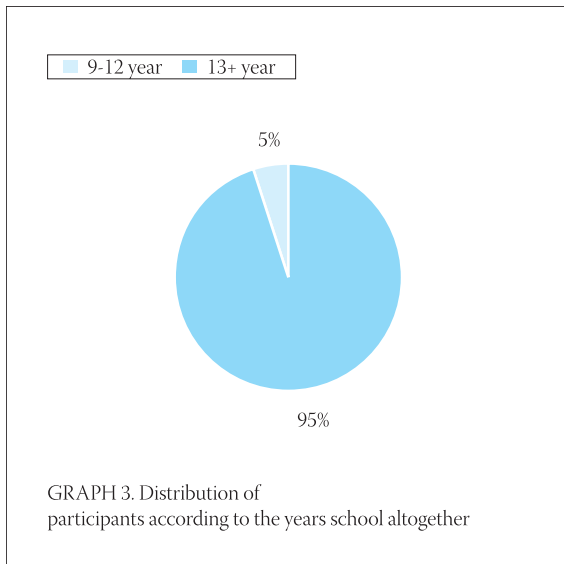
Years of smoking: 1-9; 9-19; 20 or more

Years school altogether or studied full-time: 9 years, 9-12 years, 13 or more

## RESULTS

The research encompassed 81 medical workers. The vast majority of Quit &Win participants were women (71.5%). Graph 1. The results showed that all age groups were reached by the campaign, but the highest





participation rates on average were found in the 45-54 year age group. Graph 2. The most of participants were health professionals with middle school education (81%). Only 5% of the participants were physicians. (3) Graph 3. The great number of health professionals (47.5%) smoked between 1-9 years. Research shows that 61% of participants were passionate smokers who smoked more than 14 cigarettes a day. Among them were 56.4% female smokers and 46.4% male smokers. It is surprising that 51.6% of the participants belonging to the sample had never tried to stop smoking before. Graph 4

## DISCUSSION

Quit&Win is a cost-effective evidence based smoking cessation method for population-wide public health use that also supports more broadly national tobacco control work. The Quit&Win campaigns use innovative communication methods, partnership, community organization and health service involvement. The Quit&Win model has proven to be applicative in different cultures all over the world. International Quit&Win campaigns have been carried out every other year since 1994. 77 countries and 700 000 participants took part in the Quit&Win contest in the year 2002. During Quit&Win 2002 there have also been an optional contest for health professionals. The goal is to get health professionals to stay tobacco free and this way become more motivated to act as role models and obtain better skills to do anti-tobacco work with their patients. International Quit&Win contests have rapidly grown in popularity as a practical international smoking activity. Quit&Win began in 1994 with 13 countries participating. Since then, the competition taken place internationally every other year. 1996 with 25 countries, in 1998 with

over 200 000 participants involved from 48 countries and in 2000 some 430 000 participants in 69 countries. A total of 81 medical workers in FB&H are participated in Quit&Win program 2002. The vast majority of participants were women. It is in the line with the high percentage of women in structure of medical workers in FB&H. The highest participation rates on average were found in the 45-54 year age group. The participants in that age group have been traditionally the most successful quitters. They had already health harmful smoking consequences. The most important reason to quit was in connection with prevention of serious diseases. Behaviour of medical workers is surprising in consideration of their knowledge of harmful effects of smoking. (4) We are expecting from them to take a leadership in creating safe and healthy environments and responsibility for own and patient's health. After carried campaign Quit &Win 2002 we compared gained results with results Quit &Win carried through 2000 years. There were not essential differences. Widen smoking habits among medical workers follow the general state in population. There are no essential differences in prevalent of smoking among medical workers and prevalent of smoking in population of B&H. During the whole year following the Quit&Win campaign 10-30% of the participants have stayed completely smoke-free. But also the other participants learnt for their next attempt. That campaign contains positive message for smokers. The great number of smokers (70-80%) wants to quit. Many have tried several times but often lack the support and impetus needed to abstain from tobacco consumption. Thus Quit &Win offers help to smokers who want to quit. The winners of the international prizes were drawn in Cyprus on the 7th June 2002. The winner of super prize of US 10 000 is the first prize winner of the

contest in Quebec, Canada. The six regional prizes of US 2500 went to Mauritius, Iran, Germany, Cuba, Indonesia and China. The international prize for the health professional, s contest went to Lithuania. Possibility to

win prizes has a positive appeal, and a large campaign gives encouraging support. Even if the person does not win in the draw, every successful quitter wins health.

## CONCLUSION

Quit & Win program is a positive and cost-effective way to reduce smoking among the population. Success rates in stopping smoking are lower than with very intensive and personal methods, but a much wider audience is reached and at a much lower cost. Follow-up studies have showed that after one year on an average one fifth of the participants have remained smoke-free constantly since the beginning of the Quit & Win contest. The program helps to build broad national coalitions and trends to draw positive media attention. The campaign is also a concrete channel for large international health collaboration, which is necessary considering the global nature of marketing efforts of the tobacco industry. (5)

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FIRST PAGE - A concise but informative full title of the article. Avoid abbreviations and colloquialisms.

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**Key words:** A maximum of 6 key words or short phrases suitable for indexing should be supplied.

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Number the remaining pages consecutively and type the author's(s) last name(s) at the top of each page.

Keep the Introduction brief, stating clearly the purpose of the article and its relation to other papers on the same subject. Do not give an extensive review of literature.

Provide enough information in the **Materials and Methods** section to enable other investigators to repeat the experiments. Also, note that all research described in submitted publication involving human subjects and material derived from human subjects complied with ethical principles outlined in **World Medical Association Declaration of Helsinki** - Ethical Principles for Medical Research Involving Human Subjects (initiated in June 1964, amended in October 1975; October 1983; September 1989; October 1996; October 2000).

Report **Results** clearly and concisely. Do not present the same results in tables and illustrations. Exceptionally, results and discussion can be combined in a single section. Data should be expressed in SI units.

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Start each section on a separate sheet.

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Text references should be indicated by Arabic numerals in round brackets: "the incidence is similar to that in other reports (1,2,3). Davies et al. (4) have reported ..." To avoid any delays in the editing process, authors must make every effort to see that each reference is correct and complete. Incomplete references will be returned to the principal author for completion before the manuscript is edited.

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