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MAXILLARY CRESTAL BONE LOSS AROUND BREDENT BLUESKY® IMPLANTS: ONE YEAR STUDY

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ABSTRACT

The resorption of the alveolar crest is a parameter that is frequently used in the investigation of endoosseous implants. The aim of the study was to analyze the amount of crestal bone loss of the upper jaw around Bredent BLUESKY® implants of different dimension a year after functional loading.

This study analyzed total number of 88 implants type Bredent BLUESKY®. The measurements were performed using Kodak dental software 6.11.7.0 after implantation and a year after its functional loading. The average value of the distal bone resorption around implant dimension 3.5 x 10 mm in front maxilla was 0.67 mm (± 0.098 mm), while the average mesial resorption was 0.57 mm (± 0.118 mm). The average value of the distal bone resorption around implant size 4.0 x 8 mm in maxilla lateral was 0.52 mm (± 0.176 mm), while the average mesial resorption was 0.53 mm (± 0.176 mm). Bone resorption was greater at the distal portion of the crest than mesial, although the differences were not statistically significant.

Key words: maxilla, crestal bone loss, Bredent BLUESKY® Implants.

Introduction

The ideal goal of modern dentistry is to restore the patient to normal contour, function, comfort, esthetics, speech, and health. What makes dental implant dentistry unique is the ability to achieve this ideal goal regardless of the atrophy, disease or injury of the stomatognathic system [1].

The number of dental implants used in the United States increased more than ten times from 1983 to 2002. More than 700.000 dental implants are inserted each year. The number of implants continues to increase steadily, with more than $ 150 million of implant products sold to North American dentists in 2002 compared with $ 10 million in 1983, with an expected growth sustained at 9.4% for the next several years [2, 3]. More than 90% of interfacing surgical specialty dentists provided implant treatment routinely in their practices, 90% of prosthodontics restored implants routinely, and more than 78% of general dentists used implants to support fixed and removable prosthesis compared to 65% 15 years ago [4, 5, 6].

As the implant performs its function for five years period, it may be considered as successful regarding the fact that after its removal there is no significant bone defect. Today, the criteria are stricter and require implants' survival for at least ten years. The success of implantation depends also on the quality of tissue types, materials, design and microstructure implants, wound type, degree of stress, possible corrosion, and number of other factors. It is understood that many of the implantology problems have not been definitively and satisfactorily resolved. There are many studies on the relation the body – implant: physical - chemical, experimental and clinical researches. Nowadays, there are systems made of different materials, different shapes, different micro and macrostructure, so the results ranging from extraordinary success to the complete disappointment [7].

Endoosseous implantation particularly demands finer bone structure, its structure and density. Dense bone structure, more calcified and mineralized bone, accepts better the implant than loose, unmineralized bone with plenty haemotopoietic and fat tissue [8]. The resorption of the alveolar crest is a parameter that is frequently used in the investigation of endoosseous implants. For such purposes, it is necessary to establish precisely defined reference level on the implant and to set the level of peri-implant alveolar bone in relation to that reference plan [9].

The clinical success of implants in the upper jaw is much smaller compared to implants in intercanine part called [8]. At the maxilla, the front part of alveolar crest to the second premolar is conditionally favorable region for implantation. Unfavorable region is the lateral part of the alveolar crest, including the tuber maxillae [10].

The aim of this study was to evaluate crestal bone resorption around dental implants in different regions of maxilla one year after its functional loading.

Patients and Methods

The Ethics Committee of the Faculty of Dentistry (University of Sarajevo) approved this study. All the examinees gave their informal consent.

This study analyzed total number of 88 implants type Bredent BLUESKY®. 18 implants dimension 3.5 x 10 mm were inserted in the maxilla on the right side, and 18 in the maxilla on the left side. 22 implants dimension 4.0 x 8 mm were inserted in the maxilla on the right side, and 30 in the maxilla on the left side. The implants were placed into the maxilla according to a strict surgical protocol following the manufacturer's instructions. After healing phase of three months without functional loading, gingiva former was inserted. After 14 days, gingiva former was removed and impressions were taken. The time placement of prosthetic restorations on the implants was four months after surgery. All the implants were used as an abutment of individual crowns and bridges.

Dental panoramic radiographs were made before surgery, immediately after surgery and a year after its functional loading, using Ortopantomograph type Kodak 8000 c, XJAM530. Panoramic images were calibrated using CliniView (version 5.2 Instrumentarium Imaging). The measurements were performed by comparing images using software Kodak dental software 6.11.7.0. The mesial and distal to the implant immediately after implant placement determines the highest level of bone resorption in the alveolar part, which is denoted as point A. After a year, we repeated OPG and determined the mesial and distal bone loss, which is denoted as point B. The difference

between points A and B is expressed in millimeters (mm) and indicates the level of bone resorption.

Statistical analysis

The data were analyzed using the IBM SPSS v.17 software package (descriptive statistics, paired samples t-test).

Results

The study included total number of 42 male and female patients. Among male patients, 43.5% were smokers, while 56.5% were non-smokers. Among females, 42.1% were smokers and 57.9% nonsmokers.

Among male patients, 78.3% were partially dentate, while 21.7% were totally edentulous. 94.7% females were partially dentate, only 5.3% were totally edentulous.

Table 1 shows the frequency of inserted implants in the lateral and front region of maxilla on the left and right side. It should be noted that there is no implant dimension 4.0 x 8 mm in front region of maxilla.

Region	Dimension of implant					
	3.5 x 10 mm		4.0 x 8 mm		Total	
	n	%	n	%	n	%
Maxilla right front	12	33.3	0	0.0	12	13.6
Maxilla left front	13	36.1	0	0.0	13	14.8
Maxilla right lateral	6	16.7	22	42.3	28	31.8
Maxilla left lateral	5	13.9	30	57.7	35	39.8
Total	36	**100.0**	52	**100.0**	88	**100.0**

Table 1
Frequency of inserted implant by region

The mean of the distal bone resorption around implant dimension 3.5 x 10 mm in maxilla on the right side was 0.60 mm (± 0.137 mm) with standard deviation of 0.32 mm, while the mean of mesial bone resorption was 0.50 mm (± 0.176 mm) with standard deviation of 0.36 mm. The differences between the mesial and distal resorption is not statistically significant (p = 0.254). The mean of the distal bone resorption around implant dimension 3.5 x10 mm in maxilla on the left side was 0.62 mm (± 0.118 mm) with standard deviation of 0.28 mm, while the mean of mesial bone resorption was 0.59 mm (± 0.137 mm) with standard deviation of 0.29 mm.

Testing the difference between the values between distal and mesial resorption using t-test we found no statistically significant difference (p = 0.491). The mean of the distal bone resorption around implant size 4.0 x 8 mm in maxilla on the right side was 0.52 mm (± 0.176 mm) with standard deviation of 0.41 mm, while the mean mesial resorption was 0.53 mm (± 0.176 mm) with a standard deviation of 0.40 mm. The differences between mesial and distal resorption are not statistically significant (p = 0.900). The mean of the distal bone resorption around implant dimension 4.0 x 8 mm in maxilla on the left side was 0.60 mm (± 0.157 mm) with an standard deviation of 0.44 mm, while the mean of the mesial resorption was 0.54 mm (± 0.137 mm) with a standard deviation of 0.39 mm. Testing the difference between the mean distal and mesial resorption using t-test, we found no statistically significant difference (p = 0.366).

The average value of the distal bone resorption around implant dimension 3.5 x 10 mm in front maxilla was 0.67 mm (± 0.098 mm), while the average mesial resorption was 0.57 mm (± 0.118 mm). The average value of the distal bone resorption around implant size 4.0 x 8 mm in maxilla lateral was 0.52 mm (± 0.176 mm), while the average mesial resorption was 0.53 mm (± 0.176 mm).

Tables 2, 3, 4 and **5** show the values for the level of bone resorption mesially and distally around the implant dimension 3.5 x 10 mm in different regions of the maxilla. The differences between the mean resorption on the mesially and distally sides are not statistically significant.

Implant 3.5 x 10 mm (maxilla right front)	95% CI of Mean	Standard Deviation
Distal resorption (n=12)	0.69±0.157 mm	0.26
Mesial resorption (n=12)	0.52±0.196 mm	0.36

Paired samples t-test (t= 1.428, df= 11, p= 0.181)

Table 2
The level of bone resorption mesially and distally to the implant dimension 3.5 x 10 mm - maxilla right front

Implant 3.5 x 10 mm (maxilla left front)	95% CI of Mean	Standard Deviation
Distal resorption (n=13)	0.65±0.137 mm	0.26
Mesial resorption (n=13)	0.62±0.157 mm	0.27

Paired samples t-test (t= 0.602, df=12, p= 0.558)

Table 3
The level of bone resorption mesially and distally to the implant dimension 3.5 x 10 mm - maxilla left front

Implant 3.5 x 10 mm (maxilla right lateral)	95% CI of Mean	Standard Deviation
Distal resorption (n=6)	0.42±0.294 mm	0.37
Mesial resorption (n=6)	0.47±0.314 mm	0.39

Paired samples t-test (t= -2.236, df=5, p= 0.076)
(Note, a small sample of n = 6)

Table 4
The level of bone resorption mesially and distally to the implant dimension 3.5 x 10 mm - maxilla right lateral

Implant 3.5 x 10 mm (maxilla left lateral)	95% CI of Mean	Standard Deviation
Distal resorption (n=5)	0.54±0.274 mm	0.32
Mesial resorption (n=5)	0.52±0.294 mm	0.34

Paired samples t-test (t= 0.343, df=4, p= 0.749)
(Note, a small sample of n = 5)

Table 5
The level of bone resorption mesially and distally to the implant dimension 3.5 x 10 mm - maxilla left lateral

Tables 6. and **7**. show the values of the level of bone resorption mesially and distally around implant dimensions 4.0 x 8 mm in the lateral regions of the maxilla. The differences between the mean resorption mesially and distally are not statistically significant.

Implant 4.0x0.8 mm (maxilla right lateral)	95% CI of Mean	Standard Deviation
Distal resorption (n=22)	0.52±0.176 mm	0.41
Mesial resorption (n=22)	0.53±0.176 mm	0.40

Paired samples t-test (t= - 0.128, df=21, p= 0.900)

Table 6
The level of bone resorption mesially and distally around implant dimension 4.0 x 8 mm - maxilla right lateral

Implant 4.0x0.8 mm (maxilla left lateral)	95% CI of Mean	Standard Deviation
Distal resorption (n=30)	0.60±0.157 mm	0.44
Mesial resorption (n=30)	0.54±0.137 mm	0.39

Paired samples t-test (t= 0.918, df=29, p= 0.366)

Table 7
The level of bone resorption mesially and distally to the implant dimension 4.0 x 8 mm – maxilla left lateral

Discussion

The use of dental implants for the treatment of partially or completely edentulous patients has become an integral treatment in restorative dentistry [10-14]. Endoosseous implants are commonly used. It is estimated that they represent 95% of cases. Today there are more results in the domain of experimental and clinical implantology. This shows that it is necessary to critically evaluate clinical - practical results and experience in longer period of time at a sufficient number of patients. For the success of the implantation, particular importance has the material from which the implant is made, its shape and size, then the choice of the patient, the right indication, the application of the correct surgical technique and manufacturing functional and aesthetically satisfying superstructure [15].

The present study was carried out on 42 patients with 88 implants in maxilla. All the implants showed successful tissue integration. The patients with systemic diseases have been excluded and implant prognosis was based on different implant diameters.

2008. Job et al. [16] in their study found that a crestal bone resorption around the implant in 3 months period was not statistically significant for the implants, which were inserted without removing soft tissue and amounted to 0.06 mm, while there was a statistically significant resorption mesially and distally to the implants which were inserted using technique separating soft tissue at the level of 0.4 mm.

Results of cited studies coincide with the results of this study in relation to implants placed with the technique separating the soft tissue.

In this study, mesial crestal resorption amounted in the range of 0.47 mm to 0.62 mm, and the distal resorption on the average ranging from 0.42 mm to 0.69 mm, depending on the region of the implant

dimensions 3.5 x 10 mm. Around implant dimensions 4.0 x 8 mm mesial crestal resorption was 0.47 mm and 0.62 mm, and the distal resorption was 0.42mm and 0.69 mm.

2008. Jang et al. in his research found bone resorption of 0.7 mm in the same period of evaluation like this study. Mesial crestal resorption ranged from 0.4 mm to 1.2 mm with the mean 0.76 mm when modified platform while crestal resorption of 2.1 mm to 3.1 mm on average 2.53 mm when unmodified platforms. Distal crestal resorption in modified platform ranged from 0.3 mm to 1.3 mm with the mean of 0.77 mm while in the unmodified platforms distal crestal resorption ranged from 2.2 mm to 2.9 mm, the mean 2.56 mm [17].

2010. Heinemann et al. in their longitudinal study on a sample of 147 implants size 3.7 and 4.2 mm inserted correctly in the maxilla found low crestal resorption. For implant size 3.7 mm, crestal resorption was 0.16 mm per year, while for implant size 4.3 mm, crestal resorption was 0.09 mm per year. Results of this study are different from the results of our study mostly because of different implant systems used and different implant dimensions leading towards the conclusion that cervical implant topography can significantly affect the crestal bone resorption [18].

2011. Hürzeler et al. found that the mean bone resorption within one year was 0.40 mm (± 0.12 mm) for the experimental group and 0.34 mm (± 0.29) for the group that was studied retrospectively at the control. It is important to note that the study was conducted on 5.0 mm implants and the results partly coincide with the results of our study [19].

Conclusion

Obtained results produced interesting findings showing that the resorption was mainly greater at the distal portion of the crest than mesial, although the differences were not statistically significant.

Declaration of Interest

There is no conflict of interest.

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FLUORIDE RELEASE AND CYTOTOXICITY OF RESIN-MODIFIED GLASS-IONOMER CEMENTS

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ABSTRACT

Fluoride release from glass-ionomer cements presents an important advantage in the process of prevention of secondary caries at surrounding surfaces. Biological activity of GICs can be partially determined by the quantity of released fluoride ions. The objectives of this study were: to define the quantity of fluoride ions released from the experimental resin modified glass-ionomer cements and to define the effect of fluoride ions released from the experimental RMGICs on their cyto-toxicity. Concentrations of the fluoride ions were measured indirectly, by the fluoride-selective WTW, F500 electrode potential. Statistical analyses of F-ion concentrations released by experimental RMGICs was evaluated at two time points, after 8 and 24 hours, showing statistically higher fluoride releases from RMGIC Vitrebond. To evaluate cyto-toxicity of resin modified glass-ionomer cements on NIH3T3 mouse fibroblasts, specimens were divided into groups: RMGIC GC Fuji II LC, GC Fuji Plus and Vitrebond; group 4. positive control was presented by specimens of composite Vit-l-ecence® and negative control-group 5. was presented by DMEM. Cell cultures were exposed to 10% of eluate for each single specimen and each experimental material. After the incubation period, cell metabolism was evaluated using methyltetrazolium assay. Kruskal-Wallis test and Tukey-Kramer post hoc test showed significantly more cytotoxicity of Vitrebond comparing to all other experimental materials including composite Vit-l-ecence® as a positive control. Correlation between concentrations of fluoride ion released and cytotoxic response of NIH3T3 mouse fibroblast cell line after 8 and 24 hours is high, is positive and statistically significant for Fuji II LC only.

Key words: fluoride release, resin modified glass-ionomers, cytotoxicity.

Introduction

Capability of glass-ionomer cements to act as a fluoride ion reservoir has been known for long time [1]. This characteristic of glass-ionomer cements presents an important advantage in the process of prevention of secondary caries around restorative margins as well as for surrounding surfaces [2]. Fluoride ions released by glass-ionomer cements helped in reduction of demineralization of adjacent enamel, enhancement of its re-mineralization and prevention of secondary caries by inhibition of microbial growth and metabolism [3, 4]. Fluorides represent the basic component of glass powder and if it is to be efficiently extracted by the polyacid it has to be in crystalline form as fluorite [5].

Two mechanisms have been proposed by which fluoride may be released from glass-ionomer cements. One mechanism is short term reaction presented by rapid dissolution from outer surface into solution. Second mechanism, presented with the sustained diffusion of ions trough the bulk cement, is more gradual [4]. Quantity of fluoride ions released from the glass-ionomer cements has major importance in definition of their biological activity. Resin modified glass-ionomer cements (RMGICs), hybrid version of conventional glass-ionomers, combine the main advantages of glass-ionomer cements such as adhesion to tooth structure, fluoride release and biocompatibility, with easy handling of light polymerized composites [6]. They also show some adverse properties when used as restorative materials, and the level of biocompatibility is not always satisfactory [7]. In a view of the complex chemistry and physicochemistry of RMGICs differences in the processes responsible for the fluoride release can be expected [8].

The objectives of this *in vitro* study were: To define the quantity of fluoride ions released from experimental resin modified glass-ionomer cements and to define the effect of fluoride ions released from the experimental glass-ionomer cements on their cytotoxicity on cell cultures of NIH 3T3 mouse fibroblasts.

Material and Methods

Materials and manufacture of specimens

Three resin modified glass-ionomer cements: GC Fuji II LC, GC Fuji plus (GC Corporation) and Vitrebond (3M/ESPE) were used as an experimental materials in this study. Materials were prepared at room temperature according to manufacturer's instructions, packed into open silicon rings (internal diameter 4mm and height 2 mm) between two celluloid sheets. Resin modified glass-ionomer cements: GC Fuji II LC, GC Fuji Plus (GC Corporation) and Vitrebond (3MESPE) were polymerized for 40 sec. on each surface with light activation lamp Elipar ™ FreeLight L (3MESPE) [9]. The whole sample consisted of 108 discs of RMGICs and 36 discs of composite Vit-l-ecence® (Ultradent Products, Inc. USA),

Elution samples

After sterilization, RMGICs samples were placed in 96 well tissue culture plates (Falcon MICROTEST™ 96 Tissue Culture Plate Becton Dickinson Labware). Each chamber was filled with 100µl of Dulbecco's Modified Eagle's Medium(DMEM Sigma Chemical Co. St. Louis, MO) The medium, with the immersed specimens, were maintained for 72 hours in humidified incubator at 37 °C with 95% air and 5% $CO_{2.}$ The medium was retained for toxicity testing.

Cell Culture

NIH3T3 mouse fibroblast cells (ATCC CCL 163, clone A31; American Type culture collection, Rockville, MD) used in this *in vitro* study were cultivated in experimental culture flask T-25 on DMEM (Sigma Chemical Co. St. Louis, MO), supplemented with 10% (v/v) foetal calf serum (FCS, Collaborative research, Bedford , MA) and 1% AA-liquid, (GIBCO® CO.USA) containing 10,000 units/ml penicillin in G-sodium, 10,000 µg/ml streptomycin sulphate, 25µg/ml amphotericin B as antimycotic diluted in 0,85% saline.

Cultures were incubated at 37 °C in humidified atmosphere with 95% air and 5% CO_2 until confluent. Cellular growth and medium pH were monitored daily using phase contrast microscopy and pH meter. Cells were grown to density $1x\,10^4$ cells / cm^2.

24 hours before experiment, NIH3T3 cell cultures were plated at $3x\ 10^4$ cells/ cm^2 in 96-well tissue culture plates (Falcon MICROTEST™ 96 Tissue Culture Plate Becton Dickinson Labware), containing 100 µl DMEM.

Test material and controls

After 24 hours of incubation, complete culture medium in 96 well tissue culture plates with NIH3T3

mouse fibroblast cells were replaced with 90 µl of fresh DMEM and 10 µl of extract DMEM representing 10% eluate of specimens of resin modified glass-ionomer cements previously incubated 72 hours in DMEM.

This way, all cell cultures were exposed to 10% of eluate of each single specimen and of each experimental material. Each experiment was performed using 12 representative areas, for each material as well as for positive and negative control group. Experimental dishes were incubated for 24 hours at 37 °C with 5% CO_2 and 95% air. In order to ensure reproducibility, the experiment was conducted in triplicate.

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide] cytotoxicity assay

Cytotoxicity of resin modified glass-ionomer cements, presented by GC Fuji II LC, GC Fuji Plus (GC Corporation) and Vitrebond (3MESPE), was evaluated by cell metabolic activity measured by succinic dehydrogenase (SDH) activity, which is a measure of the mitochondrial respiration of the cells [10]. Following the procedure, previously described in detail by Mossman [10], citotoxicity of three experimental RMGIC was evaluated by methyltetrazolium (MTT) assay. 24 hours after the incubation of cells with the RMGICs eluates, the basal MTT scores were obtained by spectrophotometer (Safire^2 Tecan Group Ltd.) using a test wavelength of 570 nm.

Measurement of concentration of fluoride ions

The concentration of fluoride ions in eluates of each experimental RMGICs was assayed by means of an electrode potential of ion specific electrode (WTW, F 500) in combination with referent electrode R503 / D, for all ion selective electrodes series 500. The electrode was calibrated with three standard solutions of 0, 00001 g/L; 0, 001 g/L and 0, 1 g/L of fluoride. The whole sample for the measurement of fluoride ions concentration consisted of 54 discs, 18 discs for each experimental material. Discs were divided into three groups out of which each one was divided in two subgroups consisting from three specimens of each experimental material.

After 24hrs, the test specimens were immersed in a polypropylene container PP, 25x90mm/30ml (Semikem: Cat. No.15.0597) completely covered with 5 ml distilled, de-ionized water. The containers were hermetically closed.

First measurement of fluoride concentration in eluates of three samples of each tested material was conducted after 8 hours. The other three specimens of each experimental RMGIC were eluted for 24 hours at room temperature until the moment of second measurement of fluoride concentration [11].

After 8 hours, for the first measurements, and after 24 hours for the second measurements, 5ml. of TISAB solution (total ionic strength adjustment buffer solution- Modell: TISAB; Best.Nr.:140 100; WTW D-82362 Weilheim) was added to each container. The dishes were hermetically closed and agitated at the speed of 60 Hz. for two minutes. Elutes, prepared this way were set for 30min. in order to achieve stabile solution before measurements [12].

A fluoride ion selective electrode WTW, F500, combined with reference R503/D electrode was used to quantify the amount of fluoride ion released from each specimen into the buffer solution. The fluoride ion concentrations of eluates of each experimental RMGICs were measured in triplicate.

A total amount of fluoride released (expressed in micrograms of fluoride released per gram of solution) into the buffer solution, after 8 and 24 hours was calculated from the calibration curve [11]. Each data point was the average of three samples.

Concentration of free F^- ions was determined by potentiometer methods based on mathematic formula:

$$E = E^o + \frac{RT}{nF} \ln c_{F^-}$$

Based on this formula and data obtained during the experiment, calibration curve for fluoride selective electrode was constructed. For quantitative determination of F- ions in the eluates (µg/g) standard calibration curve was obtained by plotting the peak heights of known concentration of the fluor solutions and by calibration curve for fluoride selective electrode constructed previously. Calibration diagram constructed that way gave a mathematic formula for calculation of concentration of fluoride ions expressed in µg/g which is:

$$x = \frac{112{,}77 - y}{42{,}325}$$

Statistical evaluation of cytotoxicity of RMGICs: GC Fuji II LC, GC Fuji Plus (GC Corporation) and Vitrebond (3MESPE) were performed by statistical software SPSS for Windows 15.0 (SPSS Inc. USA). Cytotoxic effect of RMGIC on NIH3T3 mouse fibroblasts were evaluated by Kruskal-Wallis test and Tu-

Vitrebond was the most cytotoxic experimental material (Z= -5.5835 ; p< 0, 0001)

Material	No. of measure-ments	Sum of ranks	Mean rank	Z-value	Median
Negative Control	12	1045.00	87.08	5.1293	0.78365
Vitrebond	12	78.00	6.50	-5.5835	0.10145
Fuji plus	12	503.50	41.96	-0.8696	0.62655
Fuji II LC	12	418.50	34.88	-1.8113	0.6262
Positive control Vit-l-escence®	12	495.00	41.25	-0.9638	0.6336

Table 1
Results of Kruskal-Wallis test of cytotoxicity of experimental glass-ionomer cements evaluated on NIH3T3 mouse fibroblast cells

The RMGIC Vitrebond showed significantly higher cytotoxicity than all other materials as well as positive and negative control

Material	Code	No. of measure-ments	Mean value	Differences between the groups
Vitrebond	B	12	0.1032417	A,C,D,E
Fuji II LC	C	12	0.61465	B,A
Positive control Vit-l-escence®	E	12	0.6246417	B,A
Fuji plus	D	12	0.635025	B,A
Negative Control	A	12	0.796475	B,C,D,E

Table 2
Tukey-Kramer test of multiple comparisons for the experimental glass ionomer cements evaluated on NIH3T3 mouse fibroblast cells

key-Kramer post hoc test. Wilcoxon test was used to determine significant differences between the concentrations of fluoride ions of each experimental material released after 8 and 24 hours of elution time. Cytotoxicity and F^- release were evaluated for significant differences by Spearman's rank correlation coefficient.

Results

Cytotoxicity:

Kruskal-Wallis test (**Table 1**) and Tukey-Kramer post hoc test (**Table 2**) for the materials evaluated on NIH3T3 mouse fibroblast cells, shows significantly more cytotoxicity of Vitrebond ($Z = -5.5835$, $p < 0,0001$). The Vitrebond (3MESPE) showed significantly higher cytotoxicity than all other materials as well as positive and negative control.

Fluoride release:

Table 3 shows descriptive statistical values of released fluoride ions from the respective RMGICs . Distributions of results do not deviate significantly from symmetry. To assess the equality of variances in three groups of experimental materials Levene's test was used and statistical difference between the variances in the experimental materials was found **(F_1 (5, 48) =20, 86, p=0, 0001; F_2 (5, 48) =13, 39, p=0, 0001).**

Values of concentration of F- ions (µg/g) released from experimental resin modified glass-ionomer cements were much higher in the 24 hours eluates for all experimental materials

Material		Min	Max	Mean	SD	Skewness		Kurtosis	
						Statistic	Std. error	Statistic	Std. error
Fuji LCII_8h	9	0,133	1,261	0,624	0,332	0,582	0,717	0,647	1,400
Fuji LCII_24h	9	0,312	2,092	1,265	0,568	-0,484	0,717	-0,627	1,400
Fuji Plus_8h	9	0,243	1,318	0,855	0,385	-0,321	0,717	-1,528	1,400
Fuji Plus_24h	9	0,920	3,112	1,731	0,738	0,706	0,717	-0,254	1,400
Vitrebond_8h	9	0,625	23,808	9,962	9,146	0,514	0,717	-1,423	1,400
Vitrebond_24h	9	1,897	33,359	12,114	10,590	1,131	0,717	0,684	1,400

Table 3
Descriptive statistic values of concentration of F- ions (µg/g) in the 8 and 24 hours eluates

Considering a statistical difference between the homogeneity of variances, for the evaluation of statistical difference in the amount of released F^- ions between two time points (8 and 24 hours) and for the three different RMGICs non-parametric statistical tests were used.

The Wilcoxon signed-rank test was used to test statistical difference in the amount of released F^- ions between two time points (8 and 24 hours) and statistical difference is significant Z= -2,897, p=0,004 so it can be concluded that the amount of released F^- ions for all experimental RMGICs was greater after 24 hours (**Figure 1**). RMGIC Vitrebond released significantly more F^- ions at each time interval than all other experimental RMGICs.

Correlation between cytotoxicity and fluoride release:

Spearman's rank coefficient between F^- released after 8 hours and cytotoxic reaction of NIH3T3 mouse fibroblast cells indicated a low, negative and statistically insignificant correlation (ρ = -0,127) while correlation between F^- released after 24 hours and cytotoxic reaction of NIH3T3 mouse fibroblast cells was negative and significant (ρ = -0,342).

Although some values of correlation coefficient are relatively high, no one reach the level of statistical significance of 0, 05 probably because of insufficient numbers of specimens in the sample (n=9).

Spearman's rank correlation coefficient between F^- released by experimental materials and cytotoxic reaction of NIH3T3 mouse fibroblast at both time points (8 and 24 hours) was statistically significant for RMGIC Fuji II LC only (8 hrs. ρ = 0,817; 24 hrs. ρ = 0,750) (**Table 4**).

Discussion

Fluoride release by RMGICs plays a major role in its selection for specific clinical application [5]. The pattern of released fluoride reflects in the greatest amount of fluoride released during the first days and then decreasing to nearly constant level [1]. Kan K.C. et al. [9] concluded that the greatest amount of F^- release occurs in the first 24 hours, being confirmed by present study were the difference in fluoride release for all experimental materials was significantly greater after 24 hours. (p= 0,004).

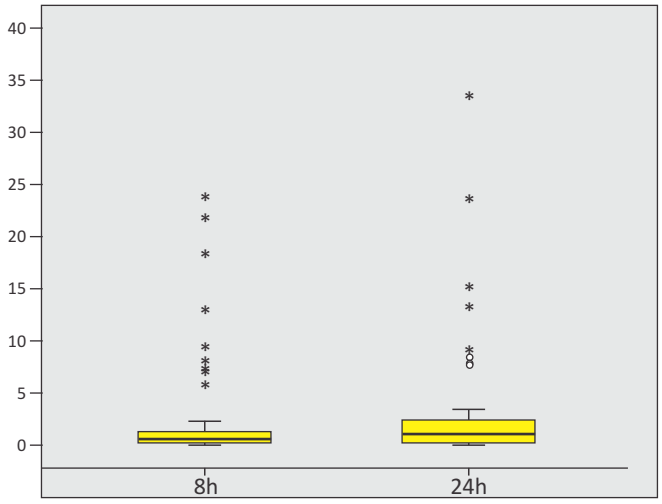

Figure 1
Box-plot distribution of the amount of released F- ions between two time points (8 and 24 hours). Median of the amount of released F- ions after 8 hours is C=0,568, which is lower comparing with the median of the amount of released F- ions after 24 hours(C= 1,0485)

Correlation coefficient demonstrated a high, positive and significant correlation between F- released after 8 hours and cytotoxic reaction of NIH3T3 mouse fibroblast cells for RMGIC Fuji II LC (ρ = 0,817). Spearman's rank correlation coefficient between F- released after 24 hours and cytotoxic reaction of NIH3T3 mouse fibroblast cells was statistically significant for RMGIC Fuji II LC only (ρ = 0,750)

Material	F- release 24hrs	Cytotoxicity NIH3T3 8hrs	Cytotoxicity NIH3T3 24hrs
Fuji II LC F-8h	0,800(**)	0,817(**)	
Fuji II LC F-24h			0,750(*)
Fuji Plus F-8h	-0,385	-0,217	
Fuji Plus F-24h			-0,561
Vitrebond F-8h	0,533	-0,233	
Vitrebond F-24h			0,250

** ρ statistically significant at 0.01
* ρ statistically significant at 0.05

Table 4
Matrice of correlation of F- release from experimental GICs and cytotoxic response of NIH3T3 mouse fibroblast cells

The present *in vitro* study evaluated the quantity of fluoride ions released from the three resin modified glass-ionomer cements: GC Fuji II LC, GC Fuji plus (GC Corporation) and Vitrebond (3M/ESPE) and defined the effect of fluoride ions released from the experimental resin modified glass-ionomer cements on their cytotoxicity.

The comparison of the cytotoxic effects of tested resin modified glass –ionomer cements clearly indicates that there are significant differences between the various materials. Results of MTT citotoxicity assay of RMGICs, on NIH3T3 mouse fibroblast cells showed intense cytotoxic effect of RMGIC Vitrebond (3M ESPE). The other RMGICs used in this study, GC Fuji II LC and GC Fuji plus (GC Corporation), showed significant decrease of cell metabolism, comparing even with composite material Vit-l-escence® (Ultradent Products, Inc. USA), which was used as a positive control. Unbound free monomers, released during the polymerization of dental composites and RMGICs, for most of the authors, are responsible for the cyto-toxic effect of these materials. There is an evidence in the literature that cyto-toxicity is related to some additional mechanisms such as short-term release of free monomers appearing during the monomer - polymer conversion [12,13]. The release of free monomers is due to irregular photo-polymerization, chemical, thermal or mechanical factors. Due to the industrial process of improving of RMGICs, the amount of unbound monomers has been decreased, but still there is no complete conversion during the polymerization process. Although the quantity of residual monomers is less than1, 5-5%, this is still sufficient for contributing to cytotoxic effects of those materials being proved *in vitro tests* [14]. Results of this *in vitro* study coincide with the results of Geurtzen W. et al. who confirmed that Vitrebond is extremely cytotoxic to cells culture, while Fuji II LC shows a moderate inhibition of cell growth [15]. The authors considered that this effect caused by Vitrebond may be mainly produced by decomposition products of the initiator diphenyliodoniumchloride, especially chlorine benzene, iodine benzene and bromide benzene which were not found in other RMGICs [15]. In the present study, the greatest amount of F^- released was showed by RMGIC Vitrebond (3M/ESPE) and those results were significantly higher comparing them with all experimental materials for both time points (8 and 24 hours). Results of this *in vitro* study coincide with the results of Mitra S.B., who demonstrated that Vitrebond was capable of long-term fluoride release without any degradation of physical properties over the time [16].

In the present *in vitro* study, the amount of F^- in RMGIC Fuji II LC (GC Corporation) 24 hours eluate was 1,265 ppm and demonstrated statistically significant relation with the cytotoxic response of NIH3T3 mouse fibroblast cells after 8 **(ρ= 0,817)** and 24 hours immersion **(ρ = 0,750).** Fuji II LC in the present study demonstrated moderate cytotoxicity which coincide with the results of Kan K.C. et al. [9], who pointed out that Fuji II LC behaved more like a resin composite.

The fact that brand formulation and material type may influence the amount of F^- ion released [17] purchase, was confirmed in present study where RMGIC Vitrebond (3M/ESPE) released significantly greater amount of F^- ions comparing with other experimental RMGICs.

With the exception of Fuji II LC (GC Corporation), the present *in vitro* study demonstrated that the concentration of F^- released by experimental RMGICs after the both 8 and 24 hours immersion had no significant effect on cytotoxic response of NIH3T3 mouse fibroblast cells. It is more likely that cytotoxic response of cell lines used in present study occurred due to unidentified toxic components which were leached out during the immersion time. However, additional, more complex experimental studies comprising large number of factors could provide more valid conclusion. Having in mind that liberation of soluble components of experimental RMGICs can occur during the polymerization process or later on, further research should be based upon identification of severely cytotoxic leachable substances and their quantification.

Conclusions

Based on the results obtained in the present in vitro study it was concluded that:

- Statistical analyses of F^- ion concentrations released by all resin modified glass-ionomers evaluated at two time points, after 8 and after 24 hours, show statistically higher fluoride releases from RMGICs: Vitrebond at each time interval than all other experimental RMGICs.

- The evaluation of citotoxicity of resin modified glass-ionomer cements on NIH3T3 mouse fibroblast cell lines showed that cytotoxic effect of Vitrebond (3MESPE) was significantly higher than all other materials as well as positive and negative control.

- Correlation Coefficient between concentrations of fluoride ion released by evaluated glass-ionomer cements and cytotoxic response of NIH3T3 mouse fibroblast cell line are relatively high, but do not reach levels of biological significance.

According to the methodology employed in the present study it can be concluded that experimental RMGICs liberated F^- as well as other soluble components which diffused into the culture medium. These components cannot be dismissed as possible cytotoxic factors which contribute to the cytotoxicity observed in this study.

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Declaration of interest

There is not any conflict of interest for this material in the manuscript for all authors, between the authors, or for any organization.

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ANALYSIS OF METALS RELEASED FROM DENTAL AMALGAM ALLOY USING INDICTIVELY COUPLED PLASMA-MASS SPECTROMETRY

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ABSTRACT

Dental amalgam is a restorative material used in dentistry from the very beginning. The basic chemical compounds of dental amalgam alloy are silver, tin and mercury. In spite of the fact that the interest or the esthetic restorative materials grow all over the world, amalgam is still the most used material. The fact that one of the main components of dental amalgam is elemental mercury causes concern of patients as well as some governmental agencies in a number of the world countries.

Aim of this paper is to analyze quantity of metals released from four dental amalgams in artificial saliva solution in vitro, and to estimate possible effects of those metals on health of patients with dental amalgam fillings.

Material and method: Experimental material is presented by four dental amalgams that differ in percentage of metals in preamalgamated alloy. Electrodes were made of those amalgams and immersed in artificial saliva solution (Duffo, Quezzada Castillo), and elute was analyzed using inductively coupled plasma - mass spectrometry (ICP-MS), for determination of quantity of mercury, silver, tin, copper and zinc isotopes.

Conclusion*:* Quantities of mercury isotopes and isotopes of other metals from dental amalgams in artificial saliva solution in vitro could not be considered potentially harmful for patients with dental amalgam fillings, regarding official statement of the World Health Organization and other governmental agencies in many countries around the world.

Key words: dental amalgam, ICP-MS, corrosion.

Introduction

Dental amalgam is a restorative material used in dentistry from the very beginning. It was distinguished as a material which is easy to handle, durable, cheap, but it has low dimensional stability and clinical behavior not fulfilling the esthetic criteria [1].

The basic chemical compounds of dental amalgam alloy are silver, tin and mercury. In the smaller quantity the alloy could be supplemented by additional copper, zinc, gold, platinum palladium, nickel, molybdenum, wolfram and the pre-amalgamated alloy by small quantities of mercury too, up to 3% [2].

Dental specialists have several options for material selection regarding the restoration of teeth. Apart from amalgam, for the restoration in the trans-canine sector resin-composite materials could be used, alloy of non-precious and precious metals, as well as glass-ionomer cements. Each one has its advantages and disadvantages. In spite of the fact that the interest or the esthetic restorative materials grow all over the world, amalgam is still the most used material. But, in the world in which number of things was enormously changed during the last hundred years, it could be strange that the dentists use materials which have not been changed significantly regarding hundred years period. Not neglecting the outlook of the restoration, it is possible for the patients to request esthetic restorative materials based on the concern regarding the presence of the mercury in the dental amalgam. Even governmental agencies in number of the countries advocate alternatives for amalgam because of the environmental reasons [3, 4].

Widely spread fear about toxicity of dental amalgam is mainly caused by cyto-toxicity of the mercury. Mercury is the most toxic heavy metal. It is strong protoplasmatic poison, penetrating into all live cells of the body. Since it represents non-polar liposoluble entity, it has fast absorption in the lungs, through the skin and mucosa membrane. The basis of mercury toxicity is its affinity towards the sulfur. The mercury is connected to sulf-hydrile groups inactivating cell's metabolism and function. Principally, the mercury could be released from amalgam in the form of mercury vapor and inhaled, or in the form of particles that could be swallowed. The researches were mainly focused on the mercury vapor release. If some portion of the mercury is released from the amalgam fillings and resorpted into the body of persons with those fillings, it is considered that the body mechanisms after shorter or longer period of time remove it mainly through renal and urinal system [2, 5, 6, 7].

Human organism is exposed to the mercury and the other metals from dental amalgam (silver, tin, copper and zinc) through evaporation, corrosive products in the swallowed saliva and direct absorption into the blood through the oral mucosa. Agency for Toxic Substances and Disease Registry as the branch of the Centers for Disease Control in USA, recognized that small quantities of the mercury from the amalgam fillings are released into the human body under the action of the factors like corrosion, chewing and teeth cracking [8, 9].

ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) is a technique used to determine trace elements in the solvents being known for almost thirty years. ICP-MS offers many benefits to laboratories performing trace metal determinations. ICP-MS instrument measures most of the elements in the periodic table. The ICP-MS accurately determines the quantity of a specific element in the material analyzed. In a typical quantitative analysis, the concentration of each element is determined by comparing the counts measured for a selected isotope to an external calibration curve generated for that element. Since ICP-MS instruments measure specific isotopes of an element, the ratio of two or more isotopes can readily be determined. Isotope ratio determinations are used in a variety of applications determining the source of a contaminant and biological tracer studies [10].

Common for different experiments carried out using ICP-MS is the fact that they allow accurate determination of enriched stable isotope-indicators in human samples, samples that already contain a large quantity of the same element, but have the natural isotopic composition. The increase in the isotopic composition, induced by enriched stable isotopes needs to be quantified, including the determination of isotope-ratio indicator and the reference isotope [11].

There are a small number of studies in which ICP-MS was used for detecting the mercury originating from dental amalgam.

Apostoli et al [12] used ICP-MS in the study aimed to asses the reference values of urinary mercury in four Italian cities, on the basis of standardized criteria by a questionnaire on personal habits, lifestyle, occupational and non-occupational exposure to mercury, medical history, number and area of dental amalgam.

Amalgam	% Ag	% Sn	% Cu	% Zn
Sample 1	70	25.7	3.3	No data available
Sample 2	70	18	12	No data available
Sample 3	41	31	28	No data available
Sample 4	70	18.5	11	0.5

Table 1
Experimental material

Component	Concentration (g/l)
NaCl	0,600
KCl	0,720
$CaCl_2 \times 2H_2O$	0,220
KH_2PO_4	0,680
$Na_2HPO_4 \times 12H_2O$	0,856
KSCN	0,060
$KHCO_3$	1,500
Citric acid	0,030

Table 2
Composition of artificial saliva (Duffo, Quezada Castillo) (14)

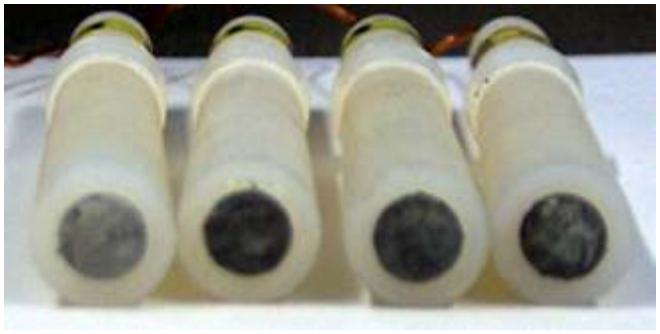

Figure 1
Amalgam electrodes

Figure 2
Agilent ICPMS 7500 ce

Shraim et al [13] evaluated concentrations of mercury and other metals in the wastewater of three dental clinics and the influent of a wastewater treatment plant in Al-Madinah Al-Munawarah using ICP-MS.

The aim of this study was to analyze the quantity of metals released from four dental amalgams in artificial saliva solution, and on the basis of these results to assess the potential impact of these metals to the health of patients having the amalgam fillings.

Material and Methods

The experimental material in this study consisted of four commercial dental amalgams, which differ in the percentage share of individual metals in pre-alloy (**Table 1**). Since those are encapsulated amalgams, mercury and pre-alloy were mixed mechanically in amalgamator, in a ratio 1:1, according to the manufacturer's instructions. Amalgam is manually condensed into molds made of polyethylene as an inert material. Moulds are cylindrical in shape, with the length of 140 mm and a diameter of 12 mm. Amalgam was condensed into a prepared dock at one end of the polyethylene mold, which is also cylindrical in shape with a diameter of 8 mm, a depth of 2 mm. Those electrodes, in fact, represented the artificial tooth. Electrodes are numbered 1-4 (**Figure 1**).

Each electrode is inserted into a freshly prepared solution of artificial saliva (Duffo, Quezada Castillo [14], **Table 2**), at room temperature for 5 days. Every 24 hours, the sample of artificial saliva in which each electrode was separately inserted was taken, and the ICP-MS analysis was performed (Agilent ICPMS 7500 ce; Agilent, Waldbronn, Germany; Software: HP ChemStation) (**Figures 2 and 3**).

Each sample of 1g elute was taken for analysis and treated with 2 ml of nitric acid (for the determination of silver). For each analysis three measurements were performed and mean value for each element calculated, being shown as the final value. Thus, we get information regarding the presence and changes of amounts of trace elements in artificial saliva solution, which are released from any of the tested dental amalgams. We determined the amount of corrosive products released in the form of ions from each amalgam alloy, expressed in g/l - parts per billion (ppb).

Figure 3
Agilent ICPMS 7500 ce

Results

Tables 3, 4, 5 and **6** show the amounts of released copper, zinc, tin and silver isotopes from four experimental samples of dental amalgams, detected by ICP-MS.

Although artificial saliva samples in which the amalgams were immersed were taken each 24 hours over five days, the analysis and interpretation of results we observe showed only the results of isotope concentration after the first 24 hours, considering the human organism. Specifically, human saliva is a very dynamic solution that is secreted during the day and after some time becomes ingested in oral cavity. None of the amalgam in the oral cavity can be continuously in contact with the same saliva during five days, but data for the first 24 hours can give us insight of the approximate quantities of certain elements that can be released from dental amalgam during the day. It is important to emphasize that in the interpretation of results we observed total amount of discharged isotopes for each individual metals - copper, zinc, silver, tin, mercury (**Table 7**). Considering data taken from Al-Salehi et al [15], that average surface of amalgam filling is 25 mm^2, and that surface of our amalgam samples is 50,24 mm^2, that daily saliva production is 770 ml, or 8 ml each 15 minutes [16], the following quantities of discharged metals from amalgam fillings during the first 24 hours were obtained (**Table 7**).

Conc.(ppb)/day	Day 1	Day 2	Day 3	Day 4	Day 5
63 Cu/ppb	24,5	112,7	29,9	37,4	48,6
65 Cu/ppb	24,4	112,0	28,5	36,4	47,8
66 Zn/ppb	341,7	92,7	50,0	50,0	144,5
68 Zn/ppb	346,7	94,9	50,0	50,0	148,4
107 Ag/ppb	6,6	10,5	5,9	9,2	10,8
109 Ag/ppb	6,7	10,6	5,9	9,4	10,9
118 Sn/ppb	50,6	93,6	166,0	290,3	404,5
120 Sn/ppb	50,2	93,3	166,2	287,1	404,5
200 Hg/ppb	7,7	6,6	5,5	4,8	8,5
202 Hg/ppb	7,9	6,6	5,8	5,5	8,6

Table 3
Concentrations of released isotopes of copper, zinc, tin, silver and mercury from amalgam sample 1.

Conc.(ppb)/day	Day 1	Day 2	Day 3	Day 4	Day 5
63 Cu/ppb	38,5	44,4	63,9	86,4	98,1
65 Cu/ppb	38,0	44,2	63,1	85,6	97,5
66 Zn/ppb	696,3	1140,0	617,2	637,0	718,5
68 Zn/ppb	705,7	1156,0	627,6	648,7	728,1
107 Ag/ppb	3,3	4,8	8,1	8,5	16,5
109 Ag/ppb	3,3	4,8	8,1	8,4	16,5
118 Sn/ppb	275,5	37,9	63,5	76,5	104,7
120 Sn/ppb	271,4	37,3	63,2	76,8	103,7
200 Hg/ppb	9,1	14,2	15,1	13,5	29,2
202 Hg/ppb	8,4	14,9	15,1	13,7	29,8

Table 4
Concentrations of released isotopes of copper, zinc, tin, silver and mercury from amalgam sample 2.

Conc.(ppb)/day	Day 1	Day 2	Day 3	Day 4	Day 5
63 Cu/ppb	34,4	45,0	49,9	61,4	75,6
65 Cu/ppb	34,1	44,3	49,1	60,1	75,0
66 Zn/ppb	221,2	261,5	50,0	17,0	50,0
68 Zn/ppb	226,4	269,9	50,0	21,5	50,0
107 Ag/ppb	3,9	12,0	3,3	6,5	8,0
109 Ag/ppb	3,9	12,1	3,2	6,5	8,0
118 Sn/ppb	46,6	146,9	579,7	108,2	145,4
120 Sn/ppb	46,6	149,8	736,2	108,5	145,7
200 Hg/ppb	0,8	5,0	5,0	1,1	5,4
202 Hg/ppb	1,1	5,0	5,0	1,5	5,2

Table 5
Concentrations of released isotopes of copper, zinc, tin, silver and mercury from amalgam sample 3.

Conc.(ppb)/day	Day 1	Day 2	Day 3	Day 4	Day 5
63 Cu/ppb	96,8	29,9	40,0	96,2	61,9
65 Cu/ppb	97,1	29,7	39,0	95,0	61,2
66 Zn/ppb	372,3	63,1	50,0	3,6	802,5
68 Zn/ppb	381,4	65,1	50,0	4,1	816,2
107 Ag/ppb	0,7	1,7	14,9	12,8	1282,0
109 Ag/ppb	0,7	1,7	14,9	12,6	1388,0
118 Sn/ppb	5,0	315,4	495,3	18,6	46,1
120 Sn/ppb	4,9	312,1	470,0	17,7	46,0
200 Hg/ppb	5,0	5,0	17,3	11,7	27,1
202 Hg/ppb	5,0	5,0	18,1	12,3	27,9

Table 6
Concentrations of released isotopes of copper, zinc, tin, silver and mercury from amalgam sample 4.

Sample / Quantity of released metal (µg)	Sample 1	Sample 2	Sample 3	Sample 4
Cu	18,73	29,30	26,25	74,30
Zn	263,76	537,19	171,50	288,79
Ag	5,07	2,52	2,99	0,54
Sn	38,62	209,55	35,71	3,80
Hg	5,97	6,71	0,73	3,83

Table 7
Amounts of metals released from average sized amalgam filling through 24 hours

Discussion

While interpreting the results it was presumed that, on the basis of the amount of detected isotopes released into artificial saliva solution from dental amalgam alloy, maximum allowable daily concentrations of these elements and they impact to the human organism can be predicted. But, we have to bear in mind all the advantages and disadvantages of in vitro studies.

From the standpoint of electrochemical corrosion, it is important to emphasize that dental amalgams, in the metallurgical point of view, are the most complex biomaterials. Dental amalgam is a dynamic material in which the reactions take place in solid state over a long time period. Since these reactions occur in a complex and variable oral environment, it is obvious that in vitro studies can only reflect the actual in vivo conditions to certain extent [17].

Determination of mercury released and adsorbed from dental amalgam in vivo is difficult and complex. Dodes [18] refers to studies of Olsson and Bergman citing the following factors as variables that affect the amount of mercury released from amalgam restorations: the number of teeth, the number of restored surfaces, basal release of mercury, the factors that increase the release, as chewing and brushing teeth, alimentary and hygiene habits, mouth breathing, the ratio of oral and nasal respiration, ingestion, absorption, inhalation, ingestion absorption, and body weight.

Most measurements of release of mercury and mercury vapor from dental amalgams in the current research were performed on dry amalgam surfaces, and they may not be relevant to the restorations of the oral environment. Amalgam restorations in the oral cavity are normally covered with a film of saliva. It was determined that the release of mercury vapor was drastically inhibited by the presence of saliva film on the surface of amalgam. The size of this inhibition casts a shadow of doubt on the application of in vitro measurements of mercury vapor from the abraded amalgam surfaces, in terms of valuation acceptable levels of mercury vapors in the respiratory environment defined by the World Health Organization (WHO) [19].

Mercury is the most noble of the main elements in dental amalgam. Under conditions dominating in the

oral cavity, silver and mercury have the lowest electrochemical activity. However, despite the limited tendency to dissolve electrochemically, these elements can form almost insoluble sulfides [17]. Okabe et al [20] refer to comprehensive review of the literature on in vivo and in vitro corrosion of dental amalgam, which states that it is not even one product of corrosion of dental amalgam in which the main constituent is mercury. Therefore, states that, regardless to the composition of amalgam, mercury released into an aqueous medium is usually kept in solution, or is re-amalgamated with residual alloy.

According to American Dental Association (ADA), mercury amalgam releases only 1-3 μg of mercury per day, comparing to 5 – 6 μg that most humans ingest daily, by food, water or air. Therefore, ADA states that 500 fillings would be necessary in order to achieve adverse effects on human organism. To the contrary, according to WHO investigations, only one filling can release between 3 and 17 μg of mercury per day [8], which is consistent to the results of this study.

Al-Salehi et al [15] state that Macker and Berglund reported that non-stimulated amount of mercury release from dental amalgam is on average 0, 4 μg per surface, daily, and that it was calculated in six different in vivo studies. Assuming that amalgam surface in vivo is 5mm x 5mm, they calculated that the amount of mercury release from one amalgam is 1, 6 $\mu g/cm^2$ per day. Assuming that amalgam surface of 1 cm^2 equals four amalgam surfaces in vivo, value of the release of mercury in our study is: Sample 1 - 23,7 $\mu g/cm^2$, Sample 2 – 26,84 $\mu g/cm^2$, Sample 3 – 2,92 $\mu g/cm^2$, and Sample 4 – 15,32 $\mu g/cm^2$. The results are within the maximum acceptable daily intake of 45 μg, prescribed by the WHO, and also within the amount of 300-500 mg per day, seven days a week, for which the WHO considers not causing any effect. The amount of 300-500 μg of mercury per day is 10 times greater than the amount ingested by patient with 12 average-sized amalgam fillings, where calculated level of fecal excretion is 60 μg [5, 21].

Particular attention should be paid to Sample 3, which released the least amount of mercury in this study. It is important to emphasize that it is amalgam with very large proportion of copper (28%), leading to the conclusion that in this way the elimination of γ_2 phase, as the *locus minoris resistentiae* from the aspect of corrosion process was achieved, and higher proportion of η phase in the process of amalgamation was achieved, thus improving corrosion resistance.

According to Al-Salehi et al, the patient should have about 160 amalgam restorations so that mercury release could reach the maximum acceptable daily intake [15, 22].

Al-Salehi et al tested influences of carbamide-peroxide [15] and hydrogen-peroxide [22], as tooth bleaching agents, on mercury release from dental amalgam, using ICP-MS. Concentrations of tested agents were 1%, 3%, 10% i 30%. Concentrations of released mercury after 24 hours on 37°C were between 360 μg/l and 1428 μg/l. Those amounts are much higher than the amounts in our investigation, probably according to different test medium and different temperature.

ADA in its report in 1998 related to the Halbach research, who examined the release of mercury from amalgam fillings in vivo in a group of 20 patients with one to forty-six amalgam surfaces in the oral cavity [23]. Dose of released mercury in that investigation was between 0, 3-13, 9 μg per day, 4, 5 μg per day on average. The same researcher came to essentially same results with another group of subjects, where average daily dose of mercury derived from amalgam was 4, 8 μg. Halbach showed that the release of mercury is linearly associated with time and the total area of restorations. The Halbach results largely correspond to the results of this study being very important, considering the fact that our study is in vitro.

Determination of quantity of mercury released from dental amalgam in various scientific studies varies significantly. It is obviously difficult to make direct comparisons of different data, due to the lack of standardization of experimental details and form of data presentation [22]. The abovementioned applies to the results of this study.

For the amount of mercury released from dental amalgam in vivo, many factors, including number and age of the restorations, amalgam type, total area and the quality of restoration, mercury measurement methods, individual sensitivity of subjects and access to data analysis, may be responsible for different results of the assessment exposure to mercury from dental amalgam. The effect of meals consumed is also important for the release of mercury, which is not consistent. It is shown for some food to reduce intra-oral mercury vapor [23].

Although the research focus regarding the potential toxicity of amalgam is oriented towards mercury, amalgam contains other substances that can be neuro-toxic in high doses. Amalgam is an alloy of mer-cury, zinc, tin, copper and silver, and each of these elements can be neuro-toxic in high doses. Analysis of data collected by research of Lobner and Asrari indi-cates that the toxicity of amalgam is not conditioned by release of mercury. Each of amalgam components (Zn, Sn, Cu, Ag) is toxic, although even high concen-trations of tin (200 μM) cause only about 15% of cell death [24].

The idea that the release of zinc from dental amalgam is toxic is not new. It has been shown that the amalgams which contain zinc are more toxic than those without zinc. Taking into account the percentage of zinc in dental amalgam, it surprises that zinc is the first toxic substance to be released. It has been shown that zinc is basic corrosion product released from amalgam. Amalgam contains enough zinc that could be responsible for toxic influence on nerve cells in culture [24].

Zinc is essential element. Daily intake of zinc is 12-15 mg. It is estimated that human body contains total amount of 2, 5 g of zinc. Poisoning due to excessive zinc ingestion is rare. Evidence of hematological, hepatic or renal toxicity was not observed in persons who ingested 12 g of elemental zinc in 2 [6].

Average sized amalgam fillings made of amalgams tested in this research release 171, 5 μg (Sample 3) to 573, 19 μg (Sample 2) of zinc per day. These amounts are far below the maximum amount allowed.

Copper is essential trace element that catalyses process of hem-synthesis and absorption of iron. It is third of the most common trace elements in human organism, after iron and zinc. Corrosion products of copper from high-copper dental amalgam alloys after exposure to saliva include oxides, chloride oxides and hydroxides, chlorides and sulphides of copper. Average daily intake of copper is between 1-3 mg, and 2-5 mg, and primary source is food. Emetic dose of copper sulfate for adults is 0, 25 to 0, 5 g, and this copper sulfate can be described as copper. Toxic concentrations of copper can be achieved after ingestion of 1g of copper. Ingestion of fluids or solid food that contains minimally 25 mg/l of copper is connected with symptoms of acute gastroenteritis. Established lethal dose of copper in untreated adults is about 10-20 g Cu [6, 25, and 26].

Average sized amalgam fillings made of amalgams tested in this research release 18, 73 μg (Sample 1), to 74, 3 μg (Sample 4) of copper per day. These amounts are far below the amount that could cause adverse effects on human organism.

Data of tin toxicity are very limited. Tin can be detected in all human tissues and accumulated amount increases with age. The largest part of the tin in the tissues is a consequence of ingestion of metals. It is estimated that daily intake is 1-38 mg, and the intake is higher with greater consumption of canned food, because the inside of the cans is coated with tin. Except for rare reports of gastrointestinal disorders, there is very little evidence of significant toxicity of tin for humans [26].

Quantities of tin released from average sized amalgam fillings made of amalgams tested in this research are between 3, 8 μg (Sample 4) to 209, 55 μg (Sample 2). There is a small possibility that this quantity of ingested tin in vivo could lead to unintended consequences.

One of the possible ways of entering the silver in the human organism is its release from dental amalgam. Soluble silver compounds are more easily absorbed than metallic or insoluble silver, and may have the potential to cause adverse effects on human organism (i.e. argyria or argyrosis). However, it is important to emphasize that the silver in any form is not considered toxic to the immune, cardiovascular, nervous, or reproductive system, and it is not considered carcinogenic [27].

Quantities of sliver released from average sized amalgam fillings made of amalgams tested in this research are between 0, 54 μg (Sample 4) to 2, 99 μg (Sample 3). Those quantities participate in very small degree in daily intake of silver in human body.

Conclusions

The quantity of release of certain metals, as isotopes, from dental amalgams was determined in this research using ICP-MS.

1. The release of all four main metals (zinc, copper, tin, silver and mercury) from tested dental amalgam alloys was proved.
2. According to our in vitro results, quantities of mercury released from each amalgam tested could not be considered as hazard to patient's health. It has

been shown that for each tested amalgam, the presence of large number of average sized amalgam fillings in oral cavity would be necessary to achieve the maximum allowed daily intake identified as such by the WHO, which is 45 μg. Since the official position of WHO is that the amount of 300-500 μg of mercury per day, seven days a week, do not lead to any adverse effects, it can be concluded that the patient could have significantly higher number of amalgam fillings in the oral cavity, made of amalgams tested, without any health effects.

3. Release of the isotopes of mercury and other metals from dental amalgam composition, in this in vitro study, does not reach the amount that could be considered potentially hazardous to the health of the patient, considering the official position of WHO and governmental agencies in many countries around the world.

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DISTRIBUTION OF WHITE SPOTS AFTER DE-BONDING IN ORTHODONTIC PATIENTS

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ABSTRACT

Fixed orthodontic appliances can interfere with removing bacterial plaques from dental surfaces which can ultimately lead to white spot formation. The aim of this study was to evaluate the quantity of white spots lesions in orthodontic patients treated with fixed orthodontic appliances during different treatment periods and to reveal the teeth most commonly affected by white spot lesions.

Materials and Methods: A total of 30 patients undergoing or scheduled for fixed orthodontic treatment were divided into two groups. The patient's sample was divided according to the duration of fixed orthodontic appliance treatment. In the first group treatment time ranged from 1 to 1.5 year, and in the second group from 1.5 to 2 years respectively. The patients were selected randomly during regular recall visits to the Orthodontic Clinic at the University Dental Clinic Center in Skopje. The patients were examined for the presence of white spot before insertion of the appliance and after its removal using visual examination.

Results: This study showed that there was a significant increase in the formation of white spot lesions along with the increase in treatment duration. The first molars were the most affected teeth by white spots followed by canines and second premolars.

Conclusion: The results of the study showed an increase of white spots formation with the increase of orthodontic treatment duration. The orthodontists should educate their patients to perform and maintain good oral hygiene by proper brushing techniques including all teeth not emphasizing anterior teeth only.

Key words: white spots lesions, fixed orthodontic appliance.

Introduction

Dental caries is a common complication of orthodontic treatment with fixed orthodontic appliances.

The clinical manifestation of early enamel caries is presented white spot lesions (WSLs). WSLs are the opaque white appearance of enamel lesion resulting from subsurface demineralization which creates pores between enamel rods and results in changes of the enamel optical properties [1, 2, 3]. Prevention of demineralization during orthodontic treatment has been a challenging issue orthodontists faced with although caries prevention measures have improved over the last years. Due to the presence of fixed orthodontic appliances, it is difficult for patients to maintain adequate oral hygiene, as orthodontic appliances increase the number of plaque retention areas on teeth surfaces that are normally less susceptible to caries development [4, 5, 6].

Fixed orthodontic appliances create stagnation areas for plaque and make tooth cleaning difficult. Brackets, arch wires, ligatures, and other orthodontic appliances complicate the use of conventional oral-hygiene measures. This often results in significant plaque accumulation around the bracket bases. Demineralization of enamel around brackets can be an extremely rapid process and appears most frequently on the cervical and middle thirds of the buccal surfaces of the maxillary lateral incisors, the mandibular canines, and the first premolars [7, 8]. De-mineralized enamel can re-mineralize after debonding under favorable conditions [9, 10].

An insight into available literature on the prevalence of WSLs revealed that most relevant studies reported the presence of these lesions at the completion of orthodontic treatment [11, 12]. Depending on the examination technique used, the prevalence of WSLs varies. Boersma et al. [13] using quantitative light fluoroscopy investigated the prevalence of WSLs at the end of orthodontic treatment and reported that 97% of patients had one or more lesions. In light of these studies, one may conclude that demineralization is a significant clinical problem resulting in an unacceptable esthetic presentation that, in some severe cases, may require restorative treatment.

Epidemiological studies found that the most commonly affected teeth are molars, maxillary lateral incisors, mandibular canines and premolars. A study by Øgaard et al. found that the level of visible plaque around the appliance, shortly after bonding, was the best predictor for white spot lesion at de-bonding [14].

Prevalence of WSLs in the literature ranges widely from (2-97%). Sagarika et al. found a prevalence of 75.6% among orthodontic patients compared to 15.6% among control group [15]. Much of the variation in detecting WSLs was due to the method of detection with the highest prevalence of demineralization found when light induced fluorescent method had been used. Similar devices mainly based on optical phenomena, like digital imaging fiber-optic trans-illumination (DIFOTI) and red laser-light induced fluorescence, are also considered as more accurate methods in relation to direct visual examination.

Material and Methods

A total of 30 patients (14 female and 16 male) undergoing or scheduled for fixed orthodontic treatment were divided into two groups. The patient's sample was divided according to the duration of fixed orthodontic appliance treatment. In the first group, treatment time ranged from 1 to 1.5 year, and in the second group from 1.5 to 2 years respectively. The patients were selected randomly during regular recall visits to the Orthodontic Clinic at the University Dental Clinic Center in Skopje. Selection criteria were: aged of 16-18 years, healthy individuals and a treatment period with fixed appliance. Only tooth surfaces gingival to the arch wire were examined for the presence of WSLs, as this is the area most prone to enamel demineralization during orthodontic treatment. The patients were seen regularly every 3-4 weeks for the orthodontic follow-up visits. During each visit, the patients were motivated to perform proper tooth brushing techniques and maintain good oral hygiene. When orthodontic treatment was finished, the bands and brackets were removed, the remaining cement and composite was removed using sharp hand instruments and all teeth were polished. The facial surfaces of the teeth were examined in order to detect white spot formation using a mirror and an explorer. Even in non extraction cases the first premolars were excluded from the study. The follo-

wing modified WSLs scoring system introduced by Gorelick et al. [16] was used for the visual examination:

Score 0 = No visible white spots or surface disruption (no demineralization),

Score 1 = Visible white spot without surface disruption (mild demineralization),

Score 2 = Visible white spot lesion having a rough-ened surface but not requiring a restoration (moderate demineralization),

Score 3 = Visible white spot lesion requiring restoration (severe demineralization).

Visual examination was performed by the same dentist for all patients. Each tooth was air-dried for 5 seconds and the buccal surface was examined visually for enamel decalcification in the portion of the crown gingival to the bracket using the following scale.

Statistical Analysis

The increasing WSL index before and after a treatment was determined. Statistical analyses were performed using SPSS version 6. Since the scoring system was used for determination, nonparametric tests were used for statistical analysis. Descriptive statistics were obtained for all groups. Kruskal-Wallis and Mann-Whitney U tests were used to compare the groups. A p-value, 0.05 was considered to be statisti-cally significant.

Results

All the results are shown in tables 1, 2, 3, 4, 5 and 6. **Tables 1 and 2** showed the descriptive statistics of white spots in upper and lower jaws of male subjects. There was an increase in the scores of white spots with the increase of treatment duration time. The first molars were affected by white spots formation more often than the other teeth followed by the second premolars and canines. **Tables 3 and 4** showed the descriptive statistics of white spots in upper and lower jaws of female subjects.

Comparison of white spots among teeth at each duration of time, right dental side versus left, upper teeth versus lower and male versus female in each treatment duration time is presented in **Table 5**. There were significant differences in the frequency of

	teeth	N	min	max	mean	SD	0	1	2	3		teeth	N	min	max	mean	SD	0	1	2	3
Upper Right	1	8	0	0	0	0	100	-	-	-	Lower Right	1	8	0	0	0	0	100	-	-	-
	2	8	0	0	0	0	100	-	-	-		2	8	0	0	0	0	100	-	-	-
	3	8	0	1	0,14	0,35	82,5	16	-	-		3	8	0	1	0,05	1,1	83	-	-	-
	5	8	0	0	0	0	100	-	-	-		5	8	0	0	0,7	0,4	83	15,6	-	-
	6	8	0	1	0,2	0,7	70	31	-	-		6	8	0	1	1	0,8	32	30	-	-
Upper Left	1	8	0	0	0	0	100	-	-	-	Lower Left	1	8	0	0	0	0	100	-	-	-
	2	8	0	0	0	0	100	-	-	-		2	8	0	0	0	0	100	-	-	-
	3	8	0	1	0,15	0,4	75,5	15	-	-		3	8	0	1	0,34	0,9	65	32	-	-
	5	8	0	0	0	0	0	-	-	-		5	8	0	1	0,6	0,5	83	15,6	-	-
	6	8	0	1	0,17	0,3	80,5	14	-	-		6	8	0	1	0,5	0,9	52	30	-	-
Total upper		80	0	1	0,11	0,25	92	7,5	-	-	Total lower		80	0	1	0,34	0,6	80	12,5	-	-

Table 1
Descriptive Analysis of White Spots in Male Group (Frequency of Score %)
Treatment time ranging from 1 to 1.5 year

	teeth	N	min	max	mean	SD	0	1	2	3		teeth	N	min	max	mean	SD	0	1	2	3
Upper Right	1	8	0	0	0	0	100	-	-	-	Lower Right	1	8	0	0	0	0	100	-	-	-
	2	8	0	1	0,3	0,4	65	32,3	-	-		2	8	0	0	0	0	100	-	-	-
	3	8	0	1	0,3	0,4	65,5	32.3	-	-		3	8	0	0	0	1	100	-	-	-
	5	8	0	0	0	0	100	-	-	-		5	8	0	0	0	0	100	-	-	-
	6	8	0	1	0,3	0,4	65,5	32,3	-	-		6	8	0	1	1	0,8	65,5	32	-	-
Upper Left	1	8	0	0	0	0	100	-	-	-	Lower Left	1	8	0	0	0	0	100	-	-	-
	2	8	0	1	0,3	0,4	65,5	32,3	-	-		2	8	0	0	0	0	100	-	-	-
	3	8	0	1	0,2	0,4	65	32,2	-	-		3	8	0	1	0,34	0,9	65,5	32	-	-
	5	8	0	1	0,2	0,4	0	32,3	-	-		5	8	0	0	0	0	100	-	-	-
	6	8	0	1	0,2	0,4	65,5	32,3	-	-		6	8	0	0	0	0	100	-	-	-
Total upper		80	0	1	0,25	0,35	75	28	-	-	Total lower		80	0	1	0,34	0,6	80	6,1	-	-

Table 2
Descriptive Analysis of White Spots in Male Group (Frequency of Score %)
Treatment time ranging from1.5 to 2 years

white spots between teeth in upper and lower right quadrant at duration [1] in female group, the first molar shows the highest frequency of white spots followed by the canine in the upper jaw. In the lower right quadrant the first molars were most commonly affected followed by canines and second premolars. In duration [2] in male group the upper arch shows significantly higher frequency of white spots than the lower. In females the lower left quadrant displayed significant difference in the distribution of white spots among teeth and again the first molars were affected by white spots more often than the other teeth followed by the canines (**Table 6**).

	teeth	N	min	max	mean	SD	0	1	2	3		teeth	N	min	max	mean	SD	0	1	2	3
Upper Right	1	6	0	0	0	0	100	-	-	-	Lower Right	1	6	0	0	0	0	100	-	-	-
	2	6	0	0	0	0	100	-	-	-		2	6	0	0	0	0	100	-	-	-
	3	6	0	2	0,14	0,35	82,5	16	-	-		3	6	0	3	0,5	1,1	83	-	-	15,6
	5	6	0	0	0	0	100	-	-	-		5	6	0	2	0,7	0,4	83	15,6	-	-
	6	6	0	2	0,7	0,9	60	32	16,1	-		6	6	0	1	1	0,8	32	32	32	-
Upper Left	1	6	0	0	0	0	100	-	-	-	Lower Left	1	6	0	0	0	0	100	-	-	-
	2	6	0	0	0	0	100	-	-	-		2	6	0	0	0	0	100	-	-	-
	3	6	0	1	0,15	0,4	82,5	16	-	-		3	6	0	1	0,34	0,9	65	32	-	-
	5	6	0	0	0	0	0	-	-	-		5	6	0	1	0,6	0,5	83	15,6	-	-
	6	6	0	2	0,67	0,4	82,5	16	-	-		6	6	0	2	0,5	0,9	52	32	15	-
Total upper		60	0	2	0,11	0,35	90	8,5	1,5	-	Total lower		60	0	3	0,34	0,6	80	13,1	6	1,8

Table 3
Descriptive Analysis of White Spots in Female Group (Frequency of Score %)
Treatment time ranging from 1 to 1.5 year

	teeth	N	min	max	mean	SD	0	1	2	3		teeth	N	min	max	mean	SD	0	1	2	3
Upper Right	1	8	0	0,2	0	0,4	100	11,5	-	-	Lower Right	1	8	0	0	0	0	100	-	-	-
	2	8	0	0,2	0	0,4	100	12,5	-	-		2	8	0	0	0	0	100	-	-	-
	3	8	0	0,1	0,14	0,35	82,5	11,5	-	-		3	8	0	1	0,5	1,3	83	12,5	-	-
	5	8	0	0,1	0	0,3	100	11	-	-		5	8	0	1	0,7	0,4	83	15,6	-	-
	6	8	0	0,3	0,7	0,45	60	12,5	-	-		6	8	0	2	1	0,8	32	32	32	-
Upper Left	1	8	0	0,1	0	0,3	100	12,5	-	-	Lower Left	1	8	0	0	0	0	100	-	-	-
	2	8	0	0,2	0	0,4	100	11,5	-	-		2	8	0	0	0	0	100	-	-	-
	3	8	0	0,2	0,15	0,3	82,5	16	-	-		3	8	0	1	0,25	0,9	65	32	-	-
	5	8	0	0	0	0	0	-	-	-		5	8	0	1	0,2	0,8	51	-	-	-
	6	8	0	0,1	0,67	0,4	82,5	11,2	5,9	-		6	8	0	3	0,5	0,9	52	32	-	11,2
Total upper		80	0	0,12	0,11	0,35	90	15,5	0,7	-	Total lower		80	0	3	0,34	0,6	83	12,1	1,3	1,8

Table 4
Descriptive Analysis of White Spots in Female Group (Frequency of Score %)
Treatment time ranging from1.5 to 2 years

Discussion

The first clinical evidence of the demineralization is the white spot lesion (WSLs), which potentially can become a cavitated carious lesion extending even into the dentin. White spot lesions are no fluoridated opacities having a more defined shape and are well differentiated from surrounding enamel which is often located in the middle of the tooth. The WSLs has been defined as "subsurface enamel porosity from carious demineralization" presenting itself as a "milky white opacity" when located on smooth surfaces. In addition to the fact that WSLs is the first step to de-

Duration	Sex	Dental arch	Sides	N	Chi-Square	Z-value
			Right	8	5,65	-
			Left	8	8,34	-
	Male	Upper	Right vs. Left	16	-	-0,48
			Right	8	8,6	-
			Left	8	3,5	-
	Male	Lower	Right vs. Left	16	-	-1,28
			Right	6	**9,4***	-
			Left	8	3,2	-
	Female	Upper	Right vs. Left	14	-	-
			Right	6	**9,7***	-
			Left	8	7,5	-
	Female	Lower	Right vs. Left	14	-	-0,15
	Male vs. Female			14	-	**-2,10***

Table 5
Comparison of White Spot among teeth at Each Duration Time
Treatment time ranging from 1 to 1.5 year

Duration	Sex	Dental arch	Sides	N	Chi-Square	Z-value
			Right	8	1,37	-
			Left	8	1,37	-
	Male	Upper	Right vs. Left	16	-	-0,06
			Right	8	3	-
			Left	8	3	-
	Male	Lower	Right vs. Left	16	-	**-1,23***
			Right	6	2,55	-
			Left	8	2,44	-
	Female	Upper	Right vs. Left	14	-	-
			Right	6	7,78	-
			Left	8	**11,4***	-
	Female	Lower	Right vs. Left	14	-	-0,15
	Male vs. Female			14	-	-0,25

Table 6
Comparison of White Spot among teeth at Each Duration Time
Treatment time ranging from 1.5 to 2 years

struction of the teeth, this enamel demineralization associated with fixed orthodontic appliances presents other significant clinical problem for the orthodontists.

Early detection of white spot lesions during orthodontic treatment is also very important, as it would allow clinicians to implement preventive measures to control the demineralization process before progression of the lesions. The Gorelick index to measure WSLs is so far the most used method to registration WSL in orthodontic patients. This index is well suited to registration of the specific area of WSL but may have limitations in measuring the extent of WSLs.

In the literature, there are conflicting reports describing the distribution of WSLs; Gorelick et al. reported that the most commonly affected tooth was the maxillary lateral incisor. On the other hand, Mizrahi [17] concluded that the maxillary and mandibular first molars were the most commonly affected teeth. In a later study, Øgaard [18] agreed with Mizrahi's conclusions. To the contrary, Geiger et al. reported that lesions occurred most frequently on maxillary lateral incisors and canines.

Detecting WSLs during active treatment can be challenging for the clinician. The clinical crown must be free from plaque and debris, and the presence of excess gingival tissue can make visualization of WSLs difficult. Furthermore, to detect incipient WSLs, the tooth must be air-dried. If these steps are not followed, a WSL could easily be overlooked. Therefore, a thorough examination of each patient should be done at each appointment, and each patient should receive a customized oral hygiene treatment regime to halt the progression of any demineralization.

The result of this study showed that there was an increase in white spot formation around orthodontic brackets when the duration time of the treatment increased. This result is in agreement with that of Zavareh et al. (19) who found that duration of treatment had a significant effect on the occurrence of white spots. Tufekci et al. [20] reported a sharp increase in the number of white spot lesions during the first 6 months of treatment that continued to rise at a slower rate to 12 months. The comparison of white spot among teeth in each quadrant showed that the first molars were most affected by white spot lesion followed by the canines and second premolars. The high incidence of white spots on first molars could be attributed to the banding of these teeth and their role in food accumulation and difficulties in maintaining good oral hygiene on banded molars. The canines and second premolars came in the second place regarding white spots formation. This may be due to the presence of hook at canine and premolar brackets and the use of force accessories such as elastic chains and coil springs aiding in plaque retention which leads to more white spot formation. The fact is that the patients usually concentrate their brushing more on anterior teeth than on posterior being in accordance to Øgaard who found that the most commonly affected teeth are the molars, maxillary lateral incisors, mandibular canines and premolars.

Most studies have also found either the maxillary laterals or maxillary canines to be the most commonly affected teeth. Although there was no difference on the right side, the maxillary left lateral incisors developed more WSL than the canines on the left side. In the mandibular arch, the canines have routinely been found as the most susceptible [21,22,23].

Conclusion

The results of this study showed an increase of white spots formation with the increase of orthodontic treatment duration.

The buccal segments were affected more than the anterior segment. The orthodontists should educate their patients to perform and maintain good oral hygiene by proper brushing techniques including all teeth and not emphasizing anterior teeth only.

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INTERPROXIMAL PLAQUE CONTROL IN PATIENTS WITH FIXED ORTHODONTIC APPLIANCES

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ABSTRACT

The aim of the study was to determine the influence of an inter-proximal cleaning on the oral hygiene status and to compare the plaque control effectiveness of an inter-proximal brushing in combination with mechanical tooth-brushing at 30 right-handed patients with brackets in the upper and in the lower arch. 15 patients used inter-proximal brushes combined with manual tooth-brushing, twice a day in all quadrants, while other 15 patients maintained oral hygiene with manual tooth-brushing technique only. Dental plaque index (DPI), Approximal plaque index (API) and Gingival bleeding index (GBI) were performed one week after the orthodontic treatment, when patients were instructed how to maintain oral hygiene and inter-dental hygiene, and also 4 weeks after orthodontic treatment. According to the results presenting statistically significant differences in the mean values of DPI, GBI and API, among the examine groups, ($p < 0,00001$), the improvements of oral hygiene could be interpreted as an outcome of the instruction and motivation given to the patients with fixed orthodontic appliances.

Key words: inter-proximal cleaning, dental plaque reduction, gingival inflammation.

Introduction

Orthodontic treatment is widely accepted treatment in dental practice, due to its positive effects on the oral-facial complex. Thus, it is becoming increasingly popular. Clinicians can offer patients functional occlusion and aesthetic improvements by using fixed orthodontic appliances. On the other side, orthodontic treatment with fixed appliances increases dental plaque amount and complicates the cleaning for the patient [1, 2].

Oral bio-film, or "dental plaque", is difficult to remove and regular brushing is often insufficient. Biofilms on dental hard and soft tissues are the main cause of dental disease [3, 4]. Orthodontic appliances severely hamper the efficacy of tooth brushing [5], reduce the self-clearance by saliva [6], change the composition of the oral flora [7], increase the amount of oral bio-film formed [8] and the colonization of oral surfaces by cariogenic bacteria [9]. These factors strongly complicate orthodontic treatment and illustrate that the need for dental biofilm control is even greater during orthodontic treatment than usual. Gingivitis, periodontitis and enamel de-calcification around fixed appliances are frequent side effects when the preventive programs have not been implemented [10, 11, 12, 13].

The link between orthodontic therapy and periodontal disease may be established if orthodontic appliances contribute to accumulation and difficult removal of sub-gingival plaque, when there is a progression of gingivitis towards more severe forms of disease. If accumulated supra-gingival plaque is left undisturbed, it will lead to gingivitis. All this implies that these patients are at greater risk for gingival and periodontal disease and caries. The factor that determines the condition of the periodontium during orthodontic treatment is the level of oral hygiene. Thus, the elimination of plaque is the main target to prevent periodontal problems [14]. In literature, numerous studies investigated the most appropriate plaque elimination method for orthodontic patients.

Regular tooth brushing is the first line of defence in controlling dental plaque. But, many studies indicated that the sole use of a toothbrush does not adequately clean all tooth surfaces. Therefore, the additional use of inter-dental cleaning aids is recommended and necessary [15]. Therefore, oral hygiene instructions should be given before the initiation of orthodontic treatment and reinforced during every visit.

A study was conducted to determine whether the instructions for maintaining oral hygiene have influence on the quality of oral hygiene and gingival health. Dental plaque index (DPI), gingival bleeding index (GBI) and Aproximal plaque index (API) were used to assess oral health at patients with fixed orthodontic appliances.

The **aim** of the study was to examine the effects of the interproximal hygiene at patients with fixed orthodontic appliances maintaining oral hygiene practicing manual tooth brushing combined with inter-proximal brushing in comparison with those patients practicing manual tooth brushing only.

Material and Methods

The study included 30 examinees, (15 male and 15 female) with the average age of 15 years, divided into two groups. The first group of 15 examinees maintained oral hygiene practicing only toothbrushing. Examinees from the second group, also 15 of them, used inter-proximal tooth brushing combined with manual tooth brushing. All examinees were selected from the Orthodontic Clinic at the University Dental Clinic Centre in Skopje, Macedonia. They were randomly selected. Silness and Loe PI and API were used to assess oral and inter-dental hygiene. Muleman GBI was used to assess gingival health. Dental indices were noted at buccal surfaces of all teeth with fixed orthodontic appliances. Control check ups were conducted at the beginning, (one week after the orthodontic treatment beginning), and four weeks after. The Bass's technique, important for plaque control in gingival parts, was explained to examiners and practiced. When needed, custom corrections were done because of the specific teeth positions and fixed orthodontic appliance. Examinees in the second group were shown and they practiced how to use inter-proximal toothbrush with different size, depending on the interproximal space for cleaning areas around the brackets, between them as well as near orthodontic arch and vestibular teeth surfaces.

Statistic analysis was done in SPSS (6) software. For DPI, GBI and API indexes one way ANOVA were used.

Results

All results are shown in tables 1, 2, 3, 4 and 5.

Table 1 shows dental plaque index (DPI) values at I group practicing only toot brushing, (p<0,012) and II group practicing tooth-brushing and inter-proximal cleaning, (p<0, 000001) one and four weeks after the beginning of orthodontic treatment.

Table 2 shows the GBI values at I group practicing only tooth brushing (p< 0,009) and II group practicing tooth brushing and inter-proximal cleaning, (p < 0,000035) one and four weeks after the beginning of orthodontic treatment.

Table 3 shows the comparison of API values at I group practicing tooth brushing only, (p<0, 00031) and II group practicing tooth brushing and inter-proximal cleaning, (p<0, 000000) one and four weeks after the beginning of orthodontic treatment.

Table 4 shows the comparison among **DPI** and **API** at I and II group four weeks after the beginning of orthodontic treatment (p<0, 000000).

Orthodontic patients	X	SD	N	df	t	p
DPI (Igr/1)	1,13	0,35				
DPI (Igr/4)	0,73	0,45	15	28	2,68	0,012
DPI (IIgr/1)	1,00	0,00				
DPI (IIgr/4)	0,26	0,45	15	28	6,20	0,000001

Analyzes of variance (ANOVA) show statistically significant differences among dental plaque index values after one week (F = 1, 69, p = 0,012), and after four weeks from the beginning of orthodontic treatment: (F = 1, 00, p = 0,000001).

Table 1 (Dental Plaque Index)
(One and four weeks after the beginning of orthodontic treatment)
(I gr: tooth brushing) and (II gr: tooth-brushing and inter-proximal cleaning)

Orthodontic patients	X	SD	N	df	t	p
GBI (Igr/1)	1,06	0,25				
GBI (Igr/4	0,66	0,48	15	28	2,80	0,009
GBI (IIgr/1)	0,93	0,25				
GBI (IIgr/4)	0,26	0,45	15	28	4,91	0,000035

Analyzes of variance (ANOVA) show statistically significant differences among dental plaque index values after one week (F = 3, 57, p = 0,009), and after four weeks from the beginning of orthodontic treatment: (F = 3, 14, p = 0, 000035).

Table 2 (Gingival Bleeding Index)
(One and four weeks after the beginning of orthodontic treatment)
(I gr: tooth brushing) and (II gr: tooth brushing and inter-proximal cleaning)

Orthodontic patients	X	SD	N	df	t	p
API (Igr/1)	59,2	16,66				
API (Igr/4)	34,4	9,81	15	28	4,96	0,00031
API (IIgr/1)	56,7	18,54				
API (IIgr/4)	15,4	3,92	15	28	8,43	0,000000

Analyzes of variance (ANOVA) show statistically significant differences among dental plaque index values after one week (F=2, 88, p = 0, 00031), and after four weeks from the beginning of orthodontic treatment (F = 022,30, p = 0,000000).

Table 3 (Approximal Plaque Index)
(One and four weeks after the beginning of orthodontic treatment)
(I gr: tooth brushing) and (II gr: tooth brushing and inter-proximal cleaning)

Orthodontic patients	X	SD	N	df	t	p
DPI (Igr/1)	1,13	0,35				
API (Igr/1)	59,20	16,66	15	28	-13,48	0,000000
DPI (IIgr/1)	1,00	0,00				
API (IIgr/1)	56,70	18,54	15	28	-11,64	0,000000

Analyzes of variance (ANOVA) show high statistically significant differences among dental plaque index values and aproximal plaque index values at the examine groups after four weeks from the beginning of orthodontic treatment. (F/I gr. = 2244, 46, p = 0, 000000), and (F /II gr. = 0, 00, p = 0,000000).

Table 4
DPI and API at I and II group four weeks after the beginning of orthodontic treatment
(I gr: tooth brushing) and (II gr: tooth brushing and inter-proximal cleaning)

Table 5 shows the comparison among **GBI** and **API** at I and II group four weeks after the beginning of orthodontic treatment (p<0, 000000).

The results noted one and four weeks after the orthodontic treatment showed statistically significant differences (p<0, 00) for all examined clinical parameters in both groups with higher significance at the second group, especially regarding high significant differences for API at the group practicing inter-proximal brushing (second group).

Discussion

In this study oral health status was evaluated in the patients with orthodontic fixed appliances. The study was conducted to determine whether the instructions for maintaining oral hygiene have influence on the quality of oral hygiene and gingival health. The hypothesis of the study was that there is a change in oral health status of the orthodontic patients receiving instructions for oral and inter-proxi-

Orthodontic patients	X	SD	N	df	t	p
GBI (Igr/1)	1,06	0,25				
API (Igr/1)	59,20	16,66	15	28	-13,50	0,000000
GBI (IIgr/1)	0,93	0,25				
API (IIgr/1)	56,70	18,54	15	28	-11,65	0,000000

Analyzes of variance (ANOVA) show high statistically significant differences among gingival bleeding index values and aproximal plaque index values at the examine groups after four weeks from the beginning of orthodontic treatment. (F/I gr. = 4168, 28, p = 0, 000000), and (F /II gr. = 5156, 71, p = 0,000000).

Table 5
GBI and API at I and II group four weeks after the beginning of orthodontic treatment
(I gr: tooth brushing) and (II gr: tooth brushing and inter-proximal cleaning)

mal hygiene maintaining oral hygiene practicing manual tooth brushing combined with inter-proximal brushing and those practicing manual tooth brushing only.

Many oral hygiene programs before the onset of the orthodontic treatment were recommended to prevent these deleterious effects [16, 17, 18]. In our study, all patients received oral hygiene education at the beginning of the study. It is well known that it becomes more difficult to keep teeth clean and maintain high oral hygiene level upon the placement of the appliances [19]. All index values resulted in decreased clinical parameter scores as expected, with statistically significant differences ($p<0,00$).

Our results are in accordance to numerous studies presenting significant increase in the quantity of dental plaque as well as of gingival inflammation in patients with fixed orthodontic appliances because of difficulties in removing dental plaque [20]. Fixed orthodontic appliances, such as orthodontic braces, arches and rings, increase the number of retention places for dental plaque accumulation. All these factors make the maintenance of oral hygiene more difficult [21].

During a fixed orthodontic appliance therapy, the technique and duration of tooth brushing and constant motivation of patients are key factors for oral hygiene maintenance [22]. Before the beginning of treatment, patients should be informed about the increased risk of caries and gingivitis and they should be instructed about the necessity of regular oral hygiene maintenance in order to minimize the risks. Ay et al. [23] have proven the thesis that oral instructions are insufficient for achieving a satisfactory level of oral hygiene orthodontists and dental hygienists must make a point of improving the level of oral hygiene of orthodontic patients.

The improvements in DPI, GBI and especially API, from one examination time point to the next result from increasing skills in using the inter-proximal brush combined with manual toothbrushes and also from instruction and motivation of the patients regarding dental hygiene [24]. The more expressed decrease in API could be associated to the baseline API having been higher than the baseline DPI and GBI, permitting a greater reduction of dental plaque accumulation (**Table 4 and 5**). The improvement in DPI, GBI and especially API in all quadrants from one examination timepoint to the next is open to these hypotheses. Clark JR. [25] has pointed out the importance of the motivation of the orthodontists for an oral health program. He stated that conversation with patients at each appointment about the effectiveness of cleaning is especially helpful. In addition to his recommendations about the feedback offered with kindness, objectivity and respect, we suggest the enforcement of the oral hygiene technique with the application under the supervision of the orthodontists.

The patients in our study developed practicing effect in using the manual Bass-tooth brushing technique, as one of the most preferable technique, and instruction in an additional aid for interproximal care. The patients tried their best to practice proper oral hygiene and make the oral hygiene parameters decreased significantly even from the baseline records. After instructing patients, they agree with the effectiveness of the Bass's tooth brushing technique and the necessities of practicing interproximal brushing for cleaning teeth more efficiently.

Conclusion

According to these results, it can be concluded that significantly lower values for DPI, GBI and API could be interpreted as an outcome of the increasing awareness of oral hygiene and greater skill in using interdental toothbrushes as dental hygiene aids in the course of time. The habit to maintain oral hygiene regulary is of great importance for maintaining gingival health throughout the orthodontic treatment and after its completion. Also, patience, persistence, training and regular check ups have great effect. All these helped in implementing obtained knowledge and skills for good oral hygiene as well as good habits by the end of orthodontic treatment.

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MICROBIOLOGICAL AND EXFOLIATIVE CYTOLOGICAL FINDING OF THE ORAL CANDIDA DUBLINIENSIS IN PATIENTS WHO ARE PSHYOACTIVE SUBSTANCES ADDICTS

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ABSTRACT

Goal: The goal of this study was to examine the presence and frequency of Candida dubliniensis in the oral cavity in psychoactive substance addicts using methods of exfoliative cytology to determine the possibility of oral precancerous conditions.

Material and methods: The research included 60 in-patients from the Institute for Alcoholism and Other Addictions of the Sarajevo Canton who were divided into two groups (alcohol addicts and substance addicts). The groups included both sexes aging 18-60. After the anamnesis, two smears were taken of the oral mucous membranes. The microbiological identification of Candida types was based on the crystallization test and cultivation on a chromatophilous basis (Chrom agar), assimilation test for yeast plants (API test), and identification test (PAL agar) for Candida dubliniensis. The second smear was taken for the exfoliative cytological test for the purpose of determining patho-histological changes.

Results: Microbiological analyses have confirmed the frequency of Candida albicans (43%) and increase in the non-albicans type irrespectively of the type of addiction (34%). The presence of Candida dubliniensis has been proven in psychoactive substance addicts (23%) and it has been confirmed that the frequency of adherence of Candida dubliniensis is in proportion to the time interval of drugs addiction. The chi-quadrate test (p=0, 55297) has confirmed that there are no significant differences in cytological findings frequencies by groups. In general, bacteria are the most present group in 70, 00 % of patients irrespectively of groups. The group of alcohol addicts, metaplasia was found in 26% of patients, and in case of drug addicts, it was identified in 18% of patients.

Conclusion: The statistical analysis has confirmed that psychoactive substances (drugs and alcohol) lead to an increase in oral Candida dubliniensis irrespectively of the type of addiction. In case in psychoactive substances use, the method of exfoliative cytology has demonstrated the presence of metaplasia – which constitutes a risk of oral precancerous conditions.

Key words: Candida dubliniensis, psychoactive substances (drugs), metaplasia.

Introduction

Oral candidiasis is an opportunistic infection caused by candida yeasts, out of which Candida albicans is the most pathogenic one and it has the capacity of pathological adherence to oral mucous membranes and causing oral diseases [1]. Other non-albicans types of high frequency in clinically manifested candidiasis are: candida dubliniensis, candida glabrata, candida tropicalis, candida crusei, candida parapsilosis, candida stelatoidea, candida kefur, candida guillermondi [2, 3]. The mentioned types are opportunists, pathogenic yeasts that may cause a whole array of lesions under certain circumstances. Candida yeasts may cause superficial candidiasis (oropharyngeal candidiasis, esophagitis, vulvovaginitis, chronic muco-coutaneous candidiasis, intertrigo and onycomicosis). Generalized candidiasis occurs as a septic disease by means of dissemination through blood and lymph and it frequently has a lethal outcome.

Virulence factors that enable yeasts to become patogenous are: the ability of yeasts to attach to the host tissue (Fonzi 2001), production of proteolytic enzymes that include highly molecular metabolites from the group of aspartyl proteases (Naglik 2003), phospholipases (Ghannoum 2000) and lipases (Stehr 2003), yeast hyphae – a morpho-genetic transformation that renders possible penetration (Fromtiling R A 2005), different immune and modular effects of yeast determinants may contribute to reduced activities of the defense system.

The authors Hazen, 1995, Hobson 2003, have divided the pre-disposition factors for the occurrence of a yeast infection in the following ones: immune-suppression caused by the use of drugs in malignant diseases treatment (cytostatics), application of anti-inflammatory drugs (corticosteroids), use of drugs against transplant rejection, idiopathic lymphocytopenia, and similar use of antibiotics and impairment of the endogenous commensal flora, through a surgery or other impairment of the protection barriers integrity of the host (e.g. burns, catheters, etc.), HIV infection, use of psychoactive substances (drugs).

A large number of epidemiological studies show an increased incidence of infections (of the mucous membrane, skin, hematogenous and disseminated infections) caused by non-candida albicans yeasts [4,5]. As of 1995, a new interesting type called Candida dubliniensis has been mentioned. As opposed to Candida albicans, it is a rare type of endogenous micro-flora of immune-suppressed patients and has reduced ability of colonization and infection (Sullivan et al. 2004). Candida dubliniensis is mostly taken from the oral cavity (buccal mucous membrane, saliva, tongue) of immune-deficient patients, primarily those that are HIV positive [6]. The colonies of Saboraud agar are soft, white and very similar to Candida albicans colonies (Larone 2002). Today, the most modern cultivation base Pal agar is used for the creation of clamidospora colonies of Candida dubliniensis with a thin membrane and higher volume (Candida albicans is a blastoconidium in a single-cell form) [7, 8, 9].

Psychoactive substance addiction is a leading social and health problem worldwide. The World Health Organization defined a drug or psychoactive substance as any natural or synthetic drug introduced into the body that may change one or more different functions of the body. Drugs are those natural or synthetic substances that may cause changes or disturbances of one or more somatic or psychological functions and are used for medical or non-medical purposes. Drugs are classified as hard and soft drugs. Hard drugs cause a strong psychological and physical addiction (heroine, cocaine). Soft drugs have medium potential to cause psychological addiction, and low level of physical addiction. They include: alcohol, amphetamines, nicotine, hallucinogens, caffeine, marijuana (hashish), organic dissolvent, etc. [10, 11, 12]. A psychoactive substance may be introduced into the body in several ways: orally (alcohol, pills), by smoking, inhaling (marijuana, cocaine, opium), snorting (cocaine, heroin), subcutaneously and intravenously (heroin, cocaine). Psychoactive substances (drugs) have great impact on oral health. Oral changes depend on the type of drugs and length of use. Changes in oral mucous membranes are not pathogenic in psychoactive substance addicts, but the following ones may be perceived: Candidiasa mucosae oris; Cheilitis angularis; Glossitis rhombica mediana; Leukoplakia; Hyperceratisis mucousae oris; Gingivostomatitis ulceromembranacea; Lingua nigra villosa; Stomatitis aphthosis recurrens (SAR); Erosiae mucosae oris; Herpes simplex; Morsicatio mucosae oris [13,14]. In psychoactive substance addicts (alcohol, drug addicts) an immune-suppression of the immune system occurs, which leads to an increase in opportunistic infections.

Researches of many authors point to the interconnectedness between the intake of psychoactive substances (drugs), length of addiction and type of drugs and the presence of oral Candida dubliniensis. Rocchi et al. 2007 proved in their work that the presence of Candida dubliniensis was related to the length of heroin use. Rooban et al. 2009 examined the oral health in addicts addicted to various drugs (opiates, cocaine, marijuana) and confirmed highly significant presence of oral Candida dubliniensis and the length of drug intake, as well as the type of drug [15,16]. Psychoactive substance addicts are risk patients for AIDS and Candida infections, the incidence of which is on continuous rise. These patients demonstrate frequent resistance to therapeutic protocol, so the imperative of the research is to isolate and confirm the importance of Candida dubliniensis by means of microbiological procedures and to establish the possibility of occurrence of precancerous conditions by means of exfoliative cytological tests.

Material and Methods

For the purpose of achieving the set goals, we have selected a sample. 60 inpatients from the Institute for Alcoholism and Other Addictions of the Sarajevo Canton and they were divided into two groups: group A – alcohol addicts, and group B – opiates addicts. The groups included both sexes aging from 18 to 60 years. After a comprehensive anamnesis, an extra-oral and intra-oral examination of oral mucous membranes was conducted. Smears were taken from the mucous membranes for microbiological identification. Two confirmatory methods were used for the identification of Candida types: the crystallization test and cultivation on a chromatophilous basis (Chrom agar). The assimilation test for yeast plants (API test) was used for the identification of non-albicans candida. By planting a positive culture on a chromatophilous basis (Chrom agar) and 24-48 hour incubation at 37°C, four types of Candida were identified based on the different color (Candida albicans, Candida tropicalis, Candida glabrata, Candida crusei).

All cultures of Candida albicans (that have been proven by the mentioned methods) were planted on a PAL agar basis for the purpose of identifying Candida dubliniensis**.** The culture had an incubation period of 24-48 hours at 30^0C. If yeast plants grew, a preparation was made and it was observed under the microscope with an enlargement of 40 x. The presence of clamidospora has confirmed that the identified yeast plants were Candida dubliniensis.

The second smear was related to the Institute for Pathology and Cytology where the exfoliative cytological test was conducted.

Results

Microbiological analysis findings

Table 1 contains the frequency (percentage) of patients in case of which the Candida types were found by examination groups and in total.

Candida albicans was present at 43% of the total patients. Non-albicans was identified at 34,3 % of the patients. Candida dubliniensis was identified at 23% of the total patients addicted to psychoactive substances (Table 1).

	ALBICANS		NON-ALBICANS						DUBLINIENSIS	
			TROPICALIS		GLABRATE		CRUSEI			
	No.	%	No.	%	No.	%	No.	%	No.	%
Group A	6	37,50	1	6,25	3	18,75	1	6,25	5	31,25
Group B	9	47,37	1	5,26	1	5,26	5	26,32	3	15,79
Total	15	42,86	2	5,71	4	11,43	6	17,14	8	22,86

Table 1
Frequency of Candida types by groups

	ALBICANS		NON-ALBICANS		DUBLINIENSIS		TOTAL
	No.	%	No.	%	No.	%	No.
Group A	6	37,50	12	31,25	5	6,25	16
Group B	9	47,37	7	36,84	3	26,32	19
Total	15	42,86	12	34,29	8	17,14	35

Table 2
Frequency of Candida types (consolidated non-albicans) by groups

In the case of group A (alcoholics), the following Candida types were identified: Candida albicans in 6 patients (37.5 %), Candida tropicalis 1 patient (6.25%), Candida glabrata 3 (19%) and Candida crusei 1 (6.2 %). In the case of group B (addicts), Candida albicans was identified in 9 patients (47.4%), Candida tropicalis 1 patient (5.3%), Candida glabrata 1 (5.3) and Candida crusei 5 (26.3%). Candida dubliniensis was identified in 8 patients, namely in group A in 5 patients (31.25%) and in group B in 3 patients (15.8%) (Table 2).

The chi-square test (*p=0,55297*) has confirmed that there are no significant differences in the frequencies of occurrence of Candida by groups. Candi-

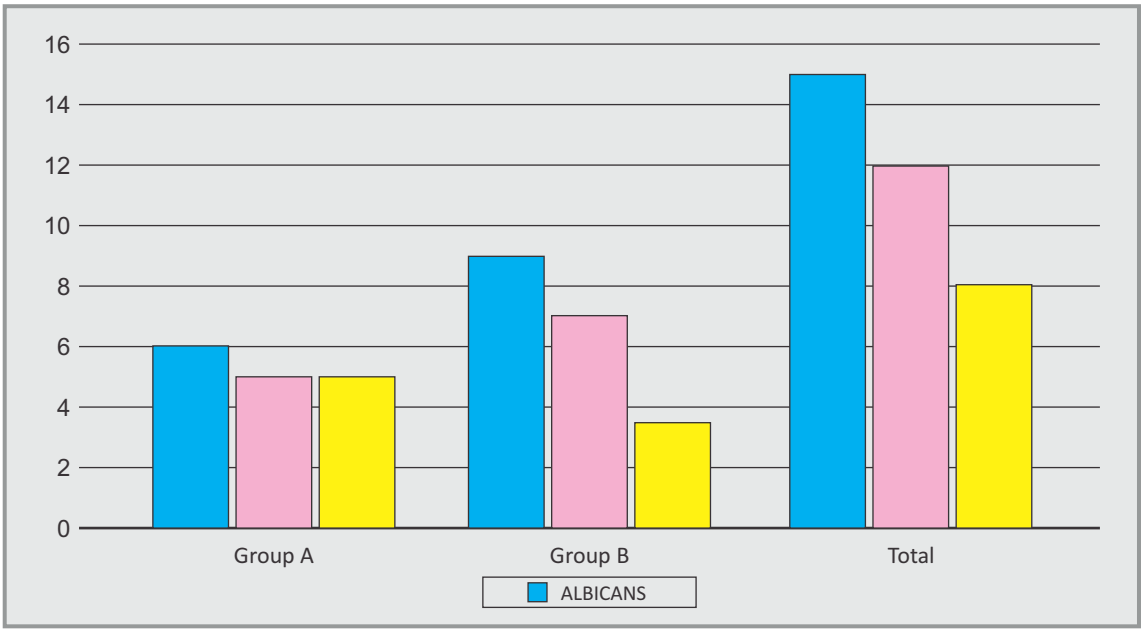

Figure 1
Presence of Candida (consolidated non-albicans) by groups

	5 - 10	10 - 15	More than 15	Total
Group A	0	0	5	5
Group B	1	2	0	3
Total	1	2	5	8

Table 3
Frequency of Candida dubliniensis according to the length of drug intake

	BACTERIA		YEAST		METAPLASIA		DYSKERATOSIS		TOTAL
	No.	%	No.	%	No.	%	No.	%	No.
Group A	15	65,22	2	8,70	6	26,09	0	0,00	123
Group B	13	76,47	2	11,76	2	11,76	0	0,00	17
Total	28	70,00	4	10,00	8	20,00	0	0,00	40

Table 4
Frequency of cytological findings

da albicans is the most frequent one in total, present in 42.86 % of the patients. In the group A, it amounts to 37.5 %, and in group B to B 47.5%.

In group B, there were 3 patients (15.8 %) that had Candida dubliniensis, whereas in the case of group A, there were 5 such patients (31.25 %) (Figure 1).

Candida dubliniensis was found in 8 patients, i.e. in case of 13.33 % of all patients. In case of group A, there were 5 patients (62.50%), whereas in group B, there were 9 of them (37.50%). In group A, all patients that had Candida dubliniensis had been drinking for more than 15 years, whereas in group B, the occurrence of Candida dubliniensis happened in the first two observed time intervals. The presence of Candida dubliniensis has been proven in case of psychoactive substance addicts (23%) and it has been confirmed that the frequency of adherence of Candida dubliniensis is in proportion to the time interval of drug addiction (Table 3).

Results of the exfoliative cytological analysis

Table 4 contains the frequency (percentage) of patients at whom bacteria, yeast and metaplasia were identified, both by examination groups and in total (Table 4).

The chi-square test (*p=0, 55297*) has confirmed that there are no significant differences in frequencies of cytological findings by groups. Bacteria are the largest group in general, namely in 70.00% of the patients. In group A, 6 patients (26.09%) had metaplasia, whereas in group B there were 2 such patients (11.76%) (Figure 2).

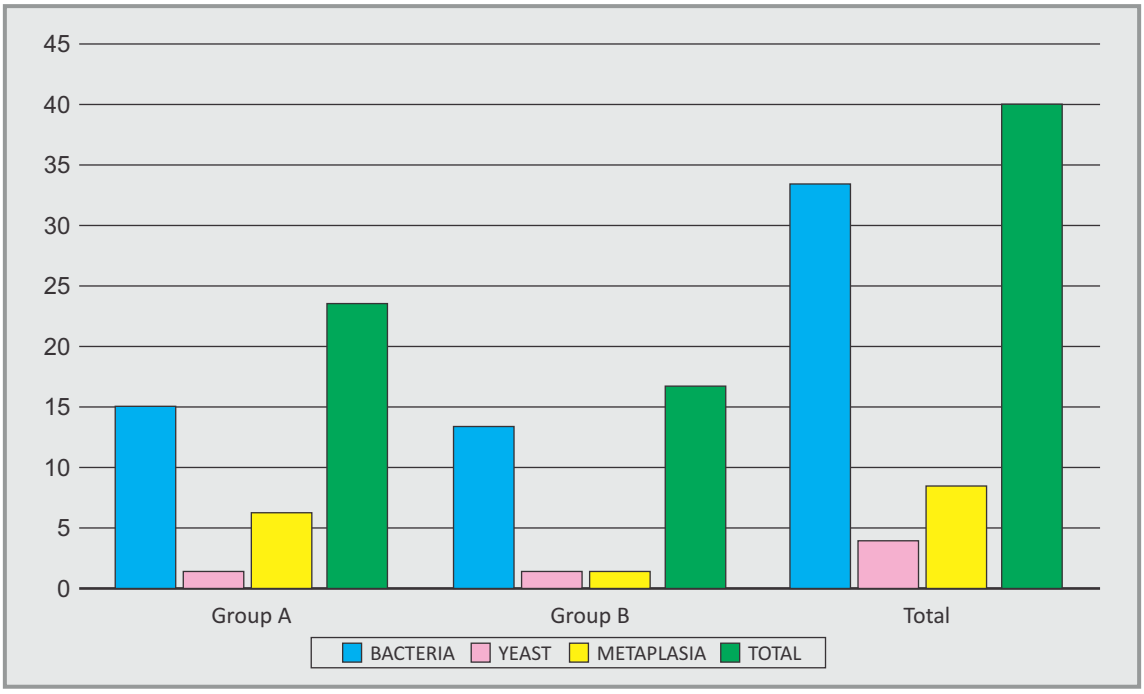

Figure 2
Presence of cytological findings by groupss

Discussion

The research over the past years has been related to Candida species, especially to the escalation of yeast infections in immune-suppressed patients, AIDS patients and patients using an immunosuppressive therapy. The Candida species frequently occurs in the oral cavity. The Candida species is an opportunistic type and will lead to an infection if there are factors of predisposition. The virulence of Candida depends on the ability to adhere to the mucous membrane of the host, production of filamentous forms of growth and release of hydrolytical enzymes. A continuous increase in yeast infection incidences, new yeast species and resistance to antifungal therapy are a challenge for research in that segment [17, 18].

Baradak (2009**)** examined in his work the clinical and microbiological state of oral mucous membranes in intravenous addicts. 60 patients participated in the study. Candida species were isolated in 50 patients. The most frequent type was Candida albicans with 70%, Candida parapsilosis 15%, Candida glabrata 7.5%, Candida tropicalis 5% and Candida dubliniensis in the case of one patient. This study has shown a prevalence of oral candidiasis in intravenous addicts [19]. The most frequent type is Candida albicans, but the non-albicans types are also on the increase, 30% [20]. Immunosuppressive conditions and anti-virus therapy are significantly related to an increase in candidiasis (Cerqueira 2010) [21]. In case of our research on a sample including 60 patients that are alcohol and drug addicts, we identified Candida albicans in 43% of patients. In group A, there were 38%, and in group B 48% respectively. With regards to the non-albicans types, the most frequent type was Candida crusei, namely in case of group B, amounting to 26%, and in group A to 6%. Candida glabrata was the most frequent type in group A, amounting to 10%, and in group B it amounted to 5.2%. Candida tropicalis was also identified being equally present in both group A and B group. The chi-square test (p=0, 55297) has confirmed that there is no statistically important difference in the occurrence of Candida by groups. The most frequent type is Candida albicans. Our results are in compliance with the results of the mentioned authors. New studies show that the occurrence of non-albicans types and Candida dubliniensis is increasing (Gery 2011).

Candida dubliniensis is a yeast species which increase was connected to the HIV infection for a long time, but its presence has been proven in case of other diseases, too [22].

Polacheck et al. (2000**)** have isolated Candida dubliniensis in case of inpatients in Jerusalem. None of the patients was HIV positive, but they all were receiving a broad spectrum of antibiotics. The high occurrence of Candida in case of psychoactive substance addicts is a result of the activity of alpha-adrenergic receptors in the blood vessels of the salivary glands that lead to vasoconstriction thus reducing the saliva production. [23, 24]. Suhail 2004 has confirmed the incidence of Candida dubliniensis in case of immune-suppressed patients. Out of 7 isolated Candida dubliniensis, 5 of them were isolated from saliva, one from a vaginal smear and one from urine. Five patients had a tumor, one patient had systematic lupus erythematodes, and one had diabetes mellitus [25]. Our research confirmed the presence of Candida dubliniensis in patients that are addicted to psychoactive substances and are not HIV positive, which is in compliance with the research of the mentioned authors. In group A (alcoholics), there were 31.25% Candida dubliniensis (in 5 patients) and 16% in group B (in case of 3 patients), which confirms that alcohol addiction with all immunological misbalances, including the local status of oral health, are an ideal medium for the occurrence of Candida dubliniensis.

According to results of both mentioned authors and our results, it is evident that Candida dubliniensis is increasing and becoming a pathogen that has the potential to colonize and cause superficial, but invasive diseases in HIV negative patients.

The impact of psychoactive substances on the occurrence of a malignant alteration has been the subject of numerous researches. Harris (2004) used a sample of 388 alcoholics and 305 alcoholics that were also drug addicts, being 40 years old on average, during a period 1994-1999, and identified frictional keratosis in 8.8% of patients, changes on the lips in 4.8% of patients, candidiasis in 38% of patients, angular cheilitis in 3% of patients, three white lesions, out of which one turned out to be a carcinoma identified during a cytological analysis. The author confirmed that the use of alcohol and drugs does constitute a risk factor for oral mucous lesions. (26) The type of drug and the length of addiction may have an impact to the occurrence of lesions and malignant changes. Rober (2008) used a sample of 100 patients who were addicts using different types of drugs to exami-

ne oral health. The patients mostly used heroin, amphetamines and marijuana. The lesions have been diagnosed clinically and processed by means of a microbiological analysis, and a statistical analysis confirmed the importance of oral mucous lesions with malign alteration and length of drug use. Laks-man (2009) also confirmed that the length of addiction and type of drugs influence the occurrence of Candida dubliniensis and precancerous conditions [27, 28].

Metaplasia is a reversible replacement of one type of mature differentiated cells in organ tissue by another type of mature cells occurring due to the abnormal position or pathological stimulus. The importance of metaplasia lies in the fact that dysplasia may occur more frequently in some areas of metaplasia (due to chronic stimulus).

Our results have shown the presence of metaplasia in 8 patients. In group A (alcoholics), 6 patients (26%) had metaplasia, and in group B (drug addicts) 2 patients (12%).

Canković (2010) confirmed the prevalence of Candida in patients with oral carcinoma. In case of 30 patients with oral carcinoma, 17% of non-albicans types were identified, 13% of Candida albicans. Statistical data have confirmed a significant degree of interrelatedness between candidiasis and age, smoking, use of drugs, alcohol and precancerous conditions [29]. The prevalence of oral mucous lesions at the Center for Addiction Rehabilitation is described by O'Sullivan (2011). The prevalence of abnormality amounted to 29% in a sample of 210 patients. The most frequent types were candidiasis 38%, aphtae 3%, lymphadenopathia 2.9%, 4 erythroplakia, 2 leukoplakia and 2 malignant lesions. The author points out the need of the obligatory cytological analysis or biopsy for the purpose of early detection of precancerous conditions [30]. Our findings have not shown the presence of dyskeratosis and carcinoma, but the presence of metaplasia has warned us that alcohol and drug addicts constitute the riskiest group of patients for the occurrence of carcinoma, and that a screening of oral tumors should be a part of the obligatory medical and dental examinations.

Conclusion

The use of psychoactive substances impacts the frequency of occurrence of Candida species (Candida albicans and non-albicans types). The presence of Candida dubliniensis in psychoactive substance addicts, irrespectively of the type of addiction, has been proven and it has been confirmed that the frequency of adherence of Candida dubliniensis is in proportion with the time interval of drug addiction. An early detection of Candida dubliniensis in patients resistant to therapy indicates a misbalance or suppression of the immune status.

In case of psychoactive substances use, the method of exfoliative cytology has shown the presence of metaplasia. Alcohol and drug addicts constitute groups of risk for the occurrence of malignant transformation of oral mucous membranes.

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PERIODONTAL STATUS IN THE PERMANENT DENTITION OF CHILDREN WITH CEREBRAL PALSY

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ABSTRACT

Introduction: Cerebral palsy is a group of permanent disorders of movements and posture resulting in physical disability caused by non-progressive disorder occurred in the developing fetal or infant brain. Children with cerebral palsy have the difficulties in maintaining oral hygiene. Periodontal diseases are common in the cerebral-palsied individuals as the result of poor hygiene, physical abilities and presence of malocclusions.

The aim: The aim of this study is to assess the periodontal status in the permanent dentition of children with cerebral palsy.

Material and Methods: A total of 44 patients aged 12 to 18 years with permanent dentition were included in this study and distributed in experimental (children with cerebral palsy, n=18), and control group (healthy individuals, n=26). The examinees were examined by a single examiner according to the WHO recommendations (Oral Health Surveys: Basic Methods). The periodontal status was assessed by using the following indexes: Plaque Index (Silness and Löe, 1964.), Gingival Index (Silness and Löe, 1964.) and Calculus Index.

Results: The mean value of Plaque Index ± SD is 4, 5 ± 3, 20 (experimental group) and 6,5 ± 3,35 (control group) (2=6,762; p=0,0799). The mean value of Gingival Index ± SD is 4, 5 ± 2 29 (experimental group) and 6, 5 ± 4, 92 (control group) (2=7,204; p=0, 0657). The mean value of Calculcus Index ± SD is 4, 5 ± 7, 37 (experimental group) (2=4,682; p=0, 1966).

Conclusion: The periodontal status (Plaque Index, Gingival Index, Calculus Index) of the children with cerebral palsy is poorer, compared with healthy individuals.

Key-words: periodontal status, children, cerebral palsy, permanent dentition.

Introduction

Cerebral palsy is a group of permanent disorders of movements and posture resulting in physical disability caused by non-progressive disorder occurred in developing fetal or infant brain. The motor dysfunction is often associated with sensory, perceptive and cognitive impairment, communication difficulties, behavioral disorders, epilepsy and secondary musculoskeletal problems [1]. Cerebral palsy is the most common cause for severe neuro-motor disturbance in children, and thus represents the significant burden for the affected child, its family, health-care and educational system and the society in general [2]. According to the American Academy of Neurology, cerebral palsy has a highest treatment cost per person [3]. The incidence of cerebral palsy is 2-2.5 per 1000 live births, making this disease the most common cause of physical disability in childhood [4].

Cerebral palsy does not cause the specific oral manifestations. However, certain conditions are more frequent or more prominent in patients with cerebral palsy, compared with general population. Characteristics of oral pathology of patients with cerebral palsy are: poor oral hygiene, pronounced periodontal diseases, more unrestored carious teeth, more extracted teeth, less dental restorations and more prominent dentofacial abnormalities compared with healthy children of the same age [5]. Children with cerebral palsy have difficulties with the maintenance of oral hygiene. Periodontal diseases are common in the cerebral-palsied individuals as the result of poor hygiene, physical abilities and presence of malocclusions. Gingival hyperplasia resulting from medication therapy contributes to the occurrence of periodontal diseases.

Numerous studies suggest that oral health is influenced by socio-economic status and cultural values in society. In physically disabled individuals the oral health care is complex. Neglecting this segment of general health may have more serious consequences in relation to healthy individuals.

The problem of oral health of children with physically and mentally disabilities in recent years is becoming more actual. Besides health reasons for oral health care, the certain formal reasons are gaining importance. In fact, in USA and many European countries, neglecting of oral health by a caregiver is considered as dental neglect, which is a form of child abuse. This kind of child abuse is punishable and can lead to the loss of child's custody.

As stated so far, the maintenance of oral health is in particularly important in children with physical and mental disabilities. This is difficult due to poor manual coordination, intellectual disability, and less importance given to oral health compared to major health problems. The timely diagnosis, dental restorations and oral rehabilitation is very important for their specific growth and development [6].

The oral health of children in Bosnia and Herzegovina is less favorable compared to economically developed countries with health care system characterized by more preventive programs and better health care for disabled children. The developed counties pay more attention to every aspect of social life for children with disabilities (their rehabilitation, education, professional training, employment and integration in society in general). The special attention is also given to medical and dental health care, respecting the saying that the disabled children also have right to smile. In future, efforts should be undertaken to improve dental care of this population: the regular check-up appointments every 3 or 6 months and the formation of specialized dental teams that will exclusively treat this vulnerable population.

Aim

The aim of this study is to assess the periodontal status in the permanent dentition of children with cerebral palsy.

Material and Methods

A total of 44 patients aged 12 to 18 years with permanent dentition were included in this study. The study sample was distributed in two clinical groups: the experimental that consisted of children with cerebral palsy (n=18), and the control group with healthy individuals (n=26). The healthy patients seeking dental care in one of the Dental Health Care

Centers in Canton of Sarajevo were randomly assigned to control group.

The examinees were examined by a single examiner, using periodontal probe and plain mouth mirror with artificial source of light at dental ward or the natural source of light in patients who were unable to be examined at dental ward. The patients were positioned towards source of natural light, avoiding direct sunlight [7].

The periodontal status is examined using periodontal probe with 0, 05 mm diameter sphere at probe tip. A controlled light pressure of 25 grams was applied during examination, which refers to pressure of WHO probe under the nail of thumb until white circle appearance. The pain during probing suggests the applied pressure is too high. It is important to keep the periodontal probe parallel to the contours of tooth. For examination of sub gingival calculus the least possible pressure should be applied, which enables the movement of probe tip along the surface of the tooth. When the probe is applied on tooth, the anatomical configuration of tooth must be followed [8].

The periodontal status was assessed using the following indexes: Plaque Index (Silness and Löe, 1964.), Gingival Index (Silness and Löe, 1964.) and Calculus Index.

Plaque Index (Silness and Löe, 1964.)

The measurement of the oral hygiene status is based on the Plaque Index, which records both soft debris and the mineralized deposits on the teeth. Cervical region of tooth is dried with three-way air dental syringe. Inspection is performed visually and by periodontal probe. The following score and criteria were applied:

0 - No plaque; plaque is not visible, and by using the probe, there is no presence on the probe tip

1 - Plaque is not visible; the probe use on tooth reveals the plaque on the probe tip

2 - Plaque is visible by naked eye

3 - Abundance of plaque by naked eye is visible on tooth and gingival margin [9].

Gingival Index (Silness and Löe, 1964.)

Gingival index assesses the condition of gingival tissue. It records and quantifies the gingival inflammation through the changesin color, bleeding intensity and the edema. The following score and criteria were applied:

0 - Normal gingiva without inflammation, there is no change in color and no edema

1 - Mild inflammation, slight change in color and slight edema but no bleeding on probing

2 - Moderate inflammation, redness, edema and bleeding on probing

3 - Severe inflammation, marked redness and edema or hypertrophy, tendency to spontaneous bleeding, ulcerations

Calculus Index

Calculus index refers to the amount of calculus on teeth and is used for assessment of oral hygiene. The following score and criteria were applied:

0 - No calculus present

1 - Presence of supra-gingival calculus

2 - Presence of sub-gingival calculus and small amount of supra-gingival calculus

3 - Presence of sub-gingival calculus and lot of supra-gingival calculus.

Results

The control group presented with Plaque Index score 0 in 42,31% of cases, score 1 in 30,77%, score 2 in 19,12% and score 3 in 7,69% of cases. The experimental group had the score 0 in 11,11%, score 1 in 33,33 %, score 2 in 50% and score 3 in 5,56% of cases. The values of Plaque Index are lower in control group compared with the experimental one. Results

Plaque Index	Control group (healthy children)		Experimental group (children with cerebral palsy)	
	Frequency	Percentage	Frequency	Percentage
0	11	42,31	2	11,11
1	8	30,771	6	33,33
2	5	9,23	9	50,00
3	2	7,69	1	5,56
Total	26	100,00	18	100,00

Table 1 Plaque Index (Silness and Löe, 1964.) of children with cerebral palsy and healthy children: 0 - No plaque; 1 - The using of probe on tooth reveals the plaque on tip of the probe, 3 - Plaque is visible by eye

Gingival Index	Control group (healthy children)		Experimental group (children with cerebral palsy)	
	Frequency	Percentage	Frequency	Percentage
0	13	50,00	3	16,67
1	9	34,62	8	44,44
2	4	15,38	5	27,78
3	0	0,00	2	11,11
Total	26	100,00	18	100,00

Table 3 Gingival Index (Silness and Löe, 1964.) of children with cerebral palsy and healthy children: 0 - Normal gingiva without inflammation, 1 - Mild inflammation, 2 - Moderate inflammation, 3 - Severe inflammation

Chi-square	6,762
DF	3
Significance level	P = 0,0799
Contingency coefficient	0,365

Table 2
Chi-square test for Plaque Index

Chi-square	7,204
DF	3
Significance level	P = 0,0657
Contingency coefficient	0,375

Table 4
Chi-square Test for Gingival Index

of Plaque Index are shown in **Table 1**. χ^2 plaque index test showed there is no significant difference in results in experimental and control group: p=0,0799, p>0,05 (**Table 2**).

In the control group the Gingival Index score 0 was presented in 50, 00% of cases, score 1 in 34, 62%, score 2 in 15, 38% cases and score 3 was not recorded. The experimental group had the score 0 in 16, 67%, score 1 in 44, 44%, score 2 in 27, 58% and score 3 in 11, 11% of cases. It is obvious that the score 0 was the most frequent in control, and score 1 in experimental group. **Tables 3** and **4** show the values of Gingival Index and χ^2- test for Gingival Index meaning that there is no significant differences between experimental and control group :p =0,0657, p>0,05.

Tables 5 and **6** show the values of Calculus Index. The score 0 is the most frequent in both experimental and control group. χ^2-test for Calculus Index showed that there is no significant differences between experimental and control group :p =0,1966, p>0,05.

Discussion

Lack of data considering the oral hygiene and the periodontal status of children with cerebral palsy is an important issue. Accessing these groups of children is great challenge, and additional difficulty is presented during dental examination due to neuromuscular in-coordination.

The mean value of Plaque Index in children with cerebral palsy is 4, 5 with standard deviation 3, 20. In healthy individuals the mean value in this index is 6, 5

Calculus Index	Control group (healthy children)		Experimental group (children with cerebral palsy)	
	Frequency	Percentage	Frequency	Percentage
0	19	73,08	8	44,44
1	3	11,54	5	27,78
2	4	15,38	4	22,22
3	0	0,00	1	5,56
Total	26	100,00	18	100,00

Table 5 Calculus Index of children with cerebral palsy and healthy children: 0 - No calculus present, 1 - Presence of supragingival calculus, 2 - Presence of subgingival calculus and a little amount of supragingival calculus, 3 - Presence of subgingival calculus and a lot of supragingival calculus.

Chi-square	4,682
DF	3
Significance level	P = 0,1966
Contingency coefficient	0,310

Table 6
Chi-square Test for Calculus Index

with standard deviation 3, 35. ^{2} Test for Plaque Index was 6,762 (significance level p=0, 0799; contingency coefficient 0,365).

Ivančić and Majstorović reported the 3, 8-4,53 values of OHI-S index in disabled children and 2,73-2,84 in healthy children in Croatia [10]. Rodrigues dos Santos et al (2003) in their study showed the significantly higher DMFI (Decayed-Missing-Filled teeth Index) and Plaque Index of children with permanent dentition with cerebral palsy in both genders, compared with healthy individuals [11].

The values of Gingival Index are lower in healthy children, compared with children with cerebral palsy. These results also support the findings that children with cerebral palsy have the poorer oral hygiene. The mean value of Gingival Index for children with cerebral palsy is 4, 5 with standard deviation 2, 29. The index for healthy children is 6, 5 with standard deviation 4, 92. The ^{2} Test for Gingival Index in permanent dentition is 7,204 (significance level p=0, 0657, contingency coefficient 0,375).

The study conducted in Riyadhevaluated the Plaque Index and Gingival Index in children with primary, mixed and permanent dentition. The higher values of Plaque Index were recorded in primary dentition and the higher values of Gingival Index in children with permanent dentition (level of significance p=0,001). The gender did not significantly alter the periodontal indexes in children [12]. These results are in accordance with numerous results of similar studies, as well as the results of our study.

The Calculus index in control group is 0 in 73, 08%, 1 in 11, 54% and 2 in 15, 38% of cases. Score 3 has not been recorded. This index in control group was present in 44, 44% of cases for score 0, 27, 78% of cases for score 1, 22, 22% of cases for score 2 and 5, 56% of cases for score 3. The mean value of Calculus Index for children with cerebral palsy is 4, 5 with standard deviation 7, 37. The ^{2} Test for Calculus Index is 4,682 (significance level p=0, 1966, contingency coefficient 0,310).

Stevanović and Jovičić evaluated the Calculus Index of children in age group 15-18 years [13]. The children with cerebral palsy had statistically more dental calculus, compared with the healthy ones (p<0,001). Statistically significant periodontal treatment needs (CPITN=2 and CPITN=3) are recorded in the patients with cerebral palsy (82, 4%) compared with healthy children (42, 6%; p<0,001). The mean of CPITN index in children with cerebral palsy is 1,752 compared with 0,308 for healthy children (p<0,001). The percentage of children with calculus in this age group was very low and amounted for 2, 86%. The mean value of Calculus Index for all subjects in this study was 0, 6508 ± 0, 4661 [13]. The results of our study correlate with previous studies. We can conclude that the differences in level of oral hygiene and periodontal status in children with cerebral palsy are getting more prominent with years. This study has focused on oral hygiene maintenance and its impact on oral health in children.

Santos et al examined the oral health condition, salivary and the microbiological parameters associated with caries in 62 children with cerebral palsy

coming from households with low socio-economic status in two dentitions: mixed (6-11 years) and permanent (11-16 years). The dental examinations were performed to measure dental caries, Plaque Index, salivary levels of Streptococcus mutants and Lactobacillus, salivary flow rate, pH of stimulated saliva, and buffer capacity of saliva. The control group comprised of 67 subjects with similar socio-economic status. The data were statistically analyzed. The results of this study showed that children with cerebral palsy had more carious teeth and the higher scores of Plaque Indexes for both genders. Microbiological examination revealed the higher level of Streptococcus mutants among children with cerebral palsy, compared with healthy children. The higher level of Lactobacilli is also recorded regardless to gender or age. The lower mean values for salivary flow rate, pH and buffer capacity of saliva were obtained in experimental group [14].

Ivančić Jokić et al in Croatia obtained value of OHI-s index 3, 8-4, 53 for disabled children and 2,73 for healthy children [15].

Martinović et al revealed that Calculus Index is significantly lower among children aged 12 and 15 in rural than in urban area, and it accounts up to 40% (0, 26 in rural compared with 0, 44 in urban) [16].

Szoke and Pedersen presented the results of their study in which the gingival bleeding score 1 was presented in 12% of 12-year old children [17]. Sulejmanagić et al (2000) examined the periodontal status of 12-year old children in Bosnia and Herzegovina: 54 % of healthy sextants, 23,87% with gingival bleeding and 17,95% of sextants with calculus [18]. Muratbegović et al (2008) in their research conducted in Bosnia and Herzegovina revealed that 43% of subjects had healthy periodontal tissues, 43% bleeding on probing and 12% with calculus [19]. The examinees from Sarajevo had healthy periodontal tissues in 54, 83%, 27,66% with gingival bleeding and calculus in 6% of subjects [20].

Conclusion

The present study observed the periodontal indexes (Plaque Index, Gingival Index, Calculus Index) of children with cerebral palsy. The results showed the oral health and the periodontal indexes are poorer in these children, compared with healthy individuals. This may be related to their medical status, poor manual coordination and intellectual disability. The present study highlighted the importance of oral hygiene maintenance in children with cerebral palsy. Oral health programs should be created and concentrated towards these children concerning their specific needs.

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THE CLINICAL APPLICATION OF PLATELET-RICH FIBRIN (PRF) IN ORAL SURGERY: REVIEW

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ABSTRACT

Current trends in oral surgery are directed towards enhancing healing process in surgical wound through manipulating the wound microenvironment. PRF is a complex regenerative scaffold that consists of fibrin matrix network with incorporation of platelets, leucocytes, cytokines, glycosaminoglycans and circulating stem cells. PRF can be considered as sn immune concentrate with multitude growth factors that are slowly released from platelets among which the most important are TGFβ-1, PDGF and IGF. At Department of Oral surgery at Faculty of Dentistry Sarajevo, PRF is used in treatment of peri-radicular defects after apicoectomy and in osseous defects after enucleating of cysts and the removal of impacted teeth. It is been used as filling material of surgical cavities with or without bone grafts. The PRF proved as simple, inexpensive and good substitute used as complement for bone grafts. The beneficial effects of therapeutic potential of clinical use of PRF at our Department are satisfactory. PRF accelerates wound closure, synchronizes healing of soft and hard tissue and acts as fibrin bandage supplemented with growth factors. PRF should be considered as surgical adjuvant that provides good mechanical support and acts as stabilizing sheath in large osseous defects after cyst enucleating. In cases of wound dehiscence due to insufficiency of soft tissues, layers of PRF provide protection for underlying tissues and enables epitelization through accelerating migration of epithelial cells. Preliminary observations of clinical effects of PRF at our Department are promising and satisfactory and could open a new era of regenerative therapy in oral surgery.

Key-words: Platelet rich fibrin (PRF), oral-surgery.

Introduction

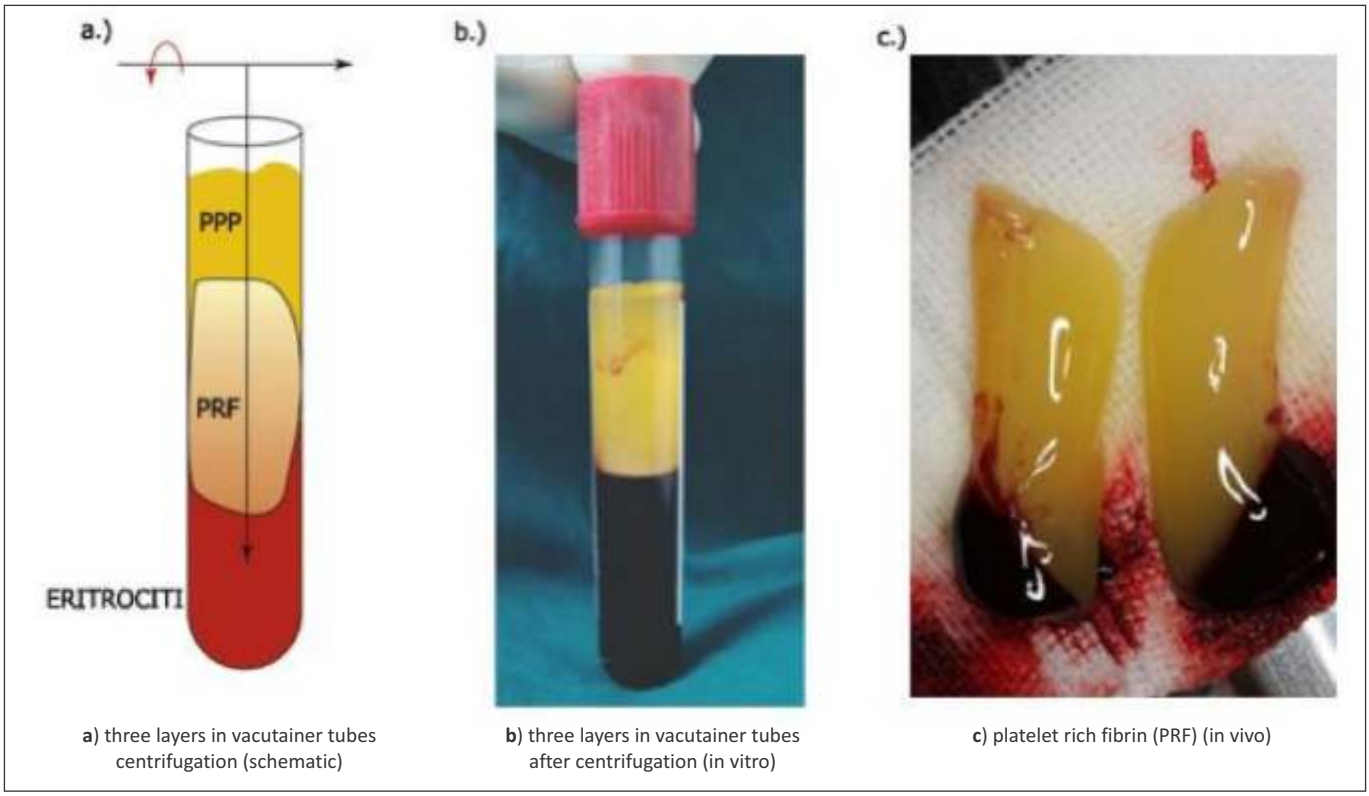
Current trends in oral surgery are directed towards enhancing healing process in surgical wound through manipulating the wound microenvironment. Regulation of inflammation and the improving of the healing capacities of oral tissues is one of the greatest challenges of clinical research. The process of healing and its regulating and mediating factors are not fully understood and many clinical trials are performed in order to reveal optimal environment for this process. The clinical application of different bioactive surgical additives that manipulate surgical wound environment is the important step in developing new therapeutic approaches in order to enhance the wound healing [1].

Platelet-rich fibrin (PRF) was introduced in 200, in France, by Choukroun as the second generation of platelet concentrate, four years after originally developed first generation - platelet-rich plasma (PRP) was presented by Whitmen. PRF was presented as an improvement of previously used PRP. Marx et al were the first to evaluate the effects of PRP (fibrin glue enriched with cytokines) in clinical conditions. They used the PRP in mandible reconstruction along with cancerous bone grafts and their findings suggested that PRP addition accelerated both degree and rate of bone formation [2]. Some of disadvantages of PRP are associated with its preparation: PRP requires quick and careful handling with use of anticoagulants and addition of bovine thrombin. However, the main disadvantage is in its massive and uncontrollable effect. Studies have shown that it releases growth factors quickly, just before the outgrowth of surrounding tissue [3].

Developed second generation PRF is characterized by simplified handling with no biochemical processing. PRF (platelet-rich fibrin) is considered as healing biomaterial with high regenerative potentiality to soft and hard oral tissues. PRF is a completely autonomous, biocompatible regenerative inductive scaffold. PRF consists of concentrated platelets trapped within fibrin meshes, which release the growth factors in a certain period of time (during seven days). High concentration of platelets is responsible for its clinical action, since platelets are autonomous source of growth factors which are responsible for immunity and healing. The PRF clot contains up to 95% of platelets, unlike natural human blood clot where they account for 5% [4].

The protocol for PRF preparation is very simple. It includes collection of whole venous blood (around 5 ml) in sterile vacutainer tubes (6 ml) without anticoagulant. The vacutainer tubes are then placed in a centrifugal machine at 3,000 revolutions per minute (rpm) for 10 min (**Figure 1**), after which it settles into the following three layers: upper straw-colored acellular plasma, red-colored lower fraction containing red blood cells, and the middle fraction containing the fibrin clot. The upper straw-colored layer is then removed and middle fraction is collected, 2 mm below to the lower dividing line, which is the PRF. The mechanism involved in this is the fibrinogen concentrated in the upper part of the tube, combined with circulating thrombin due to centrifugation to form

Figure 1
Centrifugal machine for the Platelet Rich Fibrin (PRF) at Department of Oral Surgery, Faculty of Dentistry University of Sarajevo



a) three layers in vacutainer tubes centrifugation (schematic)

b) three layers in vacutainer tubes after centrifugation (in vitro)

c) platelet rich fibrin (PRF) (in vivo)

Figure 2
The blood in the vacutainer tubes after centrifugation divided in three layers: upper straw-colored acellular plasma, red-colored lower fraction containing red blood cells, and the middle fraction containing the fibrin clot

fibrin. A fibrin clot is then formed in the middle between the red corpuscles at bottom and acellular plasma at the top. The middle part is platelets trapped massively in fibrin meshes (**Figure 2**) [5].

The PRF can be considered as reabsorbing membrane in which growth factors are released after the activation of the platelets. Fibrin mesh is a supportive matrix that releases the growth factors which have regulatory role in a tissue regeneration process. Once when they are released during wound healing, they repair the bone through stimulating the mitogenic response in the bone periosteum. The mitogenic activity of periostaeum is responsible for bone repair during wound healing [6].

PRF is a complex regenerative scaffold that consists of a homogenous 3-dimenzional organization of fibrin matrix polymerized in a tetra-molecular structure, with incorporation of platelets, leucocytes, cytokines, glycosaminoglycans and circulating stem cells [7, 8]. Fibrin is activated form of fibrinogen present in blood plasma and α-granules of platelets. Soluble fibrinogen is transformed in insoluble polymerized fibrin matrix during hemostasis [9]. The fibrin matrix present in PRF is flexible, elastic, stable and very strong [10].

Since the blood clot is the essential and fundamental for healing of oral wounds, PRF can be considered as the optimized blood clot with all significant constituents important for healing and regeneration. The biologic activity of the fibrin molecule highlights its significant cicatricle capacity since the matrix that constitutes the incorporated elements is the responsible for the real therapeutic potential of PRF. Fibrin network is slowly polymerized which makes it more coherent and better organized than natural fibrin clots. Natural and progressive polymerization enables the intimate contact of intrinsic bioactive components of PRF. Thus, PRF creates synergetic effect of its elements which efficiently directs the cells in healing process. For example, fibronectin potentates the stimulation effects from PDGF. It is considered that fibrin matrix is responsible for therapeutic action of PRF, since it is supporting element that determines its effects [11].

Fibrin matrix, as final substrate of all coagulation reactions, plays a crucial role in hemostasis since it forms cicatricial matrix at injured site. But fibrin matrix has not only healing properties, it provides supporting scaffold for structures enrolled in angiogenesis. The changes in fibrin structure markedly

affect the micro-vascular formation and in-growth thus influencing the process of angiogenesis. Fibrin acts as natural guide to angiogenesis [12].

PRF can be considered as an immune concentrate with multitude growth factors. Activated platelets release significant quantities of cytokines extrinsically retained in the fibrin architecture. Fibrin clot acts as reservoir of growth factors. The fundamental processes needed for repair of injured tissue by platelets, are their activation and de-granulation, through which they release the granule which content is important for initial phase in hemostatis and healing process.

The platelet cytokines include various cytokines among which the most important are TGFβ-1, PDGF and IGF (**Table 1**) [13]. TGFβ-1 (fibrosis agent) by stimulating osteoblasts induces massive synthesis of collagens and fibronectin. PDGF as the regulator of mesenchymal lineages migration and proliferation play essential role in physiologic cicatrisation. IGF is a cell multiplication mediator and protects cells from various apoptotic stimuli [11].

Glycosaminoglicans (heparin and hyaluronic acid) are enmeshed in fibrin polymer matrix, whose glycanic links show very strong affinity to circulating cytokines thus incorporating them in scaffold and supporting cell migrations [11]. The PRF acts as supportive matrix that enables slow release of growth factors, migration of stem cells as well as the bone morphogenesis protein in fibrin scaffold [5, 11]. Presence of natural fibrin network protects the growth factors from proteolysis [14]. This persistent release of growth factors (PDGF, TGF-β and IGF-1) improves the process of healing thought self-regulation of inflammatory process. Besides growth factors, angiogenesis is crucial for wound healing. In addition, PRF also promotes neo-angiogenesis and vascularization thus demonstrating its effect in multiple phases of wound healing. These effects are synergistic on healing process [15].

PRF induces proliferation of various cells in vitro, and shows the strongest effect on osteoblasts which are significant for regeneration of osseal defects. Because of this, PRF can be used as viable medium for

Factor	Action
Interlukin-1 (IL-1)	• key mediator of inflammation control • stimulates T-helper lymphocytes
Interlukin-6 (IL-6)	• differentiation factor for B-lymphocytes • activator for T-lymphocytes • stimulates the secretion of antibodies • supports the chain reaction leading to inflammation, destruction and remodeling
Tumor necrosis factor alpha (TNF alpha)	• activates monocytes • stimulates the remodeling capacities of fibroblasts • increases phagocytosis and neurophil cytotoxicity • modulates the expression of IL-1 and IL-6
Interlukin-4 (IL-4)	• supports proliferation and differentiation of activated B cells • supports healing by moderating inflammation • increases fibrillary collagen synthesis by fibroblast
Cytokine vascular endotheilial growth factor (VEGF)	• functions to start angiogenesis
Transforminf growth factor β1 (TGF β1)	• can induce a massive synthesis of collagen and fibronectin
Plateled derived growth factor (PDGF)	• regulates migration, proliferation and survival of mesenchymal cell lineages • plays an essential role in physiologic cicatrisation and pathogenesis of atherosclerosis and other fibroproliferative diseases
Insulin like growth factors (IGFs) 1 and 2	• cell multiplication mediator in apoptosis • exerts chemotactic effects towards human osteoblasts

Table 1
Growth factors in PRF [13]

cultivation of periostal cells [6]. Studies show that PRF enhances alveolar bone formation through several mechanisms: increasing RUNX2 expression, osteoblast differentiation, stimulating the production of osteoprotegerin and matrix mineralization and also by the stimulating alkaline phosphatase activity [16-19]. In vitro study of Li suggests that PRF can be used as successful bone augmentation material in addition to soft tissue healing on implant site [18]. PRF also promotes surrounding periodontal soft tissue regeneration through increased collagen synthesis in periodontal cells and osteoblasts, as well as proliferation and migration of periodontal progenitor cells [20]. Through regulating the interactions in healing tissue it stimulates the healing and acts protective on residual bone structure. Because the fibrin matrix is better organized with strong architecture, it can more efficiently direct stem cell migration and the healing process. Release of growth factors from PRF through in vitro studies and good results from in vivo studies leads to the optimization of the clinical application of PRF.

PRF enhances wound healing and sealing, bone maturation and regeneration and hemostasis. It can be used in order to improve graft stabilization through its mechanical properties since it acts as biological connector between bone graft particles. Its gelatinous consistency favors clot stability and creates natural membrane in surgical area. It is easy to handle with and to be placed in surgical wound. Cavities filled with PRF post oral and maxillofacial surgical procedures showed faster healing taking half time in comparison to physiologic healing [3, 21]. Studies also suggest that combination of bone grafts and PRF enhance bone density [22]. The combination of PRF with bone grafts (beta-tricalcium phosphate) provides faster healing than using grafts alone. The significant and predictable bone regeneration ater enucleating of periapical cysts is observed in these clinical cases, which was radiographic demonstrated [23, 25]. PRF acts as membrane at surgical site which promotes coherent healing and stimulate the more rapid healing. The release of growth factors and directing stem cell migration is responsible for healing properties of PRF, which has been shown in various clinical in vivo studies. In addition, PRF prevents early invagination of undesired cells thus acting like viable barrier between desired and undesired cells [13].

In oral surgery PRF can be used in:

1. wound sealing thought its effects on soft tissue healing [22]
2. treating the residual extraction sockets in order to achieve preservation of hard tissue and obtain bone volume (regeneration of bone tissue architecture) where it stimulates the new bone formation [25, 26]
3. prevention of localized osteitis after removal of mandible third molar [27]
4. enhancing palatal wound healing after free graft [28]
5. treating mandible furcating defects in patients with chronic periodontitis [29]
6. treatment of simple and multiple gingival recessions, where PRF supports gingival tissues in qualitative and quantitative sense[30]
7. infra-bony periodontal defects [31] as guided tissue regeneration membrane to affect periodontal healing,
8. sinus lift procedure and simultaneous implant placement, where PRF secures bone grafts, protects the Schneider membrane from tearing or protects it during healing when it has been torn during sinus-lift procedure, stimulate periosteum-like regenerative potential of membrane and maintain the implant on its position [32],
9. impairing better handling properties of bone grafts in bone augmentation in combination with BioOss or autologous bone [33, 34] and positive impact in the addition of PRF to bone grafts,
10. treatment of periimplant defects, where PRF has a strong regenerative potential on bone and gingiva (although is not efficient alone) [35]
11. preventing of hemorrhagic complications after tooth extractions and treating the patients on anticoagulant therapy [36]
12. as alternative to collagen membrane, where PRF shows superior effects compared with BioGide [6]
13. cleft palate repair [37].

The clinical application of PRF reveals promising new therapeutic strategies in regenerative dentistry. So far, the intrinsic properties of PRF has proven its

efficiency in periodontal surgery and implantology where is widely used for tissue engineering. It induces the soft-tissue healing and provides thick and high quality gingival tissue which is required for functional and esthetic outcome of mucogingival surgery. The high quality bone volume is important in alveolar ridge or socket preservation, and findings suggest the combination of bone grafting materials with PRF has better effect than using PRF of bone grafting materials alone. The final clinical effect of PRF is significantly altered by the material that has been used as bone substitute.

At Department of Oral Surgery at Faculty of Dentistry Sarajevo, PRF is used in treatment of periradicular defects after apicoectomy and osseous defects after enucleation of cysts and the removal of impacted teeth. It is been used as filling material of surgical cavities with or without bone grafts. In cases where osteoconductive properties of bone grafts are required, the PRF acts as stabilizer and space-maintainer thus protecting blood clot. It also improves the surgical manipulation with bone grafts and stabilizes particles of bone graft around implants or during sinus lift procedure. The PRF proved as simple, inexpensive and good substitute used as complement for bone grafts. The beneficial effects of therapeutic potential of clinical use of PRF at our Department are satisfactory. PRF has a positive effect on soft and hard tissue healing which justifies its clinical application. It accelerates wound closure, synchronizes healing of soft and hard tissue and acts as fibrin bandage supplemented with growth factors. PRF should be considered as surgical adjuvant that provides good mechanical support and acts as stabilizing sheath in large osseous defects after cyst enucleation. Unlike bone substitutes, PRF does not alter the volume of soft and hard tissues, but significantly modulate its quality and accelerates its formation. Our experience with clinical application of PRF proved its efficiency in preservation of the alveolar bone following tooth extraction: when placed in extraction socket it leads to the preservation of soft and hard tissues through accelerating of new bone formation, increasing the bone density and reducing of alveolar bone reabsorption. By preserving the alveolar ridge morphology, minimal bone augmentation procedures will be needed in order to allow future placement of dental implants. In these cases, the placement of PRF was also associated with lower incidence of postoperative surgical complications (infection, dry socket etc). In cases of wound dehiscence due to insufficiency of soft tissues, layers of PRF provide protection for underlying tissues and enables epitelization through accelerating migration of epithelial cells. Besides its regenerative potential, the immune properties of PRF also should not be underestimated. These preliminary observations of clinical effects of PRF at our Department are promising and could open a new era of regenerative therapy in oral surgery.

The main advantages of PRF are reflected through its simple preparation, low-cost and efficiency. However, some of the disadvantages of PRF are present. The amount of PRF that can be obtained from patient is limited. Also, it demands quick preparation and use. PRF must be implanted immediately since delaying can result in its dehydration and loss of growth factor content. Long-term clinical researches of efficiency of PRF are required in order to completely determine the effect of PRF on wound heeling.

Although there are numerous studies regarding the positive affects of PRF, there are some inconclusive and contradictory reports in literature considering its effects on modulating wound healing. Some authors suggest that activity of PRF is limited and short-term. Study of Suttapreyasri showed that PRF does not enhance bone formation; it just accelerates the initial soft-tissue healing in first weeks [38]. Study of Girish Rao did not show statistically significant difference in bone regeneration in extraction sockets with PRF [39]. Many histological and molecular studies on effects of PRF on wound healing should be supported with clinical findings of beneficial effects of PRF. The best clinical protocol of PRF with adjunctive material should be elucidated and defined. Extensive long-term clinical validation of PRF is needed and future studies should be conducted in order to reveal the precise role of PRF in wound healing in specific oral-surgery indications.

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DELAYED TOOTH REPLANTATION AFTER TRAUMATIC AVULSION

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ABSTRACT

Replantation refers to the insertion and temporary fixation of completely or partially avulsed teeth resulting from traumatic injury. Replantation of an avulsed tooth depends on certain clinical conditions like physiological status of periodontal ligament, the stage of root development and the length of extra-oral time. Depending on the patient's age, retention of the permanent incisor can maintain the aesthetic appearance, occlusal function and alveolar ridge height. Though the risk of progressive replacement resorption and subsequent tooth loss is high after a long dry storage, replantation provides an aesthetically acceptable permanent prosthesis at a later age. This article presents management of a case with two avulsed permanent incisors that were stored in a physiological solution for ten hours.

Key words: dental trauma, delayed replantation, tooth avulsion.

Introduction

Avulsion is defined as a complete separation of a tooth from its alveolus following a traumatic injury, which results in extensive damage to the pulp and periodontal tissues. According to the World Health Organisation's classification system, later modified by Andreasen, avulsion is classified as an injury of periodontal tissues, as well as extrusive, lateral, or intrusive luxation [1]. As a treatment option, replantation restores occlusal function and aesthetics shortly after injury. In replantation complete reestablishment of vitality of periodontal fibers is the prime objective. The percentage of success of tooth replantation has been presented to be low, ranging from 4 to 50% [2]. However, it should be emphasized that the success of tooth replantation depends on the clinical needs and patient expectations. The period of permanence of the tooth in the dental arch may be enough to meet some of the needs, such as prevention of atrophy of the alveolar ridge, allow the patients to better accept the tooth loss, and even delay the need for prosthetic solution. The success of tooth replantation depends on the maintenance of vitality of the periodontal ligament, allowing its remains adhered to the avulsed tooth to survive and recover their function. Resorption is the main cause of failure of replantations, and the prognosis of a replanted tooth is related to the type of resorption that may ultimately lead to complete destruction of the root [3]. Degeneration of the periodontal ligament depends on several factors, such as trauma, management of the root, extra-alveolar period and storage medium [4]. The need of performing endodontic treatment has been demonstrated by several authors, indicating that the pulp tissue may become necrotic and its toxins may reach the periodontal ligament through the dentinal tubules or root canal, definitely contributing to an increase in the resorption process [5].

Nevertheless, if managed appropriately, avulsed teeth with viable periodontal ligament when replanted can remain functional for some years. This article describes the management of a child with an avulsed two central maxillary permanent incisors, kept in a physiological solution for ten hours. The child has also teeth's fractures.

Case report

A 15-year-old girl was referred to the Department of Pediatric and Preventive Dentistry at the University Dental Clinical Center "St. Pantelejmon" Skopje, for treatment of avulsed teeth. She had experienced a trauma ten hours earlier and spat out her upper frontal teeth, both central incisors. The intraoral examination showed that her both maxillar incisors had been avulsed and their sockets were full with coagulum (**Figure 1**).

The avulsed teeth were kept in 0,9% NaCl solution after the injury. Radiological examination confirmed empty sockets with no other hard-tissue injury

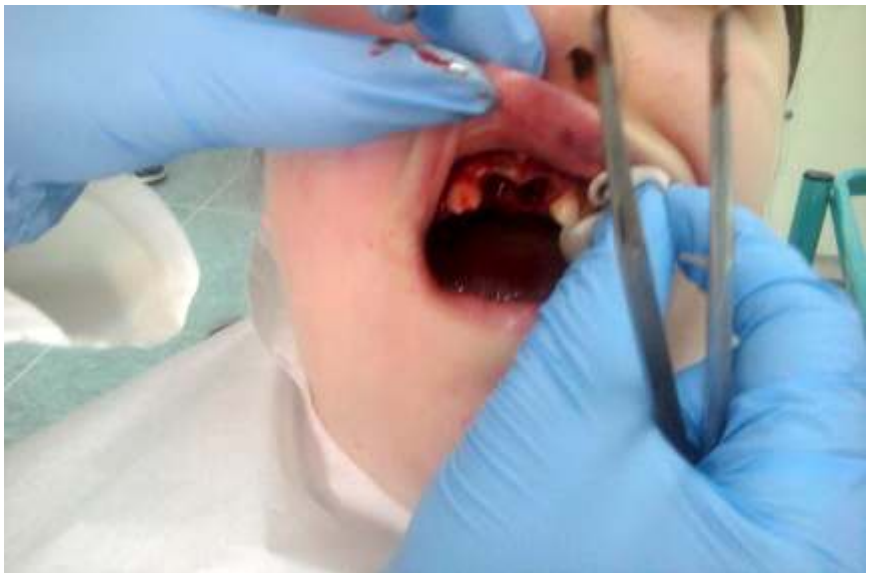

Figure 1
Avulsed maxillary central incisors

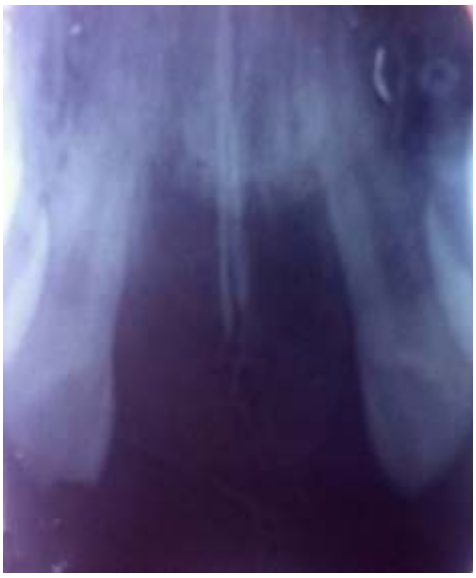

Figure 2
Pre-operative radiograph

Figure 3
Extraoral root canal instrumentation

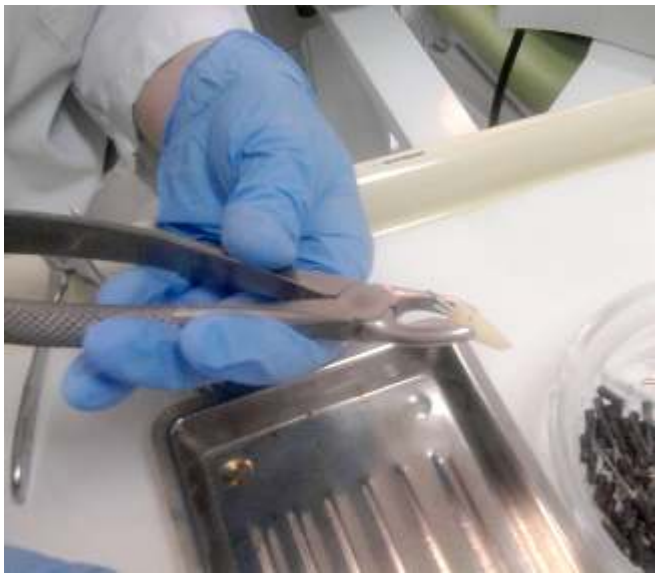

Figure 4
Drying with sterile paper

(**Figure 2**). The patient's medical history was unremarkable. All of the adjacent teeth showed a positive response to the test of vitality.

Replantation of the avulsed teeth was planned to retain teeth in the mouth for as long as possible, because of the patient's age, although the teeth were fractured and one of them had also pulpal exposition. This case was of delayed replantation and endodontic treatment was started before replantation.

Teeth surfaces were cleaned with 0.9% NaCl solution. Conventional extirpation, enlargement and cleaning of root canals were performed (**Figure 3**). Then, root canals were dried with sterile paper points (**Figure 4**) and temporarily filled with calcium hydroxide paste (**Figure 5**).

Under local anesthesia, the sockets were gently curetted to remove any coagulum, granulation tissue and pathological tissue (**Figure 6**) and irrigated with physiologic saline solution. Teeth were then soaked in 2% sodium fluoride solution for 20 minutes and replanted into their respective sockets (**Figure 7**). Once the teeth were properly seated, they were checked for alignment or occlusion (**Figure 8**) and were splinted to the adjacent teeth with a 0.5 mm

Figure 5
Temporary filling with calcium hydroxide

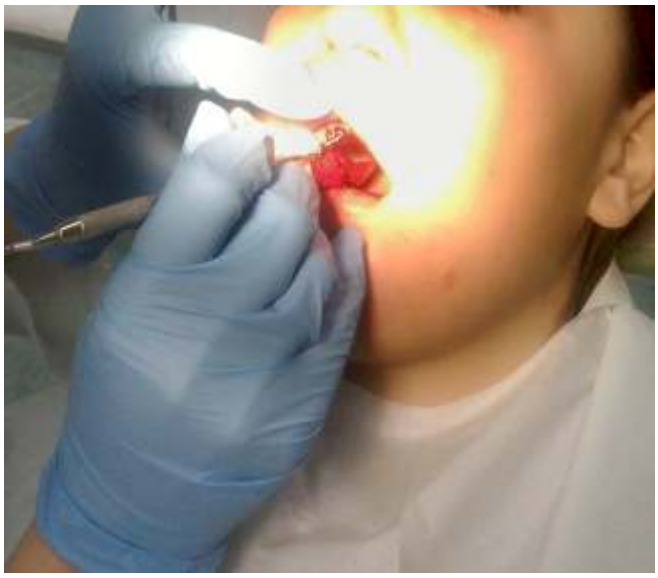

Figure 6
Socket preparation with curette

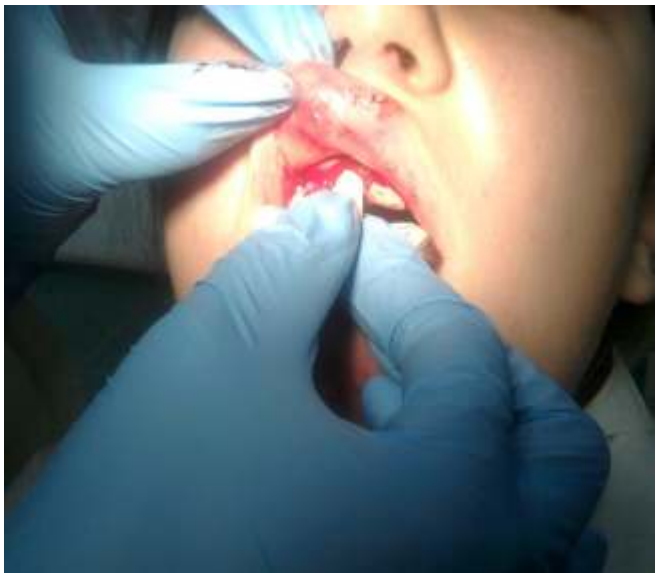

Figure 7
Replantation

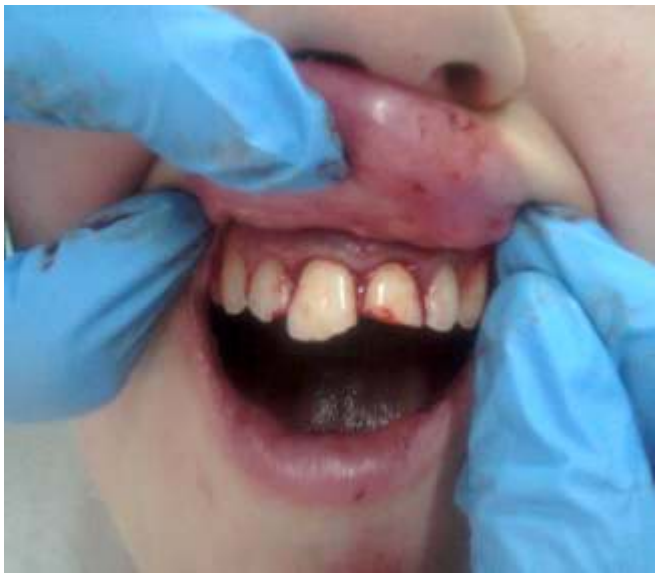

Figure 8
Clinical aspects after replantation

orthodontic stainless steel wire and acid-etch composite (**Figure 9**). Maxillary occlusal radiograph was obtained to confirm proper positioning of replanted teeth (**Figure 10**). Oral hygiene instructions were given and chlorhexidine mouthwash was recommended. A 7-day course of systemic penicillin was prescribed, and the patient was referred to the medical practitioner for an antitetanus booster.

Six weeks after the injury, before the splint was removed, the root canals were refilled with definite sealer and gutta percha points (**Figure 11**). During that period the teeth did not show any clinical symptoms like mobility, periodontal pockets or any type of root resorption.

A restoration of coronary fractures with acid-etch composite followed in order to correct aesthetic, phonetic and masticatory function, and to meet the patient's and her parents expectation of this treatment (**Figure12**). The patient came for regular controls every three months (**Figure 13**) and the follow-up period lasted for two years (**Figure 14**). During this period, the patient was asymptomatic, with no complications, and satisfied with the conservative treatment.

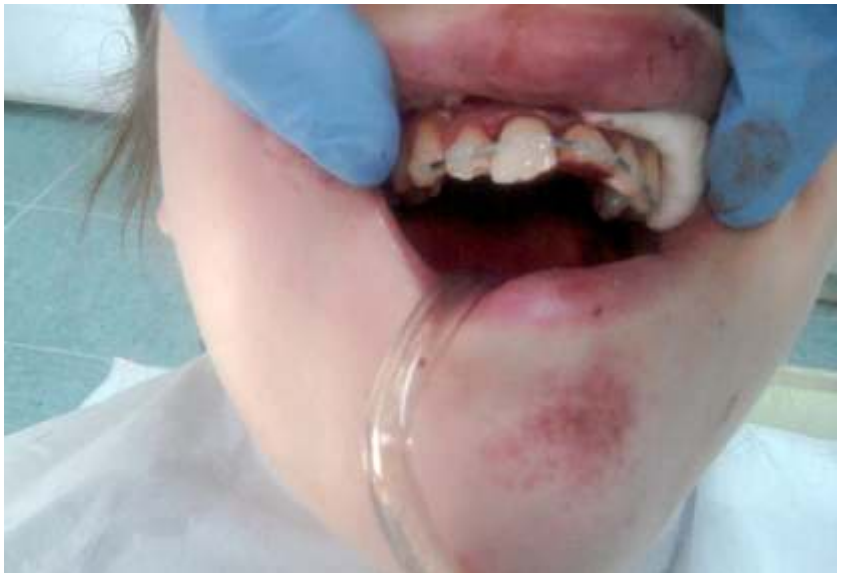

Figure 9
Stabilization with composite-wire splint

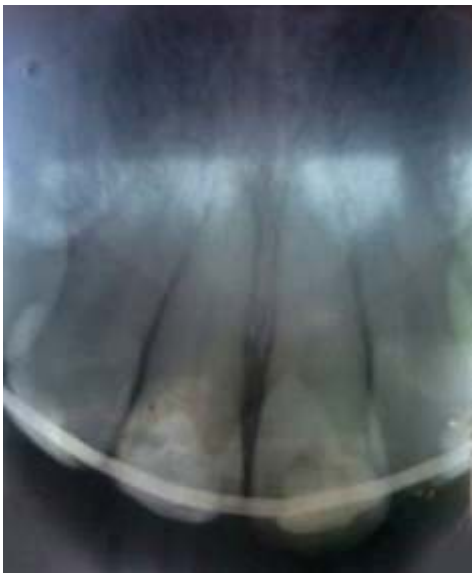

Figure 10
Radiograph after replantation

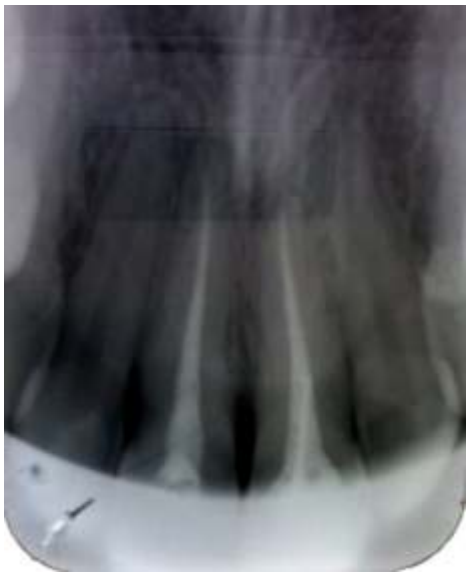

Figure 11
Extraoral root canal instrumentation

Composite restoration

Discussion

Tooth avulsion is a serious assault on the gingiva, periodontal ligament and pulp. Young permanent tooth loss leads to a severe arrest of alveolar bone formation in a growing child. Alveolar ridge would be narrow and difficult to restore in future with either a bridge or implant. Most conservative approach for managing the avulsed incisors is to replant them as soon as possible. In clinical studies, teeth replanted within 5 minutes after avulsion have the best prognosis and the chance of pulpal and periodontal healing is inversely related to the stage of root development and the extra-oral time, as well as the medium where the tooth is stored [6]. A review of literature shows that an extra-oral time of less than 15 minutes gives a greater success rate of retention [7] of replanted teeth, but if the time lapse is between 15-60 minutes, some authors advise that the root must be stored in suitable storage medium and transported to the clinic [8]. Some of the storage media that have been frequently used include Emergency Medical Tooth saver, Euro-Collins solutions, saline, pasteurized

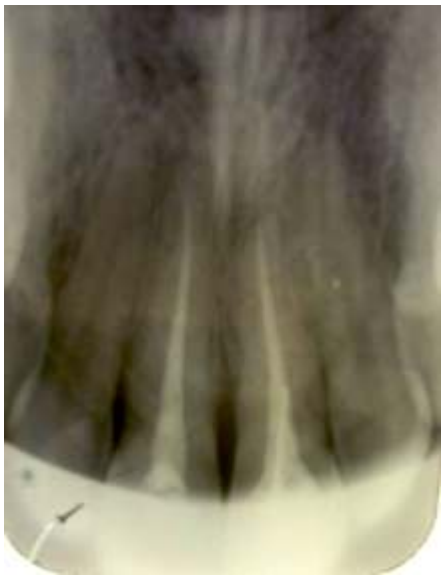

Figure 13
Radiograph after six months

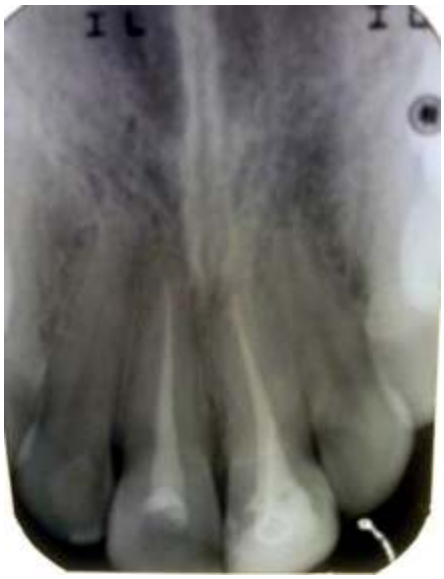

Figure 14
Radiograph after 24 months

milk, saliva, or chicken egg white [9]. Literature data shows that the average time range of replanted teeth is between 1 to 4 hours, after which the success of replantation is determined by the storage media. Success has also been reported in a study where the tooth was replanted after 8 hours, but was stored in Euro-Collins15 solution. Other studies showed that replantation after 60 minutes in no storage medium with extra-oral endodontics may result in retention of the tooth [10]. However, studies by Petrovic et al. showed that replanted dry teeth in dry storage between 15 minutes to 9 hours had low survival rates and a reduced chance of retention. But, immediate replantation is not always possible because of various unavoidable problems.

In our patient, the greatest benefit of successful replantation of an avulsed tooth was preservation of alveolar bone. Even if the replanted teeth must be extracted later, the improved alveolar development will provide better options for restoration of the site later. We could successfully achieve objectives like acceptable aesthetic appearance and occlusal function, prevention of root resorption and favorable healing for better permanent treatment in later life. Our patient was asymptomatic during the follow-up period of 24 months. This procedure gave invaluable and timely contribution to their normal and physiological growth.

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TITLE: **Fundamentals of Tooth Morphology and Dental Anthropology**

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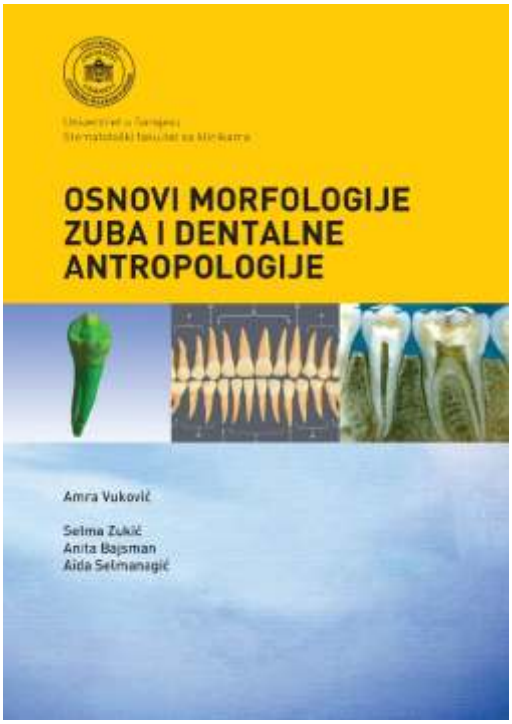

With the end of last year, from the press has come an university textbook "Fundamentals of tooth morphology and dental anthropology", published by the Faculty of Dentistry, University of Sarajevo. The authors Amra Vuković, Selma Zukić, Anita Bajsman and Aida Selmanagić have made efforts to consolidate and in a clear way present knowledge of the morphology of the teeth and the basics of dental anthropology. Considering the course of development of tooth morphology as a scientific discipline in Bosnia and Herzegovina, the authors offered to us a book that is primarily a textbook, but also will serve to all those who want to understand the connection between tooth morphology and function. This is certainly a basis for quality clinical practice, and also for a scientific approach to understanding the biology of tooth in the context of contemporary trends in biomedicine. The material is processed through seven thematic sections and chapters. In the introduction the authors write about stomatognahtic system and its components. The next chapter deals with the nomenclature in dental morphology, with the special emphasis on the morphological details of the tooth. This is followed by a chapter on the permanent and after that by a chapter on primary teeth. In the fifth thematic section authors explain the morphological and anatomical relations of the dental arches. Logical sequencing is next thematic area that describes and explains the anomalies of the teeth, dividing them into congenital and acquired. The final chapter is about dental anthropology, where is in detail explained the classification of morphological details by internationally acclaimed system. All chapters are enriched with original photographs and illustrations, as many as 121, which gives added value to this book. Reviewers of the book were distinguished professors: Professor Emeritus Hajrija Konjhodžić Raščić from Faculty of Dentistry, University of Sarajevo, Professor Hrvoje Brkić from Faculty of Dentistry, University of Zagreb and Professor Azijada Šuljak Loncarevic from Faculty of Dentistry, University of Sarajevo. Giving their opinion and a positive evaluation of the textbook reviewers wrote this: "In this textbook, the content is processed encyclopedicly ... It has a special significance for the adoption and learning of terminology - anatomical terminology of morphological details on the teeth and

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So in the end we can say that this book will serve not only to those who are bound by their work with universities, but also to clinicians and physicians during the lifelong learning in all countries of the same or similar language.

Sadeta Šečić

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