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KONTAKT / CONTACT:
Stomatološki vjesnik
Stomatološki fakultet sa klinikama
Bolnička 4a, 71000 Sarajevo
Bosna i Hercegovina

Telefon: + 387(33)443269
e-mail: glavni_urednik@stomatoloskivjesnik.ba
Web: www.stomatoloskivjesnik.ba

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Stomatološki vjesnik

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IS A pH- CYCLING MODEL A SUITABLE SIMULATOR OF ACTUAL PHYSICOCHEMICAL PROCESSES IN INITIAL CARIOUS LESIONS?

Hasić Branković Lajla*[1], Tahmiščija Irmina[1], Korać Samra[1], Konjhodžić Alma[1], Džanković Aida[1], Hadžiabdić Naida[2]

[1] Department of Dental Pathology and Endodontics, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina
[2] Department of Oral Surgery, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina

*Corresponding author

Assistant Professor
Lajla Hasić Branković, M.Sc, Ph.D,
Dental Pathology and
Endodontics specialist,
University of Sarajevo,
Faculty of Dentistry with Clinics,
Department of Dental Pathology
and Endodontics,
Bolnička 4a,
71000 Sarajevo,
Bosnia and Herzegovina
Phone: +387 (33)214 249
e-mail: lajlabrankovic@gmail.com

ABSTRACT

Artificial caries-like models were developed to understand processes in dental caries. Numerous different models were introduced during the past few decades. One of the first models was a pH-cycling model. It remains a basic model for simulation of various processes in enamel and dentin during carious lesion development.

The paper aims to present leverages and disadvantages, features and acquiring procedures for the basic pH-cycling model.

Material and methods: 60 enamel slabs made from the first and the second human premolars extracted from orthodontic reasons, were divided into three experimental groups (native enamel (NE) control group, white spot (WS)-experimental group, and demineralized enamel (DE) - negative control group). All three groups were positioned on the same experimental model. The native enamel (NE) group didn't receive any de-or remineralization treatment. WS group was submitted to a pH cycling regime to create artificial caries lesion, and demineralized enamel was created by constant demineralization during 24 hours being then submerged into distilled deionized water, without remineralization treatment.

Cross-sectional microhardness (CSMH) measurements were done through the outer peripheral layer of enamel. Measurement results are expressed as a Knoop Hardness Number (KHN) and subsequently converted into the volume of the mineral percentage.

Results: CSMH showed the formation of artificial caries like lesion with an average depth of 50-60 micrometres. Significant differences ($p \leq 0,05$) appeared in the first 40 micrometres in the surface enamel layer. Differences in enamel cross-sectional microhardness were lost in deeper portions of the enamel.

Conclusion: pH cycling models are suitable for the investigation of physical and chemical processes in enamel during early caries lesion progression. This model enables monitoring changes in depth, construction of lesions mineral profile as an additional experimental procedure in the same sample.

Keywords: artificial caries, pH-cycling model, enamel demineralization, enamel remineralization, enamel CSMH.

Introduction

pH-cycling models as simulators of actual physic-chemical processes in dental caries

In-vitro models, developed a few decades ago, have proven to be very useful models in examining the dynamics of caries formation and the appearance of carious lesions. These models realistically reflect clinical *in-vivo* conditions and provide insight into changes in the biological substrate of enamel. [1] A well-designed experimental model allows us to easily adjust the level of caries risk by changing the conditions of enamel exposure. Furthermore, enamel samples can be additionally analyzed microscopically, functionally, subjected to another type of tests or became a biological substrate for detail examination in microbiological models (for example substrate for biofilm growth), etc. [2, 3] *In-vitro* models are still the most widely used form of testing performed for this purpose. [4, 5]

Clinical studies are very expensive and pertinently time-consuming. It often takes 1-3 years to evaluate for example the preventive effect of an experimental agent. Hence, other models of shorter-term clinical studies have been developed. They are equally expensive but significantly faster to perform. [6]

Additional possibilities of testing enamel samples underwent in some experimental procedure in clinical studies are technically difficult, if not even unmanageable. [3] Clinical studies remain the gold standard for assessing the efficacy of individual agents, but due to their shortcomings, they have largely been replaced by well-controlled *in-vitro* models. [5, 6]

A well-controlled *in-vitro* model involves an experimental system precisely defining the dose-dependent sensitivity of the administered agent, as well as potential differences in efficacy and/or rate of action. [6] The models allow the isolation and emphasis of certain aspects of the carious process, such as the microbiological, biochemical or physicochemical aspects of the carious process. [3, 7] Leading among these are pH -cycling models.

A literature review shows that in the last few decades, several *in-vitro* models have been developed on which numerous experiments have been performed. Knowledge about the nature and dynamics of the carious process, the content of fluoride and other elements in it largely derive from these *in- vitro* experimental models. [4]

Various pH-cycling models have been developed for different purposes. Some are designed to evaluate demineralization, others to study remineralization and some to evaluate the effect of fluoride in these processes. [5, 8, 9] There are numerous examples of successful implementation of pH-cycling models containing enamel and dentin blocks, not just in restorative dentistry, but in dentistry in general. [10, 11, 12] They all had tremendous success in cariology. [4]

In-vitro experimental protocols, however, also show limitations.

Therefore, these models should be evaluated very carefully, especially when assessing the preventive potential of individual drugs, depending on administered doses. [13, 14] For instance, the preventive fluoride effect is observable in such models. A well-designed pH-cycling model, preconcert to simulate conditions in a specific moment of the carious process, can determine predominant component of this process is: demineralization or remineralization. Additionally, the impact of fluoride on the degree of enamel hardness, as well as the reduction of the enamel lesion depth can be evaluated. The precise chemical structure of fluoride "enriched" enamel can be also determined. [15, 16, 17, 18]

Various studies were performed to develop and adapt pH-cycling models to be more faithful representative of *in-vivo* conditions. One of the variable parameters is the substrate in pH-cycling models. Bovine enamel showed numerous advantages over humans. It is low-cost, easy to purchase and it´s chemical structure is very similar to human enamel. [13, 14, 19] Therefore, the method described in this paper is applicable for bovine enamel, likewise.

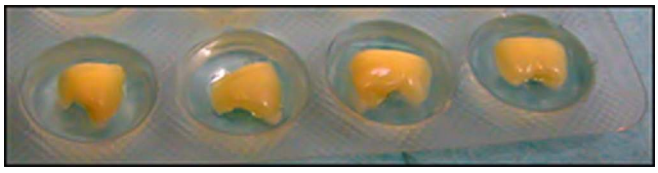
The aim of the paper is an accurate description of pH cycling protocol, as well as lesions microhardness evaluation.

Material and methods

The research was conducted based on the approval of the Ethics Committee of the Faculty of Dentistry, decision No: 09-545-3 / 11.

The research was managed on enamel slabs originating from 30 permanent premolar teeth, extracted from orthodontic reasons. The inclusive criteria were: without obvious initial carious lesions, without white spots, no enamel cracks (infractions) on the crown of the tooth. The root and crown were separated with a diamond burr, and the crown was cut in half in vestibule-oral direction. Thus, two samples of enamel were obtained from each tooth.

Enamel samples were placed in blocks of transparent self-adhesive acrylate, remaining 9 mm^2 (3 X 3 mm) free. Acrylate and samples were placed in standardized PVC blisters intended for the packaging of Paracetamol à 500 mg tablets. (**Picture 1**)



Picture 1.
Enamel blocks embedded in self-adhesive acrylate poured into PVC blisters.

The surface of the enamel blocks was polished with abrasive discs under water cooling to remove a surface layer about 30 μm thick. The aforementioned layer is naturally richer in fluorides and contains "anamnesis "of different treatments from the period of intraoral time.

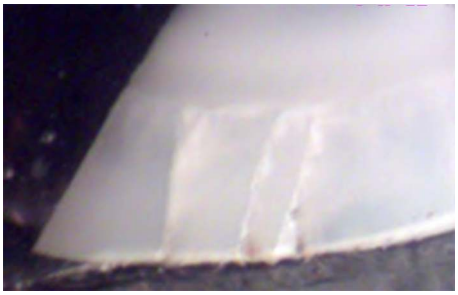
Samples were divided into 3 groups, each containing 20 samples.

Group I, "Native Enamel" (NE) was placed in the solution for remineralization, natural saliva equivalent. Samples were kept in this solution until the moment of cross-section micro hardness measure. This "positive control" group represents enamel under conditions in which it is normally found in the oral cavity. Such enamel is surrounded by saliva with a high potential for remineralization.

Twenty samples from group II, "White Spot" (WS) underwent pH-cycling regimen containing demineralization in a demineralizing solution for three hours (3h), and the rest of the time the enamel samples were submerged in the remineralizing solution, the equivalent of artificial saliva. The daily demineralization regime was established on the basis of estimated average of about person's 5 meals consumption daily. Assuming there is normal salivary flow, the pH drop after each meal lasts on average about half an hour. [5, 7]

The daily regimen was repeated cyclically for 8 days. Samples were submerged every time in a fresh solution. The amount of demineralization solution is precisely specified to prevent solution saturation with ions originating from the enamel. The experimental conditions were adjusted so that each enamel block had 20 ml of demineralization solution. After each period of demineralization, blocks were washed in a sufficient amount of distilled deionized water and then returned to the same remineralizing solution. Samples were stored at room temperature during treatment and experimental procedures. Daily regimen can be set differently, depending on what lesion depth is expected and enough for specific experiment. Total demineralization time can be shorter or longer (2-14 days). Eight day cycles were average time for lesion formation. [20, 21]

The third group "Demineralized Enamel"(DE) received demineralization protocol containing constant exposure to the demineralization solution for 24 hours, followed by immersion in distilled deionized water. The enamels from this group of samples were not "proposed" the possibility of remineralization, hence they were placed in distilled deionized water, instead of in a demineralizing solution. This group was "negative control". Total time of demineralization was same in DE and WS group (24 hours). The idea was to examine the impact of demineralization/remineralization cycle shift on the physical properties of enamel. This group ensured evidences regarding "simple" dissolution of the enamel under the acid attack. Differences between WS and DE group should provide evidences that the enamel is able to



Picture 2.
Enamel infractions that occurred after sample cutting. All these samples were excluded from further research. The image was taken using a digital camera on a stereo microscope, magnification 30X.

restore, reabsorb part of the lost minerals if it is surrounded by a medium supersaturated with minerals.

The demineralizing solution composition: 2.2 mM $CaCl_2$; 2.2 mM NaH_2PO_4, 0.05 M lactic acid in 50 mM acetate buffer with adjusted pH to 4.3 with 50% NaOH. 20,21

Remineralization solution Composition (artificial saliva): 3,9 mM Na_3PO_4; 4.29 mM $NaCl_{2;}$ 17,98 mM KCl; 1,1 mM $CaCl_2$; 0,08 mM $MgCl_2$; 0,5 mM H_2SO_4; 3,27 mM $NaHCO_3$, in destilled deionized water with pH set on 7,2. 21 The amount of artificial saliva is also specified, 10 ml of solution per each enamel block.

Sample preparation as well as microhardness measurements were performed at the Dental Materials Department on ACTA (Academic Center for Dentistry in Amsterdam; NL).

To measure micro hardness through the depth of the lesion, samples were cut perpendicular to the surface of the enamel using Isomet ™ 1000 precision saw Buehler equipped with a Mitutoyo micrometer. After polishing, 1,5mm thick samples were glued with instant cyano-acrylate adhesive to the microscope slides.

At this point, it should be noted that the cutting process was the weakest link in the preparation procedure. Certain numbers of samples were lost, either they fell out of the acrylic mold or the enamel exhibit infractions that appeared only in cross-section.

It cannot be determined with certainty whether enamel infractions occurred during sample cutting or existed before but were not visible. Due to previous, during the experiment, it was decided to examine all samples under a stereomicroscope equipped with a digital camera. (**Picture 2**) After reviewing all samples, it was decided to include only samples without visible infractions.

The largest loss of samples occurred in the third group (DE). The surface enamel layer of these samples was remarkably softened and simply lost during cutting and polishing. (**Pictures 3,4 and 5**).

CSMH (Cross-section microhardness) measurements were performed on Microhardness tester HM-124 Hardness testing machine Mitutoyo, Akashi, Japan; equipped with Knoop diamond and microscope camera MIO magnification lens 25, 100 and 200X. 22

A load of 25 g for 5 seconds was used to measure the microhardness; hence it was proven that higher loads than e.g. 50g can cause cracks in the enamel. 4

Microhardness testing was performed on every 10 μm, up to a depth of 80 μm, and ups to a depth of 200 μm every 20 μm. Measurements were performed under 100X magnification. Enamel microhardness was measured and registered in Knoop Hardness Number (KHN).

Pictures 3, 4, and 5.
Surface layer porosities in group DE. Magnification 15, 30,80 X.

Results and statistical analysis

Descriptive statistics are given in **tables 1 and 2.**

ANOVA statistical analysis of results was used for testing differences between the groups. Post-hoc LSD tests were complete to show the significance of founded differences. The mean significant difference was set at the 0,05 level and signed with an asterisk symbol. Performed tests showed statistically significant differences only in the first 50μm depth. Those results and significant differences are specified in **Table 3.**

The pH-cycling process produced a lesion with an average depth of 50-60 μm, as a global review of the results and statistical analysis demonstrate.

The most pronounced differences were in the surface layers and successively decreased in depth toward the healthy, deeper layer of the enamel (**Chart 1 and 2**).

Table 3 and Chart 3 shows the value of the volume percentage of minerals in the test groups. The mineral volume percentage was calculated based on the measured microhardness values, and a detailed procedure is explained in the "Discussion" section.

Descriptive statistics	Mean ±SD						
Depth of CSMH Measurement	10μm	20μm	30μm	40μm	50μm	60μm	70μm
NE (I group)	284,11 ± 58,40	291,22 ± 70,69	321,11 ± 58,45	320,00 ± 76,706	313,72 ± 91,842	290,06 ± 80,767	299,72 ± 92,927
WS (II group)	90,33 ± 69,44	212,27 ± 80,56	257,20 ± 89,41	279,80 ± 87,184	285,60 ± 49,231	300,27 ± 56,358	289,13 ± 65,953
DEMIN (III group)	24,46 ± 26,74	76,62 ± 56,032	106,85 ± 65,39	163,15 ± 95,994	188,23 ± 73,741	277,46 ± 73,839	296,46 ± 93,375

Table 1. Descriptive statistics for CSMH measurements in the first 70 μm.
Legend: NE-native enamel, WS-white spot lesion, DEMIN- enamel demineralized in extreme conditions, without remineralization possibility.

Descriptive statistics	Mean ±SD						
Depth of CSMH Measurement	80μm	100μm	120μm	140μm	160μm	180μm	200μm
NE (I group)	338,06 ± 144,216	320,72 ± 109,384	338,17 ± 84,244	303,44 ± 109,217	301,39 ± 104,127	332,28 ± 160,811	285,06 ± 138,858
WS (II group)	331,53 ± 62,277	349,87 ± 78,363	363,33 ± 94,827	342,67 ± 69,849	330,33 ± 55,045	366,20 ± 118,788	369,27 ± 155,924
DEMIN (III group)	270,92 ± 116,514	293,77 ± 97,616	316,15 ± 74,653	311,69 ± 83,485	317,38 ± 112,853	314,77 ± 53,176	331,31 ± 60,883

Table 2. Descriptive statistics for CSMH measurements in-depth 80-200 μm.
Legend: NE-native enamel, WS-white spot lesion, DEMIN- enamel demineralized in extreme conditions, without remineralization possibility.

Differences between groups I, II, and III	Mean Difference	Sig
NE (10) ↔ WS (10)	193,778(*)	,000
NE (10) ↔ DEMIN (10)	259,650(*)	,000
WS (10) ↔ DEMIN (10)	65,872(*)	,016
NE (20) ↔ WS (20)	78,956(*)	,003
NE (20) ↔ DEMIN (20)	214,607(*)	,000
WS (20) ↔ DEMIN (20)	135,651(*)	,000
NE (30) ↔ WS (30)	63,911	,024
NE (30) ↔ DEMIN (30)	214,265(*)	,000
WS (30) ↔DEMIN (30)	150,354(*)	,000
NE (40) ↔ WS (40)	40,200	,186
NE (40) ↔ DEMIN (40)	156,846(*)	,000
WS (40) ↔DEMIN (40)	116,646	,001
NE (50) ↔ WS (50)	28,122	,322
NE (50) ↔ DEMIN (50)	125,491(*)	,000
WS (50) ↔DEMIN (50)	97,369	,002
NE (60) ↔ WS (60)	10,211	,704
NE (60) ↔ DEMIN (60)	12,594	,653
WS (60) ↔ DEMIN (60)	22,805	,435

Table 3.
Description of statistically significant differences between the three groups given by a layer of depth.

Discussion

The natural remineralization potential of the initial carious lesion is extremely high in a solution supersaturated with necessary minerals, such as saliva. In our research, a remineralization solution was virtually identical to saliva's mineral composition.

A measurable difference in microhardness between native enamel and the untreated carious lesion is already lost at depth of 40 µm, while differences in chemical composition as well as in microstructure certainly exist.

The initial carious lesion designed in the pH-cycling model in this study probably does not have the same healing mechanism as the white spot formed *in-vivo* conditions.

The untreated initial carious lesion produced in our study apparently

Depth in µm	Volume %Mineral		
	I group NE	II group WS	III group DE
10 µm	83,77892	52,1681	32,56653
20 µm	84,68023	73,9488	48,93913
30 µm	88,35403	80,26106	55,74836
40 µm	88,22074	83,22706	66,22398
50 µm	87,46221	83,96873	70,29468
60 µm	84,53394	85,81169	82,92566
70 µm	85,74342	84,41644	85,33746
80 µm	90,36155	89,59425	82,07648
100 µm	88,30723	91,73069	85,0008
120 µm	90,37442	93,26323	87,75661
140 µm	86,20398	90,8988	87,2154
160 µm	85,95053	89,45243	87,9052
180 µm	89,68276	93,58632	87,58956
200 µm	83,9	93,93052	89,56827

Table 4.
Values of the relative enamel mineral content

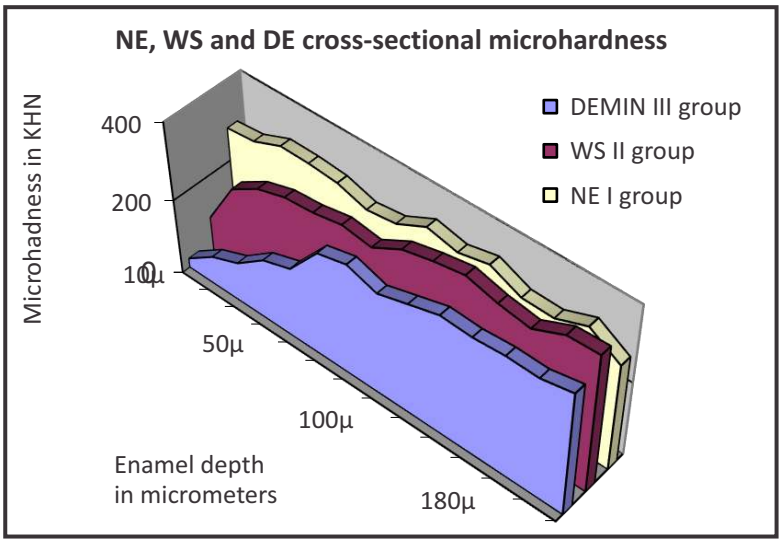


Chart 1. Microhardness for three groups given in KHN, in outer 200 micrometres of enamel.

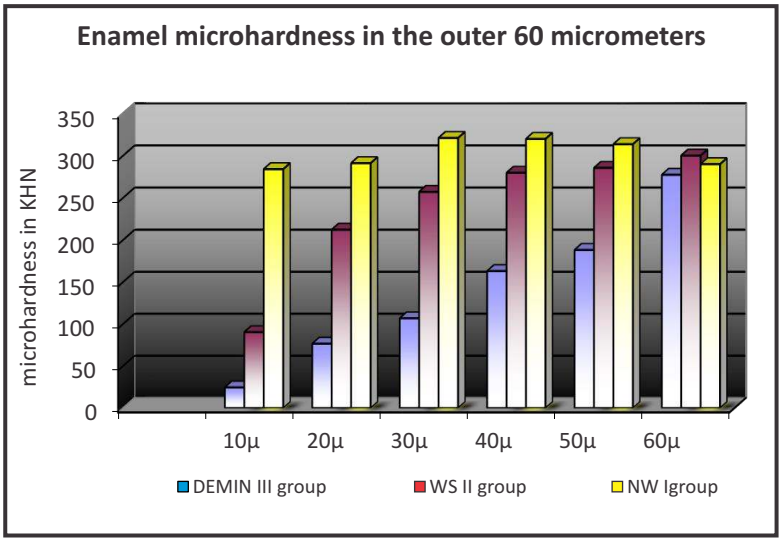


Chart 2. Enamel microhardness in outer 60 micrometres.

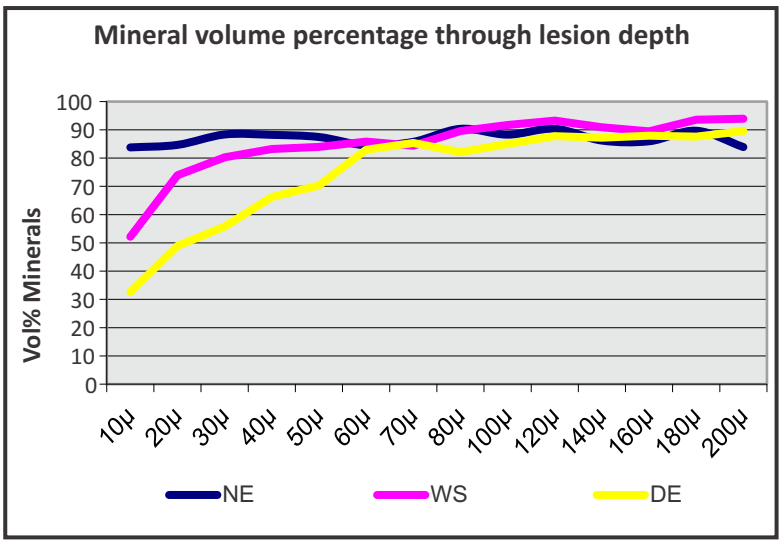


Chart 3: Changes of mineral volume content through lesions depth.

remineralizes by the same pattern as erosion does *in -vivo*. [23, 24]

It is well-known that the white spot lesion is *in - vivo* healing by forming a well-mineralized superficial zone on its surface below which is the body of the lesion with larger pores. The thickness of the well-mineralized surface zone is different according to different authors and amounts to 10-50 μm, while the body of the lesion can extend up to 350 μm into the depth of the enamel. [23, 24, 25]

To be precise, this morphological feature of the initial carious lesion was not examined by measuring the microhardness but by different microscopy methods, undoubtedly more precise than the microhardness examination.

Microhardness is only one of the physical property parameters. Other properties can be indirectly established on this basis including the mineral composition of enamel.

Measuring the microhardness in the cross-section could not prove the "body of a lesion" existence since the WS (group II) showed to be softest on the surface, but going deeper, the hardness approached the values found in native enamel.

At a depth of 100 to 160 μm the WS group shows even slightly higher hardness, but not statistically significant compared to native enamel. This can be an indication increased density zone of enamel formation, a zone generally formed below the body of the lesion.

Queiroz and associates produced an initial carious lesion about 80 μm deep, working on bovine enamel. The total demineralization time was extended to 64 hours, and the pH of the demineralizing solution was about 5. [14]

Featherstone produced a lesion with an average depth of 75 μm in a 14-day cycle with a daily demineralization period of 6 hours. [26]

Puig-Silla produced a lesion with a depth of 45 to 60μm, working also on bovine enamel, with a pH of the demineralizing solution set at 4, 4. The total duration of the demineralization cycle was 48 hours. [27]

In a 14-day cycle with a pH set on 4.3, Shirahatti produced a lesion average depth 115 to 150 μm, depending on the agent with which the enamel was pretreated before pH cycling. [28]

Referred to the above studies, the depth of the lesion was evaluated by microscope (polarized, confocal) or by microradiographs, not based on the measurement of microhardness as it was the case in our study.

Based on the above, it is reasonable to expect that our research generated similar depth lesions since the experimental conditions were similar. Hence, cross-sectional microhardness measurement as a method is not sufficiently sensitive to detect fine differences in the microstructure.

Therefore, parameters are introduced to directly connect enamel microhardness with its mineral composition, as an indicator of the microstructure. The ratio of the mentioned quantities can be expressed by the formulation established by Featherston and associates in 1983. 26:

$$\text{Volume \%Mineral} = 4,3 \times \sqrt{\text{KHN}} + 11,3$$

Therefore, calculation based on the previous formula, the cross-sectional microhardness values can be converted to the percentage mineral content of the enamel. The values of the relative enamel mineral content are given in the **table 4** and **chart 3.**

Conclusions

1. The pH cycling method proved to be valid *in - vitro* method for testing changes related to the initial carious lesion.
2. The CSMH method allows the creation of the mineral profile of the lesion and monitoring of changes in depth.
3. The natural potential for remineralization of the initial carious lesion is large, statistically significant difference in microhardness between native enamel and untreated initial carious lesion in our study is lost at a depth of 40μm.

Declaration of interest:

The authors declare no conflict of interest.

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THE INTERRELATION BETWEEN ORAL HEALTH STATUS AND SERUM GLYCATED HEMOGLOBIN LEVELS AMONG SCHOOLCHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES MELLITUS

Huseinbegović Amina*[1], Fazlić Rusmira[2], Hasanbegović Snježana[3], Bajrić Elmedin[1], Hadžić Sanja[4], Pašić Enes[4]

[1] Department of Preventive Dentistry and Pedodontics, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina

[2] Dental department of Health Centre of Sarajevo Canton, Bosnia and Herzegovina

[3] Department of Oral Medicine and Periodontology, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina

[4] University Clinical Centre Sarajevo, Endocrinology department of Pediatric Clinic, Sarajevo, Bosnia and Herzegovina

*Corresponding author

Amina Huseinbegović, PhD
Bolnička 4a
71000 Sarajevo
Bosnia and Herzegovina
e-mail:
aminahuseinbegovic@hotmail.com
Phone:+ 387 (33) 216-219

ABSTRACT

Objective: This study aimed to evaluate the effects of metabolic control on oral health status among diabetic children and adolescents.

Methods: The sample in this study consisted of 60 school adolescents diagnosed with T1DM aged 12-18 years, divided into two groups of 30 subjects each: group A consisted of patients with well-controlled glycemia and group B of those with poor metabolic control. Plaque index and Gingival index according to Sillnes and Löe, and measurement of alveolar bone loss was used to assess the level of oral hygiene and periodontal health. DMFT was used to estimate caries status.

Results: Subjects with poor metabolic control had significantly more plaque accumulations with a mean PI score of 1.87 in group B vs 1.33 in group A. Gingival inflammation was more frequent and severe in group B. Alveolar bone resorption was recorded in 46,67% subjects with poor metabolic control and in 30% subjects with well-controlled glycemia.

Mean DMFT was not significantly different between groups, i.e. 10.57 and 12.39 for groups A and B respectively, although patients in group B had significantly more extracted teeth, almost as twice as in group A.

Conclusion: Significantly higher amounts of dental plaque and more severe gingival changes in children with poor metabolic control indicate the need for early screening of risk factors for periodontal disease. More frequent extractions in these patients indicate negative health behaviors and irregular dental attendance, both of which require correction through education and motivation to establish good oral health habits.

Keywords: T1DM, metabolic control, HbA1c, periodontal health, caries, alveolar bone

Introduction

Diabetes mellitus (DM) has been recognized as a public health problem since the 1970s. [1] Between 1995 to 2010, the incidence of type 1 diabetes mellitus (T1DM)) in children under 15 years of age increased by 4.36%, and this increase was particularly pronounced after 2006 [2]. It is currently estimated that annually about 96,000 young people under 15 years of age develop T1DM globally [3]. Type 1 diabetes mellitus accounts for over 90% of diabetes that occurs in childhood and adolescence [4]. Furthermore, according to a study reported in Lancet, there is a higher increase in the incidence of this disease in developing countries or those that have just undergone economic transition. [5]

T1DM is a chronic metabolic disorder characterized by hyperglycemia. In adults as well as in pediatric patients, the metabolism of carbohydrates, fats and proteins is disrupted due to insufficient insulin secretion. The manifestation of the disease is preceded by a long-lasting, covert autoimmune process of selective destruction of pancreatic beta cells, known as pre-diabetes [6]. It is generally known that untreated uncontrolled diabetes can cause complications on almost all organs in the human body, and the most commonly affected are the heart and blood vessels, eyes, kidneys, nerves and gastrointestinal tract. These complications are mainly due to microvascular and macrovascular damage. A number of oral complications of this disease have also been described in the scientific literature, among which the most frequently mentioned are: increased caries risk, greater susceptibility to periodontal disease, more frequent opportunistic infections (e.g. Candidiasis), and xerostomia [7]. Oral complications are the result of the long-term effects of diabetes and therefore are classified as chronic complications. DM exerts its influence on periodontal tissue through vascular damage, similar to the retina, neural and renal tissue [8]. There is ample scientific evidence that children and adolescents suffering from T1DM compared with healthy children have more gingivitis as well as a higher prevalence and a more progressive form of periodontitis [9, 10, 11]. The existence of an interrelation between diabetes and periodontitis has been established in a way that diabetes increases the risk of periodontitis, but at the same time, the existing periodontal disease makes optimal glycemic control more difficult to maintain. [12, 13]

When it comes to caries, patients with T1DM have multiple risk factors that could lead to an increase in the incidence of this disease. These are primarily the acidity of saliva, i.e. pH that reaches a lower level than in healthy patients, as well as reduced salivary flow leading to slower carbohydrate clearance and increased concentration of glucose in saliva. As a consequence, elimination of dental deposits is impeded being especially expressed at night. [14, 15, 7]

Besides, due to the nature of the disease, these patients often eat more frequently thus exposing dental tissues to more frequent episodes of demineralization. [16,17]

However, reducing dietary carbohydrates to maintain glycemic control can lead to a reduced occurrence of caries in diabetics. This might explain the controversial results of numerous studies looking for answers on whether and in what way does DM influence caries experience.

Recent systematic research [18] reported that while 11 studies found higher caries rate among T1DM patients, four of them had opposite results reporting lower mean DMFT values than in healthy controls, and even 20 studies found no significant difference. Studies that attempted to evaluate the effect of the metabolic control in dental caries occurrence, also gave similarly diverse results, especially when blood concentration of glycated hemoglobin (HbA1c) cut-off of 7% has been used. [18, 19]

HbA1c is a specific retrospective index of glucose regulation in patients with diabetes mellitus. Its values for our region are determined as follows [20, 21, 22]:

- proper glycemic control — HbA1c is $\leqslant 7\%$
- poor glycemic control — HbA1c is $> 7\%$.

Given previous research results, this study aimed to evaluate the effects of metabolic control on oral health status among diabetic children and adolescents using the 7% HbA1c cut-off level.

Material and Methods

Subjects

The study sample consisted of 60 school adolescents diagnosed with T1DM aged 12-18 years. They were diagnosed at the Endocrinology department of Pediatric Clinic in Sarajevo, where the recruitment of these subjects was done.

Two groups of participants were formed, each comprising of 30 subjects: group A consisted of patients with well-controlled glycemia (HbA1c⩽ 7%) and group B consisted of those with poorer metabolic control (HbA1c>7%). The data on metabolic control were obtained from patient medical records.

Recruitment of patients was done according to exclusion and inclusion criteria.

Inclusion criteria were the acceptance to participate in the study and that T1DM was diagnosed at least two years before the study.

Orthodontic patients, those with multiple non-carious dental lesions, and those that had professionally applied topical fluorides within the previous 6 months or that used antibiotics and/or oral antiseptics during the previous 2 weeks were excluded from the recruitment process.

Thirty consecutive patients with poor metabolic control (group B) and 30 consecutive patients with well-controlled glycemia (group A) that attended the Endocrinology department for a regular check-up and met all the criteria, were referred to the Pediatric Dental Department of the Sarajevo Canton Public Health Centre.

Ethical considerations

Ethical Committee of the Faculty of Dentistry University of Sarajevo approved the study design. After the methods and aim of the research were presented to the parents/guardians, informed consent was signed. All patients received necessary dental treatment upon the examinations.

Dental examination:

Clinical examinations were performed in the dental chair, using a straight dental mirror and the World Health Organization (WHO) periodontal probe. One dentist performed all the examinations.

Dental status was assessed using the DMFT index that scores decayed – untreated carious teeth (D), missing (M), and filled (F) teeth.

The teeth with clearly visible cavitated caries lesions, those with caries adjacent to filling and all temporary fillings were recorded as decayed, whereas enamel demineralization with intact surfaces was considered intact teeth. Only teeth extracted due to caries were recorded as missing, and teeth with intact definitive fillings were denoted as filled. [23]

Oral hygiene was assessed according to the Plaque Index (PI - Silness and Löe,1964), whereas Gingival Index (GI - Löe and Silness,1963) was used to determine the condition of the gingiva. The teeth used for the evaluation were: 16, 11, 26, 36, 31, and 46, and scores were recorded for mesial, distal, buccal and lingual surface. Plaque quantity was assessed along the cervical portion of teeth.

Alveolar bone resorption was assessed using orthopantomogram x-rays since periodontal pocket probing is not indicated for patients younger than 15 years 23. Distance between the cementoenamel junction (CEJ) and the alveolar bone margin (BM) was measured, and all findings that were ⩾ 3 mm were recorded as the presence of bone resorption. If the distance was less than 3 mm it was considered as negative evidence of bone loss.

Statistical analysis

Data were analyzed using Microsoft Office Excel. Descriptive data were presented as Mean ± SD (standard deviation) or the percentages. The differences between groups were assessed by using the Student t-test. The differences were considered significant if the p value was less than 0.05.

Results

Sample

Subjects in this study were T1DM patients aged 12 to 18 years, divided into two groups according to their metabolic control. The mean age in group A (good metabolic control) was 15.97 ± 1.59 years, and 15.63 ± 1.99 in group B (poor metabolic control). There was no significant difference in respect to age between groups ($t = 0.79$, $p > 0.05$).

The average duration of T1DM in patients in group A was 8.87 ± 3.13 years, and 8.37 ± 3.12 in group B. T-test did not reveal a significant difference either ($t = 0.61$, $p > 0.05$).

Caries status

The proportion of filled and decayed teeth was almost equal in group A, 46.06%, and 45.43%, respectively, while the proportion of extracted teeth was 8.52%.

In group B, there were 46.24% decayed teeth recorded, followed by 37.37% filled teeth and almost as twice extracted teeth (16.4%) as in group A.

Mean DMFT values with its components are shown in **table 1.**

Mean DMFT score in subjects in group A was lower than in those in group B. However, the difference was not statistically significant. Patients with better metabolic control had significantly lower M component value than those with poor control ($t=2.69$, $p<0.05$). Components D and F did not differ considerably between groups.

Variable	group A (HbA1c ⩽ 7) (n = 30) Mean (SD)	group B (HbA1c > 7) (n = 30) Mean (SD)	P value
DT	4.80 (3.59)	5.73(3.16)	p >0.05
MT	0.90(1.24)	2.03(1.90)	p <0.05
FT	4.87(2.61)	4.63(2.44)	p >0.05
DMFT	10.57(3.22)	12.39(2.97)	p >0.05

Table 1.
DMFT and its components scores

Plaque Index (PI)

The mean PI score was significantly higher among subjects with poorly controlled glycemia (group B), which is shown in **table 2.**

In group A the highest individual PI score recorded was 2, while in group B this value went up to 3.

Group	n	Mean ± SD	t	P
A	30	1.33±0.66	t=2.92	P <0.01
B	30	1.87±0.73		

Table 2. Mean Plaque Index scores

Gingival Index (GI)

In total sample, healthy gingiva without inflammation was recorded in only 8.3% of subjects, mild inflammation in 28.3%, moderate inflammation in 56.7% and severe inflammation in 6.7% of subjects. (**Figure 1**.)

The mean value of the Gingival Index in group B is significantly higher than in group A. In 4 subjects with poor metabolic control Gingival Index score amounted to 3 (severe inflammation), while in patients with well-controlled glycemia the highest recorded score was 2 (moderate inflammation).

Group	n	Mean ± SD	t	P
A	30	1.37±0.67	t=2.72	P <0.01
B	30	1.87±0.73		

Table 3. Mean Gingival Index scores

Alveolar bone resorption

Subjects with poor glycemic control as compared to those with proper control tended to

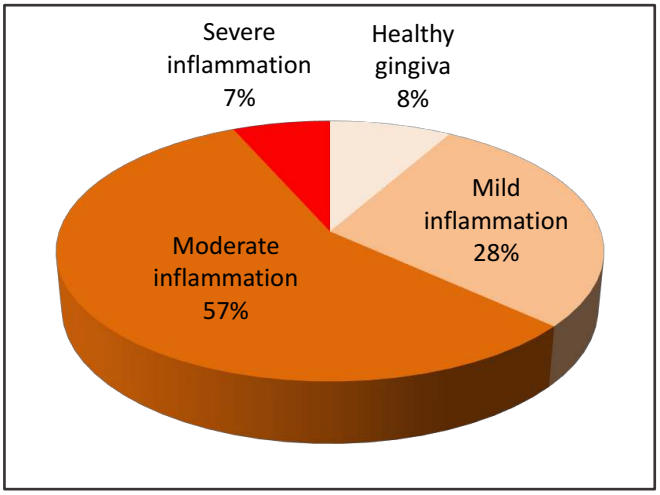

Fig. 1. Gingival index in the total sample (n=60)

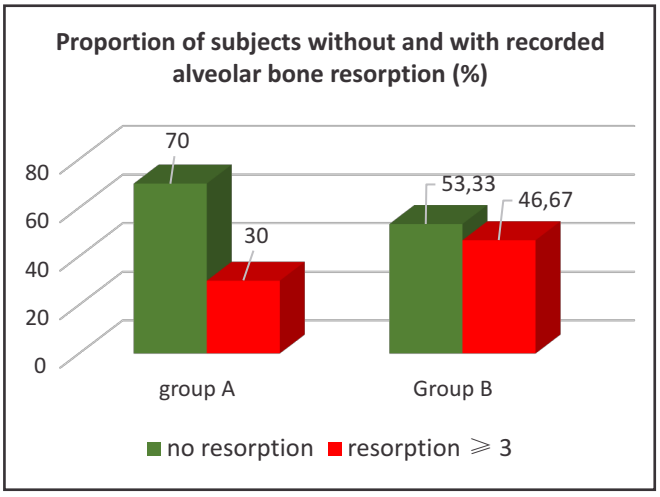

Fig 2. Alveolar bone resorption

show a higher frequency of loss of alveolar bone. The findings are shown in **Figure 2.**

Discussion

The results of this study demonstrated high DMFT values in children and adolescents with T1DM, regardless of metabolic control. The obtained DMFT values in both groups: 10.57 (SD±3.22) in well-controlled patients and 12.39 (SD±2.97) in patients with poor metabolic control, according to the WHO categorization, represent a high severity level [25]. For comparison, the average DMFT of twelve – and fifteen-year-olds in the general population in Bosnia and Herzegovina was reported to be 4.2 (SD±2.9) and 7.6 (SD±4.1) respectively [26].

Unlike a periodontal disease, which is a rather well-documented complication of DM [9, 11, 12, 13, 27], the impact of this metabolic disease on caries development, despite numerous studies, is still not fully elucidated, as the research results are very inconsistent. A recent systematic review [18], as noted in the introductory section, analyzed 35 studies comparing the association of DM and caries and reported that conclusions ranged from those that DM patients had a higher incidence, to those that diabetics had fewer caries lesions, or no difference between diabetics and the healthy controls was reported. Similar results were demonstrated by a systematic review of studies on the oral health of adolescents with T1DM, listing 5 studies indicating a significantly higher experience of caries in subjects with type 1 Diabetes Mellitus compared to healthy controls, 3 studies showing significantly lower values, and 7 studies reporting that there was no difference between the two groups [28].

Explanations could be sought in the multifactorial nature of caries, which is why different aspects of DM can affect development of this oral disease in different directions. Complex interactions of several factors such as reduced amount and flow of saliva, its buffering potential, as well as composition and quality of oral microflora should be considered. Plaque deposits, quality of nutrition and dietary patterns, socio-demographic factors, practices and attitudes related to oral health might also play an important role [7].

Considering the correlation between metabolic control levels and caries rates, which was the subject of this paper, most studies report that well-controlled diabetics have fewer decayed teeth and lower caries incidence and prevalence [29-33].

In our study, the mean value of DMFT in a group with poor metabolic control was somewhat higher compared to well-controlled patients, however, the difference was not statistically significant. This is in line with a study conducted by El-Tekeya et al. [34], which also did not indicate a significant diffe-

rence between well and inadequately controlled diabetic patients.

Accordingly, a recent meta-analysis that examined studies using a 7% HbA1c cut-off level, as we did in our study, showed no statistical difference in DMFT between patients with good and inadequate metabolic control, as well [18].

However, subjects with poor metabolic control in our study had a significantly higher rate of extracted teeth. This is similar to the results of research conducted in Hungary which showed the number of filled teeth was higher in well-controlled than in patients with poorer glycemic control [30]. These findings may suggest a lower motivation for oral health care in these patients and/or sparse dental attendance.

When it comes to periodontal indices, in our study, poorly controlled diabetic patients had significantly higher PI and GI scores. Siudikiene et al. [35] also found higher GI values in poorly controlled subjects, but the differences were not statistically significant.

Another study assessing the correlation between periodontal disease and T1DM in adolescents found that the most periodontal alterations were present in young people with $\geqslant$ 9% HbA1c [36].

Although there is a large body of scientific evidence that diabetes is a major risk factor for periodontitis, studies comparing periodontal indices in young T1 DM patients with respect to glycemic control are sparse.

In a recent Systematic review [37] authors were unable to assess the relationship between glycemic control and periodontal risk markers in children with T1DM due to heterogeneity of the studies.

However, DM has been shown to increase the risk of developing periodontal disease almost threefold compared to healthy individuals, and the risk is higher if glycemic control is poor [38]. Hyperglycemia increases the concentration of glucose in saliva and gingival crevicular fluid, promoting the proliferation of pathogenic bacteria and increases oral inflammation. Besides, the presence of elevated levels of proinflammatory mediators in the crevicular fluid of poorly controlled diabetics also leads to increased periodontal destruction. [39] In a study of experimental gingivitis, after subjects refrained from oral hygiene for 21 days, there was no difference in PI levels between type 1 diabetics and healthy controls, but diabetics developed gingivitis earlier and in a more severe form than healthy patients. [40]

In addition to the aforementioned biological factors, less favorable periodontal parameters in patients with poor metabolic control are likely to be related to behavioral factors and their overall health behavior and practices. Namely, studies examining the oral hygiene habits of insulin-dependent patients have shown that those with regular and adequate oral hygiene habits are more likely to have lower levels of HbA1c [41, 42].

Conclusion

The results of the study showed that children with poorly controlled glycemia have significantly higher amounts of dental plaque as well as more severe gingival changes than children with well-controlled diabetes. This indicates the need for early screening of risk factors for the occurrence of periodontal disease in these patients, to prevent development of a progressive form of periodontal disease.

Although metabolic control has not been found to significantly affect caries rates, more frequent extractions in patients with uncontrolled glycemia indicate negative health behaviors and irregular dental attendance, both of which require correction through education and motivation to establish good oral health habits.

Conflict of interest

The authors declare no conflict of interest.

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CAMERIERE'S THIRD MOLAR MATURITY INDEX IN ASSESSING LEGAL ADULTHOOD ON POPULATION OF BOSNIA AND HERZEGOVINA

Mlaćo Durek Jasmina*[1], Ahmić Amra[2], Mušanović Alma[3], Zukić Selma[2]

[1] Dental Department, The Public Institution Health Centre of Bugojno, Bugojno, Bosnia and Herzegovina

[2] Department of Dental Morphology, Dental Anthropology and Forensic, Faculty of Dentistry with Clinics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[3] Dental Department, The Public Institution Health Centre of Fojnica, Fojnica, Bosnia and Herzegovina

*Corresponding author

Jasmina Mlaćo Durek
The Public Institution
Health Centre of Bugojno
Wagnerova 15
70230 Bugojno

Bosnia and Herzegovina
Email: jasmina.mlaco@yahoo.com
Phone: +387(0)61597773

ABSTRACT

Introduction: Estimation of legal adulthood of an individual lacking identification documents represents one of the main challenges in modern science. This problem occurs in many European countries due to ethnic migration from countries affected by war and financial crisis. Age estimation based on dental parameters has been used for a long time in forensics. The third molar represents an important indicator for age estimation in the period from late puberty to the early twenties, due to its late development.

Aim: The aim of this study was to examine the impact of gender on the reliability and accuracy of the Cameriere's method in legal adulthood assessment based on third molar maturity index of individuals in Bosnian population.

Materials and methods: The study sample consisted of 300 digital orthopantomograms of persons aged between 13 and 24 years. The Cameriere's third molar method was used to estimate legal adulthood. (cut-off I3M<0.08). The real chronological age was used as the reference standard. Results: The sensitivity of a test for cut-off value of 0.08 was 99.4% and specificity 91.0% (95% CI). The proportion of correctly classified individuals older than 18 years was 100% for men and 98.8% for women and for minors 95.2% for male and 88% for female.

Conclusion: This research has shown that the Cameriere's method can be applied in estimation of legal adulthood in the population of Bosnia and Herzegovina, but additional research with a larger sample is needed to form population-specific standards.

Key words: Cameriere's formula, third molar maturity index, legal adulthood

Introduction

As a result of global increase in cross-border migration, many countries have recorded an increasing number of asylum seekers in recent years without valid documentation providing proof of their date of birth. [1] Minors seeking asylum in developed countries are often involved in the process of illegal migration. In order to prevent abuse of the system and protect minors, many countries have introduced procedures for determining the age when it is questionable. [2] Assessing the age of individuals often requires an integrative approach including biological anthropology, medicine, forensic dentistry and radiology. Identification of people and determination of their age based on dentition has been recognized in the field of forensic medicine and forensic dentistry and several methods based on dental changes have been developed. [3] Determining the age of persons for forensic purposes is used as an aid in identifying persons without identity data, as well as in resolving medico-legal, civil or social problems, such as the actual age of minors in adoption processes, asylum seekers and prosecution of pedophilia and other issues. [3-7]

Dental development is a more reliable indicator of biological age in children compared to other parameters. [8, 9] Age assessment procedures based on radiological examinations of dental elements are non-invasive procedures, applicable to living people, simple to perform, give reliable results, reproducible and not very expensive. [10]

Researches have shown that the third molar is the most important indicator of maturity, especially in the period from late puberty to the early twenties, because it is the last element being completed in its development. [4, 7, 10-15] Development of third molars shows a special diversity among ethnic groups and therefore it is proposed to develop specific standards for forensic purposes based on research on differentpopulations. [16, 17] Cameriere et al. published a method for determining adulthood based on the third molar maturity index and determined a specific cut-off value [3, 6, 18].

Aim: The aim of this study is to examine the influence of gender on the reliability and accuracy of the Cameriere's method for determining legal adulthood based on the third molar maturity index in persons within the Bosnian population.

Materials and methods

The sample of this study consisted of 300 digital orthopantomograms of subjects aged 13 to 24, made for other purposes (dental diagnostics and therapy). The analysis was performed retrospectively. The sample was collected in the private health institution "Dental Radiology" Mostar. Consent to use the recordings for research and scientific purposes was obtained directly from patients or, for minors, from their parents / guardians. The data used in the study are the gender, age of the patient and a teeth x-ray. The research was approved by the Ethics Committee of the Faculty of Dentistry with Clinics of the University of Sarajevo (number 02-3-4-203-8 / 2019). Inclusion criteria were: known sex and age of the individual (13-24 years), images of appropriate quality with minimal distortion, without pathology visible on the orthopantomogram. Exclusion criteria were: orthopantomograms without a specified date of birth, images without lower third molars present, image deformations, extracted or rotated third molars, fillings, crown restorations, caries, abnormalities or developmental anomalies that may result in inaccurate measurements. The real age in years was calculated as the difference between the date of recording of orthopantomograms (OPGs) and the date of birth of the subject serving as a reference standard. Dental age was estimated according to the Cameriere's method. Measurements were performed on the lower left molar, for standardization. For the analysis of the third molar maturity index, the widths of the projections of the open apices of the third lower molars (a and b) and the height of the molar (c) were recorded, using the computer program ImageJ. The third molar maturity index (I_{3M}) is defined as it follows: if the root development is complete, or if the root apices

are closed, then the index is calculated as 0. In the case when the root development is not completed, the index is calculated as the sum of the distances between the inner sides of two open apical tip (a, b) divided by the length of the tooth (c), i.e. according to the formula $I_{3M} = (a + b) / c$. Cameriere et al. proposed a cut-off value of I3M<0.08 for the determination of adults of both sexes. If I_{3M}<0.08 the person is considered to be 18 or older, and if $I_{3M} \geqslant$ 0.08 the person is considered to be under 18 years of age. Statistical data processing was performed in IBM SPSS statistical programs Statistics version 25 and MedCalc statistical software version 19.1.7.

Results

43.7% of individuals were male and 56.3% female. **Table 1** shows the number of orthopantomograms, in relation to gender and age.

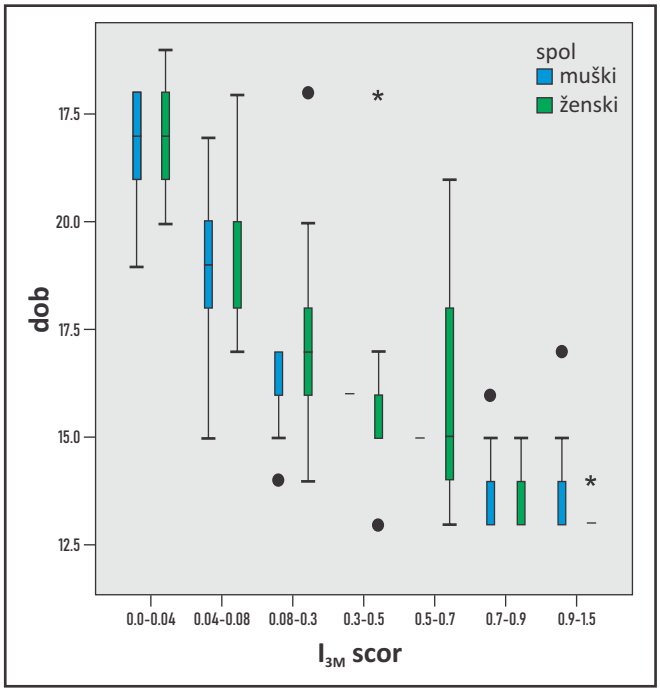
Age (years)	Male	Female	Total
13	26	40	66
14	9	7	16
15	8	13	21
16	8	4	12
17	8	10	18
18	9	14	23
19	6	3	9
20	6	13	19
21	20	18	38
22	10	21	31
23	21	25	46
24	0	1	1
Total	131	169	300

Table 1.
Distribution of subjects by age and gender in the total sample

To test the existence of significance of differences in chronological age between male and female subjects, the t test for independent samples was used. There is no significant difference in chronological age between male and female subjects classified according to I_{3M} ranks (p> 0.05). (**Table 2**) **Graph 1** shows the median and interquartile range of the chronological age of the subjects in relation to the I_{3M} ranks, while the beginnings and

3M rank	gender	N	X	SD	P*
0.0 -0.04	male	52	21.79	1.242	0.631
	female	64	21.89	1.041	
0.04 -0.08	male	21	19.10	1.640	0.900
	female	25	19.16	1.818	
0.08 -0.3	male	15	16.20	.941	0.150
	female	17	17.12	2.233	
0.3 -0.5	male	2	16.00	.000	1.000
	female	9	16.00	2.828	
0.5 -0.7	male	1	15.00	.000	0.734
	female	9	16.00	2.693	
0.7 -0.9	male	20	13.65	.933	0.275
	female	26	13.38	.697	
0.9 -1.5	male	20	13.50	1.000	0.065
	female	19	13.05	.229	

Table 2.
Chronological age in relation to I_{3M} index: number of subjects, arithmetic mean, standard deviation for males and females



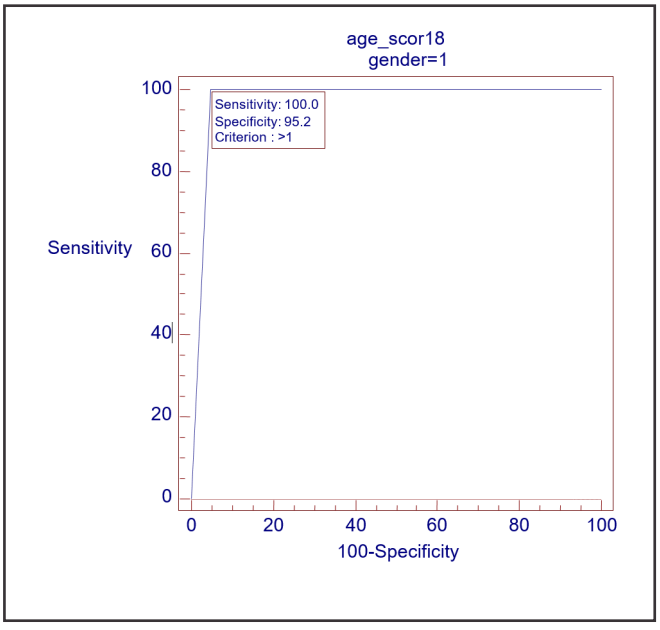
Graph 1.
The relation between chronological age and I_{3M} index ranks for males and females

Gender		Age		Total
		<18 years	⩾18 years	
male	I_{3M} ⩾0.08 (T=0)	59[c]	38[d]	62
		95.2%	4.8%	100%
	I_{3M} <0.08 (T=1)	0[b]	69[a]	69
		0.0%	100.0%	100%
	Total	59	72	131
		45.0%	55.0%	100%
female	I_{3M} ⩾0.08 (T=0)	73[c]	10[d]	83
		88.0%	12.0%	100%
	I_{3M} <0.08 (T=1)	11[b]	85[a]	86
		0.2%	98.8%	100%
	Total	74	95	169
		43.8%	56.2%	100%

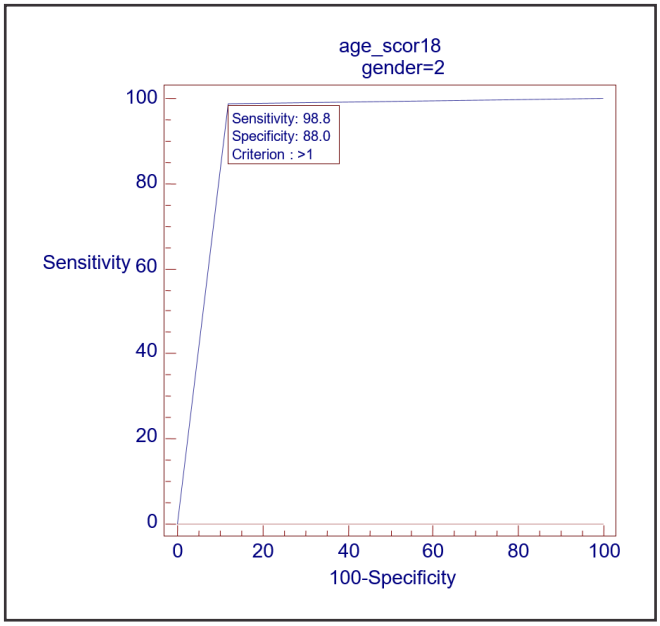
[a] – true positive [b] – false positive [c] – true negative [d] – false negative

Table 3.
Contingency table shows correct classification of I_{3M} in relation to gender

endings of the lines refer to the minimum and maximum values. Deviation values are not included in the boxplot. They are shown by dots and stars. Distribution of real age gradually



Graph 2.
ROC curve of the I3M index 0.08 for age of 18 for males



Graph 3.
ROC curve of the I3M index 0.08 for age of 18 for females

decreases as the value of the index increases (**Graph 1**). The results of the I_{3M} applicability analysis are shown in the contingency table (**Table 3**), where among subjects aged ⩾ 18 years, 69 (100%) males and 85 (98.8%) females were classified as true positive (I_{3M}<0.08). Among minors (under 18 years of age), 59 (95.2%) males and 73 (88%) females were correctly classified (I_{3M}≥0.08).

Overall sensitivity of the test was 99.4% and specificity 91.0%. (95% CI). ROC curves show sensitivity and specificity of the cut off value of I_{3M} index 0.08 in estimation of adulthood for males is shown in **Graph 2**, and for females in **Graph 3**.

Discussion

The results of this study showed that the Cameriere's method of the third molar index is a good method for assessing legal adulthood in persons of both sexes in the population of Bosnia and Herzegovina, with an accuracy of 98.8% in females to 100% in males. However, our results show that the accuracy of determination of minors

is slightly weaker, ranging from 88% for females to 95.2% for males. The Study Group on Forensic Age Diagnostic for age estimations in living persons in order to increase diagnostic accuracy recommends the use of several different examinations in one person, such as physical examination, radiological examination of the left arm, dental examination including dental status and radiological imaging of dentition. Skeletal and dental age assessment plays very important role in the assessment of chronological age.[19]

Despite the fact that development of each person is influenced by genetic, nutritional, climatic, hormonal and external factors, tooth development is considered to be independent of external factors, such as malnutrition, various diseases, mental stress and environmental factors. Even in pathological conditions, tooth development is rarely affected. [5, 20, 21] A realistic age assessment is vital to ensure that children and juveniles are properly identified and treated. [22] Age assessment methods should minimize errors that are technically unacceptable (adults classified as minors) and, if possible, exclude ethically unacceptable errors (minors classified as adults). This applies in particular to the inclusion of criminal liability of presumed juveniles. [3, 18, 23] When assessing age, it is especially important that minors are not classified as adults, as this may lead to a violation of their legal rights, including the right to asylum, exploitation by adults or health services. [24] Two methods are available to assess the age of young adults: analysis of bone morphological characteristics and radiological examination of the third molar. [5, 25, 26] Cameriere and co-workers developed a practical method for determining adulthood based on the relationship between age and the third molar maturity index, which refers to measuring the open apices of the third molar. The limit value of the index I_{3M} = 0.08 was determined. [27] In the study on the population of Bosnia and Herzegovina, the sensitivity of the test was 99.4% and the specificity was 91.0%, which is higher compared to the study of Deitos on the population of Brazil (Se 77.4% and Sp 87.4%), and Cameriere (Se 70%, Sp 98%) and approximately the De Luca study on Italians (Se 86.6% and Sp 95.7%) [4, 5, 28]. Age assessment based on third molars cannot be performed in individuals who lack third molars or are intentionally extracted, and when it is not possible to measure the apical openings or the length of the tooth itself. Some authors in their research point to the possibility that some individuals might intentionally remove their third molars to prevent age assessment. [24]

It should also be noted that there are other useful indicators for estimating age with applicable confidence intervals, which need to be used in combination for the purpose of more accurate results. [24] The final assessment of age should be a synthesis of social and medical history, clinical findings, assessment of age and age calculated by statistical methods. [29] The use of the Cameriere's index in age assessment was first examined in the population of Bosnia and Herzegovina. Analysis of the results of research on the population of Bosnia and Herzegovina shows that the third molar maturity index can be an useful method for assessing adulthood. The development of third molars shows great diversity among different ethnic groups, so development of standards for forensic purposes has been proposed. Population-specific standards will increase the accuracy of forensic age estimates based on third molar mineralization in living individuals. [16, 17] A large number of authors in their studies state the need for larger and more extensive research in order to further examine the accuracy and reliability of certain methods and the involvement of forensic dentists in the legal process of determining the age limit of 18 years.

Conclusion

Research has shown that the Cameriere's method can be used to assess legal adulthood in the Bosnian population. Gender did not significantly affect the accuracy of adulthood assessment, but additional research with a larger sample is needed to form population- specific standards.

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THE EFFECTIVENESS OF LASERS IN THE DENTINE HYPERSENSITIVITY TREATMENT

Mujić Jahić Indira[1]*, Gojkov-Vukelić Mirjana[1], Hadžić Sanja[1], Muharemović Arma[1],Pašić Enes[1], Redžepagić Vražalica Lejla[2], Jelešković Azra[2]

[1] Department of Oral Medicine and Periodontology, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina

[2] Department of Orthodntics, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina

*Corresponding author

Indira Mujić Jahić
University of Sarajevo,
Faculty of Dentistry with Clinics
Department of Oral Medicine
and Periodontology
Bolnicka 4A
71 000 Sarajevo
Bosnia and Herzegovina
Email:imujic@sf.unsa.ba
Phone:+387 (33) 214264

ABSTRACT

Introduction: Dentine hypersensitivity is a common clinical symptom in dental practice.

It is defined as a specific acute, sharp pain arising from the exposed dentine, most commonly in response to thermal, tactile, chemical and osmotic stimuli which cannot be qualified as any other type of dental pathology. The therapy uses various impregnating agents, toothpaste, gels, solutions and laser therapy.

This review aimed to compare the effectiveness of laser application in resolving dentine hypersensitivity among different desensitizing treatments.

Material and methods: the research was done using PubMed and Google Scholar search engines. The keywords were: laser therapy and dentinal hypersensitivity. Selected articles are written from 2009 through 2019. The articles were divided into three groups: dentinal hypersensitivity after bleaching, after periodontology treatment and unknown etiology. Inclusive criteria were: in all three categories we have works showing results of dentine sensitivity treatment using laser.

New technologies in dentistry, as well as more demanding patients, have brought to an application of new treatment methods in order to improve the quality of our patient's life.

Conclusion: Laser therapy has been proven effective in dentine hypersensitivity treatment of any etiology, immediately after treatment and on the long terms, concerning some dentine coatings providing short-term results. The best therapeutic effect is achieved with the combination of laser with some dentine coatings.

Keywords: laser therapy, dentinal hypersensitivity.

Introduction

"Dentine hypersensitivity is characterized by short and sharp pain occurring in the exposed dentine as a response to thermal, chemical, tactile and osmotic stimuli which cannot be attributed to any other defect in teeth or pathology". [1, 2, 3]

Although sensitivity can appear in any part of the tooth, it is most common to be in the cervical parts of the tooth on the vestibular side and on the surface of the root. [4]

Dentine hypersensitivity is a very common clinical symptom increasing in prevalence over the past few years. [5, 6, 7, 8]

The frequency of the occurrence is from 3 – 57% [4, 9, 10]. When it comes to patients suffering from periodontal disease, the occurrence of dentine hypersensitivity is, then, more common, 72 – 98% . [4, 11] Dentine hypersensitivity most often occurs between the ages of 20 to 50, and it is more common in females than in males. [4, 12]

Different factors can lead to dentine hypersensitivity. Parafunctional habits, toothbrush abrasion and microfractures caused by heavy eccentric occlusal forces belong to predicting factors. [8, 13, 14] Gingival recessions combined with abrasions, dehiscence, fenestrations, frenulum stretch, orthodontic movement, as well as gingival recessions, combined with tooth brushing methods and periodontal diseases, belong to the risk factors for the occurrence of dentine hypersensitivity. [8, 15, 16]

Many agents and approaches to treatment have been used in the prevention and treatment of dentine hypersensitivity. Professionally, the most used agents are fluorides which can reduce hypersensitivity with peripheral occlusion of the tubules and the reduction of fluid movement to/from the pulp. Patients can be prescribed with desensitization pastes with higher fluoride concentration (5000 ppm). Apart from fluoride for tubule occlusion, a large number of pastes also contain potassium salts being known to penetrate deep into dentine tubules and spread along with them to deactivate interdental nerve activity leading to the reduction of the action potential.

Additionally, we should list preparations based on HEMA/glutaraldehyde which occlude the tubules and/or stimulate protein sedimentation within them. After the application of astringents proteins can coagulate not entering the tubules, [17, 18, 19]

In clinical practice, adhesives utilizing polymerization are often used to create a protective barrier within the area of exposed dentine. Their main disadvantage is in the fact that a patient removes them by brushing only a few days after. The restorative procedure with liquid composite is taken into consideration when it comes to major damage, but also with combo glass depending on the size of the defect.

Surgical corrections are indicated in the case when a gingival recession is a cause, and they include covering the root with one of the methods of mucogingival periodontal surgery. Covering the root is not 100% successful in suppressing dentine hypersensitivity so it is often necessary to treat the "remaining" hypersensitivity with one of the listed therapy procedures.

Laser was firstly used in the treatment of dentine hypersensitivity by Matsumoto et al in 1985. Since then, many studies have been published examining the effectiveness of laser therapy used in the treatment of dentine hypersensitivity. [20, 21]

It was proven that the combination of laser therapy application and desensitizing agents provides the best results in the treatment of dentine hypersensitivity. [22]

This paper aims to show the results of dentine hypersensitivity treatment with the use of laser alone or in combination with various impregnating agents.

Material and methods

Computer research was used to examine articles published within two databases (PubMed and Google Scholar). Different combinations of keywords were used while researching: laser therapy and dentine hypersensitivity. Papers were published in the period from 2009 to 2019.

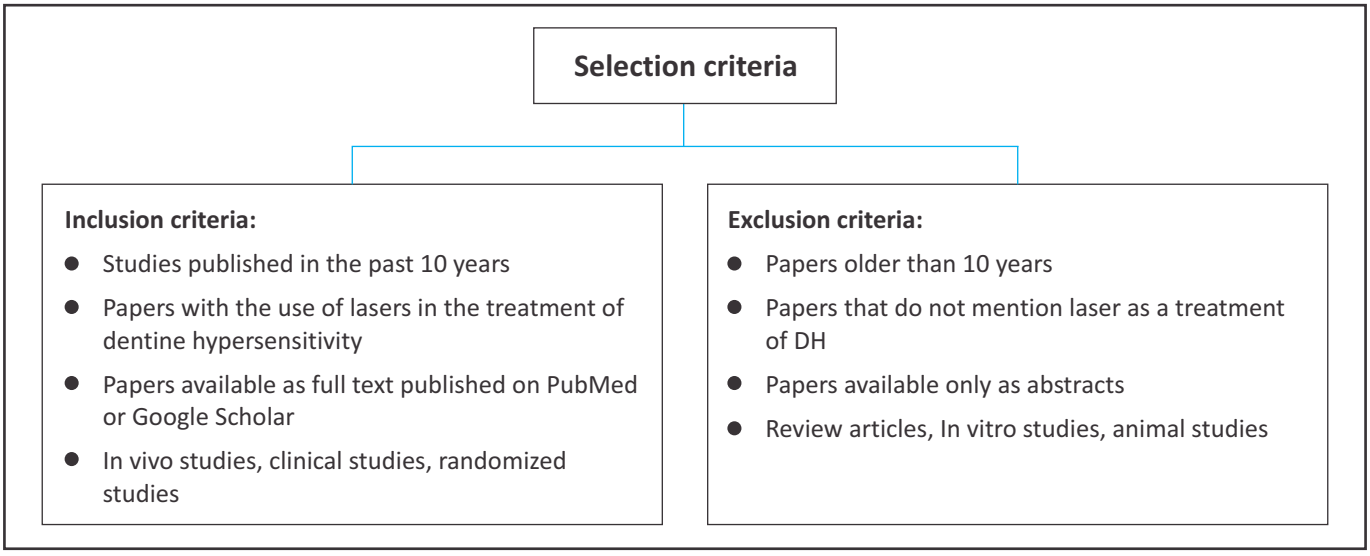

Image 1. Selection criteria

Papers were grouped into three categories: treatment of dentine hypersensitivity after teeth bleaching, after periodontal therapy and treatment of dentine hypersensitivity of unknown etiology.

The inclusion criteria and exclusion criteria based on which papers were selected are shown in schema (**Image 1**).

Results

Of 209 examined papers, 41 papers were selected according to the inclusion criteria, and 168 papers were eliminated according to the exclusion criteria (**Table 1**). Of those papers, 11 are from the group treatment of dentine hypersensitivity as a result of periodontal therapy, 6 papers are from the group treatment of dentine hypersensitivity as a result of teeth bleaching, and 24 papers are from the group treatment of dentine hypersensitivity of unknown etiology (**Table 2**).

Papers are grouped in tables and represented according to authors, year of publication, number of patients included in the study, treatment and specifications, number of visits, scales that were used for pain objectification and results (**Table 3, 4, 5**).

Database	PubMed	Google Scholar	Total
Total of examined papers	126	83	209
Papers older than 10 years	67	32	99
Papers that do not use laser	10	8	18
Review articles	11	12	23
In vitro studies	8	7	15
Only abstracts	5	8	13
Included papers	26	15	41

Table 1.
Overview of bibliography and paper selection

	Unknown etiologies	Result of periodontal therapy	Result of teeth bleaching	Total
Dentine sensitivity	24	11	6	41

Table 2.
Dentine sensitivity by categories

Author, year, reference number	Number of patients	Treatment and specifications	Number of visits	Pain objectification scale	Results
1. Chuan-Hang Yu, Yu-Chao Chang, 2013 (23)	20 patients	Er: YAG laser, 60mJ/pulse, a repetition rate of 2Hz, with water irrigation.	1	VAS (0-100mm)	Er: YAG laser desensitizing treatment can effectively reduce hypersensitivity of cervically exposed dentine.
2. Maura et al. 2019 (24)	60 patients	I. Desensitise KF 2% FGM Potassium nitrate and sodium fluoride 2% II. Clinpro XT Varnish 3M ESPE III. GaAlAs laser, 808nm	4	VAS (0-10)	A significant reduction of dentine hypersensitivity has been achieved in all three groups. There was no statistically significant difference in the reduction of dentine hypersensitivity between these three groups.
3. Yilmaz HG, Bayindir H, 2014 (25)	20 patients (60 teeth)	I.Er,Cr: YSGG laser at 0,25W II. Er, Cr: YSGG laser at 0,5W III. Placebo	1	VAS (0-10)	Both groups with the laser have shown an effective reduction of dentine hypersensitivity concerning the placebo group. However, the group treated with the 0.5 W power laser has shown better results.
4. Aghanashini et al, 2018 (26)	17 patients (40 teeth)	I. Diode laser 980nm wavelength, 320 nm core diameter optic fiber, 0,5 W output power, the 30s II. Fluor protector varnish (by Ivoclar Vivadent)	1	VAS (0-10)	Both diode laser and fluor protectors have proven effective in the treatment of dentine hypersensitivity. Diode laser, however, achieves long-term protection.
5. Umberto et al, 2012 (27)	10 patients (115 teeth)	I. 1,25%NaF, 60s II. Diode Laser GaAlAs (980nm) III. NaF gel at diode laser, the same parameters, the 60s	3	NRS (0-10)	The diode laser has proven effective in the treatment of dentine hypersensitivity, either alone, or when it is used with NaF gel, concerning group I.
6. Baba Z et al, 2018 (28)	12 patients (54 teeth)	I. Nd: YAG laser II. MI-varnish™ treatment layer	4	VAS (0-100)	Evident reduction of dentine hypersensitivity in both groups. Statistically, there is no significant difference between the two treated groups.
7. Dantas EM, Amorim FK, Nobrega FJ, Dantas PM, 2016 (29)	86 teeth	I. Fluoride varnish II. Diode laser (GaAlAs 980nm)	4	VAS (0-10)	Even though both groups displayed good results in the treatment of dentine hypersensitivity, in a short time, however, fluoride was more effective.
8. Suri I, Singh P, Shakir QJ, Shetty A, Bapat R, Thakur R 2016 (30)	30 patients (20 teeth)	I. 5% NaF II. Diode Laser (980nm GaAlAs) III. Group I + Group II IV. Placebo	1	VAS (0-10)	Although reduction of dentine hypersensitivity was achieved in all groups, 5% NaF in combination with diode laser has shown the best results.
9. Yaghini J, Mogharehabed A, Safavi N, 2015 (31)	40 patients	I. Laser toothbrush II. Non-laser toothbrush	4	VAS (0-10)	Even though both groups have shown a reduction of dentine hypersensitivity, the first group has had better results.
10. Mogharehabed A, Khatami H, Abdi Zamharir Z, et al, 2012 (32)	9 patients (60 teeth)	I. Placebo II. 5% sodium fluoride varnish III. Nd: YAG laser(1W,20Hz,120s) IV. Group II + Group III	1	VAS (0-10)	The effectiveness of 5% sodium fluoride and a laser in the treatment of dentine hypersensitivity has proven far more effective than in the placebo group. The group that was treated with the combination of NaF and laser has shown the best results.
11. Machado et al. 2019 (33)	1 patient (2 teeth)	I. Nd: YAG laser, 1060nm II. Glumma desensitizer	1	VAS (0-10)	The effectiveness has been proven in both groups equally.
12. Praveen R, Thakur S, Kirthiga M, Narmatha M 2018 (34)	23 patients (50 teeth)	I. Glutaraldehyde-based topical desensitizing agent II. Low-level diode laser (904 nm), GaAlAs	3	VAS (0-10)	A significant reduction of pain has been proven in both groups after a three-month evaluation period.

Author, year, reference number	Number of patients	Treatment and specifications	Number of visits	Pain objectification scale	Results
13. Ozlem K, Esad GM, Ayse A, Aslihan U 2019 (35)	17 patients (100 teeth)	I. Glumma desensitizer (GCA), II. Nd: YAG laser III. I+II IV: Er, Cr: YSGG laser V. IV+I	5	VAS (0-10)	Dentine hypersensitivity has been significantly reduced in all groups. Er, Cr: YSGG laser with or without GCA has proven the most effective in the treatment of dentine hypersensitivity.
14. Bal MV, Keskiner I, Sazer U, Acikel C, Saygun I 2015 (36)	21 patients (156 teeth)	I. Placebo II. Low-level laser (685nm) III. Desensitizing paste (DP) 8% arginine-calcium carbonate IV. Laser followed by DP (LLL+DP) V. DP followed by laser (DP+LLL)	1	VAS (0-10)	The application of either LLL or DP has proven effective in the treatment of dentine hypersensitivity. However, their combination has not provided an improved effect.
15. Moosavi H, Maleknejad F, Sharifi M, 2015 (37)	31 patients (62 teeth)	I. Low-power red laser (630nm) II. Placebo	1	VAS (0-10)	A significantly better reduction of dentine hypersensitivity in the group treated with the laser than in the placebo group.
16. Genovesi A, Sachero E, Lorenzi C 2010 (38)	15 patients	I. Er:YAG(2940nm)+fluoride gel II. Placebo III. Er: YAG lasers IV. Fluoride gel+placebo	1	VAS (0-10)	A statistically significant difference between groups I and II has been proven. There was no statistically significant difference between groups III and IV. Er: YAG has proven the most effective in the treatment of dentine hypersensitivity.
17. Mobadder et al, 2019 (39)	184 patients	Diode laser (980nm)	1	VAS (0-10)	Postoperative pain has reduced significantly. So, this treatment can be considered safe with a long-term effect.
18. Soares ML, Porciúncula GB, Lucena MI et al, 2016 (40)	23 patients (89 teeth)	I. Placebo II. 2% neutral fluoride gel ,60s III. Nd: YAG laser (1, 10Hz, the 60s) IV. GaAlAs laser (40mW,4J/cm², the 60s)	1	VAS (0-10)	All treatments have momentarily alleviated dentine hypersensitivity, but the results achieved in the group with a laser have shown significantly better results.
19. Lopes AO, Aranha AC 2013. (41)	24 patients (33 teeth)	I. Glumma II. Nd: YAG Laser III. I+II	1	VAS (0-10)	All treatments have proven effective in the treatment of dentine hypersensitivity, but the combination of laser and Glumma has given better long-term results.
20. Lopes AO, Eduardo C de P, Aranha AC 2015 (42)	27 patients (55 teeth)	I. Glumma II. Low-power laser (810nm) III. Low-power laser (810nm) IV. I+II V. I+III	1 1 3 3 3	VAS (0-10)	All groups have shown a reduction of dentine hypersensitivity. The Glumma group had a momentary alleviation, and the combination of glumma with the lasers has given even better results.
21. Gojkov-Vukelic et al, 2016 (4)	18 patients	Low-power diode laser (980nm)	3	VAS (0-10)	Significant effectiveness of laser has been proven in all patients.
22. Hashim et al, 2014 (43)	5 patients (14 teeth)	I. Diode laser (810nm) the 30s II. Diode laser (810nm) 1min	2	VAS (0-10)	The diode laser is effective in the treatment of dentine hypersensitivity.
23. Aranha AC, Pimenta LA, Marchi GM 2009 (44)	101 teeth	I. Glumma Desensitizer II. Seal & Protect (SP) III. Oxa-gel (OG) IV. Fluoride V. Low-intensity laser LILT (660nm)	1 1 1 1 3	VAS (0-10)	After 6 months all treatments have proven effective in the treatment of dentine hypersensitivity, without statistically significant difference between them.
24. Bilichodmath R, Kumar RV, Bilichodmath S, Sameera U 2018 (45)	8 patients (200 teeth)	I. 0.4% stannous fluoride gel II.0.4% stannous fluoride+ diode laser (810nm, non-contact mode) III. Laser only IV. 0.4% stannous fluoride + diode laser (contact mode)	1	VAS (0-10)	All groups have shown a significant reduction of dentine hypersensitivity. however, groups treated with the combination of 0.4% SnF2 and diode laser (both contact and non-contact mode) have shown better results concerning the groups treated with 0.4% SnF2 and laser separately.

Table 3. Presentation of 24 cases of dentine hypersensitivity included in the study, as a result of unknown etiology

Author, year, reference number	Number of patients	Treatment and specifications	Number of visits	Pain objectification scale	Results
1. Dilsiz A, Aydin T, Emrem G 2010 (46)	13 patients (52 teeth)	I. GaAlAs Diode laser (808nm) + desensitizer toothpaste II. Desensitizer toothpaste	3	VAS (0-10)	GaAlAs diode laser + desensitizer toothpaste has proven a bigger degree of desensitization than the control group.
2. George VT et al, 2016 (47)	20 patients	I. Ga-Al-As Diode laser (810 nm) II. Fluoride containing toothpaste	1	VRS (0-10)	After 30 days, effectiveness in the treatment of dentine hypersensitivity was significantly better in the group that was treated with a laser.
3. Sicilia A, Cuesta-Frechoso S, Suarez A, 2009 (48)	45 patients	I. Diode laser (810nm) + placebo gel II. Placebo laser + 10% potassium nitrate bio adhesive gel III. Placebo laser + placebo gel	1	VRS (0-10)	Diode laser in combination with 10% potassium nitrate bio adhesive gel has proven effectiveness in the treatment of dentine hypersensitivity. Diode laser alone has proven effective concerning the placebo effect.
4. Guney Yilmaz H, Kurtulmus-Yilmaz S, 2011 (49)	48 patients (244 teeth)	I. GaAlAs laser (810nm, 500mW, 60s, 8,5J/cm²) II. Placebo laser III. NaF varnish IV. Placebo NaF varnish	1	VAS (0-10)	Both GaAlAs laser and NaF have proven effective in the treatment of dentine hypersensitivity concerning placebo groups, without any significant statistical difference between them.
5. García-Delaney C, Abad-Sánchez D, Arnabat 2017 (50)	30 patients (120 teeth)	I. Laser (660nm) II. No laser activation	1	VAS (0-100)	A significant statistical difference has been proven in the sensitivity between groups with laser activation and no laser activation, and the laser in the first group has proven significantly more effective.
6. Pesevska S, Nakova M, Ivanovski K, 2010 (51)	30 teeth	I. Low-Level Diode laser (630-670nm) II. Topical Fluoride Varnish (Fluor Protector)	3	VRS (0-10)	The reduction of dentine hypersensitivity was significantly better in the group with a laser concerning fluor protector.
7. Raut CP, Sethi KS, Kohale B, Mamajiwala A, 2018 (52)	30 patients (99 teeth)	I. Laser without activation II. Laser AlGaInAs (940nm) +0,4%SnF2 III. Only laser	4	VRS (0-10)	Only laser or in combination with 0,4%SnF2 is effective in the treatment of dentine hypersensitivity. A significant reduction of dentine hypersensitivity was in groups II and III concerning the control group I, but there was no significant difference in the reduction of dentine hypersensitivity between groups II and III.
8. Clavijo EMA, Clavijo VRG 2009 (53)	28 teeth	I. PO 3%/Baseline; potassium oxalate 3% (OxaGel®, Kota) II. Laser/Baseline; Low-level diode laser	1	VAS (0-10)	It has been proven the both PO and laser are effective in the reduction of dentine hypersensitivity. However, the laser still provided better results.
9. Doshi S, Jain S, Hegde R, 2014 (54)	30 patients	I. GaAlAs laser (200-660nm) II. Placebo (laser without activation)	1	VRS, VAS (0-10)	A statistically more significant reduction of dentine hypersensitivity has been achieved in the first group.
10. Tabibdzadeh et al, 2018 (55)	8 patients (62 teeth)	Diode laser (980nm)	3	VAS (0-10)	Statistically significant results have been achieved after the treatment concerning the VAS scale.
11. Etemadi A, Sadeghi M, Dadjou MH 2011 (56)	40 patients	I. Low-level laser (660nm) II. Placebo (non-activated laser)	3	VAS (0-10)	Dentine hypersensitivity in the group with a laser has been reduced after the first treatment, which speaks in favour of the effectiveness of the laser in the treatment of dentine hypersensitivity.

Table 4. Presentation of 11 cases of dentine hypersensitivity, as a result of periodontal therapy

Author, year, reference number	Number of patients	Treatment and specifications	Number of visits	Pain objectification scale	Results
1. Kossatz S, Dalanhol AP, Cunha T, 2011 (57)	30 patients	I. Light-activated (470nm) II. Laser diodes (830nm) III. Non-activated	2	VAS (0-4)	Treatment in group I has proven most effective.
2. Moosavi H, Arjmand N, 2016 (58)	66 patients	I. Placebo II. LLLRL Diode laser (660nm) III. LLIL Diode laser (810)	1	VAS (0-10)	810 nm LLIL was more effective in the treatment of dentine hypersensitivity than 660nm laser. Both lasers have shown a significant pain reduction concerning the placebo group.
3. De Al meida Farhat et al, 2014 (59)	16 patients	I. LED II. LED-laser (300Mw/CCM²)	2	VRS (0-10)	The LED laser has proven ineffective in the treatment of dentine hypersensitivity after teeth bleaching.
4. De Alencar CM, De Paula B, Lamartine JNA 2018 (60)	25 patients	I. GPLACEBO: Glaser with no laser activation (placebo effect), after the teeth bleaching treatment, 5000ppm fluor preparation applied after 5 min II. GLASER-LLLT Glaser (808nm)	4	VAS (0-10)	Significant pain reduction after teeth bleaching treatment in the group treated with Glaser concerning Gplacebo.
5. de Almeida LC, Costa CA, Riehl H, dos Santos PH 2012 (61)	40 patients	I. Home bleaching with 10% carbamide peroxide, 4h/d II. 35% hydrogen peroxide, 10 min III. Quartz-tungsten-halogen light irradiation, 10mins IV. LED/laser light irradiation, 10min	3	VAS (0-10)	All of the techniques have led to sensitivity, only the Laser light irradiation technique has had a minimal sensitivity as a consequence, which speaks in favour of the fact that the laser as a treatment of choice in the therapy of dentine hypersensitivity reduction can be used for the process of teeth bleaching as well.
6. Martin J, Eduardo F, Valeria B, Andrea W, 2013, (62)	88 patients	I. H2O2 15% + TiO2 + light II. H2O2 35% + light III. H2O2 35%	3	VAS (0-10)	The sensitivity after treatment was the same in all three groups immediately after the treatment.

Table 5. Presentation of 6 cases of dentine hypersensitivity, as a result of teeth, bleaching

Discussion

With this review paper we tried to consolidate the results of published papers that examined the effectiveness of lasers in the treatment of dentine hypersensitivity, as a result of periodontal therapy, dentine hypersensitivity as a result of teeth bleaching and as a result of unknown etiology. The majority of authors addressed the treatment of dentine hypersensitivity of unknown etiology.

Treatments of used choice were Er:YAG laser of various wave lengths [25, 27, 37, 40, 41]; desensitisation agent KF2% FGMO; Potassium nitrate and sodium fluoride 2% [26, 34]; Glass ionomers – ClinPro XT Varnish 26; Photo laser, low power diode laser GaA1As 808nm [26] and 940nm [36, 49, 50, 55] and 980nm [29, 31, 42, 45]; Fluor irrigation [31, 54]; Diode laser 980nm [28, 32, 48, 58]; Fluor protect [28]; 1,25% NaF [29, 52]; 5% NaF [29, 32]; NaF gel in combination with diode laser 980nm [29, 32]; Nd:YAG 1060nm [30, 34, 35, 37, 42, 43]; dentine varnish [30]; laser toothbrushes [33]; combination of Nd:YAG and 5% sodium fluoride varnish [34]; Glumma [35, 37, 43, 44, 47]; Glutaraldehyde topical gel [36]; combination Er:YAG plus Glumma [37]; low power laser at wave lengths 685nm [38], 630 [39], 660nm [47, 53, 54, 57, 59, 61]; 2% fluor gel [42, 47]; Nd:YAG laser plus Glumma [43]; diode laser 810nm [46, 44, 51, 52, 61]; Seal protect [47]; Oxa gel [47]; 0,4% SnF2 [48]; 0,4% SnF2 plus diode laser 980nm [48]; toothpaste for sensitive teeth [49, 50].

Treatment of dentine hypersensitivity of unknown etiology

In the treatment of dentine hypersensitivity of unknown etiology, after examination and analysis of 24 papers, all authors mention that there was a statistically significant difference in the reduction of pain in teeth treated with some choice of the treatments concerning the teeth or groups that were treated by a placebo.

In the papers using only laser, a significant reduction of dentine hypersensitivity was achieved after the evaluation period. However, high power lasers provided better results in a shorter period. That difference was lost on long-term basis. In the papers using teeth laser and others using some of desensitizing agents, either varnish, coating or gels, immediately after intervention there was no statistically significant difference between laser and various desensitizing agents, but on the long-term basis after the period of evaluation teeth treated with laser still showed a positive therapeutic effect, whereas it disappeared in teeth treated with some of desensitizing agents.

The best therapeutic effect immediately after, as well as in control examinations period, proved to be a combination of a laser and some dentine coating in the treatment of dentine hypersensitivity.

Treatment of dentine hypersensitivity as a result of periodontal therapy

In the treatment of dentine hypersensitivity as a result of periodontal therapy, after examining and analysing 11 papers, it can be concluded that results are statistically significantly better in teeth treated with laser.

Treatment of dentine hypersensitivity as a result of teeth bleaching

In the treatment of dentine hypersensitivity as a result of teeth bleaching, after examining and analysing 6 papers, in all of them, treated with laser and treated with fluorine, there was a reduction of dentine hypersensitivity concerning the placebo groups. Better and long-term results have been achieved in teeth treated with laser. This speaks in favour of the effectiveness of laser in the treatment of dentine hypersensitivity and this etiology.

Both diode lasers and high-power lasers were used in the papers. Undefined was which of those two groups of lasers is more effective, and this could be the subject of some further research.

Conclusion

Laser therapy has been proven effective in the treatment of dentine hypersensitivity of any etiology, immediately after treatment as well as on long-term basis, concerning some dentine coatings providing short-term results. The best therapeutic effect is achieved with the combination of laser with some dentine coatings.

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DENTAL AGE ESTIMATION IN CHILDREN, ADOLESCENTS AND ADULTS

Selmanagić Aida[1], Ajanović Muhamed[2], Šečić Sadeta[3], Kamber-Ćesir Alma[2], Smajić Rubina[4], Dervišević Almir[5]

[1] Department of Dental Morphology with Dental Anthropology and Forensics, Faculty of Dentistry with Clinics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[2] Department of Prosthodontics, Faculty of Dentistry with Clinics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[3] Department of Oral Surgery, Faculty of Dentistry with Clinics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[4] Department of Preventive and Pediatric Dentistry, Faculty of Dentistry with Clinics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[5] Department of Maxilophyacial Surgery, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina

*Corresponding author

Aida Selmanagić
Assistant Professor, Ph.D
Department of Tooth Morphology with Dental Anthropology and Forensics
Faculty of Dentistry
University of Sarajevo,
Bolnička 4a
71000 Sarajevo

ABSTRACT

Age estimation, using forensic odontology, is a crucial step for biological identification and it plays an important role in pediatric and orthodontic dentistry, pediatric- endocrinology, archeology, paleo stomatology and in forensic dentistry as well.

Nowadays, we can use numerous methods for estimating age in various stages of life using dentition. It's very important to know that methods were developed based on the relationship between age and characteristics of tooth structure to assess the age in children and adults and which are most commonly used.

In this study we wanted to present reasons for age estimation as well as the different methods we can apply in the age assessing process.

Key words: age estimation, dental age estimation, methods for age estimation

Key words: hypersensitivity, toothpaste, pro-arginine

Introduction

Forensic Odontology is the part of dentistry which does have the fundamental role to deal with the proper handling and examination of dental evidence, and also to present the proper evaluation as well as the proper dental findings. Forensic means "court of law" in Latin language, while odontology refers to the study of teeth. [1]

The persons' identity may be established by assessing one's age, which is a procedure accepted by many anthropologists, archaeologists and forensic experts. [2]

Age estimation plays an important role in pediatric and orthodontic dentistry, paediatric-endocrinology, archeology, paleo stomatology and in forensic dentistry as well. [3, 4, 5, 6, 7]

Today, many cases such as unidentified cadavers, human remains as well as the number of remains lacking age documentation, require age determination. [8] This requires age estimation, not only for differentiating the juvenile from the adult status in criminal law cases, but especially to determine the age of a crime victim. There are requires for chronological age estimation in relation to school attendance, social benefits, employment and marriage as well [8, 9] Furthermore this procedure is necessary to be done in the cases of the state administration (adoption, motorcycle driver license, passport release etc. [10], and also when patient suffer from amnesia. [6]

Also, as the consequence of economic globalization and European integration, the number of immigrants increase in the countries with high living standard, which implies the dental age determination of the incoming population through orthopantomogram analyses. [11,12]

The UN Protocol to Prevent, Suppress and Punish Trafficking in Persons, especially Women and Children (2000) definition of human trafficking implies the exploitation of a person by means of the threat or use of force. The most recent reports state that trafficking victims were identified in 124 countries (2010–2012) what is considered like the global issue.

There is a different motivation for people trafficking, including: sexual exploitation, forced labor in, for example, catering, domestic servitude, textile production, construction, forestry, mining, forced military recruitment, begging etc. and involved victims are usually originating from less developed countries.

Children are vulnerable to trafficking and they are also involved in criminal activities, often with no proper identity documents. [13]

According the UN guidelines the different age groups take over the different kind of law responsibility. It implies the correct age of subject have to be assessed.

The interest in the age assessment of living young persons with no birth certificates are available, is not a recent phenomenon and does have a long history. For example, in ancient Rome adolescents were considered mature enough to be involved into military service, as soon as their second molars had fully emerged. [14]

In England's history there is also one of the well-known attempts using teeth as the indicator of age where juvenile work and criminality were serious social problems when dentist Edwin Saunders, was the first to publish information regarding dental implications in age assessment by presenting a pamphlet entitled „ Teeth A Test of Age "to the English Parliament in the 19 th. century. [15]

Age estimation is a complex and challenging task. It's the main aim is to find the best optimal method for legal, medical age estimation which is in the same time simple, reliable and reproductive, that can be applied in both, living and in the decreased persons as well. [15]

There are many various methods for age estimation today. Andre Luiz Bergamo et al. (2016) in their extensive investigation made conclusion that the techniques based on the relationship between age and characteristics of tooth structure to estimate the age in children and adults are developed and most commonly used. [16]

So, dental age estimation methods may be classified as:

1. According to the degree of development of the dentition:
   - Methods applied to the forming dentition
   - Methods for the adult fully formed dentition.
2. According to the technique of investigation:
   - Clinical or visual
   - Radiographic
   - Histological
   - Physical and chemical analysis.

Then, methods to be employed in dental age determination using dentition can be grouped into 3 phases:

1. Age estimation in prenatal, neonatal and early postnatal child
2. Age estimation in children and adolescents
3. Age estimation in adults.

In the literature we can find description of several techniques addressing age determination in adults. Different methods are classified into three categories: morphological, biochemical as well as radiological methods. [15, 16, 17]

We can also make difference between non-invasive and invasive methods in the process of age assessment in permanent dentition:

1. Non-invasive methods:
   - Age assessment using Gustafson and Koch method
   - Age assessment using Schour and Massler method
   - Age assessment using third molar development
   - Age assessment using physiological changes of the teeth structure during the period of use (chromogenic changes of teeth, attrition, periodontal destruction, apposition of secondary dentin and/or cementum).
2. Invasive methods:
   - Age assessment using Gustafson method
   - Age assessment using quantification of tooth cementum annulation
   - Age assessment using aspartic acid racemization. [14]

When we talk about age of person, we make distinction between biological, chronological and dental age. [18]

In many cases, chronological age and biological age may not to be same, because of their developmental variations. [15] And it is well known that somatic development is connected to chronological age. [19]

The most commonly used developmental indicators include:

- Tooth development and eruption- Dental age
- Skeletal maturity- Skeletal age
- Sexual development- Sexual age
- Body height and weight [19,20,21]

Some investigators have suggested the applicability of the bone age determination method, while others choose advantages of the dental age determination method. They underline that teeth mineralization is less affected by external factors. Yet, many authors have advocated to combine both type of methods in different populations. [22] Also, dental maturity is more relevant in comparison to the teeth emergence into the oral cavity, so it is less affected by nutritional and endocrine status. [15,23]

Teeth represent useful material for age estimation [8, 24] as being the most resistible structure in the human body able to survive every disaster such as fire accidents, bomb blast, plane crashes, mass disasters etc. [25] So, everyone have to be aware of dental evidence importance for forensic age determination procedures. [26]

Moreover, dental post-mortem data can help us in predicting the age of person from approximately 18-20 weeks in utero until the last tooth is lost. [26]

In the line with recommendations suggested by the international and interdisciplinary Study Group on Forensic Age Diagnostics established in 2000., in Berlin, every expert's finding should comprise three independent evaluations by forensic experts in the relevant disciplines:

- Clinical examination- the recording of body measurements and an evaluation of signs of sexual maturity.
- Skeletal examination- X-ray examination of the left hand.
- Dental examination- dentition status and evaluates on orthopantomogram. [12, 30, 31, 32]

Methods for age estimation in children and adolescents include:

1. Schour and Massler's method (1941)
2. Nolla's method (1960)
3. Morrees, Fanning and Hunt method (1963)
4. Demirjian's method (1973)
5. Open apex method (Cameriere method)
6. 3rd molars [1]

We can assess the dental age amongst children with greater accuracy, so many teeth are undergoing development and calcification simultaneously. But this accuracy is in descending with the completion of a person's dental development. [6, 24, 26]

Although development process of teeth is one of the most valuable biomarkers for age estimation in childhood [27], age estimation is especially difficult after the age of 14 because all of the teeth, except unerupted ones, are in the process of completing their apical formation, in 16 or 17 years. [28] So, the third molars are staying to continue maturing to later age only. [26]

Furthermore, according the suggestions of Study Group on Forensic Age Diagnostic, one essential criterion for dental age assessment is the evaluation of third molar mineralization and eruption. [12, 29, 30, 31]

„ Dentes sapientes "show the greatest frequency of agenesia, also their variations in shape, size, position, time of maturation and time of eruption and tend to appear earlier in males than females. [32, 33] Robinson (1993.) described these characteristics of third molars with this aphorism: „The only thing we can predict about a third molar is its unpredictability. " [34]

Although the third molars show great variability in their dentition, they stay like the most reliable biologic indicator available for age determination during the middle teens and early twenties, [6, 33] because they are the latest teeth to initiate and complete development. [26, 34]

Conclusion

There are many different methods for age estimation today. But age estimation is a complex and challenging task so it's the main aim is to find the best optimal method for legal, medical age estimation which is simple, reliable and reproducible and that can be applied in both, living and in decreased persons as well.

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ROOT CANAL TREATMENT MODIFICATION AT PATIENT UNDERGOING LONG-TERM BISPHOSPHONATE AND CYTOSTATIC THERAPY

Hasić Branković Lajla[1]*, Tahmiščija Irmina[1], Korać Samra[1], Džanković Aida[1], Hadžiabdić Naida[2]

[1] Department of Dental pathology with endodontics; Faculty of Dentistry University of Sarajevo.

[2] Department of oral surgery with implantology, Faculty of Dentistry University of Sarajevo.

*Corresponding author

Assistant Professor
Lajla Hasić Branković, M.Sc, Ph.D,
Dental Pathology and Endodontics specialist,
University of Sarajevo,
Faculty of Dentistry with Clinics,
Department of Dental Pathology and Endodontics,
Bolnička 4a,
71000 Sarajevo,
Bosnia and Herzegovina
Phone: +387 (33)214 249
e-mail: lajlabrankovic@gmail.com

ABSTRACT

Introduction: In order to prevent osteonecrosis in a patient undergoing bisphosphonate therapy, American Association of Endodontists (AAE) developed a protocol for dental treatment. There are not any precise recommendations whether root canal treatment is indicated if there is an extensive periapical lesion.

Case report: The paper presents root canal treatment of teeth 36 with apical periodontitis and sinus tract at a 39 year old patient on long-term bisphosphonate therapy and complex health issues: Sy. Sjögren, osteoporosis, hypothyreosis, temporomandibular joint dysfunction. The modification of root canal treatment emerged as consequences of:

1. Increased risk of osteonecrosis as a result of long-term bisphosphonates therapy,
2. Impossible rubber-dam placement due to a constant cough impulse caused by Sy. Sjögren, resulting in risk of mucous irritation with irrigants,
3. Temporomandibular joint dysfunction requiring shortening appointment duration,
4. Modification of the inter-appointment canal medication due to cytotherapy that patient simultaneously receives,
5. Significant obstruction of the root canals established during the treatment. According to previous, the appointments duration were shortened using a single-file technique, adequate chemical treatment with 5.25% NaOCl in gel form (lower risk of mucosal irritation) and intracanal medication by a combination of Ca(OH)2 and chlorhexidine.

Control X-ray showed satisfactory signs of apical healing. The final success evaluation requires an extended observation period, due to the possibility of subsequent osteonecrosis associated with bisphosphonate therapy.

Conclusion: The number of patients on bisphosphonate therapy increases daily with simultaneously decreasing age limit for osteoporotic changes.

This requires serious clinical research and development of more precise endodontic protocols.

Keywords: bisphosphonates, osteoporosis, Sy.Sjögren, root canal treatment.

Introduction

Although American Association of Endodontists (AAE) has a protocol for dental treatment of patients submitted to bisphosphonate therapy, there are not any precise recommendations if endodontic therapy is indicated while a pathological process of endodontic etiology is already present in the bone. [1, 2, 3]

Bisphosphonates (BPs) are the principal therapy for osteoporotic changes. They are proscribed worldwide, nowadays at a relatively early age, probably due to advanced diagnostic procedures. Besides this, BPs are adjuvant therapeutics for cancer patients with metastatic changes in bones. Like any other medication, BPs shows serious side effects. Osteonecrosis of the jaw is one of them. It is the main concern with important medical and dental implications. [4, 5, 6]

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) occurrence varies between 0% and 28% of all patients on BPs therapy. [4, 7, 8]

Patients on BPs therapy have increased risk of developing BRONJ after tooth extraction. Therefore, the dentist should escape or delay tooth extraction as much as possible. [4] According to the literature, the healing rate of periapical lesions in patients undergoing BPs therapy is not different than in general population. Root canal treatment is recommended as a non-surgical alternative, especially with modern endodontics methods. [9]

Many patients simultaneously receive chemotherapy and/or corticosteroid therapy, due to their main disease (cancer, for example). [10, 11, 12, 13]

It is well-known how chemotherapy and corticosteroid treatment can interfere with root canal therapy. [4, 12, 14, 15]

In the same time, root canal treatment can trigger BRONJ as a consequence of soft tissue damage, which can occur during rubber dam placement, and /or apical extrusion of infected debris. [4, 16]

In this particular clinical case, our second big concern was a fact that the patient has Syndrome Sjögren. Implications of dry mouth syndrome on caries prevalence and its complications are well documented.

Some clinical recommendations for BRONJ risk-reducing procedures couldn't fully comply as a result of Syndrome Sjogren. [4, 16]

For example, a rubber dam placement was difficult cause of constant cough impulse. The patient couldn't use chlorhexidine mouth rinses as a precaution of infection, due to her extreme mucosal sensitivity. Irrigants selection and usage were limited for the same reason.

Temporomandibular joint dysfunction was an additional aggravating circumstance.

Case Report

The paper presents a report of a possible modification of standard endodontic therapy protocol in a female patient with complex health problems: Sy. Sjögren, Osteoporosis, Hypothyreosis, Temporomandibular joint dysfunction.

Anamnestic data:

In 2008 a patient was diagnosed Sy. Sjögren as well as sensitive polyneuropathy. Osteoporosis was discovered shortly after. The patient was submitted to continuous corticosteroid therapy (Medrol 4 mg) since then. Bisphosphonates were administered shortly after, in the form of Bonviva (ibandronic acid), one dose per month.

Recently, the rheumatologist additionally proscribed 400mg of Endoxan, in the form of 6 boluses administered intravenous one per month. A problem occurred on tooth 36 between the second and third cycle of chemotherapy.

After a short period of intense pain, a fistula appeared next to the tooth.

Clinical findings:

The tooth crown of lower left first molar was restored with a rather poor composite filling. The tooth was slightly sensitive on percussion. Sinus-

tract was present in the time of examination. Thin gutta-percha point was positioned in the sinus tract and X-ray was made. (**Figure 1**)

Rubber dam couldn't be placed due to constant cough impulse. Through the access cavity, orifices of four root canals were exposed. (**Figure 2**)

Root canals were extremely narrow. The initial glide-path was achieved with small hand path-finders (ISO#.06 and .08). Canals had to be hand-instrumented till width ISO# 15.

Regarding a TMJ dysfunction, followed by a difficult mouth opening, we tried to achieve as short as possible visit duration. "Single-file" machine -drive rotary technique was reasonable selection. The endo motor was used in continuous rotating mode. Torque was set on 2 Ncm, at speed of 250 rpm.

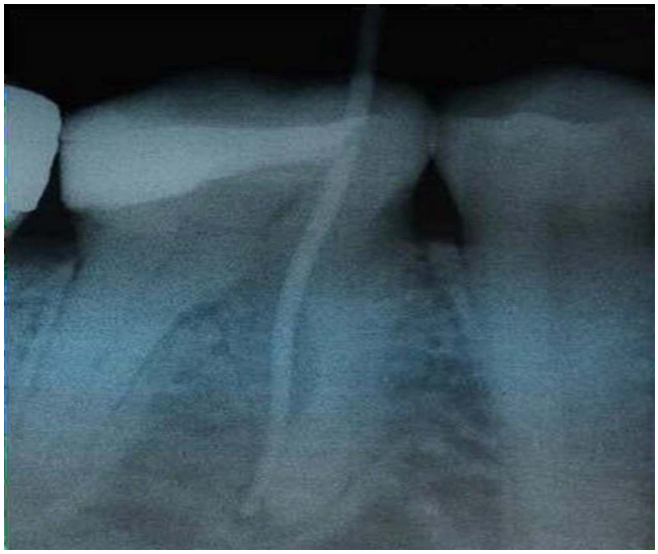

Figure 1. Initial X-ray. Thin gutapercha point was inserted into sinus tract.

Figure 2. Indirect view of the entrances to root canals.

"Single-file" "T-One File Gold" (Global top Inc. ® Co) was used during operation. An adequate chemical debridement was achieved by using 5.25% NaOCl in the gel form, decreasing the risk of mucosal irritation (Chloraxid 5.25%, Cerkamed, Pl) (**Figure 3**.). A gel form of NaOCl doesn't smear over mucosa.

Figure 3. Cholaxid gel, adopted from https://cerkamed.com/product/chloraxid-525-gel/

Gel 17% EDTA "Endo-Prep Gel" and a combination of 15% EDTA and 10% urea peroxide (Endo-Prep Cream, Cerkamed, Pl) were used as lubricants needed for rotary instrumentation. Extended inter-seance medication was performed by combining the Ca $(OH)_2$ and chlorhexidine-based gel prepared by manual mixing Calcipast and GlucoHex 2% Gel, (Cerkamed, Pl) in a 1:1 ratio.

Inter-seance root canal medication was adapted to the rhythm of chemotherapy (10 days before next, and 20 days after the previous bolus of Endoxan). The sinus tract was closed after the first session, although biomechanical treatment of canals was not completed in a satisfactory degree.

Considering sclerosis and difficulties to keep mouth open for a long time period, canals couldn't be instrumented enough in the first appointment. As a result of temporomandibular dysfunction, as well as, constant cough impulse, work had to be constantly interrupted to give the patient an opportunity to rest her joints. Saliva was controlled simply by weak saliva ejector. Strong saliva ejector was used only in phases of copious irrigation. A burning sense of dry mouth additionally impeded procedure. The patient was allowed to use 2-3 drops of D3 vitamin every 10 minutes or so, to keep

her mucosa protected from irritants. Same precaution measures repeated in the successive appointments.

The canals were further instrumented by each subsequent session. Medication was repeated at monthly intervals three times. After completion of cytotherapy, we decided to definitive obturation. Canals were obturated with the sealer and gutta-percha points gauge ISO # 25 / .07 in "single-cone" technique ("Primary" gutta-percha point, Gapadent Co., Ltd.). (**Figure 4**)

Control X-ray showed adequate obturation accuracy of the root canals (**Figure 5**).

The tooth was restored with direct composite filling in the next session (**Figure 6**).

Discussion

The therapy was successfully completed, regardless of relative unfavorable prognosis and objective difficulties during clinical work. In principle, osteonecrosis is more common in a mandible than in maxilla. [1, 2] Complications are more common in combination with steroid therapy, which our patient receives caused by polineuropathy and Sy. Sjögren. [4]

Risk of root canal treatment failure is significantly higher in patients undergoing chemotherapy. [18]

Risk of BRONJ development is higher as BPs therapy is longer. [4, 18]

Regardless of the high comorbidity and objective difficulties during the work, the classical endodontic treatment with few adjustments showed an acceptable result.

This confirmed the fact that patients on long-term BPs therapy can expect a suitably periodontal healing rate after conventional root canal treatment. [19]

In this particular clinical case, recommended endodontic protocol [4] needed a few adjustments.

Chlorhexidine mouthwash rinse was too aggressive, so we decided to skip this step. Aseptic conditions were not established cause rubber-dam placement was impossible.

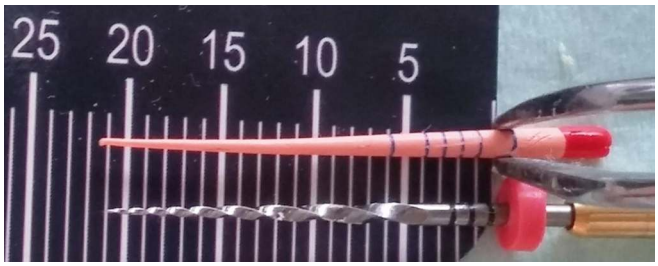

Figure 4. "Single-file" "T-One File Gold"(Medium) endo-file and matching “Primary" gutta-percha point (ISO # 25 / .07), Gapadent Co., Ltd,Corea.

Figure 5. Control X-ray after definitive obturation.

Figure 6. Final composite restauration.

A gel form of NaOCl showed good cleaning properties. Simultaneously, it had a low irritant effect on the mucosa. We used Nickel-titanium single file in rotary mode to avoid reciprocating

systems due to their possible apical debris extrusion. [4] Single-cone obturation technique minimizes the risk of overfilling or overextension. The requirement for a single visit endodontic was impossible to achieve due to TMJ dysfunction. Bisphosphonates are associated with osteonecrosis, but there is not enough documentation concerning the root canal obstructions related to long-term BPs therapy.

Conclusion

After completion of endodontic therapy, control X-ray showed satisfactory signs of apical periodontium healing. However, the final evaluation of endodontic therapy success, in this case, will only be possible through the next follow-up period since there is a possibility of osteonecrosis associated with bisphosphonate therapy.

Regardless of the high comorbidity and objective difficulties during the work, classical endodontic treatment showed good results.

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