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Analysis of the mineral composition of hypomineralized first permanent molars

Analiza mineralnog sastava hipomineralizovanih prvih stalnih molara

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Abstract

Background/Aim. Hypomineralization of molars and incisors (molar-incisor hypomineralization – MIH) is defined as enamel hypomineralization of systemic origin of one or more of the four first permanent molars, which may be associated with changes in the maxillary, and less frequently in the permanent mandibular incisors. The aim of this study was to investigate the mineral content in hypomineralized teeth as a contribution to under-standing the origin of these changes, which will be important for effective restorative approach. **Methods.** A total of 10 extracted first permanent molars diagnosed with MIH were used in the study as the experimental group, and intact first premolars extracted for orthodontic reasons were used as the control group. A certain surface of hypomineralized and healthy enamel and dentin was analyzed using a scanning electron microscope equipped with an energy-dispersive spectrometer (SEM/EDS). **Results.** By conducting quantitative chemical analysis of the distribution of the basic chemical elements, it was found that the concentration of calcium (Ca) and phosphorus (P) was significantly higher in healthy enamel (Ca = 28.80 wt%, and P = 15.05 wt%) compared to hypomineralized enamel (Ca = 27.60 wt% and P = 14.32 wt%). Carbon (C) concentration was statistically significantly higher in hypomineralized enamel (C = 11.70 wt%) compared to healthy enamel (C = 10.94 wt%). Hypomineralized and healthy enamel did not differ significantly regarding the ratio of calcium and phosphorus concentrations whereas the ratio of calcium and carbon concentrations was statistically significantly higher in healthy enamel compared to hypomineralized enamel. **Conclusion.** Concentration of the main chemical elements, primarily calcium and phosphorus, is significantly reduced in hypomineralized enamel whereas carbon concentration is increased compared to healthy enamel.

Key words:
tooth demineralization; molar; dental enamel; dentin; elements; microscopy, electron, scanning.

Apstrakt

Uvod/Cilj. Hipomineralizacija molara i sekutića (*molar-incisor hipomineralisation* – MIH) definiše se kao hipomineralizacija sistemskog porekla jednog ili više od četri prva stalna molara, koja može biti udružena sa promenama na maksilarnim, a nešto ređe na mandibularnim sekutićima. Cilj ovog rada bio je da se istraži mineralni sadržaj hipomineralizovanih zuba kao doprinos razumevanju nastanka ovih promena, što će biti od značaja za efikasniji restaurativni pristup. **Metode.** U istraživanju je korišćeno 10 izvađenih prvih stalnih molara sa dijagnozom MIH, kao eksperimentalna grupa, a kao kontrolna grupa zuba korišćeni su prvi intaktni premolari izvađeni iz ortodonskih razloga. Određena površina hipomineralizovane i zdrave gleđi i dentina analizirana je pomoću elektronskog mikroskopa opremljenog energetskim disperzionim spektrometrom (SEM/EDS). **Rezultati.** Kvantitativnom hemijskom analizom raspodele osnovnih hemijskih elemenata, utvrđeno je da je koncentracija kalcijuma (Ca) i fosfora (P) bila statistički značajno veća u zdravoj gleđi (Ca = 28,80% tež. i P = 15,05% tež) u odnosu na hipomineralzovanu gleđ (Ca = 27,60% tež. i P = 14.32% tež). Koncentracija ugljenika (C) bila je statistički značajno veća u hipomineralizovanoj gleđi (C = 11,70% tež) u odnosu na zdravu gleđ (C = 10,94% tež). Zdrava i hipomineralizovana gleđ nisu se statistički značajno razlikovale u odnosu koncentracija kalcijuma i fosfora, dok je odnos koncentracija kalcijuma i ugljenika statistički bio značajno veći u zdravoj gleđi u odnosu na hipomineralizovanu gleđ. **Zaključak.** Koncentracija osnovnih hemijskih elemenata, pre svega kalcijuma i fosfora, značajno je snižena u hipomineralizovanoj gleđi, a koncentracija ugljenika povišena u poređenju sa zdravom gleđi.

Ključne reči:
zub, demineralizacija; molari; zub, gleđ; dentin; elementi; mikroskopija, elektronska, skenirajuća.

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Introduction

Hypomineralization of molars and incisors (molar-incisor hypomineralization – MIH) [1] is defined as enamel hypomineralization of systemic origin of one or more of the four first permanent molars, which may be associated with changes in the maxillary, and less frequently in the mandibular permanent incisors [1].

This condition has been found in children throughout Europe and is considered to be a significant problem in terms of dental health in many countries [2].

Former studies indicate that hypomineralization changes in the first permanent molars and incisors are the result of the interruption of the enamel formation process from about 37 weeks to 3 years of age. Obstacles during the formation process of enamel matrix will manifest as quantitative or morphological defects (hypoplasia or hypoplastic changes) [3], while interruptions in the process of mineralization or maturation can produce morphologically normal, but structurally or qualitatively defective hypomineralized enamel [4].

Hypomineralized teeth which are not part of some other structural anomaly have been noticed in everyday dental practice since a long time ago. Hypomineralization is clinically manifested as a disturbance in translucency of enamel (enamel opacities). Defective opaque enamel is of normal thickness, with smooth surface and can be whitish, white-yellow or yellow-brown and the defect typically has a clear line between affected and healthy enamel. These changes are usually localized to the vestibular surface of incisors and cusps of molars [5, 6].

Hypomineralized molars, depending on their degree of hypomineralization, are brittle, fragile and, as such, liable to posteruptive chipping of enamel caused by chewing forces. Teeth affected by MIH are sensitive to thermal, mechanical and chemical stimuli [7–9].

It has been discovered that mechanical characteristics in MIH teeth such as hardness and modulus of elasticity are significantly lower than in healthy enamel [10, 11].

Hypomineralized enamel surface is substantially disorganized, its content of inorganic compounds, primarily Ca/P ratio, is reduced by 5–20%, and increased content of carbon and carbonates can be noted if compared to normal enamel. Enamel in the cervical area of a tooth usually retains normal structure [12–14].

While biological and physicochemical factors or the ones of environmental origin that lead to these disorders still remain unclear, the effects of the changes in mechanical properties of hypomineralized enamel, as well as reduced concentration of basic inorganic compounds are quite clear. The microstructure of hypomineralized enamel in permanent teeth has been described in several studies to have less organized prismatic structure with wide interprismatic zones [15–17].

Numerous studies, using different methods for studying morphological, chemical and mechanical properties of hypomineralized enamel compared to normal enamel of permanent teeth, show that there is a significant difference between the two [13, 14, 16, 18].

The fact that some basic analyses of the enamel in the first permanent molars with the diagnosis of MIH in some researches show changes in chemical and mineral composition of the enamel, the aim of this study was to examine the mineral composition of hypomineralized teeth as the contribution to understanding the causes of these changes, which will be of significant importance to some more efficient and restorative approach.

Methods

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Pristina, with the headquarters in Kosovska Mitrovica.

A total of 10 extracted first permanent molars diagnosed with severe MIH were used in the study as the teeth of the experimental group. The increased risk of posteruptive enamel loss and failure of restoration, rapid development of caries, unpredictable behavior of the remaining enamel, the failure to achieve an adequate level of analgesia, dental fear and anxiety of children, were all the justifying reasons for extraction of first permanent molars diagnosed with severe MIH and in combination with orthodontic treatment applied upon the extraction.The optimal time for extraction is the beginning of calcification and bifurcation of the root of the mandibular second molar at the age of 8.5–9.5 years. If extraction is performed after that time, a problem of space and ill-positioning of the second permanent molar may incur.

Intact first premolars extracted for orthodontic reasons were used as the teeth of the control group, since healthy first permanent molars were not indicated for extraction and thus difficult to collect for evaluation. According to the literature, there is no evidence that the enamel structure of premolars is different from the enamel structure of molars [16].

We used the teeth of children aged 8.5–10 years for whom we received written parental consent to participate in the study, and who had previously been fully informed, orally and in writing, about the aims of the study.

The extracted teeth were fixed in 4% formalin and kept until preparations and measurements in test tubes without identification, so that none of the teeth could point to a specific patient.

The analysis of the chemical properties of the enamel was performed in the Laboratory for Scanning Electron Microscopy with Energy-Dispersive Spectrometry (SEM-EDS) at the Faculty of Mining and Geology, University of Belgrade.

Specific surface was examined by means of a scanning electron microscope (SEM) type JEOL JSM-6610LV at 20 kV. Relative amounts of the measured chemical elements were calculated using Energy-Dispersive System-EDS, model X-Max Large Area Analytical Silicon Drift connected with INCA Energy 350 Microanalysis System, with the detection limit of 0.1 mas. % and resolution of 126 eV.

For the initial preparation of samples, the coronal part of the selected teeth was separated from the roots using a diamond disc, and then the crown was cut into a buccal and oral part in the mesial-distal direction, with constant cooling

at the speed of 6000 rev/min. The resultant of the tooth crown was put into epoxy resin, so that the longitudinal section of enamel and dentin was visible. The obtained portion was polished on the surface on one side with the fine silicon carbide paper, and finally with 9 and 1 μm diamond paste. Polishing was needed to obtain highly polished surfaces for a quantitative chemical analysis. Then the samples were sprayed and coated with the carbon layer of 15–25 nm thickness, so that the surface could be electron transparent for the penetrating electron beam. The device SCD005 LEICA model was used for carbon vapor deposition of the samples. The samples for the SEM analysis had to be previously cleaned by plunging into ethanol and keeping in an ultrasonic bath for a few minutes, and then dried under an ordinary lamp. The sample was then fixed on the holder and placed on the table in the scanning electron microscope chamber. The signals from the sample, *via* the detectors placed in the sample chamber, were transferred electronically to a computer, which is a part of an energy-dispersive system, for EDS analyses. The image of back-scattered electrons (BSE) and secondary electrons (SE) can also be transferred to a computer for EDS analysis, making it possible to choose points for analysis directly on the image, and the analysis can be at a single point or along a selected line.

Quantitative analysis of the distribution of chemical elements in enamel and dentine was done by applying "dotted" analysis at various distances of previously determined line of analysis. For analysis, 31 points were selected on each examined section both in the enamel and the dentine as follows: the first examined point was randomly selected in dentine and the following 30 points from the enamel-dentine border in occlusal direction through the enamel section surface (Figures 1 and 2). The following methods of statistical processing were used to test the results: Mann-Whitney *U*-test, Student's *t*-test and χ^2 test.

Results

Basic chemical elements of both healthy and hypomineralized enamel and dentin were distributed by conducting quantitative chemical analysis. Basic chemical elements plus oxygen whose content had been calculated by stoichiometric calculations were detected. The results of the analysis are displayed in Table 1.

Basic chemical elements analysis of healthy enamel showed that the mean value of Ca concentration was 28.80 wt% whereas the mean value of calcium concentration in hypomineralized enamel was 27.60 wt%. It is observed that the concentration of calcium was significantly higher in healthy enamel compared to hypomineralized enamel ($F = 7.24$; $p = 0.014$). Analysis of the distribution of calcium concentration indicated the values in enamel layers, going from the enamel-dentine border occlusally, to be as follows: the first 12 of the analyzed spectra were of no statistical significance compared to hypomineralized enamel. The spectra was used to label the relative mass distribution *per* element within the examined point, the same way as operated and defined by EDS spectrometer. The values of calcium concentration in healthy enamel in other spectra compared to hypomineralized enamel were statistically significantly higher ($p < 0.05$).

The mean value of phosphorus concentration in healthy enamel was 15.05 wt% and in hypomineralized enamel 14.32 wt%. Phosphorus concentration was statistically significantly higher in healthy enamel compared to hypomineralized enamel ($F = 6.09$; $p = 0.024$). Analysis of the results confirmed that the distribution of phosphorus concentration in enamel layers, going from the enamel-dentine border occlusally, at the first 14 analyzed spectra, was of no statistical significance, whereas at the following 16 analyzed spectra these values were significantly higher in healthy enamel compared to hypomineralized enamel ($p < 0.05$).

The mean value of carbon concentration in healthy enamel was 10.94 wt% and in hypomineralized enamel 11.70 wt%. Carbon concentration was statistically significantly higher in hypomineralized enamel compared to healthy enamel ($F = 6.22$; $p = 0.023$). Analysis of the results of carbon concentration distribution indicated that, go-

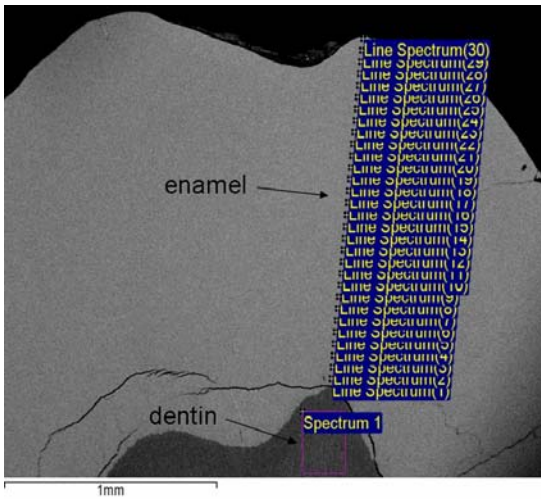

Fig. 1 – Selected area for energy dispersive spectrometry (EDS) analysis – hypomineralization of first permanent molars (molar incisor hipomineralization group).

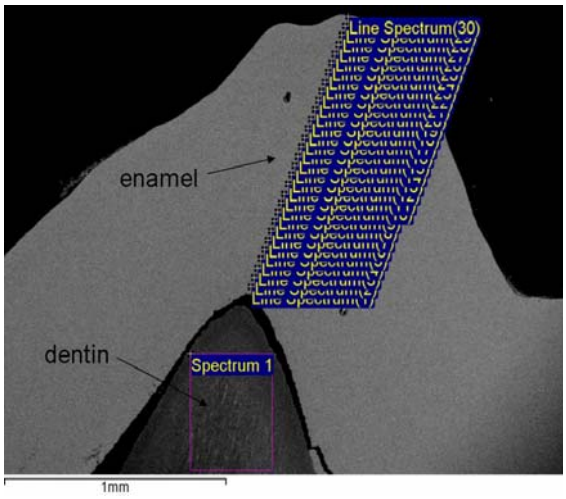

Fig. 2 – Selected area for energy dispersive spectrometry (EDS) analysis – first permanent premolars (healthy enamel group).

Table 1

The concentration profile of chemical elements of hypomineralized and healthy tooth enamel and dentin expressed in weight percentage

Spectrum	Calcium (%)		Phorporus (%)		Carbon (%)		Oxygen (%)	
	MIH	HE	MIH	HE	MIH	HE	MIH	HE
Spectrum 1	14.21	14.35	8.30	8.41	9.62	9.03	44.53	44.72
Line Spectrum (1)	27.69	28.07	14.51	14.37	11.71	12.51	44.56	43.68
Line Spectrum (2)	27.80	27.97	14.68	14.95	10.78	10.86	44.34	43.98
Line Spectrum (3)	28.15	28.76	14.97	15.02	10.98	10.97	44.74	44.04
Line Spectrum (4)	27.66	28.53	14.56	14.83	11.52	11.20	44.72	43.15
Line Spectrum (5)	27.61	28.42	14.46	14.84	11.78	11.26	44.67	44.42
Line Spectrum (6)	28.00	28.17	14.94	15.19	10.89	10.53	44.86	44.32
Line Spectrum (7)	27.55	27.98	14.85	15.00	11.01	10.98	44.74	43.90
Line Spectrum (8)	28.15	28.31	14.52	15.11	11.72	11.07	44.79	44.29
Line Spectrum (9)	28.16	28.14	14.44	14.72	10.56	10.47	45.07	44.08
Line Spectrum (10)	27.92	28.08	14.34	14.79	11.01	11.22	44.86	44.12
Line Spectrum (11)	28.09	28.15	14.90	15.08	11.84	11.26	45.13	44.21
Line Spectrum (12)	27.18	27.63	14.23	14.74	12.08	11.02*	45.19	43.98
Line Spectrum (13)	27.14	28.61*	14.98	15.02	12.12	11.10*	45.00	43.97
Line Spectrum (14)	27.24	28.61*	14.13	14.64	11.96	11.08*	44.98	44.13
Line Spectrum (15)	27.19	28.39*	13.36	14.80*	11.63	11.24	44.02	44.00
Line Spectrum (16)	27.17	28.53*	13.83	14.93*	11.66	11.11	45.02	43.90
Line Spectrum (17)	26.53	28.65*	14.08	15.01*	11.94	10.76*	45.00	43.86
Line Spectrum (18)	26.73	28.63*	13.71	14.78*	11.92	10.95*	44.98	43.60
Line Spectrum (19)	27.23	28.94*	13.97	15.19*	11.94	10.75*	44.97	43.86
Line Spectrum (20)	26.36	28.70*	13.89	15.04*	11.90	10.97*	45.08	43.60
Line Spectrum (21)	26.52	27.91*	14.16	15.18*	11.99	10.76*	44.67	43.64
Line Spectrum (22)	27.06	28.82*	13.84	15.24*	11.62	10.75*	44.73	43.21
Line Spectrum (23)	27.03	27.70*	14.59	15.40*	11.68	10.45*	44.85	43.29
Line Spectrum (24)	26.71	28.26*	13.73	15.42*	11.82	10.46*	44.35	43.30
Line Spectrum (25)	26.98	28.32*	14.86	15.39*	11.33	10.48*	44.49	43.04
Line Spectrum (26)	26.87	28.58*	13.74	15.55*	11.45	10.26*	44.21	43.31
Line Spectrum (27)	26.40	28.22*	13.79	15.53*	11.33	10.27*	44.11	43.06
Line Spectrum (28)	26.88	28.56*	14.82	15.51*	11.61	10.30*	43.97	42.71
Line Spectrum (29)	26.73	28.93*	13.82	15.78*	10.99	9.96*	44.10	42.84
Line Spectrum (30)	26.95	28.75*	13.60	15.74*	11.11	10.04*	44.72	43.77
Mean	27.06	28.80*	14.32	15.05*	11.70	10.94*	44.62	43.17
Std. deviation	1.47	1.03	0.78	0.06	1.06	0.95	1.25	0.09
Max.	28.16	28.94	14.97	15.78	12.12	12.51	45.19	44.42
Min.	26.36	27.63	13.36	14.37	10.56	9.96	43.97	42.71

***Statistically significant ($p < 0.05$); MIH – molar incisor hipomineralization group; HE – healthy teeth group.**

ing from the enamel-dentine border occlusally, at the first 12 analyzed spectra, these values were of no statistical significance, as well as in the 15th and 16th analyzed spectrum, whereas at the following examined points these values were significantly lower in healthy enamel compared to hypomineralized enamel ($p < 0.05$).

The experimental and control groups of teeth did not differ significantly when the concentration of calcium and phosphorus was considered, as well as the carbon in dentin ($F = 3.30$; $p = 0.087$).

Analysis of the ratio of calcium and phosphorus concentration in healthy enamel was 1.91, whereas the ratio of calcium and phosphorus concentration in hypomineralized enamel was 1.90. Analysis of the ratio of calcium and carbon concentration in healthy enamel was 2.65, and the ratio of calcium and carbon concentration in hypomineralized enamel was 2.39. Healthy and hypomineralized enamel did not differ significantly in terms of statistics considering the ratio of calcium and phosphorus concentration ($F = 3.30$; $p = 0.087$), whereas the ratio of calcium and carbon concentration was statistically significantly higher in healthy enamel compared to hypomineralized enamel ($F = 6.14$; $p = 0.023$) (Table 2).

Table 2

The ratio of calcium/phosphorus and calcium/carbon concentrations of healthy and hypomineralized tooth enamel of the examined teeth

Parameter	Ca-P		Ca-C	
	MIH	HE	MIH	HE
number of teeth	10	10	10	10
mean	1.90	1.91	2.39	2.65
SD	0.05	0.04	0.36	0.28
p	< 0.000		< 0.000	

MIH – molar incisor hipomineralization group; HE – healthy teeth group; SD – standard deviation.

Discussion

In previous studies a number of different methods have been used for studying chemical and mineral properties of hypomineralized enamel in teeth with MIH [12, 16, 18–21].

A number of studies analyzing the enamel in permanent first molars diagnosed with MIH have shown changes in chemical composition, as well as reduction in mineral composition [9, 12, 13]. A reduction in the volume of mineral content by 20% indicates the presence of higher concentration of organic compounds in the area of hypomineralized enamel [14]. It is assumed that in hypomineralized teeth the removal of the remaining proteins (amelogenin, enamelin, etc.) may be incomplete [22–24].

In this study, analysis of basic chemical elements in enamel shows that in terms of statistics the concentration of calcium and phosphorus is significantly higher in healthy enamel compared to hypomineralized enamel. The finding of lower levels of calcium and phosphorus in hypomineralized enamel compared to healthy one is in line with the research conducted by Fagrell et al. [20]. Similar results have been shown in other studies, too [12, 18, 19].

Analysis of the results of carbon concentration shows that the values are significantly higher in hypomineralized enamel compared to healthy enamel.

Jälevik et al. [13] assumed in their study that increased carbon content, determined by means of radiographic microanalysis, may be the result of increased carbonate concentration, or an increase in organic components of the enamel.

Increased carbon content in hypomineralized enamel compared to normal enamel has been proved in other studies, too [12, 17, 19, 20].

Analysis of healthy and hypomineralized enamel shows that there is no significant difference regarding calcium and phosphorus concentration.

Fagrell et al. [20] also found in their study in 2010 that calcium / phosphorus ratio was not significantly different between normal and hypomineralized enamel.

This was in contrast to the previous study conducted by Jälevik et al. [18]. However, other studies have also found a stable calcium/phosphorus ratio (Ca/P), despite some differences in the level of mineralization.

Different results in the literature have been discussed by Mahoney et al. [10, 17], leaving some room for further discussion. The explanation for the stable calcium/phosphorus ratio (Ca/P) is in what it actually represents. It is assumed that in hypomineralized enamel with a higher content of organic matter, phosphorus analyzed by means of radiographyc microanalysis, possibly derives not only from hydroxyl apatites, but also from organic matter.

In this study, the ratio of calcium and carbon concentration is statistically significantly higher in healthy enamel compared to hypomineralized enamel.

As well as in ours, the research conducted by Fagrell et al. [20] indicated that the average value of carbon was significantly higher in hypomineralized enamel compared to normal one, whereas calcium/carbon ratio had significantly lower values in hypomineralized enamel compared to normal.

This study confirms the findings of other researchers – hypomineralized first permanent molars do not only have morphological changes but also reduced concentration of basic chemical elements in the basis of their defects.

A reduced concentration of basic chemical elements of hypomineralized enamel in first permanent molars explains the reduced resistance of the affected enamel to pressure during mastication and inadequate retention of the material during restoration.

Further studies of mineral content, ultrastructure and mechanical properties of hypomineralized teeth would be useful for understanding the origin of these phenomena and would help with the selection of restorative materials.

Conclusion

This study confirms the findings of other researchers – hypomineralized first permanent molars do not only have morphological changes but also a reduced concentration of basic chemical elements in the basis of their defects. By conducting quantitative chemical analysis of the distribution of the basic chemical elements, it was found that, in terms of statistics, the concentration of calcium and phosphorus was significantly higher in healthy enamel when compared to hypomineralized enamel. Carbon concentration was significantly higher in hypomineralized enamel compared to healthy enamel. Healthy and hypomineralized enamel do not differ significantly regarding the ratio of calcium and phosphorus concentration whereas the ratio of calcium and carbon concentration is significantly higher in healthy enamel compared to hypomineralized enamel.

Further studies of the mineral composition of hypomineralized teeth would contribute to obtaining a clearer etiology of the changes on MIH affected teeth as well as the influence on mechanical resistance during the mastication process. Furthermore, clearer knowledge on irregularities of structural integrity of hypomineralized tooth enamel would lead to better diagnostics, prevention and treatment of this malformation on the first permanent molars and incisors.

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