The mineral content of the hard dental tissue of mesiodens

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Objective. Mesiodens is the most common form of supernumerary tooth mainly located between the maxillary central incisors. Its etiology is not completely understood but both genetic and environmental factors are assumed. The degree of mineralization and inorganic element content in hard tooth tissues is poorly understood as well as is the durability and suitability for allo- and auto-transplantation. Therefore aim of this study was to examine the content of inorganic elements.

Materials and Methods. This study included 26 mesiodens teeth and 26 normal central incisor teeth as controls. All specimens were prepared for SEM/EDS analysis which was aimed at specific sites on the enamel, dentine and cementum in order to evaluate the weight percentage and ratio of important inorganic elements.

Results and Conclusion. The results showed that there was a difference in the weight percentage of selected inorganic elements (calcium, phosphorus, oxygen, carbon, magnesium and sodium) in all three types of dental hard tissues but the differences were mostly expressed in the cementum tissue. The statistical analysis showed that the differences were marginally significant especially for calcium and phosphorus values and ratio in the enamel and dentine. The carbon and magnesium content in all three hard tissues showed the most differences, but overall, the hard tissues mineral content of the mesiodens did not differs significantly from healthy teeth.

Key words: mesiodens, elemental composition, dentin, enamel, cementum

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INTRODUCTION

Tooth development is a complex process of mineralization of connective tissues that depends on various genetic control mechanisms and biochemical cellular reactions. Supernumerary teeth have become interesting to clinicians and researchers in last decade due to their auto- and allo-transplantation potential as well as a good source of stem cells[1-3]. The overall prevalence of the supernumerary tooth in permanent dentition is from 1.5–3.5% and mesiodens contribute around 80% of cases (ref.[4]). Mesiodens is the most common form of the supernumerary tooth mainly located just between the maxillary central incisors[5]. Often the mesiodens has a typical appearance (conical shape) and with all morphological characteristics of the normal tooth, but it sometimes is atypical with altered form as a result of defective development from the remnants of the tooth bud[6,7]. Both, genetic and environmental, factors have been considered and several different theories such as the atavism, dichotomy theory and genetic syndromes have been suggested to explain the occurrence of the mesiodens[8-10]. Regardless of the reason, its development follows that of other normal permanent teeth, while its mineralization frequently occurs later than homologous teeth which means that inductive factors (stimuli) for mesiodens teeth are weaker and somewhat impaired[11,12]. A number of studies have shown that the normal tooth possess calcium (Ca), phosphorus (P), and oxygen (O), as indicated by the formula for hydroxyapatite (HAP), also carbon (C), magnesium (Mg), sodium (Na), as well as several trace elements[13-15]. On the other hand, a survey of the literature related to the mineral content of the supernumerary teeth as well as mesiodens provided no results. Given that, the degree of mineralization, ratio of inorganic elements as well as the presence of trace elements in hard dental tissues of the mesiodens are important facts when susceptibility to tooth decay and erosion are evaluated, the aim of our study was to examine the mineral content of the enamel, dentin and cementum of the mesiodens.

MATERIAL AND METHODS

All procedures in the study were approved by the Ethics Committee. The study included 26 mesiodens teeth and 26 central incisor teeth. All specimens were obtained from different patients. Clinical diagnosis of the mesiodens was confirmed with X-ray imaging and these teeth, extracted for esthetic or orthodontic reasons, were used as the experimental group, while the control group consisted of central incisor teeth extracted for orthodontic reasons. Teeth from both groups were from the permanent dentition, from healthy patients of both sexes without caries, periapical granulomas and orthodontic treatment. Patients were 25–50 years of age from whom we received

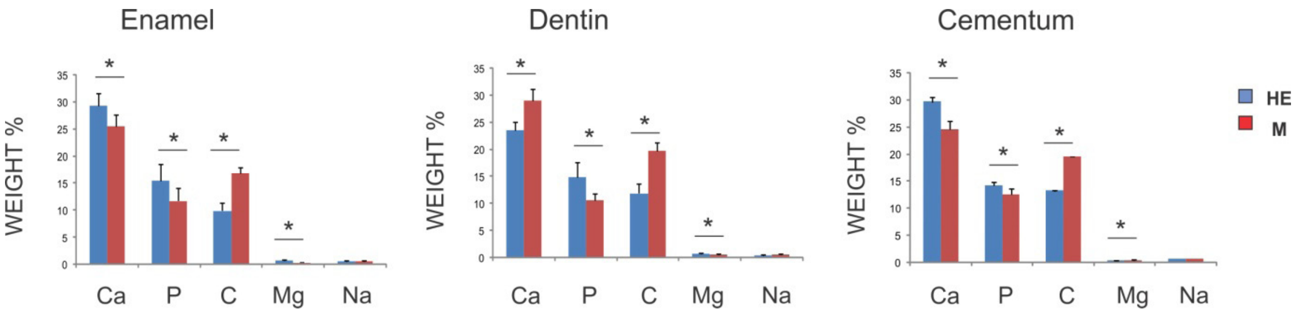

Fig. 1. Distibution ofbasic chemical elements in healthy and mesiodens enamel, dentine and cementum in weight percentage. HE- healthy teeth; M-mesiodens; (*)-significantly

written consent to participate in the study and who had previously been fully informed, orally and in writing, about the aims of the study. During the initial preparation of samples, the teeth were cut into a lingual and labial part and separated using a diamond disc with constant cooling at the speed of 6000 rev/min. The surface of the obtained parts was first polished with fine silicon carbide paper and then with diamond paste. Diamond polishing was essential to remove the debris and to obtain glossy surfaces needed for a quantitative chemical analysis. The enamel surface of each tooth was polished for 5 min with a rubber cup mounted at a low-speed hand piece (KaVo, Biberach, Germany) and under constant deionized water irrigation in order to remove the remnants of the carbide paper. Abrasive substances were not used. Finally, the teeth were rinsed again in an ultrasonic bath before storing them in labeled plastic tubes with 10% formalin solution for 3 days for further analysis. Although fixing solution for a prolonged period could affect the mineral composition of the teeth, in our experiment the fixation period was too short to manifest that effect.

Energy dispersive spectrometry (EDS) analysis

Energy dispersive spectrometry is a specific method for determining the concentration of chemical elements in the substratum. Samples for the EDS analysis were previously cleaned by ethanol and kept in an ultrasonic bath for a several minutes and then air dried. The samples were then fixed on a holder with carbon paste (to the bottom of the specimens) and gold vapor was applied, so that the surface would be electron transparent for the penetrating electron beam. Such samples were then analyzed with scanning electron microscope equipped with energy-dispersive system for EDS analyses. The analysis of the chemical elements of the enamel, dentin and cementum was performed in the Laboratory for Scanning Electron Microscopy with Energy-Dispersive Spectrometry (SEM-EDS) Surface of the specimens 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 XMax Large Area Analytical Silicon Drift connected with INCA Energy 350 Microanalysis System, with the detection limit of 0.1 mass percentages and resolution of 126 eV. Optimal magnification was set on 500. The SEM/EDS analysis was directed to specific regions of the teeth: for the enamel the three sites selected were (the surface, 0,5mm below the surface and 0.5 mm distal from the dentin-enamel junction). For dentin crown and root parts were selected and for cementum 0,1mm from the root apex. In order to assure the same location of analysis for all the specimens, selection of every line of interest was performed by aiming the EDS X-ray at the exact same distance from the marker point (e.g. tooth surface, enamel-dentin junction and root apex) on the low-power microscopic image of the examined tooth.

Statistics

Quantitative analysis of the distribution of chemical elements in enamel, dentin and cementum were done by applying "dotted" analysis at a previously determined line of analysis. The chemical and elemental composition of each tooth samples were tested for normal distribution (Gaussian distribution) using the Shapiro-Wilk test. The following were used to process the: Mann-Whitney U-test, Student t-test and X^2 test. The level of significance was set as ($P<0.05$). The results are presented as means ± standard deviation (SD). For statistical analysis SPSS, IBM Analytics, USA software, version 22.0 was used.

RESULTS

Results of the comparative quantitative analysis of the basic chemical elements (plus oxygen whose content had been calculated by stoichiometric calculations) in hard tissues of the healthy teeth and mesiodensare displayed in Table 1.

Enamel analysis

Analysis of healthy enamel showed that the mean value of Ca concentration was 29.33 wt% whereas the mean value of calcium concentration in mesiodens enamel was 25.59 wt%. It was observed that the concentration of calcium was significantly higher in healthy enamel compared to mesiodens enamel ($P<0.05$). The mean value of phosphorus concentration in the enamel of the teeth from the control group was 15.46 wt% and in mesiodens enamel 11.78 wt%. Carbon concentration was statistically significantly higher in mesiodens enamel compared to healthy enamel ($P<0.05$). Regional distribution of the carbon showed that the concentration of this element sharply decreased from the dentin-enamel border towards the surface (Table 2). The mean value of magnesium

Table 1. EDS results from enamel, dentine and cementum.

ELEMENT	ENAMEL		DENTIN		CEMENTUM	
	HE	M	HE	M	HE	M
Calcium (%)	29.33 ± 2.33	25.59 ± 2.01	23.55 ± 1.55	29.10 ± 2.03	29.62 ± 1.44	24.54 ± 1.51
Phosphorus (%)	15.46 ± 3.04	11.78 ± 2.26	14.94 ± 2.62	10.59 ± 1.26	14.17 ± 0.87	12.47 ± 1.60
Carbon (%)	9.95 ± 1.40	20.64 ± 2.18	11.93 ± 1.76	19.78 ± 1.52	13.21 ± 0.71	19.50 ± 1.10
Oxygen (%)	44.48 ± 1.95	44.15 ± 2.10	48.15 ± 2.59	39.33 ± 2.64	41.96 ± 1.02	42.28 ± 1.89
Magnesium (%)	0.72 ± 0.16	0.35 ± 0.12)	0.76 ± 0.14	0.60 ± 0.11	0.28 ± 0.06	0.34 ± 0.12
Sodium (%)	0.58 ± 0.10	0.61 ± 0.11	0.48 ± 0.07	0.59 ± 0.10	0.68 ± 0.12	0.62 ± 0.21
Ca/P ratio	1.97 ± 0.55	2.26 ± 0.50	1.63 ± 0.35	2.78 ± 0.38	2.10 ± 0.19	2.00 ± 0.30
Ca/C ratio	3.02 ± 0.49	1.53 ± 0.15	2.23 ± 0.37	1.48 ± 0.17	2.25 ± 0.21	1.26 ± 0.12

Data in weight %. HE – healthy teeth group; M – mesiodens teeth group

concentration in enamel of the teeth from the control group was 0.72 wt% and in mesiodens enamel 0.35 wt%. Magnesium concentration was statistically significantly higher in healthy enamel compared to mesiodens enamel (P<0.05). The mean value of sodium concentration in healthy enamel was 0.58 wt% and in mesiodens enamel 0.61 wt%. There was no statistically significant difference-between the concentration of sodium in healthy enamel compared to mesiodens enamel (P>0.05).When the concentration of calcium and phosphorus was considered, the experimental and control group of were significantly different, as well as carbon in enamel (P<0.05) (Fig. 1).

The ratio of calcium and phosphorus concentration in healthy enamel was 1.97:1, whereas this ratio in mesiodens enamel was 2.26:1. Analysis of the ratio of calcium and carbon concentration in healthy enamel was 3.02:1 and the ratio of calcium and carbon concentration in mesiodens enamel was 1.53:1. Healthy and mesiodens enamel did not differ significantly in terms of statistics considering the ratio of calcium and phosphorus concentration (P=0.083), whereas the ratio of calcium and carbon concentration was statistically significantly higher in healthy enamel compared to mesiodens enamel (P=0.000) (Table 1).

Dentine analysis

Analysis of healthy dentine showed that the mean value of Ca concentration was 23.55 wt% whereas the mean value of calcium concentration in mesiodens dentine was 29.10 wt%. It is shown that the concentration of calcium was significantly higher in mesiodens dentine compared to healthy dentin (P<0.05). The mean value of phosphorus concentration in healthy dentine was 14.94 wt% and in mesiodens dentine 10.59 wt%. Phosphorus concentration was statistically significantly higher in healthy dentin compared to mesiodens dentine (P<0.05).The mean value of carbon concentration in healthy dentine was 11.93 wt% and in mesiodens dentine 19.78 wt%. Carbon concentration was statistically significantly higher in mesiodens dentine compared to healthy dentine (P<0.05). The mean value of magnesium concentration in healthy dentine was 0.76 wt% and in mesiodens dentine 0.60 wt%. Magnesium concentration was statistically significantly higher in healthy dentine compared to mesiodens dentine (P<0.05). The mean value of sodium concentration in healthy dentine was 0.48 wt% and in mesiodens dentine 0.59 wt%. Sodium concentration was not significantly higher in mesiodens dentine compared to healthy dentin (P>0.05). The concentration of dentine calcium, phosphorus and carbon was significantly different between groups (P<0.05) (Fig. 1).

Table 2. Carbon content in wt% in different zone of enamel of healthy and mesiodens teeth.

Zone of enamel	Carbon (weight %)	
	Healthy teeth	Mesiodens teeth
Close to DEJ	14.10 ± 1.83	39.20 ± 3.64
Middle zone	9.12 ± 1.36	15.58 ± 1.94
Surface zone	6.63 ± 1.01	7.14 ± 1.14
Mean±SD	9.95 ± 1.40	20.64 ± 2.18

The ratio of calcium and phosphorus concentration in the dentine of the teeth from the control group was 1.63:1, whereas the ratio of calcium and phosphorus concentration in mesiodens dentine was 2.78:1. Analysis of the ratio of calcium and carbon concentration in healthy dentine was 2.23:1, and the ratio of calcium and carbon concentration in mesiodens dentine was 1.48:1. Healthy and mesiodens dentine were statistically significantly different for ratio of calcium and phosphorus concentration (P<0.05). The ratio of calcium and phosphorus concentration was statistically significantly higher in mesiodens dentine compared to healthy dentine. The ratio of calcium and carbon concentration was statistically significantly higher in healthy dentine (P<0.05) (Table 1).

Cementum analysis

Chemical elements analysis of healthy cementum showed that the mean value of Ca concentration was 29.62 wt% whereas the mean value of calcium concentration in mesiodens cementum was 24.54 wt%. We observed that the concentration of calcium was significantly higher in healthy cementum compared to mesiodens cementum (P<0.05). The mean value of phosphorus concentration in healthy cementum was 14.17 wt% while in mesiodens cementum was 12.47 wt%. Phosphorus concentration was statistically significantly higher in healthy cementum compared to mesiodens cementum (P<0.05). The mean value of carbon concentration in healthy cementum was 13.21 wt% and in mesiodens cementum 19.50 wt%.

Carbon concentration was statistically significantly higher in mesiodens cementum compared to healthy cementum ($P<0.05$). The mean value of magnesium concentration in healthy cementumwas 0.28 wt% while in mesiodens cementum was 0.34 wt%. Magnesium concentration was statistically significantly higher in mesiodens cementum compared to healthy cementum ($P<0.05$). The mean value of sodium concentration in healthy cementum was 0.68 wt% and in mesiodens cementum 0.62 wt%.There was no statistically significant difference between the concentration of sodium in healthy cementum compared to mesiodens cementum ($P>0.05$). The cementum levels of Ca, P and C were statistically significant between groups ($P<0.05$) (Fig. 1).

Ratio of calcium and phosphorus concentration in healthy cementum was 2.10:1, whereas the ratio of calcium and phosphorus concentration in mesiodens cementum was 1.96:1. Analysis of the ratio of calcium and carbon concentration in healthy cementum was 2.25:1, and the ratio of calcium and carbon concentration in mesiodens cementum was 1.26:1. Healthy and mesiodens cementum did not differ significantly for calcium and phosphorus concentration ($P>0.05$), whereas the ratio of calcium and carbon concentration was statistically significantly higher in healthy cementum compared to mesiodens cementum ($P<0.05$) (Table 1).

DISCUSSION

Scientific and clinical interest in supernumerary teeth and mesiodens has risen sharply in the last decade mostly due to reports in which researchers showed their successful clinical use in auto- and allo-transplantation procedures and identified these teeth as a good source of stem cells[1-3]. Previously, supernumerary teeth were considered mostly as an orthodontic and esthetic problem. Clinical utilization of these teeth in transplantation procedures potentiates the importance of the quality and durability of the hard dental tissues, especially in terms of their mineral content. In the available literature a number of the studies have been published on epidemiology, etiology and clinical manifestations associated with supernumerary teeth and mesiodens, but not on inorganic content[5,8]. In our study, the results showed that the concentration of calcium and phosphorus is higher in healthy enamel and cementum compared to mesiodens while in the dentine the calcium is higher, but phosphorus is lower. Nevertheless, overall the in incisors are very similar which is in correlation with the previous findings[16,17]. It is well known that when enamel ratio of Ca/P is under 1,33 the tooth is more susceptible to decay, but in our investigation neither normal incisors, nor the mesiodens belong to that category[18,19]. Our results showed a significant different in carbon content between groups. Analysis of the carbon concentration shows that the values are significantly higher in mesiodens enamel compared to healthy teeth, but regional distribution shows that concentration of mesiodens carbon abruptly decreases from the DEJ to the surface (with end-value very near to the normal teeth) which is in correlation to the previous findings[7,20]. This increased carbon presence in mesiodens is the result of persistence of remains of organic matter, probably due to alteration in maturation during which proteins are reabsorbed[21]. Bozal et al.[21] and Jalevik et al.[22] reported that decreased carbonate content strengthens the enamel. Crombie et al.[23] claim that carbonate is a substitute for the phosphate group in the HAP. Normal or lower carbon concentration contributes to enamel hardness. One more conclusion is that mesiodens is not susceptible to accelerated decay although there are no comprehensive data on the incidence of the mesiodens caries[24-26]. In relation to the Mg differences found, magnesium is "trace" element that is absorbed, but not incorporated into the hydroxyapatite structure. Our results showed significantly (twice) lower amounts of magnesium in mesiodens enamel compared to normal teeth. Wychowański et al.[27], suggest that magnesium is detrimental to enamel due to increased susceptibility to acid influence. We conclude that lower mesiodens enamel magnesium content in fact contributes to its resistance. This study showed increased calcium and decreased phosphorus content so the ratio of these elements is markedly in favor of calcium (up to 2.78:1). Our findings are in correlation with the report of Akgun et al.[17] who found that mesiodens dentine is more calcified that normal dentine although exact values are at variance. On this matter we managed to find one very short report of Caihong et al.[28] who stated the opposite, but our sample was larger. The dentinal Ca/C ratio examination in our study showed that this ratio is lower in mesiodens than in healthy teeth, but the total amount of these elements was in fact increased. This is important because some authors claims that the very low carbon content in dentine may reduce its ability to withstand the mechanical pressure from the enamel, to become brittle and develop micro-cracks[29,30]. In a conclusion we can state that out study showed that, although there are some differences in overall mineral content and the ratio of the important inorganic elements of the hard tissues of the mesiodens and normal incisors, these differences are not highly significant. Therefore, auto- and allo-transplantation of mesiodens is valid given that durability is similar to normal teeth.

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