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Lead concentration in hard dental tissues – SEM/EDS analysis

Irena Kuzmanović Radman[1], Adriana Arbutina[2], Renata Josipović[1], Aleksandra Đeri[1]

[1]University of Banja Luka, Faculty of Medicine, Department of Restorative Dentistry and Endodontics, Study program Dentistry, Banja Luka, Republic of Srpska, Bosnia and Herzegovina;
[2]University of Banja Luka, Faculty of Medicine, Department of Orthodontics, Study program Dentistry, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

SUMMARY

Introduction Currently, one of the most important ecological issues is exposure to lead in environment, since it is a metal with evident toxic effects on human organism. Hard dental tissues are suitable structures for assessing long-term effects of exposure to toxic metals.
The aim of this paper was to determine the concentration of lead in hard dental tissues of a rat with experimentally induced DM using SEM/EDS analysis, after 14 and 30 days of exposing animals to lead.
Material and methods The study was conducted in rats of Wistar strains divided into the three groups. The first group consisted of 8 rats (128 molars and premolars of the upper and lower jaws) with experimentally induced DM, taking lead in the course of 14 days at the concentration of 1500 ppm; the second group included 8 rats (128 molars and premolars of the upper and lower jaws) taking lead in the course of 30 days at the concentration of 1500 ppm, while the third control group consisted of 5 healthy rats (80 molars and premolars of the upper and lower jaws). Experimental animals received lead-acetate every day at the concentration of 1500 ppm via water ad libitum. In these animals, diabetes mellitus was induced by Alloxan. The teeth samples were analysed using scanning electron microscopy (SEM). EDS analysis determined the mass fraction of lead and other elements in hard dental tissues.
Results No lead was detected in a single tooth layer in the teeth of rats that received lead in drinking water in the course of 14 days. The average values of the mass fraction of lead, calcium, and phosphorus in enamel of teeth of rats receiving lead in the course of 30 days amounted to: lead 0.36%, calcium 15.48%, and phosphorus 10.62%. Lead was registered only in enamel.
Conclusion Lead was detected in enamel only in rats receiving lead in the course of 30 days while it was not detected in teeth after the course of 14 days.
Keywords: lead; enamel; dentin; SEM/EDS analysis

INTRODUCTION

Hard dental tissues consist of several different minerals that together with calcium represent major macromineral and therefore represent ideal tissues for assesing the long-term effects of exposure to toxic metals [1, 2, 3]. Exposure to lead is significant health problem in many countries because it is associated with the impact on general health (anemia, hypertension), or the pathology of bones and teeth, including dental caries [4, 5, 6].

Although lead levels in hard dental tissues are useful indicators of lead exposure, information on its time effects and lead compounds in dental tissues is very limited. Some studies have shown that the influence of lead on developing teeth does not have to be related only to its cytotoxic effects but also to interaction with proteins and enzymes of extracellular matrix. It has also been confirmed that the pulp metabolism is significantly delayed in some metabolic disorders such as diabetes [7, 8].

There are reports in the literature indicating that the presence of lead in chemical composition of enamel can alter its ultrastructure and lead to its damage. Thus, Gomes et al. in their study found in pre-school children's teeth, who lived in the industrial city area, higher lead concentration in enamel than in children who lived outside this area [9]. Data from ancient populations revealed high prevalence of hypoplastic enamel with high levels of lead in bones and teeth. Correlation between the presence of lead in dental tissues and clinical alteration of enamel was noticed in the form of discoloration. An increase of enamel hypoplasia has been confirmed in children exposed to high lead concentrations [10, 11, 12].

In vitro studies have shown that the presence of lead during amelogenesis can lead to ultrastructural alterations of enamel associated with modifications in physicochemical relationship and making enamel more sensitive to demineralization [13]. It has been found that the content of metal in teeth (in the population in areas polluted with lead) was associated with an increased incidence of caries lesions. However, the correlation between the influence of lead and development of dental caries in hard dental tissues is still subject of numerous studies [14].

Moss et al. confirmed correlation between lead exposure during dentin formation and increased caries prevalence [15], and Martin et al. found that lead affects formation of caries but only in deciduous teeth [16]. Gomes et

Address for correspondence: Irena KUZMANOVIĆ RADMAN, Bulevar Vojvode Petra Bojovića 1a, 78000 Banja Luka, Republika Srpska, Bosnia and Herzegovina; *irena.radman78@gmail.com*

al. investigated association between lead concentrations in deciduous teeth enamel and found no connection between lead and dental caries in children's teeth living in industrial zone [9].

Cenić-Milošević et al. examined correlation between the concentration of lead in extracted teeth of the inhabitants of Pančevo and Belgrade (among members of different age groups) and concluded that one of the possible causes of tooth loss and damages caused by caries was long-term exposure to lead [17]. Barmes and Ludmgh found correlation of lead concentration in the teeth and tooth decay, and concluded that subjects with high lead concentration had more teeth with dental caries. It has also been confirmed that lead, as a cytotoxic agent, can have an effect on ameloblasts, i.e. an alteration in the amount of proteins and delay in amelogenesis [18]. Gerlach et al. concluded that lead increases the concentration of proteins and slows down enamel mineralization of incisors of rats that consumed water contaminated with lead [19]. Tvinnereim et al. found association between lead exposure during dentin formation and increased sensitivity to caries in rats' teeth [20].

The aim of this paper was to determine using SEM/EDS analysis the concentration of lead in hard dental tissues of rats with experimentally induced DM after 14 and 30 days of animal exposure to lead.

MATERIAL AND METHODS

Wistar strain rats (21) and 336 teeth were selected for the experiment due to similarities in the physiology of dental pulp of rats and pulp physiology of human teeth. The study was approved by the Ethics Committee of the Institute of Dentistry of the Faculty of Medicine in Banja Luka. All rats were divided into the two groups: the first group consisted of 8 rats (128 molars and premolars of the upper and lower jaws) with experimentally induced DM taking lead in the course of 14 days at a concentration of 1500 ppm. The second experimental group consisted of 8 rats (128 molars and premolars of the upper and lower jaws) taking lead in the course of 30 days at a concentration of 1500 ppm. The control group consisted of 5 healthy rats (80 molars and premolars of the upper and lower jaws). The protocol of experimenatly induced diabetes mellitus in rats included the use of Alloxan solution which was applied intraperitoneally and the protocol for lead intoxication included the intoxication of adult rats using lead – acetate at the concentration of 1500 ppm via water ad libitum. All animal procedures, nurturing, experimental treatments, sacrificing without pain and stress were conducted in accordance with the guidelines for the care of animals used for experimental research („Guide for the Care and Use of Laboratory Animals", 1996 National Academy Press, Washington, DC). After decapitation, upper jawbones of rats were separated from soft tissues and stored in fixative (10% neutral buffered Formalin) and then prepared for the SEM-EDS analisys. The tooth samples were cut and polished with a diamond disc through the middle of the tooth in the medio-distal direction in order to expose the cross-section of the enamel zone and dentine mass. Recording and analyses were performed using the Scanning electron microscope (JEOL JSM 6460LV) and connected OXFORD INCAx-sight spectrum analyzer. For the purposes of this analysis, images were obtained using Back-scatter or Primary emissions of reflected electrons in Compo mode (BEIc), as it proved to be the most useful emphasizer of the enamel zone and dentine mass. The samples were observed at an acceleration of 20kV at a working distance (WD) of 10 mm, at the angle of incidence that was suitable for the inclination of polished surface of premolars and molars. The general image was given in clear magnification of 35x, and for the purposes of more precise EDS analysis, the magnification of 100x was used. The obtained results were analysed and processed statistically.

RESULTS

Table 1 shows the average values of the mass fraction of phosphorus, calcium, and lead in the teeth parts of all examined groups. The average values of the mass fraction of phosphorus in the teeth of the rats receiving lead in the course of 14 days was highest in the area of enamel - dentine junction (15.61%), dentin (13.96%) and the lowest values were in enamel (13.92%). In the rats receiving lead in the course of 30 days, the highest mass fraction was found in dentin (13.96%), then in enamel – dentin junction (21.91%), and the lowest in enamel (10.62%).

The highest average values of calcium, in teeth of the rats receiving lead through drinking water in the course of 14 days, were in enamel – dentin junction (25.66%), then in enamel (23.28%), and the lowest in dentin (22.35%). In the rats receiving lead in the course of 30 days, the mass fraction was 20.13% in the area of enamel – dentin junction, 15.48% in enamel, and 21.74% in dentin.

No lead was detected in any of the tooth layers in the teeth of rats receiving lead through drinking water in the course of 14 days. It was detected only in enamel of rats receiving lead in the course of 30 days (0.36).

Analysis of the obtained values of the mass fraction of certain elements in enamel indicated statistically significant difference only in the values of calcium ($p<0.05$). Also, lower values were recorded in the group of rats receiving lead through drinking water in the course of 14 days compared to the group of rats receiving lead in the course of 30 days. There was no statistically significant difference in the mass fraction of other elements (Table 1, Figure 1).

The relation between obtained values of the mass fraction of examined elements in all tooth parts was estimated by the Spearman's correlation coefficient. Statistically significant negative correlation between the mass fraction of calcium and mass fraction of lead was found, as well as statistically significant positive correlation with the mass fraction of phosphorus. The existence of statistically significant negative correlation between the mass fraction of lead and mass fraction of calcium was also found (Table 2).

Table 1. The average values of the mass fraction of certain elements in hard dental tissues in the examined groups
Tabela 1. Prosečne vrednosti masenog udela pojedinih elemenata u delovima zuba kod ispitivanih grupa

	Hard dental tissue Deo zuba	Group Grupa											
		Pb 14 days Pb 14 dana						Pb 30 days Pb 30 dana					
		N	X̄	SD	Med	Min	Max	N	X̄	SD	Med	Min	Max
Wt% P	Enamel Gleđ	12	13.92	3.39	14.66	8.14	19.22	7	10.62	4.49	10.90	1.36	14.51
	EDJ GDG	11	15.61	2.40	15.87	10.24	18.77	8	12.91	4.54	13.43	3.50	18.23
	Dentin	12	13.95	2.13	13.86	10.55	17.40	6	13.95	3.06	14.50	9.08	17.10
	Pulp Pulpa	0	.	.	.	.	.	1	16.55	.	16.55	16.55	16.55
	Total Ukupno	35	14.46	2.74	14.68	8.14	19.22	22	12.63	4.18	13.43	1.36	18.23
Wt% Ca	Enamel Gleđ	12	23.28	5.68	23.02	14.37	31.76	7	15.48	8.26	17.10	.67	25.88
	EDJ GDG	11	25.66	4.35	25.28	15.98	31.73	8	20.13	10.82	20.72	1.92	37.40
	Dentin	12	22.35	4.52	23.11	14.69	27.79	6	21.74	8.49	21.65	9.70	33.57
	Pulp Pulpa	0	.	.	.	.	.	1	29.10	.	29.10	29.10	29.10
	Total Ukupno	35	23.71	4.96	24.86	14.37	31.76	22	19.50	9.33	19.38	.67	37.40
Wt% Pb	Enamel Gleđ	12	.00	.00	.00	.00	.00	7	.36	.62	.00	.00	1.30
	EDJ GDG	11	.00	.00	.00	.00	.00	8	.00	.00	.00	.00	.00
	Dentin	12	.00	.00	.00	.00	.00	6	.00	.00	.00	.00	.00
	Pulp Pulpa	0	.	.	.	.	.	1	.00	.	.00	.00	.00
	Total Ukupno	35	.00	.00	.00	.00	.00	22	.11	.37	.00	.00	1.30

EDJ – enamel-dentin junction
GDG – gleđno-dentinska granica

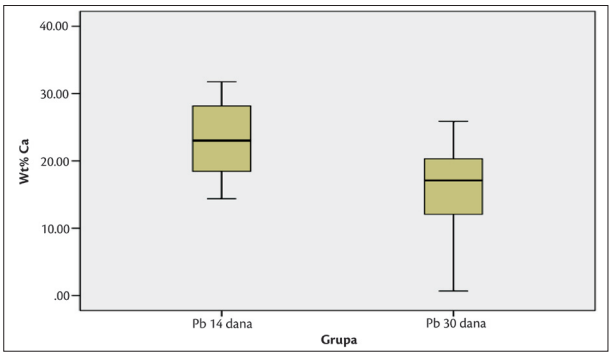

Figure 1. Mass fraction of calcium in enamel in the examined groups
Slika 1. Maseni udeo kalcijuma u gleđi u ispitivanim grupama

Table 2. Spearman's correlation coefficient of the mass fraction of certain elements in hard dental tissues
Tabela 2. Spirmanov koeficijent korelacije masenih udela pojedinih elemenata u tvrdim zubnim tkivima

Spearman's Rho Spirmanov koeficijent		Wt% P	Wt% Ca	Wt% Pb
Wt% P	Ro	1.000	.927**	-.249
	P	.	.000	.062
	N	57	57	57
Wt% Ca	Ro	.927**	1.000	-.272*
	P	.000	.	.040
	N	57	57	57
Wt% Pb	Ro	-.249	-.272*	1.000
	P	.062	.040	.
	N	57	57	57

** Correlation is statistically significant at the probability level of 0.01
** Korelacija je statistički značajna na nivou verovatnoće od 0,01
* Correlation is statistically significant at the probability level of 0.05
* Korelacija je statistički značajna na nivou verovatnoće od 0,05

DISCUSSION

Although the level of lead in hard dental tissues is useful indicator of lead exposure, information on its effects is very limited. Some heavy metals can replace calcium in hydroxyapatite crystals, and therefore the assessment of lead levels in the tooth should not be based solely on the absolute content of this metal, but also its relation to calcium. The aim of one of the more recent studies was to examine the presence of cadmium and lead in deciduous teeth in children suffering from celiac disease and food allergy in the industrial areas of Polland. Using flame atomic absorption spectrophotometry, it was confirmed that these metals were mainly accumulated in deciduous teeth. It has also been observed that toxic heavy metals in teeth remained in a dynamic balance with normal tooth formation, i.e. that they were replaced by calcium in hydroxyapatite crystals [1–4, 15]. Orzechowska-Wylęgała et al. came upon similar results. They found significantly

higher percentage of lead and cadmium compared to calcium in deciduous teeth of children with celiac disease and food allergies compared to the teeth of healthy children [21].

In their study, Malara et al. examined the content of lead and cadmium from tobacco smoke in deciduous teeth of children, whose parents were smokers, in relation to calcium using the method of atomic absorption spectrophotometry. The study found that toxic heavy metals accumulated in the teeth remained in a dynamic balance with normal tooth content (heavy metals replaced calcium in hydroxyapatite crystals) [22].

The results of these studies indicate that lead can replace calcium in hydroxyapatite crystals, which is consistent with the findings of our study. Analysing the obtained values of the mass fraction of certain elements it was noticed that the highest average value of calcium in the rats's teeth receiving lead through drinking water in the course of 14 days was in the area of enamel–dentin junction, then in enamel, and the lowest was in dentin. The lowest average value of calcium in the teeth of rats receiving lead in the course of 30 days was in the area of enamel-dentin junction. Lead in the teeth of rats who received it in the course of 30 days was observed in enamel only, indicating possibility of replacing calcium by lead in the area of enamel-dentin junction and enamel.

Liu et al. who analyzed the mass fraction of elements in enamel and dentin using induced mass spectrometry reported similar results. The samples were human teeth (third molars) collected in ambulances in Taiwan. The obtained results showed detected P in enamel with the mass fraction of 2.19%, Ca (27.91%), and Pb (0.72), and the concentrations of P, Ca and Pb were higher in dentin than enamel. Also, Ca/P ratio was constant [23].

The results of our study and the values of the mass fractions of P, Ca and Pb are consistent with the study conducted by Arora et al. where decreased mass fraction of Ca (24.35%) and P (12.41%) were found in the teeth of patients exposed to lead. They concluded that the concentration of lead in enamel and dentin has been increasing over the years, indicating that teeth can be realiable biomarker of lead contamination [24].

In our study, the SEM analysis protocol was performed on the same segments of all teeth (enamel, enamel-dentin junction and dentin) and lead was not detected in significant concentration. In the experimental groups, such alterations can be explained by the presence of carious lesions or the enamel defects with accumulated lead, detected in the surface parts of enamel.

The study of Bercovitz et al. included the analysis of the presence of lead in the teeth of children and adults using atomic absorption spectrophotometry, indicating higher lead concentration in children's teeth [25]. Youravong et al. examined enamel and dentin in children with high lead concentration in blood using the secondary ion mass spectrometry (SIMS) and x-ray microanalysis. These methods confirmed higher lead concentration in the area between dentin and pulp. X-ray microanalysis could not detect lead while the secondary ion mass spectrometry detected it in dentin near the border with pulp. SEM analysis with multi-element detectors is also a reliable method that can be effective in conventional chemical testing since it does not require standard protocols for sample preparation and it is faster [10].

Anttila examined lead concentration in enamel of deciduous incisors of children in Finland, Askola, a rural area, rich in radon. The study utilized Proton Induced X-rays, and the results showed that lead concentration (8.8+/-6.6 ppm) in enamel was similar to those found in other areas of Finland, indicating that radon does not cause significant increase in lead levels in enamel [26].

The results of these studies are consistent with the results obtained by Appleton, who examined lead concentration in rats's teeth using the SEM analysis. X-ray analysis showed the localization of lead in the form of, so-called, lead line as well as rapid drop of intracellular calcium that was replaced by lead ions [27]. Some studies reported similar results when administering an intravenous lead-acetate injection to mice caused response in dentin by forming so-called lead lines. This was associated with rapid but temporary increase in serum calcium and phosphorus due to the fact that lead replaced calcium and phosphorus in hydroxyapatite crystals [25, 10].

In our study, phosphorus was detected by SEM analysis and the analysis of the obtained results showed that phosphorus could have been replaced by lead, in addition to calcium, in hydroxyapatite crystals.

Issa's results showed that lead was detected in all molars from the experimental group. Higher lead concentration was found close to gingival part of the tooth than occlusal part, with a significant decrease in calcium concentration. These results are consistent with other studies that also have shown lower lead concentration in enamel. It should be emphasized that experimental animal species (rats) in the study by Issa et al. were given lead in the course of 60 days (30 mg / L) through drinking water and the rats in our study were given lead-acetate at the concentration of 1500 ppm (in water ad libitum) in a much shorter period. Much lower values of the mass fraction of lead and solely in the enamel also explain this [28].

Results that were not consistent with ours are the findings of Grobler et al. who examined pregnant female rats who received lead through drinking water during pregnancy and during lactation. Lead concentration in molars was tested by the atomic absorption spectrophotometry. It was found that the highest embedded concentration of lead in dental tissues was in females who drank water with the highest lead concentration. However, this research was conducted by applying high concentrations of lead in water that does not normally happen in the nature [29].

CONCLUSION

Based on the obtained results of this study, it can be concluded that the mass fraction of lead in the teeth of rats who received lead in the course of 30 days, was detected only in enamel, while lead in the teeth of rats who received lead in the course of 14 days was not detected in

any tooth structure. The mass fraction of calcium and phosphorous in enamel of rats who received lead in the course of 30 days was lower than in rats receiving lead in the course of 14 days.

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Koncentracija olova u tvrdim zubnim tkivima – SEM/EDS analiza

Irena Kuzmanović Radman[1], Adriana Arbutina[2], Renata Josipović[1], Aleksandra Đeri[1]

[1]Univerzitet u Banjoj Luci, Medicinski fakultet, Katedra za bolesti zuba, Studijski program Stomatologija, Banja Luka, Republika Srpska, Bosna i Hercegovina;

[2]Univerzitet u Banjoj Luci, Medicinski fakultet, Katedra za ortopediju vilica, Studijski program Stomatologija, Banja Luka, Republika Srpska, Bosna i Hercegovina

KRATAK SADRŽAJ

Uvod Izloženost olovu u životnoj sredini je danas jedna od važnijih ekoloških tema, s obzirom na to da se radi o metalu sa izrazitim toskičnim efektima na ljudski organizam. Čvrsta tkiva zuba predstavljaju dobre strukture za procenu dugoročnih efekata izlaganja toksičnim metalima.

Cilj ovog rada je bio da se SEM/EDS analizom odredi koncentracija olova u tvrdim zubnim tkivima pacova sa eksperimentalno izazvanim DM-om, nakon 14 i 30 dana izlaganja životinja olovu.

Materijal i metode Istraživanje je sprovedeno kod pacova soja Vistar podeljenih u tri grupe. Prvu grupu je činilo osam pacova (128 molara i premolara gornje i donje vilice) sa eksperimentalno izazvanim DM-om koji su uzimali olovo tokom 14 dana u koncentraciji od 1500 ppm, drugu grupu osam pacova (128 molara i premolara gornje i donje vilice) koji su uzimali olovo tokom 30 dana u koncentraciji od 1500 ppm, dok je treću kontrolnu grupu činilo pet zdravih pacova (80 molara i premolara gornje i donje vilice). Eksperimentalne životinje su svakog dana dobijale olovo-acetat u koncentraciji od 1500 ppm putem vode *ad libitum*. Dijabetes melitus kod ovih životinja je indukovan aloksanom. Uzorci zuba su analizirani skening elektronskom mikroskopijom (SEM). EDS analizom je određen maseni udeo olova i ostalih elemenata u tvrdim zubnim tkivima.

Rezultati U zubima pacova koji su dobijali olovo u vodi za piće tokom 14 dana nije detektovano olovo ni u jednom sloju zuba. Prosečne vrednosti masenih udela olova, kalcijuma i fosfora u gleđi zuba pacova koji su dobijali olovo 30 dana iznosile su: za olovo 0,36%, za kalcijum 15,48% i za fosfor 10,62%. Olovo je registrovano samo u predelu gleđi.

Zaključak Olovo je detektovano u zubima pacova koji su dobijali olovo toko 30 dana i to samo u gleđi, dok olovo u zubima pacova koji su ga dobijali u vodi za piće tokom 14 dana nije detektovano ni u jednom sloju zuba.

Ključne reči: olovo; gleđ; dentin; SEM/EDS analiza

UVOD

Čvrsta zubna tkiva se sastoje od nekoliko različitih minerala koji sa kalcijumom predstavljaju glavni makromineral pa zato predstavljaju idealna tkiva za procenu dugoročnih efekata izlaganja organizma toksičnim metalima [1, 2, 3].

Izloženost olovu je danas značajan zdravstveni problem u mnogim zemljama jer je ono povezano sa uticajem na opšte zdravlje (anemija, hipertenzija), odnosno na patologiju kostiju i zuba, uključujući pre svega karijes zuba [4, 5, 6].

Iako su nivoi olova u tvrdim zubnim tkivima korisni pokazatelji izloženosti olovu, informacije o njegovom vremenskom delovanju i jedinjenjima olova u tkivima zuba su vrlo ograničene. U nekim studijama se pokazalo da uticaj olova na zube u razvoju ne mora biti povezan samo sa njegovim citotoksičnim efektima već i sa međusobnom interakcijom sa proteinima i enzimima ekstraćelijskog matriksa. Potvrđeno je takođe da je metabolizam pulpe značajno usporen u nekim metaboličkim oboljenjima, kao što je dijabetes [7, 8].

Postoje izveštaji u literaturi koji ukazuju na to da prisustvo olova u hemijskom sastavu gleđi može promeniti njegovu dentalnu ultrastrukturu i dovesti do oštećenja gleđi. Tako je i Gomes sa saradnicima u svojoj studiji utvrdio u zubima predškolske dece koja su živela u industrijskoj oblasti grada veće koncentracije olova u gleđi nego kod dece koja su živela van ove oblasti [9].

Podaci drevnih populacija otkrili su visoku rasprostranjenost defekata hipoplastične gleđi kod populacije koja je imala visoke nivoe olova u kostima i zubima. Uočena je povezanost između prisustva olova u dentalnom tkivu i kliničkih promena u gleđi u vidu diskoloracije i potvrđeno da je hipoplazija gleđi povećana kod dece izložene visokim koncentracijama olova [10, 11, 12].

In vitro studije su pokazale da prisustvo olova u toku amelogeneze može dovesti do ultrastrukturnih promena gleđi, koje mogu biti povezane sa modifikacijama u fizičko-hemijskom odnosu i time gleđ učiniti osetljivijom na demineralizaciju [13].

Jedno od istraživanja je pokazalo da je sadržaj metala u zubima (kod stanovništva u oblastima zagađenim olovom) povezan sa povećanom incidencom karijesa. Međutim, povezanost uticaja olova i nastanka karijesa u tvrdim zubnim tkivima još uvek je predmet brojnih istraživanja [14].

Moss i saradnici su potvrdili povezanost između izloženosti olova u vreme obrazovanja dentina i povećane zastupljenosti karijesa [15], a Martin i saradnici su zaključili da olovo utiče na nastanak karijesa samo kod mlečnih zuba [16]. Gomes i saradnici su procenjivali povezanost između koncentracije olova u gleđi mlečnih zuba i nisu pronašli povezanost između olova i dentalnog karijesa kod dece u industrijskoj zoni [9].

Cenić-Milošević i saradnici su pokušali da utvrde korelaciju između koncentracije olova u izvađenim zubima stanovnika Pančeva i Beograda (kod pripadnika različitih starosnih grupa) i zaključili da je jedan od mogućih uzroka gubitka zuba i karijesnih oštećenja upravo dugotrajna izloženost olovu [17].

Barmes i Ludmgh su ustanovili korelaciju koncentracije olova u zubima i zubnog kvara, i zaključili da su ispitanici sa visokim koncentracijama olova imali veći broj karijesnih zuba.

Potvrđeno je takođe da olovo kao citotoksični agens može dovesti do uticaja na ameloblaste, to jest promena u količini proteina i kašnjenja u amelogenezi [18].

Gerlach i saradnici su zaključili da olovo povećava koncentraciju proteina, a usporava mineralizaciju gleđi kod sekutića pacova koji su pili vodu sa prisutnim olovom [19].

Tvinnereim i saradnici su uočili vezu između izlaganja olovu u trenutku formiranja dentina i povećanja osetljivosti na karijes kod zuba pacova [20].

Osnovni cilj ovog rada je bio da se SEM/EDS analizom odredi koncentracija olova u tvrdim zubnim tkivima pacova sa eksperimentalno izazvanim DM-om, nakon 14 i 30 dana izlaganja životinja olovu.

MATERIJAL I METOD RADA

Za uzorak su odabrani pacovi soja Vistar, zbog velike sličnosti u fiziologiji pulpe zuba pacova sa fiziologijom pulpe humanih zuba. U eksperiment je uključen 21 laboratorijski pacov soja Vistar, odnosno 336 zuba. Studija je odobrena od strane Etičkog komiteta Zavoda za stomatologiju Medicinskog fakulteta u Banjaluci. Eksperimentalne grupe pacova su podeljene u dve grupe: prvu grupu je činilo osam pacova (128 molara i premolara gornje i donje vilice) sa eksperimentalno izazvanim DM-om koji su uzimali olovo tokom 14 dana u koncentraciji od 1500 ppm. Drugu eksperimentalnu grupu je činilo osam pacova (128 molara i premolara gornje i donje vilice) koji su uzimali olovo tokom 30 dana u koncentraciji od 1500 ppm. Kontrolnu grupu je činilo pet zdravih pacova (80 molara i premolara gornje i donje vilice). Protokol eksperimentalno indukovanog dijabetes melitusa kod pacova je uključivao primenu rastvora aloksan, koji je aplikovan intraperitonealno, a protokol za intoksikaciju olovom je obuhvatao intoksikaciju adultnih pacova olovnim acetatom u koncentraciji od 1500 ppm putem vode *ad libitum*. Sve procedure na životinjama, negovanje, eksperimentalni tretman, žrtvovanje bez bola i stresa izvedeni su u skladu sa Smernicama za brigu o životinjama u eksperimentalnim istraživanjima (Guide for the Care and Use Laboratory Animals, 1996 National Academy Press, Washington, DC). Nakon dekapitacije, gornjovilične kosti pacova su odvajane od mekih tkiva, pohranjene u fiksativ (10% neutralni puferovani formalin) i potom su u uzorku zuba pripremljeni za SEM/EDS analizu. Uzorci zuba su sečeni i polirani dijamantskim diskom kroz sredinu zuba u mediodistalnom smeru kako bi se eksponirao poprečni presek zone gleđi i dentinske mase. Snimanje i analize su urađeni na Skening elektronskom mikroskopu (JEOL JSM 6460LV) i priključenom OXFORD INCAx-sight spektralnom analizatoru. Za potrebe ove analize slike su dobijene Back-scatterovanom ili Primarnom emisijom odbijenih elektrona u Compo modu (BEIc) jer se pokazalo da najkorisnije ističe zone gleđi i dentinske mase. Uzorci su posmatrani pri ubrzanju od 20kV na radnoj distanci (WD) od 10 mm i pod upadnim uglom koji je bio primeren nagibu polirane površine premolara i molara. Opšti snimak dat je u preglednom uvećanju 35×, a za potrebe preciznije EDS analize upotrebljeno je uvećanje 100×. Dobijeni rezultati su analizirani i statistički obrađeni.

REZULTATI

U Tabeli 1 prikazane su prosečne vrednosti masenih udela fosfora, kalcijuma i olova u delovima zuba kod svih ispitivanih grupa. Prosečne vrednosti masenih udela fosfora u zubu pacova koji su dobijali olovo 14 dana bila je najveća u predelu gleđno-dentinske granice (15,61%), potom u predelu dentina (13,96%) a najmanja u predelu gleđi (13,92%). Kod pacova koji su dobijali olovo 30 dana najveći maseni udeo je uočen u dentinu (13,96%), zatim u oblasti gleđno-dentinske granice (21,91%), a najmanja u gleđi (10,62%).

Prosečne vrednosti udela kalcijuma u zubima pacova koji su dobijali olovo u vodi za piće tokom 14 dana bila je najveća u predelu gleđno-dentinske granice (25,66%), potom u gleđi (23,28%) i najmanja u dentinu (22,35%). Kod pacova koji su dobijali olovo 30 dana maseni udeo u oblasti gleđno-dentinske granice je iznosio 20,13%, u gleđi 15,48%, dok je u dentinu iznosio 21,74%.

U zubima pacova koji su dobijali olovo u vodi za piće tokom 14 dana nije detektovano olovo ni u jednom sloju zuba. Kod pacova koji su dobijali olovo 30 dana, ono je bilo detektovano samo u gleđi (0,36).

Analiza dobijenih vrednosti masenog udela pojedinih elemenata u gleđi je ukazala da postoji statistički značajna razlika jedino u vrednostima kalcijuma ($p < 0,06$). I ovde su manje vrednosti zabežene u grupi pacova koji su dobijali olovo u vodi za piće tokom 14 dana u odnosu na grupu pacova koji su dobijali olovo tokom 30 dana. U masenim udelima ostalih elemenata nije dobijena statistički značajna razlika (Tabela 1, Slika 1).

Povezanost dobijenih vrednosti masenog udela pojedinih elemenata u određenim delovima zuba procenjivana je Spiromanovim koeficijentom korelacije. Uočeno je da postoji statistički značajna negativna korelacija između masenog udela kalcijuma i masenih udela olova i statistički značajna pozitivna korelacija sa masenim udeom fosfora. Takođe je uočeno da postoji statistički značajna negativna korelacija između masenog udela olova i masenog udela kalcijuma (Tabela 2).

DISKUSIJA

Iako je nivo olova u tvrdim zubnim tkivima koristan pokazatelj izloženosti olovu, informacije o njegovim efektima u tkivima zuba su vrlo ograničene. Pojedini teški metali mogu zameniti kalcijum u kristalima hidroksiapatita pa se zato i procena nivoa olova u zubu ne bi smela bazirati samo na apsolutnom sadržaju ovog metala već i na njegovom odnosu prema kalcijumu. Jedna od savremenijih studija imala je za cilj da ispita zastupljenost kadmijuma i olova u mlečnim zubima kod dece koja pate od celijaklije i alergije na hranu u industrijskim oblastima Poljske. Upotrebom plamene atomske apsorpcione spektrofotometrije uvrđeno je da su se ovi metali najviše akumulirali u mlečnim zubima. Takođe je uočeno da su toksični teški metali u zubima ostali u dinamičnom balansu sa normalnom građom zuba, tj. da su zamenjeni kalcijumom u hidroksiapatitnim kristalima [1–4,15].

Do sličnih rezultata su došli Orzechowska-Wylęgała i saradnici, koji su utvrdili da u mlečnim zubima dece sa celijaklijom i alergijama na hranu postoji znatno veći maseni udeo olova i kadmijuma u odnosu na kalcijum nego u zubima zdrave dece [21].

U svojoj studiji Malara i saradnici su ispitivali sadržaj olova i kadmijuma iz duvanskog dima u mlečnim zubima dece čiji su roditelji pušači, odnosno njihov odnos sa kalcijumom, metodom atomske apsorpcione sektrofotometrije. Studija je utvrdila da su toksični teški metali koji se talože u zubu ostali u dinamičnom

balansu sa normalnim sadržajem zuba (teški metali su zamenili kalcijum u hidroksiapatičnim kristalima) [22].

Rezultati navedenih studija ukazuju da olovo može zameniti kalcijum u kristalima hidroksiapatita, što je u skladu i sa nalazima ovog istraživanja. Analizom dobijenih vrednosti masenog udela pojedinih elemenata uočeno je da je prosečna vrednost udela kalcijuma u zubu pacova koji su dobijali olovo u vodi za piće tokom 14 dana bila najveća u predelu gleđno-dentinske granice, potom u gleđi i najmanja u dentinu, a da je prosečna vrednost masenog udela kalcijuma u zubu pacova koji su dobijali olovo 30 dana bila najniža u oblasti gleđno-dentinske granice. Prosečna vrednost masenog udela olova u zubu pacova koji su dobijali olovo 30 dana uočena je samo u gleđi, što ukazuje na mogućnost zamene kalcijuma olovom u oblasti gleđno-dentinske granice i gleđi kod testiranih pacova.

Do sličnih rezultata su došli i Liu i saradnici, koji su sproveli studiju u kojoj su određivali maseni udeo elemenata u gleđi i dentinu pomoću indukovane masene spektrometrije. Uzorke su činili humani zubi (treći molari) sakupljeni u ambulantama u Tajvanu. Dobijeni rezultati su pokazali da je u gleđi detektovan P čiji je maseni udeo iznosio 2,19%, zatim Ca (27,91%) i Pb (0,72), a koncentracija P, Ca i Pb je bila veća u dentinu nego u gleđi. Takođe je i odnos Ca i P bio konstantan [23].

Rezultati ovog istraživanja i vrednosti masenih udela P, Ca i Pb su saglasni sa istraživanjem Arore i saradnika, gde je takođe uočen smanjen maseni udeo Ca (24,35%) i P (12,41%) u zubima pacijenata izloženih uticaju olova. Arora i saradnici su zaključili da koncentracija olova u gleđi i dentinu raste sa godinama i ukazuju da zub može biti pouzdan biomarker za olovo [24].

U ovom istraživanju je protokol SEM analize bio sproveden na istim segmentima svih zuba (gleđi, gleđno-dentinskoj granici i u dentinu), a olovo nije detektovano u značajnoj koncentraciji. U eksperimentalnim grupama ove studije ovakve promene se mogu objasniti prisustvom karijesnih šupljina ili defekata gleđi u koje se nataložilo olovo, a koje je SEM analizom detektovano u površinskim delovima gleđi.

Studija Bercovitza i saradnika je obuhvatala analizu prisustva olova u zubima dece i odraslih pomoću atomske apsorpcione spektrofotometrije i ukazala na veću koncentraciju olova u dečjim zubima [25].

Youravonga i saradnici su ispitivali gleđ i dentin kod dece sa visokom koncentracijom olova u krvi metodom sekundarne jonske masene spektrometrije (secondary ion mass spectrometry-SIMS) i mikroanalizom x-zracima. Ove metode su ukazale na vidljiv nivo olova u dentinu na granici sa pulpom. Mikroanaliza x-zracima nije mogla detektovati olovo, dok ga je sekundarna jonska masena spektrometrija detektovala u dentinu blizu granice sa pulpom. SEM analiza sa multielementnim detektorima je takođe pouzdana metoda koja može biti efikasna kod konvencionalnog hemijskog ispitivanja jer ne zahteva standardne protokole pripreme uzoraka i kraće traje [10].

Anttila je ispitivala koncentraciju olova u gleđi mlečnih sekutića dece iz Askole, ruralnog područja, najbogatijeg radonom, u Finskoj. Ispitivanje je rađeno pomoću protonske indukcije x-zracima, a rezultati su pokazali da je koncentracija olova (8,8 ± 6,6 ppm) u gleđi zuba bila slična nalazima iz drugih oblasti Finske, ukazujući na to da radon ne utiče na značajan porast nivoa olova u gleđi zuba [26].

Rezultati ovih istraživanja su saglasni sa rezultatima Appletona, koji se bavio ispitivanjem olovne linije u zubima pacova pomoću SEM-a. Analizom uz pomoć x-zraka je uočena lokalizacija olova u vidu tzv. olovne linije i brzi pad intracelularnog kalcijuma, koji je bio zamenjen jonima olova [27]. Do sličnih rezultata su došle neke studije koje su pokazale da se davanjem intravenske injekcije olovo-acetata miševima javlja odgovor u dentinu zuba, formiranjem tzv. olovne linije. Ovo je bilo povezano sa brzim, ali privremenim porastom serumskog kalcijuma i fosfora, tj. činjenice da olovo zamenjuje kalcijum i fosfor u kristalima hidroksiapatita [25, 10].

U ovoj studiji je takođe SEM analizom detektovan fosfor, a analiza dobijenih rezultata je pokazala da je olovo pored kalcijuma moglo zameniti i fosfor u kristalima hidroksiapatita.

Rezultati Issa su pokazali da je olovo detektovano u svim molarima iz eksperimentalne grupe, a veća koncentracija olova je uočena uz gingivalnu nego uz bukookluzalnu ivicu, uz značajno smanjenje koncentracije kalcijuma. Ovi rezultati su u skladu sa ovim istraživanjima jer je i ovde uočena manja koncentracija olova u gleđi zuba. Treba naglasiti da su eksprimentale životinje (pacovi) u studiji Issa i saradnika dobijali olovo 60 dana (30 mg / L) u vodi za piće, a pacovi u našoj studiji dobijali olovo-acetat u koncentraciji 1500 ppm (u vodi *ad libitum*) u mnogo kraćem periodu. To objašnjavaju i mnogo manje vrednosti masenog udela olova i to samo u gleđi [28].

Rezultati koji nisu bili u skladu sa našim su nalazi Groblera i saradnika, koji su ispitivali trudne ženke pacova koje su dobijale olovo u vodi za piće tokom trudnoće i tokom laktacije. Koncetracija olova u molarima je ispitivana atomskom apsorpcionom spektrofotometrijom i utvrđeno je da je olovo najviše ugrađeno u zubno tkivo kod ženki koje su pile vodu sa najvećom koncentracijom olova. Međutim, ovo istraživanje je urađeno primenom visokih koncentracija olova u vodi koja se inače ne nailazi u životnoj sredini [29].

ZAKLJUČAK

Na osnovu dobijenih rezultata ovog istraživanja može se zaključiti da je maseni udeo olova u zubima pacova koji su dobijali olovo 30 dana bio detektovan samo u gleđi, dok olovo u zubima pacova koji su dobijali olovo tokom 14 dana nije detektovano ni u jednom sloju zuba.

Maseni udeo kalcijuma u zubima pacova koji su dobijali olovo 30 dana u oblasti gleđi je bio niži u odnosu na vrednosti udela kalcijuma u gleđi zuba pacova koji su dobijali olovo tokom 14 dana, a maseni udeo fosfora u zubima pacova koji su dobijali olovo tokom 30 dana bio je najmanji u gleđi, te je bio niži nego u gleđi zuba pacova koji su dobijali olovo tokom 14 dana.