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Sadržaj mangana u trajnim zubima

The Manganese Content of Human Permanent Teeth

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Sažetak
Svrha studije bila je istražiti i usporediti sadržaj mangana u uzorcima zuba pojedinaca s različitih zemljopisnih lokacija. **Materijal i postupci:** Na području dvaju kosovskih gradova – Kline i Mitrovice, te u Grazu u Austriji bilo je skupljeno sedamdeset i šest uzoraka zuba od pojedinaca (u dobi od 18 do 65 godina). Ukupno je bilo 40 zuba od pacijenata i 47 od pacijentica.Koncentracija mangana mjerila se korištenjem induktivne spektrometrije (ICP-MS-a). **Rezultati:** Srednja vrijednost koncentracije mangana kod mještana Kline iznosila je 2,03 ± 1,44 mg/kg, kod stanovnika Mitrovice 1,42 ± 0,71 mg/kg, a kod stanovnika Graza 1,01 ± 0,85. Sadržaj mangana kod Klinjana i Mitrovčana bio je statistički znatno veći od onoga u zubima stanovnika Graza ($p < 0,05$). Nije bila ustanovljena statistički veća razlika u koncentraciji mangana u zubima između muških i ženskih ispitanika ($p > 0,05$). **Zaključci:** Koncentracija mangana bila je veća u uzorcima zuba stanovnika Kosova negoli Austrijanaca.

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Ključne riječi
mangan; elementi u tragovima; zubi

Uvod

Mangan (Mn) element je u tragovima i vrlo je važan u ljudskom i životinjskom metabolizmu (1). Mnogo ga je u okolišu i najčešće ima oksidacijsko stanje +2 i +7. Budući da utječe na metabolizam glukoze i lipida, stvaranje vezivnoga i koštanoga tkiva, te na rast i reprodukciju, njegov manjak ili višak mogli bi rezultirati važnim kliničkim učincima. Dnevne potrebe ljudi za manganom iznose 2 do 3 mg, a dopuštena koncentracija u krvi je 10 μg /L (2). Najveća dopuštena koncentracija mangana i drugih elemenata u tragovima važan su čimbenik i za ljudske zube. Najveća dopuštena koncentracija mangana u vodi iznosi 1 do 1,5 mg/L (3).U kristali-

Introduction

Manganese (Mn) is the trace element which plays an important role in the human and animal metabolism (1). It is widely spread in the environment and the most common oxidation states are +2 and +7. Since manganese influences in the glucose and lipid metabolism, connective and bone tissue formation, and the growth and reproduction system, the defficient or high amounts of manganese could bring serious clinical effects. Daily human needs for manganese is 2-3 mg, allowed concentration of Mn in blood is until 10 μg/L (2). The maximal allowed concentration of Mn in Trace elements like manganese have an important role for human health. The maxi-

ma hidroksilnog apatita pronađeno je više od 49 elemenata, pa i mangan, uglavnom u malim postocima (4). Mnogi znanstvenici istaknuli su da u strukturama zuba nisu pravilno raspoređeni elementi u tragovima. Koncentracija mangana veća je u vanjskim slojevima cakline nego na caklinsko-dentinskom spojištu (5). Gierat i Kucharzewsha (6) pronašli su veće koncentracije mangana u trajnim zubima negoli u mliječnima. Koncentracija elemenata u tragovima u korelaciji je s karijesom. Aluminij (Al), željezo (Fe) i stroncij (Sr) su karijesni inhibitori, a mangan (Mn), bakar (Cu) i kadmij (Cd) karijesni promotori (7). Stermer i suradnici (8) istražili su količinu mangana u 51-om uzorku zuba iskopanom kod gotičke crkve Svetog Olafa u Trondheimu u Norveškoj. Koristeći se analizom atomske raščlambe (AAS-a) potvrdili su da povećana koncentracija mangana rezultira obojenjem zuba crnim. Zaključili su da je mangan, vjerovatno u obliku oksidnih naslaga iz tla, bio uzrok *takvom* obojenju.

Sadržaj mangana u zubima pojedinaca s Kosova u literaturi još nije obrađen. Zato je naše istraživanje bilo usmjereno na koncentraciju mangana u dvama gradovima (Klini i Mitrovici). Osim toga razine mangana bile su izmjerene i na uzorcima iz Austrije (Graz).

Materijali i načini

Istraživanje se temeljilo na analizi 86 trajnih zuba (izvađeni zbog različitih indikacija) dobivenih od osoba s boravištem u dvjema državama. U Kosovu su zubi bili ekstrahirani u ordinacijama u Mitrovici (n = 31) i Klini (n = 32), a u Austriji u Grazu (n = 23). Zubi s ispunima i endodontskom terapijom nisu se obrađivali. Oni s karijesom bili su očišćeni nakon ekstrakcije. Svaki izvađeni zub odvojeno je bio stavljen u posudu s 10-postotnom formalinskom otopinom. Nakon toga su bili oprani četiri puta po 10 minuta vodom (Milli-Q 18,2 MΩ.cm) i etanolom (voda/etanol/voda/etanol) u ultrazvučnoj kupelji. Zatim su osušeni zaleđivanjem preko noći u polipropilenskim epruvetama od 15 ml. Osušeni zubi usitnjeni su tučkom na veličinu manju od 2 mm. Svaki uzorak bio je razgrađen u kopiji pomoću uređaja visokotlačne mikrovalne razgradnje (MLS ultra CLAVE II) - uzorci od 250 mg bili su razgrađeni u 5 ml dušične kiseline (HNO3 p.A. sub boiled) tijekom 40 minuta na 250^0C. Za osiguranje kvalite-

mum allowed level of Mn in water is 1-1.5 mg/L (3). In hydroxyapatite crystals of enamel are found more than 49 elements, one of them was Mn, mostly in very small percentage (4). Many researches showed that trace elements were not equally distributed in tooth structures. Mn concentration is higher in outer surface of enamel than in enamel-dentin border (5). Gierat-Kucharzewsha (6) found higher Mn concentrations in permanent than in primary dentition. Trace element concentrations are in correlation with the presence of dental caries. Aluminium (Al), Iron (Fe) and Strontium (Sr) are caries inhibitory, while Manganese (Mn), Copper (Cu) and Cadmium (Cd) are caries promoting trace elements (7). Stermer et al. (8) have examined the manganese content in 51 teeth from excavations of the gothic church St. Olav in Trondheim. Using the atomic absorption analysis (AAS) they confirmed the increased manganese content in blackish stained enamel. They have concluded that manganese, probably in the form of an oxide deposited from the soil**,** was the cause of the blackish staining.

However, the manganese level in the teeth of individuals from Kosova was less investigated. Therefore, the aim of our study was to measure the manganese concentrations in teeth samples from individuals living in two places in Kosova (Klina and Mitrovica). Furthermore, the manganese level in teeth samples was also measured in individuals from Austria (Graz).

Materials and methods

The research was conducted in 86 permanent teeth (extracted for various indications) from individuals living in two countries. In Kosova, teeth were extracted from habitants of Mitrovica (n = 31) and Klina (n = 32), and in Austria from Graz (n = 23).The teeth with filling and endondotic treatment were excluded. The teeth with caries were cleaned after extraction. Every extracted tooth was put in special container containing 10% formalin solution. Then the teeth were washed consecutively four times for 10 minutes with water (Milli-Q 18.2 MΩ.cm) and ethanol (water/ethanol/water/ethanol) in an ultrasonic bath. After that, they were freeze-dried over night in 15 ml polypropylene tubes. Dried teeth were broken up with an agar mortar and pestled to a grain size smaller than 2 mm. Each tooth sample was digested in duplicate in a pressurized microwave digestion unit (MLS ultra CLAVE II), taking 250 mg of tooth sample that were digested in 5 ml of nitric acid (HNO3 p.A. sub boiled) for 40 min-

te podataka, prazni uzorci (n = 3) i standardni materijal za referenciju (NIST SRM 1400 Bone ash) (n = 3) bili su uključeni u svaku razgradnju paralelno s uzorcima zuba. Razgradni produkti bili su razrijeđeni do 50 ml u polipropilenskim epruvetama. Prije mjerenja razgrađeni zubi još su dodatno razrijeđeni 1: 5 na dan mjerenja. Količina mangana mjerila se induktivno spojenim plazmatskim masenim spektrometrom (7500c ICP-MS, Agilent®, Waldbronn, Njemačka). Kako bi se korigirao utjecaj sadržaja suspenzije, svakom su uzorku dodane poznate koncentracije germanija i indija (100 μg/l) kao interni standard. Rezultati su prikazani kao aritmetička sredina, median i standardna devijacija. Razina statističke znatnosti bila je postavljena na 0,05.

utes at 250^{0}C. To ensure data quality, blank samples (n = 3) and standard reference material (NIST SRM 1400 Bone ash) (n = 3) were also included in each digestion run together with teeth samples. Digests were diluted to 50 ml in polypropylene tubes. Before the measurement, the digested teeth solutions were further diluted 1: 5 on the day of measurement. The manganese was analyzed by using inductively coupled plasma mass spectrometer (7500c ICP-MS, Agilent®, Waldbronn, Germany).To correct for any matrix suppression effects, a known concentration of Germanium and Indium (100 μg/l) were added to each sample as an internal standard. Results were expressed as mean, median, and standard deviation. The level of significance was 0.05.

Rezultati

Koncentracija mangana u zubima pojedinaca s područja Kosova i Austrije

Aritmetička sredina koncentracije mangana najviša je bila u uzorcima zuba iz Kline (2,3 ± 1,44 mg/kg) (raspon 0,4 – 5,9 mg/kg). U Mitrovici je iznosila 1,42 ± 0,71 mg/kg (raspon 0,4 – 2,9 mg/kg), a u Grazu 0,97 ± 0,85 mg/kg (raspon 0,1 – 3,4 mg/kg) (Tablica 1., Slika 1.). Koncentracija mangana u uzorcima iz Kline bila je znatno viša nego u dru-

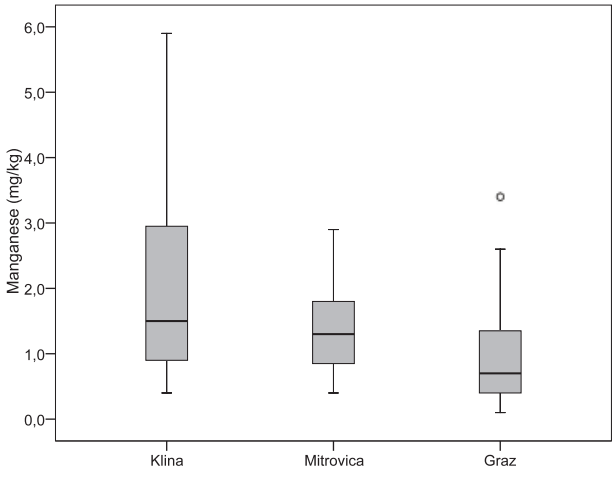
Results

Manganese concentrations in teeth of individuals from Kosova and Austria

The mean manganese concentrations in teeth samples are highest in Klina (2.03 ± 1.44 mg/kg) (range 0.4 – 5.9 mg/kg). In Mitrovica, the mean manganese concentration was 1.42 ± 0.71 mg/kg (range 0.4 – 2.9 mg/kg), and in Graz 0.97 ± 0.85 mg/kg (range 0.1 – 3.4 mg/kg) (Table 1, Figure 1). The teeth manganese concentrations were signifi-

Tablica 1. Koncentracija mangana (mg/kg) u trajnim zubima pojedinaca s područja Kosova (Klina, Mitrovica) i Austrije (Graz). N je broj ispitanih uzoraka svake skupine.
Table 1 Distribution of manganese concentrations (mg/kg) in permanent teeth of individuals from Kosova (Klina, Mitrovica) and Austria (Graz). N is the number of participants in each group.

Područje • Region	N	Min.	Max.	Median	Ar. sred. • Mean	St. dev.
Klina	32	0.4	5.9	1.5	2.03	1.44
Mitrovica	31	0.4	2.9	1.3	1.42	0.71
Graz	23	0.1	3.4	0.7	0.97	0.85



Slika 1. Koncentracija mangana (mg/kg) u trajnim zubima pojedinaca s područja Kline, Mitrovice i Graza. Kvadrati predstavljaju medijan i raspon (minimum-maksimum) za svaku skupinu.
Figure 1 Manganese concentrations (mg/kg) in permanent teeth of individuals from Klina, Mitrovica, and Graz. Boxes represent median and range (minimum-maximum) per each group.

ga dva analizirana područja – u Mitrovici i u Grazu ($p < 0,05$). Ističemo da su u Mitrovici koncentracije mangana u zubima više bile nego u uzorcima iz Graza ($p < 0,05$).

Mangan i spol

Utjecaj spola na razine mangana u zubima nalazi se u Tablici 2. Nije uočena statistički veća razlika između uzoraka zuba muškaraca i žena ($p > 0,05$) (Slika 2.).

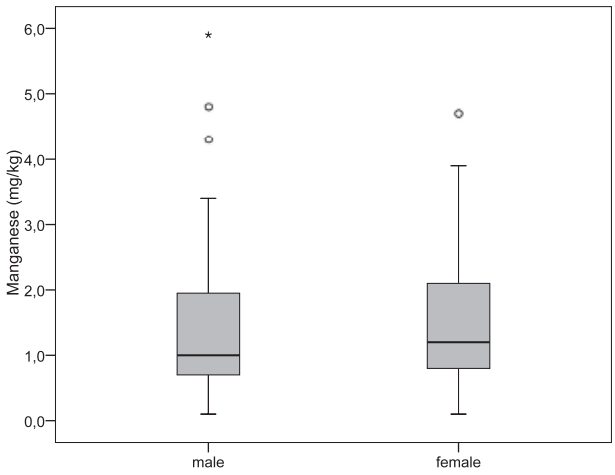
cantly higher in Klina than in both other places including Mitrovica and Graz ($p < 0.05$). However, in Mitrovica, the teeth manganese levels are higher than in teeth samples from Graz ($p < 0.05$).

Manganese and gender

The influence of sex on manganese teeth level was presented in Table 2. There are no significant differences in overall teeth manganese concentrations between female and male subjects from locations included in this research ($p > 0.05$) (Figure 2).

Tablica 2. Koncentracija mangana (mg/kg) u uzorcima trajnih zuba muškaraca (40) i žena (46)
N je broj ispitanih uzoraka svake skupine
Table 2 Manganese concentrations (mg/kg) in permanent teeth from male (40) and female (46) tested
N is the number of participants in each group

G	N	Min.	Max.	Median	Ar. sred. • Mean	St. dev.
muški • male	40	0.1	5.9	1.0	1.53	1.27
ženski • female	46	0.1	4.7	1.2	1.52	1.03



Slika 2. Koncentracija mangana u trajnim zubima (mg/kg), ovisno o spolu
Figure 2 Distribution of manganese concentrations (mg/kg) in permanent teeth according to sex.

Rasprava

Naši rezultati dokazali su da se elementi u tragovima akumuliraju u tvrdim zubnim tkivima i to je već opisano u literaturi. Također potvrđuju i rezultate ostalih autora o taloženju u zubima. (9-14). Prema našim spoznajama, to je prvo istraživanje u kojem se proučavalo razine mangana u uzorcima zuba s područja Kosova.

Rezultati su pokazali da je najveća koncentracija mangana u Klini (2,03 ± 1,44), a zatim u Mitrovici (1,42 ± 0,71mg/kg), te u Grazu (0,97 ± 0,85 mg/kg) (Tablica 1., Slika 1.). Koncentracija mangana u zubima statistički je mnogo veća u Klini nego u druga dva istražena područja – u Mitrovici i Grazu ($p < 0,05$). Ističemo da su razine mangana u zubima u Mitrovici veće nego u Grazu ($p < 0,05$). Nije

Discussion

Our results showed that trace elements as manganese have affinity to accumulate in human dental hard tissues as was reported previously. These results also are in agreement with the results of other authors about the tooth deposition affinity (9-14). To our knowledge, this is the first study about the manganese level in teeth samples from Kosova.

Our results showed that the mean manganese concentration in teeth samples was highest in Klina (2.03 ± 1.44) followed by Mitrovica (1.42 ± 0.71mg/kg), and in Graz (0.97 ± 0.85 mg/kg) (Table 1, Figure 1). The teeth manganese concentrations were significantly higher in Klina than in both other places Mitrovica and Graz ($p < 0.05$).However, in Mitrovica, the teeth manganese levels were

ustanovljena statistički znatna razlika u koncentraciji mangana u zubima muškaraca i žena uključenih u istraživanje ($p > 0,05$) (Tablica 2.).

Nekoliko stručnjaka istraživalo je razine mangana u zubima. Lane i Peach su izvijestili o srednjoj vrijednosti koncentracije mangana od 3,5 ppm (odgovara 3,5 mg/kg) u populaciji Oxforshirea (12). U Poljskoj je srednja vrijednost koncentracije mangana varirala od 4,6 μg/g (≈ 4,6 mg/kg) u Beskidu do 5,9 μg/g (≈ 5,9 mg/kg) u Katovicama (13). Premda je usporedba naših podataka s ranije objavljenima dosta složena zbog različite metodologije, razlika u tipu zuba, te u spolu i starosti testiranih uzoraka, naši su rezultati ipak u skladu s objavljenim razinama mangana u uzorcima zuba. No, pomoću instrumentalne neutronske aktivacijske analize (INAA), Iyengar i Tandon dobili su niže vrijednosti koncentracija mangana u humanim zubima (od 0,13-1,29 mg/kg) (15).

Nekoliko autora mjerilo je koncentraciju mangana u caklini i dentinu. Koncentracija u caklini bila je vrlo niska (0,08-20 ppm, odgovara 0,08-20 mg/kg), a u dentinu viša (od 0,6 to1000 ppm) (4). Prema Anđićevu mišljenju (5), vrijednosti mangana u vanjskoj caklini su 0,45-1,98 ppm i više su od već objavljenih (0,34 – 0,79 ppm) (1).

Naši rezultati pokazuju znatnu razliku u koncentracijama mangana u uzorcima zuba između pojedinaca s prebivalištem u dvjema kosovskim regijama u odnosu prema onima u Austriji. U Klini su količine mangana možda veće zbog rudnika boksita u okolici. Zbog toga su potrebna daljnja istraživanja sadržaja mangana i ostalih elemenata u tragovima u biološkim uzorcima (uključujući i zube) i onima iz analiziranih područja kako bi se bolje mogli objasniti dobiveni rezultati.

higher than in teeth samples from Graz ($p < 0.05$). There are no significant differences in teeth manganese concentrations between female and male subjects included in this research ($p > 0.05$) (Table 2).

Several studies have investigated the manganese level in teeth samples. Lane & Peach reported the mean Mn concentrations of 3.5 ppm (equivalent 3.5 mg/kg) at population of Oxfordshire (12). In various regions of Poland, the mean Mn concentration ranged from 4.6 μg/g (≈ 4.6 mg/kg) in Beskid to 5.9 μg/g (≈ 5.9 mg/kg) in Katovica (13). Although the comparison of our results with previously published papers is rather complex due to different analysis methodology, difference in tooth type, and in the difference in sex and age of tested individuals, our results are generally in agreement with reported values for Mn levels in teeth samples. However, using instrumental neutron activation analysis (INAA), Iyengar & Tandon reported much lower Mn concentration in human teeth (from 0.13-1.29 mg/kg) (15).

Several authors have measured the manganese concentrations in enamel and in dentin. Mn concentrations in enamel are very small (0.08-20 ppm, equivalent 0.08-20 mg/kg), while in dentine are higher (from 0.6 to1000 ppm) (4). According to Anđić (5), the manganese values in outer enamel of 0.45-1.98 ppm were higher than those (0.34 – 0.79 ppm) reported previously (1).

Our results showed the significant difference in Mn levels in teeth samples between individuals living in two regions of Kosova and in Austria. The higher Mn level in Klina than in other investigated locations may be due to the bauxite mines situated near the Klina suburb. Therefore, further investigation of manganese and other trace elements level in biological (including teeth) and environmental samples collected in this location should be done to explain these results.

Abstract

Aim: The aim of this study was to investigate and compare the manganese concentrations in teeth samples of individuals from different geographical locations. **Material and Methods:** Eighty-six teeth samples were collected from adult (18-65 years) individuals living in two towns in Kosova (Klina and Mitrovica), and in Austria (Graz). Total of 40 and 46 teeth samples were taken from male and female individuals, respectively. Manganese concentrations in teeth samples were measured by using inductively plasma mass spectrometry (ICP-MS). **Results:** The mean manganese concentration in permanent teeth from Klina residents is 2.03 ± 1.44 mg/kg, from Mitrovica residents 1.42 ± 0.71 mg/kg, and from Graz residents 1.01 ± 0.85. Manganese concentrations in teeth of individuals from Klina and Mitrovica are significantly higher than in Graz ($p < 0.05$). There was no significant difference between manganese levels according to sex in overall tested groups ($p > 0.05$). **Conclusions:** Manganese concentrations were higher in teeth samples from Kosova than in samples from Austria.

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Key words
Manganese; Trace elements; Tooth

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