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Original scientific paper

Lead Concentrations in Teeth from People Living in Kosovo and Austria

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A B S T R A C T

The objective of this study was to compare lead concentrations in 86 human permanent teeth extracted from residents of three different geographical regions. The study included 31 permanent teeth from residents of Mitrovica (Kosovo), 32 from Klina (Kosovo) and 23 from Graz (Austria). The concentrations of lead were measured using Agilent 7500c inductively coupled plasma mass spectrometer (ICP-MS) (Agilent, Waldbronn, Germany). The comparisons between groups were based on the geographic area, age and gender. The highest lead level was found in teeth extracted from Mitrovica residents (22.3 mg/kg), followed by Klina (3.2 mg/kg), and Graz (1.7 mg/kg). Lead levels in teeth from Mitrovica residents are significantly higher (p<0.0001) than in other two groups, possibly due to environmental contamination with lead. Overall results in this study support the concept that tooth lead level may present an important indicator in evaluating environmental exposure of human population to heavy metals.

Key words: *lead concentration, permanent teeth, ICP-MS, pollution*

Introduction

Increased industrialization in the world is associated with the extraction and distribution of large amounts of toxic metals from their natural deposits causing human population exposure[1]. Among various toxic elements, lead is one of the highest prevalence in the nature. Kosovo's industrial zones are affected by polluted air, water and soil due to environmental pollution (various technological units without filters, untreated waste, leaking). In the absence of a functional industry, however, these areas present sources of environmental pollution. Heavy metals particularly lead, pose a risk for population living in Mitrovica area. Lead levels in air, water and soil between various geographical areas differ significantly. The most important factors which influenced on lead levels in environmental samples are both industrial and urban development and life style[2]. Evaluation of toxic effects of heavy metals on humans living in polluted areas is usually based on analysis of heavy metals in blood, urine, hair, teeth and fingernails[3–5].

Lead is one of the major environmental pollutants and the teeth have a capacity to accumulate lead from the environment[6]. Therefore, teeth are a good indicator of long-term exposure to lead from polluted environment and many researchers exploit this feature[7–11]. The lead concentration in teeth is influenced by age and type of tooth, geographical area and the socio-economic status[12]. Many authors have studied the influence of age and gender on lead accumulation in human teeth. It has been demonstrated that lead concentration increased over time in dentin and pulp, excluding enamel[13]. A significant relation exists between lead concentration in teeth and patients' age[14,15], but no significant difference were found between genders[14,16]. In the study of lead accumulation in permanent dentition in Erfurt area which included suburban residents[17], lead concentrations were related to patient's age. Additionally, lead concentrations in teeth depend on the lead level in food and in air. They found no significant differences between sex and investigated areas of Erfurt and surroundings. Spatial distribution of lead in the dentine of human primary teeth can be used to obtain temporal information of lead exposure during the pre and neonatal periods[18]. Associations between lead exposure and dental caries have been reported, but only weak evidence, for an association of low-le-

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vel exposure with dental caries was found[19]. The objective of this study was to measure the lead concentration in human permanent teeth between three different geographic areas and to evaluate the influence of environment pollution on lead accumulation in permanent teeth. The area of Mitrovica (Kosovo) was recognized as a heavily polluted place with several heavy metals. Therefore, the analysis of lead in teeth from this location was compared with the remaining two relatively unpolluted areas (Klina and Graz, both rural and urban zones, respectively).

Materials and Methods

A total of 86 human permanent teeth, extracted for various reasons were used for analysis of lead. The first studied group included 31 extracted permanent teeth from Mitrovica. The second and the third group included 32 and 23 permanent teeth sampled from Klina (Kosovo) and Graz (Austria), respectively. The epidemiological data included information on the patients' age, gender, dental chart and place of residence. Only teeth without fillings and/or endodontic treatment were considered in the study. The decayed teeth were cleaned following extraction. Each extracted tooth was placed in separate containers containing 10% formaldehyde. Teeth samples were inspected to be free of fillings and debris. Then they were washed in water and ethanol four consecutive times for 10 minutes (Millie-Q 18.2 MΩ.cm) (water/ethanol/water/ ethanol) in an ultrasonic bath. They were then freeze-dried over night in 15 ml polypropylene tubes. Dried teeth were crushed with an agar mortar and pestle to a grain less than 2 mm in size. A 250-mg of tooth sample was digested in 5 ml of nitric acid (HNO_3 p.A., sub boiled) for 40 minutes in a pressurized microwave digestion unit (MLS ultra CLAVE II). Each tooth sample was digested in duplicate. Digests were diluted to 50 ml in polypropylene tubes. Reference material (NIST SRM 1400 Bone Ash) and blanks were digested in each digestion run together with teeth samples and included for evaluation of results. On the day of measurement, the digested teeth solutions were further diluted (1:5) and then quantitatively analyzed for lead using an Agilent 7500c inductively coupled plasma mass spectrometer (7500c ICP-MS, Agilent, Waldbronn, D). In addition, monitor solutions (containing the lead of known concentration) were also analyzed regularly throughout every run of tooth samples to allow recalculation of sample concentrations in case of instrumental drift. Results were expressed as mean, median and geometric mean. As a measure of variance standard deviation and geometric standard deviation were used. Difference between lead concentrations in three locations was calculated by Student's t-test. The level of significance was set up at $p<0.05$. Pearson correlation was carried out to test relationship between lead concentration in teeth and age of residents in three locations (Graz, Klina, Mitrovica).

Results

Table 1 shows the arithmetic, geometric means and medians of lead concentrations in permanent teeth from patients of Mitrovica, Klina and Graz. Results also presented standard deviation, geometric standard deviation and minimum – maximum values. The mean overall lead levels in teeth of patients from Mitrovica, Klina and Graz were 22.3 mg/kg, 3.18 mg/kg and 1.69 mg/kg, respectively. Lead concentrations in permanent teeth collected in Mitrovica were significantly higher than in both Klina and Graz ($p<0.0001$). A significant higher lead values were also found in teeth of patients from Klina than in Graz participants ($p<0.05$). There was a statistically significant positive correlation between lead concentrations in teeth of residents of relatively lower polluted areas of Klina and Graz ($r=0.82$). However, relationship between

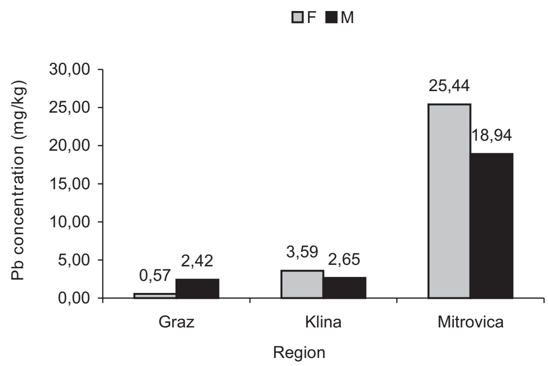

Fig. 1. Concentration (mg/kg) of lead – gender distribution.

TABLE 1
LEAD CONCENTRATION (MG/KG) IN PERMANENT TEETH FROM DIFFERENT REGIONS

Place	Graz	Klina	Mitrovica
No.	23	32	31
Arithmetic mean	1.69	3.18	22.3
Arithmetic standard deviation	1.96	2.3	22.85
Median	0.7	2.1	14.7
Geometric Mean	1.06	2.53	12.33
Geometric standard deviation	2.5	1.97	3.27
Minimum – Maximum value	0.37–8.24	0.97–8.94	1.31–76.18

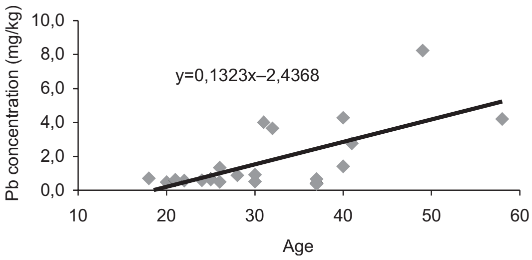

Fig. 2. Correlation and Regression line between age and lead concentration – Graz.

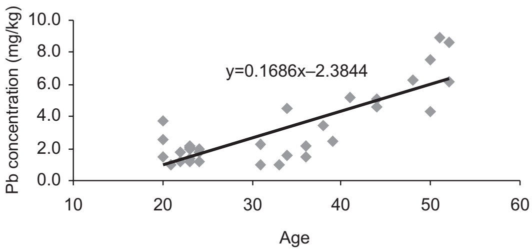

Fig. 3. Correlation and Regression line between age and lead concentration – Klina.

lead concentrations in teeth of patients from Mitrovica and other two regions (Klina, Graz) was not significant. Figure 1 shows the lead distribution in teeth samples according to gender of participant in three locations. There were not significant difference in lead levels according to gender in teeth collected in Mitrovica and Klina ($p>0.05$). However, the concentration of lead in teeth of males from Graz was significantly higher than in females. Figures 2–4 show relationship between age of residents and lead concentrations in their teeth. Lead concentrations in teeth samples from Klina and Graz increased linearly with the age of participants. However, different pattern was observed for lead levels in teeth taken in Mitrovica (Figure 4). In this group, lead concentrations in teeth slightly decreased in relation to age and there were no significant relationship between these two parameters.

Discussion and Conclusion

Comparison of the results form this study with those reported previously is quite complex due to geographic differences in sampling of tooth, pollution parameters, size of population sample, tooth type and sample preparation (whole tooth, only enamel or dentin)[10–12,20]. However, several papers have been selected for comparison with results of our study. Results of this study show that the highest lead concentrations in human permanent teeth were in Mitrovica (22.3 mg/kg), followed by Klina (3.1 mg/kg) and Graz (1.6 mg/kg) (Table 1). These results show that lead concentrations in Mitrovica are much higher than reported values for lead in other high polluted regions[21–24]. Such high concentration may be explained with the fact that Mitrovica is an industrial area with various technological units: large smelters, refineries, flotation, battery factory and sulphuric acid production facility. Vegetables and crops grown in Mitrovica area were reported to contain increased values of lead[25] compared to unpolluted areas. High lead concentrations were found in fresh foodstuffs (such as spinach, onions and potatoes)[26]. Therefore, ingestion of these foodstuffs from Mitrovica area could result in higher lead ingestion by consumers. This was supported by previous reports that ingestion of lead by foodstuffs in Mitrovica residents is three times higher than is the maximum lead level recommended by Word Health Organization (WHO). Results of high lead levels in teeth of Mitrovica residents are in accordance with results obtained previously which also reported higher lead levels in the environment with industrial contamination[27,28]. Results of the present study show a statistically significant positive correlation between age and lead concentrations in teeth collected in Klina and Graz (Figure 2 and 3). These results are in agreement with other papers that previously demonstrated the significant relationship between lead level in teeth and patient's age[14,15,29–31]. Investigations in Poland (Katovica) reported that lead levels were higher in people older than 30 years than in younger participants[3]. However, results of this paper showed that in high polluted are of Mitrovica, the kinetics of lead accumulation in teeth is different than in relatively unpolluted locations of Klina and Graz (Figure 4). There were no relationships between age and lead levels in teeth of Mitrovica participants, and a slightly decreasing trend of lead level was observed in patients older than 40 years (Figure 4). The mean concentrations of lead do not vary significantly between the ages 20–50, but increased rapidly at ages 51–60[32]. Furthermore, higher lead levels were found only in teeth of Mitrovica participant younger of 40 years than in the respective age groups of participant from Klina and Graz. It could be due to saturation of body with lead in Mitrovica residents exposed to continuous environmental pollution. Poor hygiene of food stored in an open environment could also influence on lead intake. These results are in agreement with other reports which reported high lead level in areas polluted with heavy metals[27,33–36]. No statistically significant difference of lead exposure between urban and suburban regions was found[37]. Results of the present paper show that higher lead concentrations were found in females patients from Mitrovica (25.4 mg/kg) than in males (18.4 mg/kg) but these differences were not significant ($p>0.05$). In Klina, lead values were about 8 times lower in teeth samples of both female and male participants than in Mitrovica par-

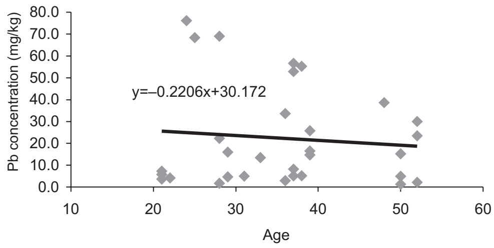

Fig. 4. Correlation and Regression line between age and lead concentration – Mestrovic.

ticipants. The lead levels in teeth of female and male participants from Graz were about 40 and 8 times lower than in respective groups from Mitrovica area, respectively. According to gender, difference in lead levels between female and male participant from Klina were not significant, similarly was found for Mitrovica participants (Figure 1). These results are in agreement with other studies which also reported that lead levels in human teeth were not influenced by gender[15,16,30,37]. However, by contrast in Mitrovica and Kline, lead level in teeth collected in Graz were significantly higher in male (2.4 mg/kg) than in female (0.5 mg/kg) participants ($p<0.05$). This may be attributed to the professions of male participants in this group which included work at open polluted environments (traffic regulators, construction workers, street janitor, etc).The results of the present study indicated that teeth may serve as an indicator of environmental pollution with heavy metals, including lead. Our results also show that lead levels in human teeth are related to the industrial contamination, and further epidemiological investigations are needed to explain the mechanisms and health impact of lead and other heavy metal accumulation on human health.

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KONCENTRACIJA OLOVA U ZUBIMA POPULACIJE KOSOVA I AUSTRIJE

SAŽETAK

Cilj ove studije bio je usporediti koncentraciju olova u 86 stalnih zubiju stanovnika tri različite geografske regije. Studija je obuhvatila trajne zube 31 stanovnika Mitrovice (Kosovo), 32 stanovnika Kline (Kosovo) i 23 stanovnika Graza (Austrija). Koncentracije olova su mjerene spektrometrom Agilent 7500c (Agilent, Waldbronn, Njemačka). Skupine su uspoređivane s obzirom na geografsko područje, dob i spol. Najviša kocentracija olova zabilježena je u zubima ispitanika iz Mitrovice (22,3 mg/kg), zatim iz Kline (3,2 mg/kg) i naposljetku iz Graza (1,7 mg/kg). Koncentracija olova u zubima ispitanika iz Mitrovice je značajno viša ($p<0,0001$) od ostale dvije skupine, najvjerojatnije zbog zagađenja okoliša olovom. Rezultati ove studije podupiru teoriju da razina olova u zubima predstavlja važan indikator pri procjeni utjecaja izlaganja ljudskih populacija teškim metalima.