

LEAD LEVELS IN CHILDREN DECIDUOUS TEETH ARE ASSOCIATED WITH PARENTS' EDUCATION STATUS AND DAILY DAIRY CONSUMPTION: AN IRANIAN EXPERIENCE

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Abstract

Lead is one of the most hazardous metals, which causes many deleterious effects, including mental retardation, especially in children. Primary teeth are used for the determination of lead body burden, especially in children. Dust particulates and floated soil that come by dusty weather are strongly involved in the body burden caused by lead. This paper aims to study the lead contents present in the deciduous teeth of children in three cities with different climates located in Sistan and Baluchistan province, Iran. Teeth samples were collected from dental clinics with the help of the Dentistry Faculty of Zahedan University of Medical Sciences from three cities: Zahedan, Chahbahar and Zabol. Lead content of the samples was quantified using atomic absorption spectrophotometry. A questionnaire was used to gather personal data and respective information, such as age, sex, place where they live (urban and rural), city of residence, mouth health status, parents educational levels, parents job, dairy daily intake and types of toys. The results of this study showed that there is a significant relationship among parents' education level, the type of toys material and the teeth lead level (TLLs). Mean concentration of lead in the three cities was $32.65 \mu\text{g/g} \pm 3.91$ with no meaningful differences between them. The mean average age of children was 8.28 ± 0.18 (36.7% boy and 63.3% girl). Our results showed that TLLs were significantly lower in the children with well-educated parents – with a p-value of 0.013.

Rezumat

Plumbul este unul dintre metalele cele mai periculoase care provoacă numeroase efecte nocive, inclusive retard mintal, mai ales la copii. Se consideră că dinții primari pot fi utilizați pentru a determina încărcarea cu plumb a organismului, în special la copii. Particulele de praf și de sol care se întâlnesc în atmosfera poluată contribuie, într-o proporție crescută, la acumularea plumbului în organism. Această lucrare își propune să studieze conținutul de plumb din dinții temporari ai copiilor din trei orașe cu climă diferită situate în provinciile Sistan și Baluchistan din Iran. Probele de dinți au fost colectate cu ajutorul Facultății de Stomatologie din cadrul Universității de Științe Medicale din Zaheda, de la clinicile stomatologice din trei orașe: Zahedan, Chahbahar și Zabol. Conținutul de plumb din probe a fost cuantificat folosind spectrometria de absorbție atomică. Un chestionar a fost utilizat pentru a colecta date personale și informații precum vârsta, sexul, locul în care trăiește (urban sau rural), orașul de unde au fost recoltate probele, nivelul de sănătate al gurii, nivelul de educație al părinților, locul de muncă al părinților, consumul zilnic de produse lactate și tipurile de jucării. Rezultatele acestui studiu au arătat că există o relație semnificativă între nivelul de educație al părinților, tipul de jucării care aparțin copiilor și nivelul de plumb din dinți. Concentrația medie de plumb în cele trei orașe a fost de $32,65 \mu\text{g/g} \pm 3,91$, fără diferențe semnificative între orașe. Vârsta medie a copiilor a fost de $8,28 \text{ ani} \pm 0,18$ (36,7% băieți și 63,3% fete). Rezultatele noastre au arătat că un conținut de plumb la nivelul dinților semnificativ mai mic este corelat cu copiii proveniți din părinți bine educați ($p = 0,013$).

Keywords: deciduous teeth, dusty weather, lead, paediatric lead exposure

Introduction

Lead is one of the most toxic elements that may accumulate in the body, especially in children [20, 40]. Based on the previous studies, one of the most abundant sources of lead intoxication in human is tetra ethyl lead; however, new data revealed that other sources such as water, food and especially soil, could potentially be considered as notable sources of intoxication with heavy metals particularly lead compounds [22, 23]. Global warming and its consequences including drought and strong winds, has caused dusty weather that carry dust for long distances. This kind of weather is accompanied by a considerable amount of fine dust particulates spread in the region and affects many cities in western parts of Afghanistan, eastern parts of Iran and also other parts of the world. In Iran the cities Zabol, Zahedan and Chabahar are exposed to windy and dusty weather that may increase the exposure to lead. Zabol is a city located in south east of Iran and famous for its "120 day winds" which bring a heavy load of dust to the region. Jawad Mohmand *et al.* investigated the presence of toxic metals in dust samples from rural, urban and industrial areas of Pakistan and found high levels of Zn, Mn, Cu, Cr, Ni, Co, and Cd, suggesting a potential health risk especially for children [19]. Coal mining areas are usually polluted with the dust contaminated with Pb, Al and Zn, which represents a health risk for people living in those areas. An outdoor-dust survey carried in an industrial area in the Western part of the "Bassin Minier de Provence", France, showed that Zn and Pb occur at low-average levels but mainly in bio-accessible forms that are associated with health risks [43]. Mark Patrick Taylor *et al.* in a study on soil, surface dust and post-play hand wipes from different playgrounds from Australia's oldest lead-zinc mining city of Broken Hill, showed that lead concentrations were consistently elevated and pose a serious risk of harm for children [28].

Lead affects many organs and tissues such as blood and the central nervous system. Compared to adults, children are more vulnerable to lead toxicity due to their higher blood lead levels (BLL). Absorption of lead from pulmonary and gastrointestinal system in children is faster and higher than in adults. In addition, lead accumulates at a lesser extent in children's bone compared to adults. Therefore, BLL would be higher in children. As they eat and drink -per unit- more than adults, nutrition deficiency (e.g. vitamin D) is more common in children than adults and haematological and neurological disorders in children happen at lower levels of lead [34]. Furthermore, children are in the developmental stages. All these factors

indicate that children would be more vulnerable to lead toxicity.

Lead can induce motor dysfunction, cognitive deficit and impairment of mental development [20]. Nicolescu *et al.* conducted the first study from Central and Eastern Europe dealing with links between environmental exposure of children to neurotoxic metals and attention deficit hyperactivity disorder (ADHD). They showed that attention deficit could be an important basic adverse effect of lead in children [37]. In addition, it is believed that intelligence quotient (IQ) in children has a direct relationship with BLL and an increase of 1 µg/mL in BLL results in a 1 degree decrease in the IQ in range of < 10 µg/mL [21, 24]. Several biological samples including blood, plasma, urine, hair, nail, primary and permanent teeth and saliva may be used to determine lead body burden [8]. To evaluate lead levels in children's body, it was suggested to use a non-invasive method using decayed shed primary teeth. Furthermore, children who are 4 - 10 years old are more vulnerable. Teeth represent one of the most available and reliable samples for analysing lead levels as mineralization occurs over time. In teeth, lead is retained over time and it could be analysed easier as compared to bones where it is also more stable [8]. Both primary and permanent teeth are useful for lead determination but deciduous teeth are preferred as they are more readily available in comparison to permanent teeth. Many studies have confirmed that teeth have been successfully used as an index of lead accumulation in the body as well as an indicator for environmental pollution [8, 39]. Thomas J. Shepherd *et al.* showed that laser ablation Pb isotope analysis of deciduous teeth could be used together with histological analysis to identify the time, source and duration of exposure to lead in childhood [47]. This study was conducted in Sistan and Baluchistan province, south-east of Iran, in three different cities: Zabol, Zahedan and Chabahar.

Materials and Methods

Data collection

Ninety teeth samples (from 90 children with the mean age of 8.28 ± 0.18 year; based on the written consent obtained from their parents) were collected with the help of the Faculty of Dentistry of Zahedan University of Medical Sciences, Faculty of Pharmacy of Zabol University of Medical Sciences and International University of Chabahar and were stored at 4°C until the time of analysis. All samples were harvested, cleaned with detergents in distilled water (DW) and immersed in 10% HNO₃ (Merck, Germany), overnight. Then, samples were washed thoroughly with DW and rinsed with deionized

water. Only no carious teeth without fillings were used in this study.

Questionnaire

A questionnaire was used to collect data from each person. Information in the questionnaire were categorized in terms of age, sex, place of living, dairy product intake, child birth order, parents' education, family monthly income and place of living (i.e. rural or urban).

Sample preparation

In order to remove organic material, each tooth was first cleaned with a solution of 3% H₂O₂ and then washed several times with DW. Teeth were dried in an oven at 50°C and weighed. Afterwards, they were dissolved in an aliquot of 3 mL of 70% HNO₃ and 1 mL of 70% perchloric acid. Each mixture was poured in 50 mL beaker and dried. Then, the resultant was rinsed with a solution of 1% HNO₃, made up to 10 mL, and then kept in a vial.

Analysis of samples

High-purity water obtained from a double-purification system Milli-RO and Milli-Q (Millipore, USA) was used for the final washing of vials and preparation or dilution of samples and standards. For preparation of standards or samples, 65% HNO₃ was used throughout the study. For method calibration, the standard solutions of 1 mg/mL were used (Merck, Pb, Cat. No.19776). For routine quality control, the ICP Multi Element Standard I (toxic elements, No. 15474, Merck, Germany) was used. All standard solutions for analysis were prepared in 0.5% HNO₃ (v/v). Washing solution for auto-sampler capillary was 0.2% (w/v) aqueous solution of Triton X-100 (Merck, Germany) with the addition of 0.1% (v/v) nitric acid.

Analytical characterization

Samples were analysed using an atomic absorption spectrometer (5100 ZL, Perkin Elmer, USA) with hollow-cathode lamps. Lead concentrations were calculated using flameless atomic absorption spectrometry (GF-AAS). The samples and modifiers were injected with the use of an AS-70 auto-sampler. Auto-sampler aspiration sequence in all methods included 2 µL of washing solution aspirated before all the others, modifiers and sample. The peak area was measured as an analytical signal. Each experiment was conducted in duplicate, and the mean of two results was used to calculate the lead concentration.

Statistical analysis

In this study, SPSS version 15 (SPSS Institute, Inc., Chicago, IL, USA) package was used for statistical analysis. For data with normal distribution, a two-way ANOVA method was used for comparison of means. To investigate the influence of different variables including age, sex, parents' education, etc., the Pearson test was used. Qualitative variables

have been described and attributed to numbers and percentages. For each group set, statistical parameters including the mean, standard deviation (SD) and median of lead concentrations were calculated and reported. Due to the presence of outlier data, the median was considered as the main estimator. Due to non-normal distribution, a non-parametric statistical procedure was applied to infer whether differences exist among the medians of population samples or subgroups using two-tailed p-value. Multiple linear regressions examined the relation between correlated variables and the level of lead concentration. This analytical method determines the relative effect of one variable while controlling the effect of other variables.

Results and Discussion

Atomic absorption spectrophotometry is the most common method used for determination of lead concentration in biological samples such as teeth [1]. In the current study, a sample of 90 deciduous teeth from children aged between 5 to 10 years old were collected and analysed. From these samples, 59 teeth obtained from children living in urban areas and 31 samples belonged to children living in sub-urban and rural areas. Also, 63.3% of samples belonged to females and 51.2% of teeth samples belonged to children who were the first child in family. Table I presents a summary of data with the number of samples and percentiles used in this study in terms of sex, residence area, parents education level, parents occupational status, health status and oral health of the children as well as data regarding daily dairy intake and type of children toys. Analysis of lead in the deciduous teeth of children aged between 5 to 10 years old showed that lead levels are higher compared to other countries [4, 9, 13,14, 16, 29]. It was clarified that median TLL in our three cities was higher as compared to other cities around the world. A study that was done in Boston, USA reported a TLL of 3.3 µg/mg [29]. Three different studies in England reported TLL values between 4 and 12 µg/mg [6, 7, 27]. Other studies reported different TLLs as in Germany 2 µg/mg [13], in India 4.3 [12], Mexico 5 [26], Denmark 10 [16], Scotland 9 [3], New Zealand 6.24 [14] and Taiwan 4.6 µg/mg [42]. All these results, compared to our data, were much lower. In the current study, some environmental and physiological factors including parental socio-economic status, number of children, children living place, amount of dairy consumption, toy types, age and sex as well as teeth-related factors including type of teeth and intensity of teeth decay were considered.

The three cities in which the current study was conducted, have fairly similar climate patterns;

however, small differences exist between them e.g. Zahedan and Zabol are dry and hot, while Chabahar is a harbour with a hot and humid weather. The most important climatic factor of Zabol is its windy weather, commonly called “120 day winds” that is accompanied by high levels of loading particles in air [32, 33]. There were no significant differences observed in TLL among the three considered cities (28.6 ± 4.7 , 27.7 ± 4.5 and 26.9 ± 5.9 for Zahedan, Zabol and Chabahar, respectively) ($p > 0.05$). In

addition, there was no significant difference between girls and boys samples ($p > 0.05$). Previous studies showed that blood and teeth lead levels are correlated with living places, type of teeth, dairy products consumption as well as order of child’s birth [1, 39]. High lead levels in children induce severe, even fatal toxic outcomes and high lead levels are associated with growth retardation and impairment of behavioural and cognitive developmental in children [44].

Table I

Basic characteristics and lead concentration in tooth from the studied children

Characteristic	N	%	Mean \pm SD	Median	p - value
Gender					
Male	33	36.70	38.12 ± 57.55	24.84	0.382
Female	57	63.30	29.48 ± 16.51	23.68	
Residency area					
Urban	59	65.60	33.43 ± 23.68	23.68	0.065
Rural	14	15.60	37.60 ± 21.32	32.84	
Sub-urban	17	18.90	25.88 ± 12.65	24.07	
Father education level (n = 83)					
Illiterate	0	0	----	----	0.013
Primary	22	26.50	28.24 ± 18.68	23.10	
High-school	23	27.70	24.38 ± 67.73	24.47	
2yrs academic	11	13.30	31.37 ± 13.80	32.84	
4yrs academic	16	19.30	33.60 ± 20.93	26.92	
More than 4 years	11	13.30	22.37 ± 04.05	21.27	
Mother education level					
Illiterate	6	06.70	92.37 ± 129.75	34.32	0.505
Primary	25	27.80	26.66 ± 11.28	24.09	
Diploma	30	33.30	28.94 ± 11.60	24.05	
2yrs academic	13	14.40	27.33 ± 12.64	23.68	
4yrs academic	12	13.30	30.20 ± 22.79	19.86	
More than 4 years	4	04.40	31.69 ± 17.61	23.75	
Oral health (n = 84)					
Satisfactory	15	17.90	26.79 ± 7.23	24.47	0.303
Moderate	62	73.80	32.54 ± 43.23	23.75	
Unhealthy	7	08.30	35.00 ± 17.36	41.61	
Brushing time/day (n = 85)					
No brushing	36	42.40	39.11 ± 55.55	24.22	0.774
One’s a day	41	48.20	27.23 ± 11.93	24.09	
More than one	8	09.40	23.67 ± 06.62	21.19	
Dairy products consumed (n = 81)					
1 serving a day	41	50.60	24.05 ± 08.04	24.07	0.829
1-2 serving a day	12	14.80	29.28 ± 15.51	24.37	
3 serving a day	13	16.00	27.73 ± 15.38	15.38	
3-4 serving a day	15	18.50	56.08 ± 92.90	26.86	
Toy materials					
Plastic	38	63.30	39.22 ± 54.17	25.99	0.061
Non-plastic	22	36.70	24.34 ± 11.75	20.62	
	Mean	SD	β coefficient	95% CI	p - value
Age (year)	08.28	0.18	-4.17	-1.85, 0.06	0.066
Birth order (n = 84)			3.86	-1.23, 8.95	0.135
Body Mass Index (kg/m ²) (n = 56)	18.66	0.91			
Monthly income (Rials) (n = 76)	8421052.63	572778.13			
Lead concentration in teeth ($\mu\text{g/g}$)	32.65	03.91			

Based on Table I, the mean lead concentration in teeth of boys was found to be higher than that of girls (38.12 ± 57.55 and 29.48 ± 16.51 $\mu\text{g/g}$ for boys and girls, respectively). The median was

slightly higher for boys (less than 1 μg) ($p = 0.382$). In the univariate analysis, no statistically significant differences were found between genders, levels of mother education, oral health status, brushing, dairy

products consumption, children age and their birth order.

Small differences were observed in lead concentrations among children residing in urban ($33.43 \pm 23.68 \mu\text{g/g}$), rural ($37.60 \pm 21.32 \mu\text{g/g}$) and sub-urban ($25.88 \pm 12.65 \mu\text{g/g}$) areas. The calculated median for samples obtained from rural areas was found to be higher than that of urban and sub-urban areas. Medians were 23.68, 32.84 and 24.07 for urban, rural and sub-urban areas, respectively. However, these differences were not statistically significant at a confidence level of 95%, but it was statistically significant at a confidence interval of 93% ($p = 0.065$). This finding strongly indicates that in the rural regions the levels of environmental lead is higher than in urban and sub-urban areas.

The results of this study showed that there is a correlation between parents' educational level and lead concentration levels. Educational status of the parents was categorized in different levels including illiterate, primary, high school, and 2, 4 and more than 4 years of academic studies. However, no clear relationship was observed between levels of parent's education and lead level, but comparing literate and illiterate parents, showed that TLL are much higher in children with illiterate parents. Lead levels in the teeth samples of children with illiterate mothers found to be up to three folds more than those in children with educated mothers. It indicates that in children with well-educated

mothers, the amount of TLL is lower which is consistent with other studies [35]. However, in some other studies the results were controversial [11, 39]. The average lead concentration in teeth extracted from children playing with plastic toys ($39.22 \pm 54.17 \mu\text{g/g}$) was found to be significantly higher than that in teeth of children playing with non-plastic toys ($24.34 \pm 11.75 \mu\text{g/g}$) as well as the population's mean value ($32.65 \pm 3.91 \mu\text{g/g}$). However, the difference was not statistically significant at a confidence level of 95%, but it was statistically significant at a confidence interval of 93% ($p = 0.061$).

In this study, TLL was in the range reported by others studies (3-53 $\mu\text{g/g}$); however, it was among studies with high TLLs ($32.65 \mu\text{g/g} \pm 3.91$) [15]. Furthermore, lead is mineralized over time and it accumulates in some tissues such as teeth. In addition, TLL in elder people would be greater than in younger people. TLL under 50 $\mu\text{g/g}$ were not considered as toxic [15, 38]. Therefore, in the current study, our results showed that TLL was found to be in non-toxic range. In a similar study conducted in Jordan, it was shown that TLL in smokers are greater than in non-smokers (31.89 and 31.02 $\mu\text{g/g}$, respectively) [1]. Results of this study, consistent with other studies, showed that daily dairy consumption does not have a significant effect on the TLL [5, 25].

Table II

Multiple linear regression results for a model of associated variables that were predictors of teeth lead level

Characteristic	β	95 % CI		p - value
Gender	-9.072	-35.038	16.895	0.485
Residency area	-8.596	-25.254	8.061	0.304
Father education level	3.417	-7.776	14.611	0.541
Mother education level	-11.917	-23.117	-0.0717	0.038
Oral health	-11.567	-38.878	15.744	0.398
Brushing time/day	-18.946	-37.406	-0.485	0.045
Dairy products consumed	9.536	-6.040	25.112	0.224
Toy materials	-30.540	-57.691	-3.388	0.028
Age (year)	-9.897	-20.107	0.312	0.057
Birth order (n = 84)	14.371	3.709	25.033	0.009

The multivariate analysis of relationships between lead concentration and basic characteristics revealed the following results:

It was observed that the level of mothers' education (with a β equal to -11.917 at 95% CI: -23.117, -0.0717 and a p-value of 0.038), toy materials (with a $\beta = -30.540$; 95%CI: -57.691, -3.388; $p = 0.028$) and the birth order ($\beta = 14.371$; 95%CI: 3.709, 25.033; $p = 0.009$) significantly influenced the mean average of the lead concentrations in the samples. However, it must be mentioned that the number of brushing times during a day ($\beta = -18.946$; 95%CI: -37.406, -0.485; $p = 0.045$) and the children age ($\beta = -9.897$; 95%CI: -20.107, 0.312; $p = 0.057$)

had a relatively significant concordance with the average amount of lead concentration. The results of the multivariate analysis are presented in the corresponding Table II.

Some factors are responsible for higher TLL in these cities including their special weather conditions, utilization of leaded gasoline, painting, drinking-water problems, presence of mines and smelters as well as agricultural issues.

One of the most important origins of lead is the dust [39]. These cities have a windy and dusty weather. Furthermore, due to global warming and lack of precipitations, dust is spread widely and can change the weather conditions substantially.

Therefore, lots of fine dust particles with a high lead content are dispersed in the atmosphere. Moreover, leaded gasoline is another important source of lead [17, 45]. In fact, currently in Iran, gasoline which is supplied by petrochemical companies does not meet global standards [46] and also, there is no control over vehicles emissions. Though many years ago, addition of lead to paints was prohibited, still there are a lot of old buildings in the region and children are in contact with leaded paint. In a study performed in south west England, Andrew Turner *et al.* showed that in 221 out of 271 exterior paints analyses, lead was detected and this represents a potential risk of exposure to paint particles especially for children [2]. Another source of lead is drinking water [30]. Old drinking water pipelines, which are still in use for water delivery, and the presence of lead service lines represent a source of lead exposure for children in various parts of the world such as Washington DC and Montreal [10, 30, 36]. Drinking water in the north of Sistan and Baluchistan province is provided by the Hirmand River in Afghanistan which delivers drinking water for two cities Zahedan and Zabol. The river crosses places with lead minerals and it might get polluted by industrial and agricultural run-offs [41]. In addition, the lack of drinking water in the region has resulted in the use of other untreated water resources such as rivers or underground water that could be potentially polluted with lead. Moreover, long-lasting drought has led to lack of fresh water and due to using water from origins with greater contamination of heavy metals such as lead, such intoxications are probable.

Ingestion of food contaminated with lead is considered as another source of lead exposure. Determination of the concentration of heavy metals in vegetables grown in two areas which are historically known to be contaminated with these substances in Romania, showed elevated levels of Pb and Cd were found in carrots and yellow onion [31]. Higher incidence of lead poisoning, especially in children, is found in China, where in the mining zones the concentration of lead in vegetables grown there is usually higher than the national tolerance limits [48]. Eating fish from polluted waters represents another source of lead exposure because heavy metals are persistent and non-biodegradable and may be bio-accumulated in water-organisms. A study conducted on fish and water samples from a river near a city with intense industrial activity in Romania, revealed that the level of lead in these samples is elevated compared to samples from other areas [19].

Conclusions

In conclusion, the results of this study showed that teeth are valuable indicators of lead levels in the

body. Determination of lead contents in teeth samples revealed an important relationship between environmental exposure factors and accumulation of lead in the body. Our result showed that TLLs in children that are living in a zone with windy and dusty weather from Iran are greater than normal values which can mainly be attributed to the type of toys material and parents' educational level.

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Conflict of interest

The authors declare no conflict of interest.

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