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Relating tooth- and blood-lead levels in children residing near a zinc–lead smelter in India

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Background. Lead toxicity particularly affects children because of their increased capacity for absorption and retention. Blood-lead (BPb) levels reflect recent exposure and are of limited value in predicting neurotoxicity, whereas in teeth, lead accumulates over a long period of time and provides an integrated record of lead exposure from intrauterine life until the teeth are shed.
Aim. The present study aimed to relate tooth-lead (TPb) and BPb levels in children residing near a zinc–lead smelter in India, and to evaluate the effectiveness of primary teeth as bioindicators of life-long lead exposure.
Design. The lead levels in primary teeth and blood of 100 children aged between 5 and 13 years, living in the proximity of a zinc–lead smelter were measured by atomic absorption spectrophotometry. The mean levels were tabulated based on village, age, sex and tooth type, and analysed statistically.
Results. The mean BPb level was significantly influenced by proximity to the lead source, but not by age or sex. There was no consistent pattern of correlation between BPb and TPb levels.
Conclusion. Primary teeth showed significantly high lead levels compared to blood; they reflect cumulative exposure to lead and prove to be better indicators of body lead burden.

Introduction

Lead is a heavy metal that exists in relatively small quantities in the earth's crust. It has no biological value and is not a required nutrient[1]. Human activities and extensive use of lead in industry have resulted in its redistribution in the environment leading to contamination of air, water, and food and thereby a significant rise in lead concentration in human blood and body organs[1].

Lead toxicity affects several organ systems including the nervous, haemopoietic, renal, endocrine, and skeletal systems. Paediatric lead poisoning is associated with an increased risk of undesirable effects, by virtue of children being in the growth phase and because of their increased capacity for absorption and retention[1–3]. Studies have shown that prolonged pre-school exposure to low doses of lead in childhood results in reduction of IQ scores[4].

Exposure to this metal can be evaluated by measuring lead in blood, teeth, hair, and bone which are then used to estimate body lead burden[1]. Most studies looking at lead exposure among children have used blood-lead (BPb) levels as a marker of exposure[3,5]. Lead in the blood has a short half-life of 30 days and reflects recent exposure and, therefore, is of limited value in predicting neurotoxicity[3].

Teeth accumulate lead over a long period of time and provide an integrated record of lead exposure from intrauterine life until the teeth are shed. Because the dental hard tissues are relatively stable, metals deposited in teeth during mineralization are, to a large extent, retained. Unlike in bone, there is no turnover of apatite in teeth which are, therefore, the most useful material for studying past lead exposure. Primary teeth may thus be used as indicators of long-term lead exposure during early life[6–8].

In India, several studies[9] have been undertaken to determine the BPb level, but data pertaining to tooth-lead (TPb) level is lacking.

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Also, the correlation between TPb and BPb levels has not received sufficient attention. This prompted us to carry out this study with the aim of comparing primary TPb and BPb levels in children residing near a zinc–lead smelter in Dariba village, Rajasthan, India, and evaluating the effectiveness of primary teeth as bioindicators of life-long lead exposure.

Methodology

The present study was carried out to evaluate lead levels in primary teeth as indicators of lead exposure in children from villages located in and around a zinc–lead smelter in Dariba, Rajasthan, India.

The study group consisted of 100 children in the age group of 5–13 years, residing in any of five villages located within a radius of 4 km from the zinc–lead smelter. Each of these children had at least one healthy primary tooth nearing exfoliation or requiring extraction for therapeutic purposes. The children were grouped into three for convenience of sample collection, based on age and time of tooth exfoliation as follows: (i) 5–8 years (ii) 9–11 years, and (iii) 12–13 years.

Collection of tooth and blood samples

A total of 100 primary teeth (incisors, canines, and molars) were collected from the study group, after obtaining written informed consent of the parents.

Two millilitre of venous blood was collected from each subject, using disposable syringes, and promptly transferred to a lidded glass vial. Before clotting could occur, the reagent ethylene diamine tetra acetic acid, which binds to lead in blood and facilitates its separation at the next stage, was added in equal volume to the blood and the mixture was shaken for 2 min[10].

Analysis of tooth lead

To prevent sample contamination with exogenous lead, all laboratory glassware was cleansed with detergent and double-distilled water; they were then immersed in a 2-M HNO_3 overnight and washed several times with double-distilled water before a final rinse with deionized water[1].

Each tooth was cleaned and soaked in a 3% solution of hydrogen peroxide to remove organic material, after which it was washed several times with double-distilled water and deionized water, air dried and weighed. The tooth was then dissolved in 3 mL of 70% HNO_3 and 1 mL of 70% perchloric acid ($HClO_4$) in a 50-mL beaker. The mixture was heated slowly until a clear, colourless solution was obtained, which was then evaporated until dry. The digest was then rinsed with distilled water, filtered if cloudy, made up to 10 mL and shaken[1].

The lead concentration in the final digested solution was determined by using Flame Atomic Absorption Spectrophotometer (AAS) with electrothermal atomization (Varian Inc., Palo Alto, CA, USA). The specifications of the instrument were: lamp current 9.0 mA, wavelength 217.0 nm, band pass 0.5–1.0 nm, ash temperature 800°C and atomization 2300°C without temperature control[1].

Analysis of blood lead

The blood sample was mixed thoroughly by inverting the sample container 15 times. A 3-mL aliquot of the blood sample was immediately dispensed into a centrifuge tube. Ammonium Pyrrolidine Dithio Carbamate solution (0.5 mL) was added to the tube, and the tube was capped and inverted 15 times. The tube was then allowed to stand for 5 min, after which 3 mL of *n*-butyl acetate was added to the tube. The tube was capped again and shaken for a minimum 3 min at a rate sufficient to ensure mixing of the organic layer and blood. The tube was then centrifuged at 3000 revolutions/min for 2 min. The organic layer was aspirated into the flame of the AAS and absorbance was recorded[10,11].

Statistical analysis

The values obtained were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS-15) software for windows. Group comparison between males and females was carried out by using the Student's *t*-test. Analysis of variance was used to assess group comparison for tooth type, age, and village. A critical value of $P < 0.05$ was considered statistically significant.

Results

The present study was carried out to determine and correlate the lead levels in blood and teeth of 100 children, all residents of villages located in the vicinity of a zinc–lead smelter.

An analysis of the BPb and TPb levels of children from the five villages showed that the mean BPb levels were highest in Village 1 which was located closest to the zinc–lead smelter, whereas the mean TPb levels were highest in Village 4. Intra-village comparison of the mean BPb and TPb levels showed significant differences ($P < 0.05$) between the two in the case of Villages 1 and 4, and highly significant differences ($P < 0.001$) in the remaining three villages, with the TPb levels being much higher than the BPb levels in all the villages (Table 1).

Highly significant differences ($P < 0.001$) were observed in BPb levels between the five villages, wheareas differences in TPb levels were found to bear no significance statistically ($P > 0.05$) (Table 2).

An inter-village comparison of BPb levels revealed that differences in BPb between Village 1 and the other villages were highly significant statistically ($P < 0.001$), with the exception of Village 5 where the difference was only significant ($P < 0.05$). A comparison of BPb levels in Villages 3 and 5 also revealed a statistically significant difference ($P < 0.05$) (Table 3).

When mean BPb and TPb levels in boys were compared to those in girls, no significant differences were observed between the sexes in either of the parameters studied. However, the BPb–TPb differences within both gender groups were of high statistical significance ($P < 0.001$) (Table 4).

Of the tooth types studied, although the primary canines had the highest concentrations

Table 2. Comparison by village of BPb and TPb levels.

	d.f.	Sum of squares	Mean square	*F*	*P*
BPb	4	65.37	16.09	6.36	0.000*
TPb	4	54.47	13.86	1.05	0.38

BPb, blood lead; TPb, tooth lead.
*Highly significant.

Table 3. Inter-village comparison of BPb levels.

Village	Village	Mean difference BPb	*P*
1	2	7.52	0.001**
	3	8.53	0.000**
	4	8.75	0.000**
	5	4.86	0.032*
2	3	1.00	0.484
	4	1.23	0.502
	5	2.66	0.167
3	4	0.23	0.883
	5	3.67	0.027*
4	5	3.89	0.054

BPb, blood lead.
*Significant.
**Highly significant.

Table 1. Intra-village comparison of BPb and TPb levels (μg/dL).

Village	Distance from Pb source (km)	*N*	Mean ± SD		Difference	*P*
			BPb	TPb		
1	0.5	9	15.11 ± 5.62	33.68 ± 22.59	18.75	0.034*
2	2.0	17	7.59 ± 4.36	29.66 ± 23.28	22.07	0.001**
3	2.5	48	6.58 ± 5.26	43.13 ± 30.48	36.55	0.000**
4	3	14	6.36 ± 4.52	51.55 ± 66.58	45.20	0.027*
5	4	12	10.25 ± 5.33	50.49 ± 25.87	40.24	0.000**

BPb, blood lead; TPb, tooth lead.
*Significant.
**Highly significant.

Table 4. Intra-group comparison of BPb and TPb levels based on gender.

		Mean ± SD			
Sex	*N*	BPb	TPb	Difference	*P*
Male	67	8.19 ± 5.69	40.02 ± 38.99	31.83	0.000*
Female	33	7.39 ± 5.41	46.18 ± 28.31	38.78	0.000*

BPb, blood lead; TPb, tooth lead.
*Highly significant.

of lead, followed by the incisors and the molars, the differences were not of statistical significance. When these TPb levels were compared with the BPb levels of the children from whom the individual tooth types were obtained, highly significant differences were observed ($P < 0.001$) (Table 5).

In the three age groups studied, no significant differences were found between the groups either in BPb levels or in TPb levels. However, the BPb–TPb differences within each age group were of high statistical significance ($P < 0.001$) (Table 6).

Discussion

Debate continues over the nature, magnitude, and persistence of the adverse effects on human health of low-level exposure to environmental lead. Generally, lead poisoning occurs slowly from the gradual accumulation of lead in bone and tissues after repeated exposure. Left untreated, lead poisoning can damage many internal organs including the kidney and nervous system[1–4]. Owing to the possibility of permanent impairment, lead poisoning is particularly dangerous during the critical development periods of infants and young children.

In India, lead has been used in industry and as a gasoline additive for many decades.

Table 6. Intra-group comparison of BPb and TPb levels based on age.

		Mean ± SD			
Age groups (years)	*N*	BPb	TPb	Difference	*P*
5–8	36	8.14 ± 5.46	38.96 ± 20.82	30.82	0.000*
9–11	46	7.65 ± 5.82	44.85 ± 45.37	30.720	0.000*
12–13	18	8.22 ± 5.48	41.08 ± 32.80	32.86	0.001*

BPb, blood lead; TPb, tooth lead.
*Highly significant.

Case reports and case series of lead poisoning have been published, as have surveys of BPb and TPb levels in hospital and clinic populations. Epidemiologic studies of elevated BPb levels in specific occupational groups such as jewellery workers, traffic police, and papier-mâché workers have also been reported[9]. Surveys of industrial workers, performed by the National Institute of Occupational Health, have demonstrated mean BPb levels in excess of 40 μg/dL in iron-foundry workers, glaze workers, and battery-plant workers[9].

It is well-recognized that in the past, processing of lead–zinc and zinc–lead ores in smelters has resulted in widespread contamination of the environment and has severely affected the health of the community. Studies have reported significantly higher BPb levels[12–15] and TPb levels[13] in children residing near lead factories/mines compared to those of children residing away from the lead source. Thus, the present study comprised of five villages located in the vicinity of a zinc–lead smelter in Dariba, Rajasthan, India.

Paediatric lead poisoning is associated with an increased risk of adverse effects in a variety of target organs, with the central nervous, haematopoietic, and renal systems receiving the greatest attention[16,17]. Exposure to lead is

Table 5. Intra-group comparison of BPb and TPb levels based on tooth type.

		Mean ± SD			
Tooth type	*N*	BPb	TPb	Difference	*P*
Primary incisors	36	8.17 ± 5.42	40.67 ± 21.06	32.50	0.000*
Primary canines	29	6.79 ± 5.12	46.77 ± 50.10	39.98	0.000*
Primary molars	35	8.63 ± 6.11	39.56 ± 34.33	30.94	0.000*

BPb, blood lead; TPb, tooth lead.
*Highly significant.

estimated by measuring levels of lead in the blood (μg/dL). The US Center for Disease Control and Prevention (CDC) has set a 'level of concern' for children at 10 μg/dL. However, studies have provided evidence of the possibility of very harmful effects at even levels of exposure as low as 5 μg/dL. Hence, no level of lead exposure can be considered safe enough[3,16].

Blood-lead levels primarily reflect recent exposure (i.e., over the last 3–5 weeks) and correlate poorly with lead levels in shed primary teeth[17]. Shed primary teeth can be used as indicators of long-term lead exposure during early life because much of lead deposited in teeth during mineralization is retained. The metabolism of lead is affected by the same factors that affect calcium metabolism, with a tendency to 'follow the calcium stream'. Mineralized tissues are thus long-term storage sites for lead[2,3]. Mean dentine lead levels increase with age and duration of exposure to high levels of lead[17].

Primary teeth provide a readily accessible bone biopsy, hence the concentrations of lead in the whole primary teeth, the enamel, or the dentin (particularly circumpulpal) have served as proxy measures for skeletal lead, and thus for total body lead burden, in epidemiologic studies of childhood lead toxicity. Also, the lead burden of children is more pronounced than that of adults and higher lead levels have been reported in primary teeth than permanent teeth[18–21]. Hence, in the present study, primary teeth that were either shed or nearing exfoliation were analysed for lead levels.

Considering the advantages of using teeth to assess lead exposure, the relation between TPb and BPb levels deserves more attention, and several studies[7,22] have already attempted to determine the same. However, in the face of a severe paucity of such data pertaining to the Indian population, it is vital that data be collected, correlated, and compared with that of different populations. This study was an attempt to assess the significance of the correlation, if any, between TPb and BPb levels in 5- to13-year-old children living in the area of a zinc–lead smelter in Dariba, Rajasthan, India.

Several methods have been used for the determination of lead in teeth, such as high-resolution gamma spectrometry, X-ray emission spectrography, mass spectrometry, AAS, and anodic stripping voltametry[21]. Of these, AAS has received wide attention because of its sensitivity (especially graphite furnace AAS) and is considered one of the most reliable techniques for the analysis of trace elements[1,2,21].

The results showed that, among the villages studied, only Villages 1 and 5 had a mean BPb level greater than 10 μg/dL, which is the 'level of concern' as given by the CDC and the OSHA[3,16]. Children from Village 1 had a mean BPb level significantly higher than the rest. This could be attributed to its proximity to the lead-smelter and is in keeping with the findings of another study[12]. However, the variation in mean TPb levels in all the villages studied was not significant. This could be explained by the fact that exposure to lead is not consistent and different children may be exposed to different and varying levels of lead over a period of time.

Variations were observed in the BPb levels of children included in the study, which bore no statistical significance, based on age, sex, and tooth type. These variations could be attributed to the fact that BPb levels are indicative of only the current exposure[3].

Blood-lead and TPb levels do not seem to depend on gender. Although our results showed that BPb levels were higher in males and TPb concentrations were higher in females, the differences were not significant. These findings concur with those of other studies[3,6].

Some researchers have reported that TPb levels increase with age[6,23–25]. However, no association in the present study was observed between dental lead and age. This is in keeping with the findings of other studies which suggest that exposure levels from various environmental and dietary sources might contribute more than age to the accumulation of lead in teeth[3,6,24,26].

In the present study, the primary canines were observed to contain the highest concentrations of lead followed by the incisors and molars, although the differences were not statistically significant. These findings are in accordance with the findings of some studies[4]. However, other studies have reported that TPb concentrations showed a falling gradient from the incisors to the molars[1,3,26]. These minor variations could be explained, first, by the difference in morphology and size between the various tooth types and second, by the different but overlapping times of mineralization of these teeth. The difference found between the tooth groups may thus be due to variations in exposure to the metal during tooth formation[3,6,20,26].

In the present study, significant differences were observed between the mean BPb and TPb levels. These observations are expected since it is likely that children would receive the preponderance of their total lead exposure during a narrow time interval and hence, the pattern of correlation between BPb levels at particular ages and TPb levels might be different.

The lack of a consistent pattern of correlation between the BPb and TPb levels of the study population led us to conclude that our observations may be the result of a lack of homogenous study samples. Although our results were in accordance with those of studies undertaken in other countries[3,6], further research of different Indian populations of varying ethnicities is necessary to corroborate these results.

Further, studies need to be carried out on carious teeth as higher lead concentrations have been reported in carious than in non-carious teeth[26].

Conclusion

The following conclusions could be drawn from the present study:

1. Blood-lead concentration was higher in children residing in closer proximity to the zinc–lead smelter, whereas TPb was not influenced by minor increase/decrease in distance from the lead source within the area of the study.
2. The BPb concentrations were independent of age and sex while, TPb concentrations, although significantly higher than the BPb levels, was independent of age, sex, and tooth type.
3. The TPb level being significantly higher than BPb level is indicative of the cumulative exposure to lead as against the BPb which reflects recent exposure. Hence, TPb can be considered a better biologic indicator of the overall exposure.
4. No consistent pattern of correlation existed between the BPb and TPb levels in the population studied.

It was concluded that although no correlation is found between the TPb and BPb levels, in view of the limitations of the present study, more studies with larger sample sizes, using more homogenous and standard parameters and in different ethnic populations of India are needed to substantiate the results of the present study. However, it is proposed that primary TPb level be substituted as the biologic indicator of lead exposure of the child.

What this paper adds

- Hitherto unavailable data pertaining to blood- and tooth-lead levels of a group of Indian children.
- Further basis for using primary teeth to determine total body burden of lead.

Why this paper is important to paediatric dentists

- This paper can contribute to the paediatric dentist's role in promotion of public health. The paediatric dentist needs to be aware of environmental pollutants that can adversely affect general and dental health. Further studies are underway that aim to determine the effects of lead, if any, on the oral and dental tissues.

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