

Lead level, enamel defects and dental caries in deciduous teeth

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Keywords

Lead. Dental enamel. Dental caries, epidemiology. Dental enamel hypoplasia.

Abstract

Objective

To verify the relationship between lead concentration in the enamel of deciduous teeth and the presence of enamel defects and, consequently, with dental caries among preschool children.

Methods

The sample consisted of 329 preschool children in Piracicaba, State of São Paulo: 132 attending municipal kindergartens close to industrial plants and 197 attending kindergartens in non-industrial areas. This sample belonged to an initial study made between 2000 and 2001, in which the lead concentration was obtained by means of enamel biopsy. Oral clinical examination of the children from both regions was performed to verify the prevalence of enamel defects, using the Developmental Defects of Enamel (DDE) Index of the World Dental Federation (FDI), and of dental caries, using the decayed, missing and filled surfaces (dmfs) index of the World Health Organization. The chi-squared test and relative risk calculation were utilized in relation to a significance level of 5%, considering each region separately.

Results

Among the children from the non-industrial area, there was a higher proportion with dental caries among those with higher lead concentrations in deciduous teeth ($p=0.02$). This was not, however, observed among the children from the industrial area ($p=0.89$). There was an increased relative risk (RR) of caries among the children from the non-industrial area, but this was not seen among the children from the industrial area. No relationship was observed between the presence of lead and enamel defects.

Conclusions

No data was found that would give evidence of a relationship between lead concentration and enamel defects in either of the areas studied. No relationship was found between lead and dental caries in the industrial area, thus emphasizing that more studies of such relationships are needed.

INTRODUCTION

In an earlier study,¹⁰ it was found that preschool children living in an industrial region of the city of Piracicaba, State of São Paulo, presented greater lead (Pb) concentrations in their dental enamel than did those living in a non-industrial region. There are reports in the literature indicating that the presence of

lead in the chemical composition of dental enamel may alter dental ultrastructure, thereby giving rise to defective enamel. It was therefore considered important to investigate such relationships among this same population of preschool children.

Within this context, *in vitro* studies have shown that the presence of lead during amelogenesis may cause

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alterations to the ultrastructure of the enamel,⁸ given that ameloblasts are cells that are extremely sensitive to environmental alterations. Such alterations may, in turn, be associated with modifications in the physico-chemical behavior of the enamel,¹⁵ thereby making it more susceptible to demineralization.

Thus, some studies have indicated that there is an association between the presence of lead in dental tissue and clinical alterations in the enamel. Such variations may relate to coloration⁹ or hypoplasia.¹ However, these studies have not been sufficient for establishing a clear relationship between the presence of lead and such enamel defects.

A relationship between the presence of lead in the dental tissue and increased dental caries, albeit indirect, has been reported by some researchers.^{2,9,17} Nonetheless, the mechanism through which this takes place has not been well established yet.

Because of the divergences in the literature and scarcity of data on this subject among Brazilian communities, an epidemiological investigation was made in order to determine the relationship between the presence of lead in dental enamel, enamel defects and caries, in Brazilian children's deciduous teeth. Thus, the objective of the present work was to verify the relationship between the concentration of lead present in the enamel of preschool children's deciduous teeth and the prevalence of enamel defects and caries.

METHODS

In order to determine which regions of the city of Piracicaba, State of São Paulo, to be studied, the following bodies were firstly consulted to obtain information on lead pollution in the city: Piracicaba Municipal Department of the Environment, the Environmental Sanitation Technology Company (Companhia de Tecnologia de Saneamento Ambiental - Cetesb) and the Center for Nuclear Energy in Agriculture of the University of São Paulo (CENA/USP). These bodies reported the existence of a car battery factory in an industrial district located in the Dois Córregos region, which was taken as the industrial region for the study. The kindergartens located in this industrial region were then found by using a map of the city. Kindergartens in another region known as Campestre, which does not have industrial plants and was taken as the non-industrial region, were also localized.

The calculation of the sample size¹³ was done by starting from prior knowledge of the prevalence of enamel defects among preschool children in these two

regions (the industrial and non-industrial regions), through data collected during a survey in 1999.⁵

The schools health sector of the Piracicaba municipal authorities furnished listings of the children enrolled in the ten kindergartens in the two regions selected for the study. There was a total of 421 children: 209 in the industrial region and 212 in the non-industrial region.

Because of losses, the sample consisted of 329 preschool children of both sexes, aged four and five years old. The adapted Frias Matrix¹⁶ was utilized for the age calculations. In the sample, there were 197 children from six kindergartens in the non-industrial region and 132 children from four kindergartens in the industrial region.

The delineation of the study was single-blind. The scheduling of the children for them to participate in the study was done by another researcher. It was thus avoided that the examiner would have prior knowledge about the children.

The participants were attended at consultation offices in the university dental laboratory.

Some modifications to the original biopsy technique³ were made so as to adapt it to the present sample. These modifications were made after performing a pilot study.¹⁰

The teeth were submitted to professional prophylaxis, using a Robinson-type brush, rubber cup and pumice, followed by washing and drying using air and gauze and relative isolation using cotton wool rolls. These procedures were accomplished by an assistant so that the researcher would not touch any metallic surface before performing the biopsy.

Adhesive tape with a central perforation of 1.6 mm in diameter (± 0.03) was firmly stuck onto the vestibular surface of one of the upper central deciduous incisors (51 or 61) of each participant in the study, thereby demarcating the area of the biopsy. 5 μ l of 1.6 mol/l HCl prepared in 70% glycerol (v/v) was deposited onto this area of exposed enamel. The drop of acid was agitated for 20 seconds, using the end of the pointer, and was then aspirated and transferred to a collecting tube containing 200 μ l of MILLI-Q ultrapurified water. To recover the residual acid and dissolved enamel, 5 μ l of ultrapurified water was placed on the enamel, agitated for 10 seconds and added to the biopsy solution in the collecting tube. The lead and phosphorus concentrations were determined from this solution, in the laboratory. The phosphorus con-

centration was used for estimating the mass of the enamel removed by the biopsy. After concluding the biopsy, the tape was removed and the tooth was washed with water for 30 seconds, dried using air jets and again isolated, in order to receive the application of fluoride gel.

Control biopsies were performed on the bench surface or on the caps of the pointer supports, so as to check the lead contamination in the working environment during the procedures. All the material utilized and the bench on which the instruments and pipettes were laid out were cleaned using a 10% solution of nitric acid (HNO_3), in order to remove possible prior lead contamination. The collecting tubes were also previously washed using 10% nitric acid: immersed in the solution for 24 hours and then rinsed 20 times using ultrapurified water and dried under a hood that had been decontaminated from metals.

The examinations were conducted under artificial light, with drying of the surface using air and gauze, using a clinical mirror and a CPI (Community Periodontal Index) probe. The prevalence of caries was described using the dmfs index, which consists of summing the decayed, missing and filled surfaces of the deciduous teeth, according to the criteria of the World Health Organization (WHO),¹⁹ and the prevalence of enamel defects using the DDE (developmental defects of enamel) index, which describes the different types of defects, from opacity to hypoplasia.⁶ The examinations were conducted by a single calibrated examiner, and the intra-examiner error was calculated. Around 10% of the total sample was randomly selected for reexamination, with calculation of the intra-examiner error during data collection.

To avoid possible evaporation and loss of sample volume, it was decided to dehydrate the samples by placing them in a desiccator with anhydrous calcium chloride (CaCl_2) for 36 hours. This procedure was tested previously to verify that no alterations would occur. At the time when the lead and phosphorus assays were performed, the samples were rehydrated using 210 μl of ultrapurified water, agitated and divided into two parts, for lead and phosphorus assaying.

The lead determination was performed using a graphite furnace atomic absorption spectrometer (GFAAS), calibrated using standard solutions containing 0 to 100 ppb of lead. The sample with 490 μl of a solution of 0.2% (w/v) $\text{NH}_4\text{H}_2\text{PO}_4$ with 0.5% (v/v) Triton X-100 and 0.2% HNO_3 .

The analysis of the phosphorus concentration was done in order to determine the quantity of enamel

removed from the tooth surface and the depth of the biopsy, so that the quantity of lead could be calculated in μg per g of enamel removed (ppm). The phosphorus was assayed via the Fiske & Subbarow colorimetric method.⁷ Thus, 105 μl of sample, 295 μl of MILLI-Q ultrapurified water and 83 μl of molybdcid acid were pipetted together and agitated. After 10 minutes, 33 μl of reducer were added and the mixture was again agitated. After 20 minutes, the color intensity was measured in a spectrophotometer (Beckman DU-70) at 660 nm. The apparatus was calibrated using samples that presented the following known phosphorus concentrations: 0.58 $\mu\text{g}/\text{ml}$, 1.16 $\mu\text{g}/\text{ml}$, 2.32 $\mu\text{g}/\text{ml}$, 4.65 $\mu\text{g}/\text{ml}$ and 9.30 $\mu\text{g}/\text{ml}$.

Considering that human enamel contains 17% phosphorus by weight, this was the starting point for determining the mass of enamel removed. To determine the thickness of enamel removed by the biopsy, it was assumed that the enamel had a density of 2.95 g/cm^3 .

Information on the socioeconomic level of the children studied was collected by applying a questionnaire to each mother or the person responsible for the child, with questions relating to family income and the mothers' education level. This information was collected by the health agents of the kindergartens.

The statistical analysis was done using the SAS (Statistical Analysis System) and Epi Info version 5.0 statistical software. Correlation analysis, the chi-squared test and calculations of relative risk (RR) in relation to a significance level of 5% were utilized, and each region was considered separately.

The present research was approved by the Research Ethics Committee of the Dentistry School of Piracicaba, Universidade Estadual de Campinas (FOP/Unicamp) (protocol n^o. 29/99), in accordance with Resolution 196/96 of the National Commission for Research Ethics (CONEP).

RESULTS

The reliability parameters were acceptable and in accordance with the recommendations from the World Dental Federation (FDI)⁶ and WHO¹⁸ for the percentage concordance and from Bulman & Osborn⁴ for the Kappa concordance index. The training showed an intra-examiner percentage concordance of 97.6% and Kappa of $k=0.8$ for the caries. For the enamel defects, the percentage concordance was 97.7%. During the data collection, the percentage concordance of the intra-examiner error was 99% for the caries, with

Kappa of $k=0.9$, and 90% concordance for the defects.

The sample loss was 36.8% in the industrial region and 7.1% in the non-industrial region.

The average value (\pm standard deviation, SD) of phosphorus found in the enamel biopsy samples from the exposed group (industrial region) was $4.1 \mu\text{g/ml}$ (± 1.5) and $4.4 \mu\text{g/ml}$ (± 1.6) from the non-exposed group (non-industrial region). The average masses of enamel removed (\pm SD) were $0.24 \mu\text{g}$ (± 0.095) and $0.25 \mu\text{g}$ (± 0.097), respectively. There was no statistical difference in the depths of the biopsies (\pm SD) between the groups from the two regions ($p>0.05$): these depths were $4.1 \mu\text{m}$ (± 1.5) for the exposed group and $4.4 \mu\text{m}$ (± 1.6) for the non-exposed group.

A negative correlation between lead concentration and the total number of enamel defects was found in the non-industrial region ($r^2=-0.1497$ and $p=0.0357$), while such a correlation was not observed in the industrial region ($p>0.05$). When the total number of defects was discriminated, there were no correlations ($p>0.05$) between lead concentration and hypoplasia, lead concentration and demarcated opacity and lead concentration and diffuse opacity, either in the industrial or non-industrial region.

The results for the relationship between lead concentration and enamel defects (presence/absence) in the industrial and non-industrial regions are expressed in Table 1.

According to Table 1, no statistically significant difference was found between the regions ($p>0.05$) with regard to the presence/absence of enamel defects.

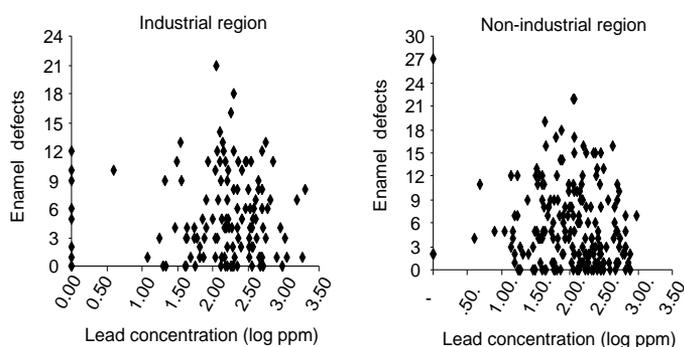


Figure 2 - Distribution of dental enamel defects according to lead concentration in the enamel of children in the industrial and non-industrial regions. Piracicaba, SP, 2001.

The relative risks between lead concentration (140 and 220 ppm) and enamel defects (presence/absence) were 1.01 ($0.87<RR<1.16$) and 1.03 ($0.89<RR<1.19$), respectively, for the industrial region, and 1.03 ($0.91<RR<1.16$) and 0.99 ($0.86<RR<1.14$), respectively, for the non-industrial region, thus showing that there was no risk ($p>0.05$).

The distribution of enamel defects according to the lead concentration found in the enamel of deciduous teeth of the children from the industrial and non-industrial regions is represented in the Figure.

There was no correlation between lead concentration and caries in either region ($p>0.05$).

The relationship between lead concentration and caries (presence/absence) in the samples from the industrial and non-industrial regions is shown in Table 2.

There was a statistically significant difference for the proportions of individuals with presence of caries and lead concentrations of more than 140 and 220 ppm, in relation to those with presence of caries and lead concentrations of less than or equal to 140

Table 1 - Lead concentration in the enamel of preschool children's deciduous teeth, according to region and the presence or absence of enamel defects (ED). Piracicaba, 2001.

Lead concentration ($\mu\text{g/g}$)	Region			
	Industrial (N=132)		Non-industrial (N=197)	
	Presence of ED	Absence of ED	Presence of ED	Absence of ED
>140*	68 (51.6%) ^a	12 (9.0%)	71 (36.0%) ^a	13 (6.6%)
≤140	44 (33.4%) ^a	8 (6.0%)	93 (47.2%) ^a	20 (10.2%)
Total	112	20	164	33
>220**	45 (34.0%) ^a	7 (5.3%)	47 (23.9%) ^a	10 (5.0%)
≤220	67 (50.8%) ^a	13 (9.9%)	117 (59.4%) ^a	23 (11.7%)
Total	112	20	164	33

Values followed by the same letter, in the same column, indicate that there was no statistically significant difference according to the chi-squared test ($p<0.05$).

*Median value of lead concentrations

**Average value of lead concentrations

and 220 ppm, only for the non-industrial region ($p=0.0223$ and $p=0.0141$, respectively).

There was an increased risk of caries among children from the non-industrial region who had lead concentrations of more than the median and average: 1.40 ($1.05<RR<1.88$) and 1.46 ($1.10<RR<1.93$), respectively. This was not seen among the children from the industrial region: 0.98 ($0.67<RR<1.43$) and 0.89 ($0.60<RR<1.32$).

There was a statistically significant difference in family income ($p<0.0001$) and education level ($p<0.0001$) between the groups from the industrial and non-industrial regions: these levels were greater in the industrial region.

DISCUSSION

The lead concentrations in the deciduous teeth were analyzed in two ways (median and average), because no defined reference values exist in the literature for the concentrations in dental enamel that would reflect a condition of lead poisoning or, furthermore, would relate to enamel defects and caries. The studies in the Brazilian literature have established reference values only for blood and have reported the importance of verifying lead levels as a routine examination.¹⁴ However, blood measurements indicate acute lead contamination, thus differing from dental enamel, which indicates past contamination.

Nonetheless, no relationship was found between any of the lead concentrations analyzed in the present study and enamel defects in the deciduous teeth. The results from the correlations and the relative risks between lead concentrations and enamel defects, when these existed, were weak. For the non-industrial region, for which the correlation was negative, this relationship ought to be

better investigated, considering that this result diverges from some reports in the literature.^{1,10} However, the latter reports did not produce conclusive results and reported the relationship between lead in dental tissue and enamel defects in an indirect manner. Moreover, in examining the Figure of the present study, it can be seen that, in the industrial region, there were more cases of children with enamel defects even in the absence of lead concentration, thereby indicating that other factors must be contributing towards the presence of these defects. Besides this, the visualization of the industrial and non-industrial regions in the Figure allows the observation that, where there were greater lead concentrations, there were greater numbers of enamel defects in the industrial region than in the non-industrial region.

With regard to the relationship between lead and caries, surprisingly, a greater proportion of individuals with the presence of caries at lead concentrations of more than the median and average was observed in the non-industrial region. However, it was seen that, in the industrial region, both the mother's education level and the family income were greater than those in the non-industrial region. These may have acted as confounding factors that could have exercised a role that perhaps was more important than the environmental lead contamination in the industrial region. Although the characterization of socioeconomic levels from the education network has been utilized by other researchers,¹² in the present work this appears not to have been a variable that homogenized the groups in relation to similarity of socioeconomic level. Another matter that may have interfered was the greater sample loss in the industrial region.

One important epidemiological finding was that the relative risk of having caries was greater among the children living in the non-industrial region who presented lead concentrations of more than 140 and

Table 2 - Lead concentration in the enamel of preschool children's deciduous teeth, according to region and the presence or absence of dental caries. Piracicaba, 2001.

Lead concentration (µg/g)	Region			
	Industrial (N=132)		Non-industrial (N=197)	
	Presence of ED	Absence of ED	Presence of ED	Absence of ED
>140*	36 (27.3%) ^a	44 (33.4%)	48 (24.4%) ^a	36 (18.3%)
≤140	24 (18.1%) ^a	28 (21.2%)	46 (23.3%) ^b	67 (34.0%)
Total	60	72	94	103
>220**	22 (16.7%) ^a	30 (22.8%)	35 (17.8%) ^a	22 (11.2%)
≤220	38 (28.7%) ^a	42 (31.8%)	59 (29.9%) ^b	81 (41.1%)
Total	60	72	94	103

Values followed by different letters, in the same column, indicate that there was a statistically significant difference, according to the chi-squared test ($p<0.05$).

*Median value of lead concentrations

**Average value of lead concentrations

220 ppm in their deciduous teeth. This result reaffirms the tendency reported in the literature towards a relationship between presence of lead in dental tissue and increased prevalence of caries.^{2,9,17}

No data were found that would give evidence of a relationship between the lead concentration in the enamel of deciduous teeth and enamel defects, in either of the regions studied. However, a relationship was found between lead and caries in the non-industrial region, thus emphasizing the need for more studies on such relationships, taking into consideration the importance of socioeconomic factors in caries.

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