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A *plateau* detected in lead accumulation in subsurface deciduous enamel from individuals exposed to lead may be useful to identify children and regions exposed to higher levels of lead ☆, ☆☆

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Abstract

In a previous study, we showed 4 times more lead in surface deciduous enamel (1.9–5.9 μm) of a notoriously contaminated area (Bauru, São Paulo State, Brazil) in comparison to samples from a region with no lead contamination described (Ribeirão Preto, São Paulo State, Brazil). The samples from the more superficial enamel (1.9–3.18 μm) showed higher amounts of lead and the highest variability, while in the subsurface enamel (3.18–5.9 μm) a *plateau* in lead content was detected in children living in the contaminated environment (around 600 μg/g). Here we expand our previous study, and use only samples obtained from subsurface enamel (Ribeirão Preto, $n = 186$; Bauru, $n = 20$). We tried to distinguish regions with more children with lead above the threshold of 600 μg/g of lead in enamel. We tested whether differences in the percentage of children with "high" lead ($\geqslant 600$ μg/g) could be observed among the different Kindergartens studied in Ribeirão Preto. We also tested whether these results were different from the ones provided by conventional comparison of the data. Ribeirão Preto showed almost 4 times less lead than Bauru ($p < 0.0001$), and a statistically significant difference was found only in Ribeirão Preto between Kindergarten 2 and 5 ($p < 0.01$). Twelve percent of the children from Ribeirão Preto had "high" lead, while 55% of the children from Bauru did so. However, when we looked at the percentages of children with "high" lead in each Kindergarten, and compared them, a whole new picture emerged, in which we could see children with "high" lead concentrated mainly in 3 Kindergartens from Ribeirão Preto, with Kindergarten 5 with 33% of the children with "high" lead, being statistically different from all Kindergartens, except 4 and 6. The threshold of 600 μg/g of lead in subsurface enamel was tentatively settled here after the *plateau* seen in exposed children, and enabled us to identify locations with more children exposed to a higher amount of lead.

Keywords: Lead; Enamel; Deciduous teeth; Enamel biopsy; Plateau

1. Introduction

Needleman demonstrated in many studies that teeth could be used to assess lead exposure when children with different backgrounds of exposure to lead were compared (Needleman and Bellinger, 1991; Needleman et al., 1972, 1974, 1979, 1990). In some of these studies, lead content in dentine of deciduous teeth was determined, since dentine lead closely represents lead stored in bones, a compartment

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of long-term storage (Hu et al., 1998; Todd et al., 1996). Over the past decades, dentine lead content was used in many studies to indicate exposure to lead in archeological, environmental and epidemiological studies (Grandjean and Jorgensen, 1990; Gulson, 1996; Rabinowitz et al., 1993). However, what dentine lead levels are associated with different degree of exposure or degree of neurological damage remains to be determined. The main disadvantage of deciduous teeth dentine as a biomarker of exposure to lead is the need to collect large numbers of teeth from a population and the laboratory steps necessary to gain access to inner dentine. While dentine lead levels can only be determined in shed deciduous teeth, lead content of enamel can be estimated in samples obtained by a clinical procedure that is easily performed in 4–6-year-old children. The use of this procedure enables researchers to gain access to lead content data of surface enamel of a large number of children from a population (when parents are appropriately informed on the procedure, and give their informed consent). Recent studies of our group using this procedure to obtain superficial samples of deciduous enamel (Almeida et al., 2007;Gomes et al., 2004) coincide with a new interest in the use of enamel as a marker of exposure to lead (Barbosa et al., 2005; Ericson, 2001 Rinderknecht et al., 2005; Uryu et al., 2003). However, details on how lead accumulates in surface enamel are not known, particularly in deciduous surface enamel, and this precludes the development of a strategy to use deciduous enamel as a marker of exposure to lead.

Lead is found in higher levels in surface enamel, and a gradient has been described in permanent human teeth, with lead levels steadily decreasing from surface to inner enamel (Arora et al., 2005; Brudevold et al., 1977; Brudevold and Steadman, 1956; Purchase and Fergusson, 1986). Some studies showed a relationship between the lead in surface enamel and environmental lead exposure in permanent teeth (Cleymaet et al., 1991a–e) and in deciduous teeth (Almeida et al., 2007; Gomes et al., 2004). These studies reported lead concentration in surface enamel in agreement with the environment where people lived.

In a previous study we described how lead accumulates in surface deciduous enamel (1.9–5.9 μm). These data were collected *in vivo* from a large sample size ($n = 247$, children from Ribeirão Preto, a city with no record of environmental lead contamination, and $n = 26$, children from Bauru, which lived in an area notoriously contaminated with lead). In each of these children (4–6 years old) one enamel microbiopsy was done in one upper deciduous incisor, and the sample obtained was used for determination of lead and phosphorus. Samples from both regions were stratified in categories of close biopsy depths based on the biopsy depth quartiles. Median lead contents were statistically different when Ribeirão Preto (206 μg/g) was compared with Bauru (786 μg/g) ($p < 0.0001$), whereas biopsy depth was not different (3.9 μm in Ribeirão Preto and 3.8 μm in Bauru). Pearson's correlation coefficient between biopsy depth and $\log_{10}$ of lead content was −0.29 (Ribeirão Preto) and −0.18 (Bauru). Differences in lead content were statistically significant between the children living in Ribeirão Preto and Bauru in all quartiles of biopsy depth, showing that the lead accumulation in deciduous teeth surface enamel is related to the environment where people live, and, in contrast to permanent teeth, lead does not show a steady decrease in lead content in the subsurface enamel (Almeida et al., 2007). In that study we described a *plateau* in lead content in subsurface deciduous enamel ($\geqslant 3.18$ μm depth) of children living in an area notoriously contaminated with lead. This *plateau* appeared because lead values medians from the three groups of deepest biopsies (3.18–5.9 μm) were all close to 600 μg of lead per g of enamel in children from the contaminated area, while these lead values medians steadily decreased in the children living in the apparently non-contaminated city (from 278 to 121 μg/g). Since the outermost enamel has direct contact with saliva, and is probably the most susceptible area of enamel to small fluctuations in lead contents over time, we thought that this subsurface enamel (3.18–5.9 μm) might be analyzed separately, and that the *plateau* might be a useful indication of the exposure of a child or group of child to lead.

Therefore, the aim of this study was to test whether we would find different results of lead in enamel from the children from seven Kindergartens from Ribeirão Preto, SP, Brazil, by distributing the children as having "high" or "low" exposure to lead based on the cut-off value of 600 μg/g of lead in enamel.

2. Materials and methods

2.1. Sample population

The data analyzed in this study comprise enamel subsurface samples from deciduous teeth collected *in vivo* from 4- to 6-year-old children living in Ribeirão Preto, SP, Brazil ($n = 186$) and Bauru, SP, Brazil ($n = 20$). While Ribeirão Preto is a city in which no contamination with lead has been described so far (either in scientific journals or by the local health authorities), the sample population in Bauru was selected in the area nearby a battery plant that worked until January 29, 2002, which heavily contaminated the environment (de Freitas et al., 2007). Further details on the study design, population and chemical analysis of lead and phosphorus are found in our previous work (Almeida et al., 2007). Briefly, a sample from surface enamel was obtained by a microbiopsy technique (Gomes et al., 2004), and lead was expressed as μg/g of enamel, and enamel mass was calculated assuming an enamel density of 2.95 g/cm^3 and a phosphorus content of 17.4% (Koo and Cury, 1998; Weidmann et al., 1967). Only samples with phosphorus determined in triplicate were included in the analysis. Data for lead and phosphorus were initially obtained from 247 children from Ribeirão Preto and 26 children from Bauru, and the data were stratified according to quartiles of biopsy depths. Since lead levels are extensively described in the literature to accumulate in surface enamel, no normal distribution of lead contents was expected (nor found) in relation to biopsy depth, and therefore initially median and quartile values were described, as well as non-parametric statistics used for comparisons. A steady decrease in lead content medians was seen in samples from the Ribeirão Preto children from the outer to the inner quartile of biopsy depth, while a *plateau* was observed in lead content medians from the Bauru children in the subsurface enamel (3.18–5.9 μm),