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Lead in saliva from lead-exposed and unexposed children

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ABSTRACT

Introduction: Whole blood is used for diagnosis of lead exposure. A non-invasive method to obtain samples for the biomonitoring of lead contamination has become a necessity. This study 1) compares the lead content in whole saliva samples (Pb-saliva) of children from a city with no reported lead contamination (Ribeirão Preto, São Paulo State, Brazil) and children of a region notoriously contaminated with lead (Bauru, São Paulo State, Brazil), and 2) correlates Pb-saliva with the lead content in the enamel microbiopsy samples (Pb-enamel) in the case of these two populations.

Methods: From a population of our previous study that had included 247 children (4- to 6-year-old) from Ribeirão Preto, and 26 children from Bauru, Pb-saliva was analyzed in 125 children from Ribeirão Preto and 19 children from Bauru by inductively coupled plasma mass spectrometry (ICPMS). To correlate Pb-saliva with Pb-enamel, we used Pb-enamel data obtained in our previous study. The Mann-Whitney test was employed to compare the Pb-saliva data of the two cities. Pb-saliva and Pb-enamel values were then Log_{10} transformed to normalize data, and Pb-saliva and Pb-enamel were correlated using Pearson's correlation coefficient.

Results: Median Pb-saliva from the Ribeirão Preto population (1.64 μg/L) and the Bauru population (5.85 μg/L) were statistically different ($p<0.0001$). Pearson's correlation coefficient for Log_{10} Pb-saliva *versus* Log_{10} Pb-enamel was 0.15 ($p=0.08$) for Ribeirão Preto and 0.38 ($p=0.11$) for Bauru.

Conclusions: A clear relationship between Pb-saliva and environmental contamination by lead is shown. Further studies on Pb-saliva should be undertaken to elucidate the usefulness of saliva as a biomarker of lead exposure, particularly in children.

1. Introduction

Lead is usually analyzed in whole blood for biomonitoring exposure in human populations. However, blood collection is an invasive procedure that causes discomfort, especially in children (Barbosa et al., 2005). Saliva collection is easier than blood collection for large population screening studies, and therefore it has been suggested by some authors as a possible

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biomarker of lead exposure (Silbergeld, 1993; Koh et al., 2003; Nriagu et al., 2006).

Indeed, Pb-saliva may indirectly reflect the amount of lead in plasma (Omokhodion and Crockford, 1991), and in recent years plasma lead levels has gained considerable attention (Smith et al., 2002; Barbosa et al., 2006; Bergdahl et al., 2006). The plasma reflects the extracellular fluid in the body (Guyton and Hall, 2000), thus allowing access to the true amounts of lead to which cells are exposed. The saliva, in turn, is formed by the active transport of water and ions from plasma (Humphrey and Williamson, 2001).

Studies from the past decade described plasma concentrations in lead workers and controls (Schütz et al., 1996), as well as in unexposed and exposed children (Bergdahl et al., 1999), showing that plasma lead concentrations are reliable.

In this sense, this study aimed at: (1) comparing Pb-saliva in samples from a city with no apparent lead contamination (Ribeirão Preto, São Paulo State, Brazil) with samples from an area notoriously contaminated located in the city of Bauru (Bauru, São Paulo State, Brazil), and (2) correlating Pb-saliva with Pb-enamel in these two populations.

2. Methods

2.1. *Sample population*

The study proposal was approved by the Ethics Research Committee (FORP/USP protocol no. 2003.1.353.58.7), according to Resolution 196/96 of the National Ethics Research Committee. Parents signed an informed consent prior to inclusion of their child in the study.

The children studied here were the same that participated in our previous study (Costa de Almeida et al., 2007) that had included 247 children (4- to 6-year-old) from Ribeirão Preto, São Paulo State, Brazil (city with no reported lead contamination), and 26 children living in a region highly exposed to lead (Barbosa et al., 2006; de Freitas et al., 2007) during the running of a battery plant located less than 1000 m from their residences in Bauru, São Paulo State, Brazil. Further details on the study population in this previous study are available elsewhere (Costa de Almeida et al., 2007). The median blood lead level in children (6 to 8 years-old) from Ribeirão Preto is 2.0 μg/dL (manuscript in preparation), while the median blood lead level in children (<13 years-old) living in the contaminated area in Bauru was 7.3 μg/dL, and 37% of these children presented blood lead level above the action limit established by the World Health Organization (10 μg/dL) (de Freitas et al., 2007).

2.2. *Saliva sample collection*

Whole non-stimulated saliva was collected from 125 children living in Ribeirão Preto and 19 children from Bauru. The reason why a smaller population was included in the present study is that some children failed to spit (probably due to different motor control of muscles in children at this age).

After thoroughly rinsing the child's mouth with tap water, 5 mL of non-stimulated whole saliva was collected into 50 mL Falcon tubes. The samples were frozen at -20 °C until analysis was performed. All the saliva collections were carried out between 8 and 11 a.m.

2.3. *Enamel microbiopsy technique and chemical analysis*

The detailed enamel microbiopsy procedure and chemical analysis are described in detail in Costa de Almeida et al. (2007).

2.4. *Saliva analysis*

The saliva samples were analyzed by inductively coupled plasma mass spectrometry (ICPMS) (Perkin Elmer Elan DRC II) following the method proposed by Barbosa et al. (2006). The method's quantification limit was 0.02 μg/L.

2.5. *Blood Lead Data*

To explore the possible association between Pb-saliva and Pb-blood, we examined whether Pb-saliva correlated with Pb-blood in the contaminated region (Bauru) using blood data derived from the periodic evaluations performed by the Epidemiological Surveillance System.

The blood samples were collected in three distinct periods: 1) diagnostic assessment; 2) confirmation assessment for the children who had Pb-blood higher than 10 μg/dL (both collected in 2002); 3) evaluation of children after environmental control measures were taken (between September 2003 and October 2004). The saliva samples were collected in August 2004, and the blood samples from the same children had been collected between May 2002 and December 2002, before environmental control measures aiming at decreasing lead content in the environment had taken place. To correlate Pb-saliva with Pb-blood, only the data concerning Pb-blood obtained during the confirmation assessment (second collection) and Pb-blood data obtained after the environmental control measures (third collection) were used. The data from the confirmation assessment were employed in the analysis because the collection time was closest to the saliva collection time.

2.6. *Statistical analysis*

The distribution of Pb-saliva and Pb-enamel was analyzed for normality and lead distribution was not normal in the Ribeirão Preto population. The Mann-Whitney test was used to compare Pb-saliva between the two cities. Moreover, Pb-saliva and Pb-enamel values were Log_{10} transformed to normalize data for testing correlation between Pb-saliva with Pb-enamel. Only this correlation was performed using Pearson's correlation coefficient.

A probability level of 5% was considered statistically significant. Statistical analyses were performed using the Graph Pad Prism Program (Version 3.0).

3. Results and Discussion

This study shows there is a clear relationship between Pb-saliva and environmental contamination by lead, as judged from data obtained for populations with different histories of lead exposure.

Fig. 1 shows Pb-saliva (range and quartiles) found in children from Ribeirão Preto and Bauru. Pb-saliva in the