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INORGANIC COMPOUNDS

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Evaluation of the use of salivary lead levels as a surrogate of blood lead or plasma lead levels in lead exposed subjects

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Abstract We conducted a study to evaluate the use of parotid salivary lead (Pb-saliva) levels as a surrogate of the blood lead (Pb-B) or plasma lead levels (Pb-P) to diagnose lead exposure. The relationship between these biomarkers was assessed in a lead exposed population. Pb-saliva and Pb-P were determined by inductively coupled plasma mass spectrometry, while in whole blood lead was determined by graphite furnace atomic absorption spectrometry. We studied 88 adults (31 men and 57 women) from 18 to 60 years old. Pb-saliva levels varied from 0.05 to 4.4 μg/l, with a mean of 0.85 μg/l. Blood lead levels varied from 32.0 to 428.0 μg/l in men (mean 112.3 μg/l) and from 25.0 to 263.0 μg/l (mean 63.5 μg/l) in women. Corresponding Pb-Ps were 0.02–2.50 μg/l (mean 0.77 μg/l) and 0.03–1.6 μg/l (mean 0.42 μg/l) in men and women, respectively. A weak correlation was found between Log Pb-saliva and Log Pb-B ($r = 0.277$, $P < 0.008$), and between Log Pb-saliva and Log Pb-P ($r = 0.280$, $P = 0.006$). The Pb-saliva/Pb-P ratio ranged from 0.20 to 18.0. Age or gender does not affect Pb-saliva levels or Pb-saliva/Pb-P ratio. Taken together, these results suggest that salivary lead may not be used as a biomarker to diagnose lead exposure nor as a surrogate of plasma lead levels at least for low to moderately lead exposed population.

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Introduction

Whole blood lead (Pb-B) levels have been used as the primary indicator to diagnose lead exposure throughout the last five decades (ATSDR 1999). The degree to which Pb-B reflects the labile, toxic fraction of lead in the circulation is not yet known. It has been suggested that plasma lead (Pb-P) represents a more relevant index of exposure to the distribution of and health risks associated with Pb than Pb-B does. Indeed, from a physiological point of view, we can assume that the toxic effects of Pb are primarily associated with Pb-P, because this fraction is the most rapidly exchangeable one in the blood compartment (Barbosa et al. 2005; Fleming et al. 1999; Hu et al. 1998). However, the use of plasma requires an invasive sample collection, which causes discomfort and trauma, especially in children. Since Pb-P may be directly excreted into saliva, Pb-saliva may indirectly reflect the fraction of lead in plasma, which is the most diffusible fraction of lead in blood (Haeckel 1993). Moreover, saliva may be collected non-invasively, which is of special interest (Aguirre et al. 1993). Despite these facts, studies evaluating the use of salivary lead levels to diagnose lead exposure are still scarce. Furthermore, only a few of them evaluated the correlation between Pb-saliva and Pb-B or Pb-P levels.

While the idea of measuring Pb-saliva is attractive, some authors advocate that saliva has yet many limitations as a biomarker for biomonitoring lead exposure

(Koh et al. 2003). Moreover, Pb-saliva levels reported in the literature are conflicting. This fact is probably due to large variations in its ion content throughout the day, in addition to changes in salivary flow rates before, during and after meals. Variations also arise depending on the manner in which saliva collection is stimulated (or not), and on the nutritional and hormonal status of the individual (Silbergeld 1993). In addition, the very low levels of Pb present in saliva serve to limit the range of suitable analytical techniques that can be used to get reliable data.

Salivary lead measurement is usually performed with the collection of whole saliva. However, it represents secretions from the major and minor glands. On the other hand, individual gland secretions such as that from parotid saliva are superior to whole saliva for many compositional analyses because whole saliva contains nonsalivary elements such as desquamated epithelial cells, food debris, bacteria, gingival crevicular fluid and leukocytes. In addition, whole saliva is much more susceptible for flow variations and contamination during collection when compared to parotid saliva (DiGregorio et al. 1974).

Thus, the aim of this paper is to evaluate the relationship between parotid Pb-saliva levels and Pb-B or Pb-P levels in an adult population low to moderately exposed to lead. We also evaluate whether Pb-saliva may be used as a surrogate of Pb-P levels in the same population, by assessing their saliva/plasma lead ratio.

Methods

Subjects

This study was approved by our institutional review committee and each subject provided written informed consent. Volunteers, aged from 18 to 60 ($n = 88$), 57 women (age range 18–60; mean 34.2 ± 10.4) and 31 men (age range 18–60; mean 36.6 ± 12.4) living in the city of Bauru, São Paulo, Brazil, comprised the studied population. Most of them were highly exposed to lead, from air and soil, during the running of a battery plant located near their income area. Although the battery plant was closed in 2002, part of this population is still exposed, indoor or outdoor, due to constant deposition of lead on soil and vegetation surrounding their houses.

Materials

High purity de-ionized water (resistivity 18.2 mΩ cm) obtained by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout. All reagents used were from high purity analytical grade. All chemical solutions used for Pb determination were stored in high-density polypropylene bottles. Whole blood and plasma were stored in 2 ml eppendorffs at −80°C. Eppendorffs, falcon tubes, plastic bottles, autosampler cups and glassware materials were cleaned by soaking in 10% v/v HNO_3 for 24 h, rinsing five times with Milli-Q water and dried in a class 100 laminar flow hood located inside the class 10000 clean room.

Sample collection

Saliva was collected from each volunteer from the parotid gland using a modified Carlson–Crittenden collector. This collector consists of a cup with an inner and outer chamber (Navazesh 1993). The inner chamber is attached to a plastic tubing that carries saliva to a previously ultra-cleaned (2 ml) eppendorff. The outer chamber is attached to a rubber bulb device via plastic tubing for suction inducing. After sample collection, 20 µl of a concentrated suprapured HNO_3 was added to each eppendorf and immediately frozen at −80°C until used for analysis.

Venous blood samples were collected from each volunteer in two evacuated tubes of 6 ml containing lyophilized heparin (Vacuntainer BD, trace metals free). Before collection, the skin of the volunteer was cleaned with alcohol and MilliQ water. The first fraction of blood was used to determine the Pb-B content and the second was used for plasma collection.

Blood samples were immediately centrifuged 800×*g* for 6 min at room temperature) for plasma collection to avoid transference of lead from erythrocytes. Each plasma fraction was then pipetted into two ultra-cleaned eppendorffs (2 ml) and immediately frozen at −80°C until used for analysis.

Both samples were collected from each volunteer in the same day and always from 8 to 10 a.m. to reduce circadian contributions (Tenovuo 1989). Subjects were asked to come for sample collection after at least 12 h of fasting, oral hygiene and smoking.

Sample analysis

Blood samples were analyzed by graphite furnace atomic absorption spectrometry (Varian SpectrAA 220) following the method proposed by Zhou et al. (2002). Briefly, 100 µl of blood samples were diluted 1:10 with a solution containing 0.2 % v/v HNO_3 0.5 Triton X-100. Then, 12 µl of the resulted sample was delivered into the graphite tube with graphite platforms previously coated with W-Rh permanent modifier. Calibration was performed against lead aqueous solutions. The method detection limit is 0.7 µg/dl. To evaluate the accuracy of the results, NIST 955 whole blood Standard Reference Material and Blood Reference Materials produced by the New York State Department of Health as part of their Interlaboratory Program of Proficiency Testing were analyzed before and after 10 ordinary samples.

Plasma and saliva samples were analyzed by inductively coupled plasma mass spectrometry (Perkin Elmer 6100) following the method proposed

by Schutz et al. (1996) with little modifications. Briefly, 300 µl of plasma were diluted 1:10 with a solution containing ammonia (0.04 mol/l), disodium ethylenediaminetetracetic dihydrate (Na_2EDTA; 200 mg/l) and Triton X-100 (100 mg/l). The method detection limit for lead was 0.02 and 0.03 µg/l for saliva and plasma, respectively.

To check the saliva lead data for accuracy, we compared the lead results of samples with Pb levels higher than 1.5 µg/l ($n = 12$) by using the inductively coupled plasma mass spectrometry (ICP-MS) method with those obtained by an alternative method with graphite furnace atomic absorption spectrometry (GF AAS). There was no statistical difference between each pair of values (*t* test, 0.05). To validate Pb-P data, Serum Reference Materials produced by the New York State Department of Health as part of their Interlaboratory Program for Proficiency Testing were analyzed before and after 10 ordinary samples.

Hemoglobin plasma levels were obtained by each sample to check for possible hemolysis. Hemolized plasma samples were excluded from the final data.

Statistical analysis

Pb-B, Pb-saliva and Pb-P values were log transformed to normalize data. The Pearson's correlation (r, P) was calculated for associations between Log Pb-saliva with Log Pb-B, Log Pb-P, time of exposure and age, grouped or not by gender. Multiple regression analysis was used to calculate the inter-relationship of all the parameters considered (independent variables) with Pb-saliva or Pb-saliva/Pb-P ratio as the dependent variable.

Results

Pb-saliva levels found in the studied group varied from 0.05 to 4.4 µg/l, with a mean of 0.85 µg/l. Pb-saliva levels varied from 0.05 to 4.4 µg/l (mean 0.86 µg/l, $n = 31$) in male volunteers and from 0.05 to 3.9 µg/l (mean 0.84 µg/l, $n = 57$) in females. Corresponding Pb-Bs were 32.0–428.0 µg/l (mean 112.3 µg/l) and 25.0–263.0 µg/l (mean 63.5 µg/l) in men and women, respectively. Plasma lead levels were 0.02–2.50 µg/l (mean 0.77 µg/l) for men and 0.03–1.60 µg/l (mean 0.42 µg/l) for women.

Relationship between Pb-saliva and Pb-blood or Pb-plasma levels

We found practically similar correlation coefficients between Pb-saliva and Pb-B levels ($r = 0.277$, $P = 0.008$) and between Pb-saliva and Pb-P levels ($r = 0.280$, $P = 0.006$) as shown in Fig. 1. The same correlation was also evaluated with data separated by gender, showing stronger correlations for male volunteers compared to females. In males, for example, the correlation between Log Pb-saliva and Log Pb-B was 0.316, $P < 0.005$ and between Log Pb-saliva and Log Pb-P was 0.41, $P = 0.02$. However, there was no statistic significant correlation between Log Pb-saliva and Log Pb-P for female volunteers ($r = 0.17$, $P = 0.151$).

The %Pb-saliva/Pb-B ratio varied from 0.07 to 5.40% for whole volunteers with a mean of 1.33% for males and 1.51% for females, while Pb-saliva/Pb-P ratio varied from 0.20 to 18.0 (mean 4.5) for male and from 0.20 to 12.1 (mean 4.6) for female volunteers. Table 1 shows summary statistics for the subject's data.

Discussion

To our knowledge, this study is the first to describe the relationship between lead levels in saliva with those levels in blood and plasma in a wide adult population exposed to lead. Moreover, this study is also the first to describe the Pb-saliva/Pb-P ratio and whether age and gender are factors that might influence this ratio.

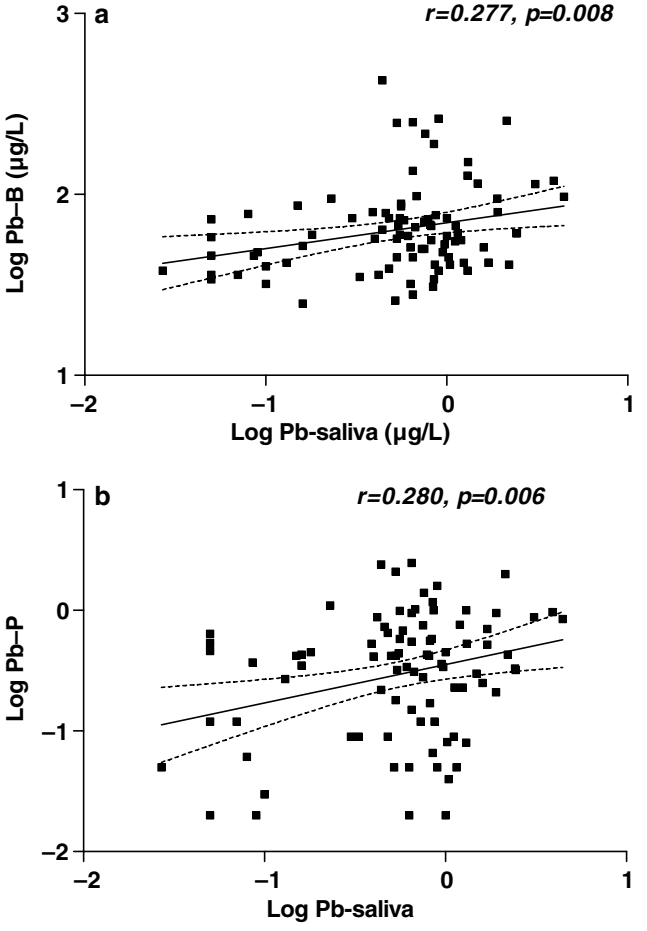

Fig. 1 Scatter diagrams to show the correlation between Pb-saliva and **a** Pb-B, and **b** Pb-P. (log-normal values; $n = 88$)

Table 1 Descriptive statistics of variables under study for the subjects

Category	Mean	Median	SD	Min	Max
Subjects ($n=88$)					
Women age ($n=57$)	34.2	32	12.4	18	60
Men age ($n=31$)	36.6	35	10.4	18	60
Pb-saliva (μg/l)					
Women	0.84	0.75	0.7	0.05	3.9
Men	0.86	0.63	0.9	0.05	4.4
Pb-P (μg/l)					
Women	0.42	0.36	0.32	0.03	1.60
Men	0.77	0.46	0.83	0.02	2.50
Pb-B (μg/l)					
Women	63.5	37.6	56.0	25	263
Men	112.3	74.0	98.2	32	428
Pb-saliva/Pb-B ratio (%)					
Women	1.51	1.22	1.2	0.07	5.40
Men	1.33	0.74	1.9	0.07	4.57
Pb-saliva/Pb-P ratio					
Women	4.6	1.8	6.8	0.20	12.1
Men	4.5	2.0	9.9	0.20	18.0

Max maximum, *Min* minimum, *SD* standard deviation

While other studies had suggested stronger association between Pb levels in saliva and those in plasma or blood (Silbergeld 1993; P'an 1981; Brodeur et al. 1983; Omokhodion and Crockford 1991; Timchalk et al. 2001), our results show a weak correlation between Pb-saliva and Pb-B ($r=0.277$, $P=0.008$) or Pb-P ($r=0.280$, $P=0.006$). One of the reasons for this difference must be due to the distinction in the blood lead levels of the volunteers evaluated in those studies with our volunteers. For example, the study made by P'an (1981) was on a population with much higher levels of Pb-B, and if only subjects with comparable (to this study) levels of blood lead were compared, the relationship between Pb-saliva and Pb-B in the P'an study would also be weak.

Some studies are suggesting that Pb-P levels may better reflect the toxicologically labile fraction of circulating Pb that is more freely available for exchange with target tissues than do Pb-B levels (Barbosa et al. 2005). This suggestion is consistent with the toxicokinetic characteristics of readily exchangeable Pb, and it has also been substantiated by recent data indicating that % Pb-P/Pb-B ratio are more strongly associated with bone lead levels than are Pb-B levels (Cake et al. 1996). Plasma lead levels may also reflect endogenous lead stored in bone for years, which in course may be released back to the bloodstream during the bone remodeling process (Barbosa et al. 2005). However, the use of plasma requires an invasive sample collection, which causes discomfort and trauma, especially in children.

It has been argued that Pb in saliva is the direct excretion of the Pb fraction in diffusible plasma, i.e., the fraction not bound to proteins (Omokhodion and Crockford 1991). Despite the associations reported in the literature, the older saliva Pb concentrations are quite high, and the values vary from study to study (Fung et al. 1975; Gonzalez et al. 1997). Fung et al. (1975), for example, reported that mean salivary Pb is 31% of mean blood-Pb among nine children, in whom the mean blood-Pb was 420.0 μg/l. In another study, 24 adults with a mean blood Pb of 86.0 μg/l had a mean salivary Pb level that was 56% of the blood-Pb level (Omokhodion and Crockford 1991). In contrast, our results show much lower levels of lead in parotid saliva (mean of 0.85 μg/l). It can be pointed out that our findings are in agreement with recent data reported by Koh et al. (2003) and Wilhelm et al. (2002) in exposed and unexposed subjects, respectively. Koh et al. (2003) determined Pb in saliva and blood from 82 exposed adults using electrothermal atomic absorption spectrometry (ET AAS). The mean blood-Pb reported was 266.0 μg/l and the mean salivary Pb was 7.7 μg/l (i.e., 3% of the mean blood-Pb). Wilhelm et al. (2002) found that the Pb content in the saliva of unexposed children was lower than 1.5 μg/l. One reason for this discrepancy on the Pb-saliva levels between earlier studies and more recent ones may be due to contamination during sample collection and/or analysis in the former.

We found a percentage Pb-saliva/Pb-B ratio varying from 0.07 to 5.40% and Pb-saliva/Pb-P ratio from 0.20 to 18.0. Koh et al. (2003) also observed comparable percentage Pb-saliva/Pb-B ratios in their study with exposed lead workers. However, they did not measure plasma lead levels. On the other hand, in a recent publication, Timchalk et al. (2006) found Pb-saliva/Pb-B ratio ranging from 0.01 to 0.06 in rats treated with a single dose of 100 mg/kg/day lead acetate.

A high intersubject saliva/plasma lead ratio in this study was observed. This variation was higher among male volunteers compared to females. However, multiple regression analysis shows no influence of age, gender and time of exposure on Pb-saliva levels or Pb-saliva/Pb-P ratio. Other studies have stated high intersubject saliva/plasma ratio variability for other elements such as lithium with a low intrasubject variability (Siegel 1993). Unfortunately, we did not evaluate the intrasubject variability in our study. However, if Pb behavior is assumed to be similar to lithium, one may advocate that salivary monitoring might only be a useful predictor of plasma concentration irrespective of whether a saliva/plasma ratio is established for each subject (Siegel 1993).

In conclusion, while the idea of measuring Pb-saliva for monitoring lead exposure is attractive, our findings suggest that parotid saliva is neither a suitable biomarker to diagnose lead exposure nor a surrogate of Pb-plasma levels, at least for low to moderately exposed populations such as that evaluated in this study.

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References

Agency for Toxic Substances and Disease Registry (ATSDR) (1999) Toxicological profile for lead. US Department of Health and Human Services, Public Health Service, July

Aguirre A, Testaweintraub LA, Banderas JA, Haraszthy GG, Reddy MS, Levine MJ (1993) Sialochemistry—a diagnostic-tool. Crit Rev Oral Biol Med 4:343–350

Barbosa Jr F, Tanus-Santos JE, Gerlach RF, Parsons PJ (2005) A critical review of biomarkers for monitoring human exposure to lead: advantages, limitations and future needs. Environ Health Perspect 113:1669–1674

Brodeur J, Lacasse Y, Talbolt D (1983) Influence of removal from occupational lead exposure on blood and saliva lead concentrations. Toxicol Lett 19:1992–1999

Cake KM, Bowins RJ, Vaillancourt C, Gordon CL, McNutt RH, Laporte R, Webber CE, Chettle DR (1996) Partition of circulating lead between serum and red cells is different for internal and external sources of lead. Am J Ind Med 29:440–445

DiGregorio GJ, Firko AP, Sample RG, Bobyock E, McMichael R, Chernick WS (1974) Lead and aminolevulinic acid concentrations in human parotid saliva. Toxicol Appl Phramacol 27:491–493

Fleming DEB, Chettle DR, Weber CE, O'Flaherty EJ (1999) The O'Flaherty model of lead kinetics: an evaluation using data from a lead smelter population. Tox Appl Pharmacol 161:100–109

Fung HL, Yaffe SJ, Mattar ME, Lanighan MC (1975) Blood and salivary lead levels in children. Clin Chim Acta 61:423–424

Gonzalez M, Banderas JA, Baez A, Belmont R (1997) Salivary lead and cadmium in a young population residing in Mexico city. Toxicol Lett 93:55–64

Haeckel R (1993) Factors influencing the saliva/plasma ratio of drugs. Ann NY Acad Sci 694:129–142

Hu H, Rabinowitz M, Smith D (1998) Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms. Environ Health Perspect 106:1–8

Koh D, Ng V, Chua LH, Yang Y, Ong HY, Chia SE (2003) Can salivary lead be used for biological monitoring of lead exposed individuals? Occup Environ Med 60:696–698

Navazesh M (1993) Methods for collecting saliva. Ann NY Acad Sci 694:72–78

Omokhodion FO, Crockford GW (1991) Lead in sweat and its relationship to salivary and urinary levels in normal healthy-subjects. Sci Total Environ 103:113–122

P'an AYS (1981) Lead levels in saliva and in blood. J Toxicol Environ Health 7:273–280

Schutz A, Bergdahl IA, Ekholm A, Skerfving S (1996) Measurement by ICP-MS of lead in plasma and whole blood of lead workers and controls. Occup Environ Med 53:736–740

Silbergeld EK (1993) New approaches to monitoring environmental neurotoxins. Ann N Y Acad Sci 694:62–71

Siegel IA (1993) The role of saliva in drug monitoring. Ann N Y Acad Sci 694:86–90

Tenovuo JO (1989) Human saliva. Clinical chemistry and microbiology. CRC Press, Boca Raton, pp 1–19, 91–92

Timchalk C, Poet TS, Lin Y, Weitz KK, Zhao R, Thrall KD (2001) Development of an integrated microanalytical system for analysis of lead in saliva and linkage to a physiologically based pharmacokinetic model describing lead saliva secretion. Am Ind Hyg Assoc J 62:295–302

Timchalk C, Lin Y, Weitz KK et al (2006) Disposition of lead (Pb) in saliva and blood of Sprague–Dawley rats following a single or repeated oral exposure to Pb-acetate. Toxicology (in press)

Wilhelm M, Pesch A, Rostek U, Begerow J, Schmitz N, Idel H, Ranft U (2002) Concentrations of lead in blood, hair and saliva of German children living in three different areas of traffic density. Sci Total Environ 297:109–111

Zhou Y, Zanão RA, Barbosa Jr F, Parsons PJ, Krug FJ (2002) Investigations of W-Rh permanent modifier for the determination of Pb in blood by electrothermal atomic absorption spectrometry. Spectrochim Acta part B 57:1291–1300