Environmental exposure to lead and oxidative stress biomarkers among healthy children in La Plata, Argentina

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ABSTRACT

Introduction. Environmental exposure to lead is still a major public health problem, especially in children. Oxidative stress may be a primary mechanism associated with toxicity. The objective of this study was to measure blood lead levels (BLLs) in children aged 1 to 6 years exposed to lead in La Plata and suburban areas and their relation to oxidative stress biomarkers.

Population and methods. Cross-sectional, analytical study. Clinically healthy children aged 1 to 6 years were analyzed. BLLs, antioxidant enzyme activity, and extent of lipid peroxidation were measured. The statistical software package R, version 3.5.1, was used.

Results. A total of 131 children participated; their median age was 2.33 years. The geometric mean of BLLs was 1.90 μg/dL; 32% showed a measurable BLL and 3%, BLLs ≥ 5 μg/dL (international reference). The comparison of oxidative stress biomarkers based on BLLs showed a significant difference in median thiobarbituric acid reactive substances (TBARS): 12.0 versus 10.0 nmol MDA/mL of plasma; $p = 0.02$. In addition, the correlation between BLLs and TBARS was positive ($r = 0.24$; $p = 0.012$).

Conclusions. Most children had a BLL below the limit recommended by international agencies; although such BLLs do not affect antioxidant enzyme activity, they can induce lipid peroxidation. These results demonstrate the usefulness of this biomarker as an early diagnosis tool to assess subtoxic lead effects.

Key words: *lead, oxidative stress, antioxidants, lipid peroxidation, children.*

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INTRODUCTION

Lead is a non-essential metal for humans, although widely distributed in nature, both due to its natural production and its industrial use.[1] Most studies carried out in Latin America refer to exposure from specific sources. For this reason, the challenge in this region lies in identifying lead effects on populations of children exposed to unknown sources or resulting from environmental exposure to lead over long periods of time.[2,3]

Due to the convincing evidence about the toxic effects of lead, still in low levels, reference values reduced in recent decades. In 2012, the Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) advised reducing the recommended value from 10 μg/dL to 5 μg/dL,[4] a value that has to be reviewed periodically because several multicenter studies concluded that there is no safe lead level for immature bodies.[5,6]

Lead causes multisystemic effects; the central nervous system is the main target of toxicity, especially if exposure occurs during development, and this becomes a causative factor of neurobehavioral alterations.[7,8]

To date, no single process to account for its toxicity has been described, although several studies suggest that oxidative stress may be a primary mechanism associated with toxicity.[9-11] The emergence of a redox imbalance may result from a direct

effect of lead on cell membranes, inducing lipid peroxidation, development of oxygen reactive species and/or depletion of the antioxidant defense system.[12-14]

Based on the preceding, the objective of this study was to measure blood lead levels (BLLs) in children aged 1 to 6 years exposed to lead in La Plata and suburban areas and their relation to oxidative stress biomarkers.

POPULATION AND METHODS

An observational, cross-sectional, analytical study was conducted in male and female, clinically healthy children aged 1 to 6 years who attended the Pediatric Clinic at the Health Observatory of the Pediatric Research and Development Institute (Instituto de Desarrollo e Investigaciones Pediátricas, IDIP) of Hospital de Niños Sor María Ludovica of La Plata between May 2014 and March 2015. It is worth noting that La Plata, the capital city of the province of Buenos Aires, Argentina, is located near one of the largest oil refinery industrial sites in South America. Children with diagnosed chronic conditions, acute conditions and/or infections at the time of the study, genetic disorders and neurological history, or moderate or severe malnutrition were excluded.

The sample was selected by convenience, in a non-probabilistic fashion. The sample size was estimated to establish a 0.3 correlation between BLLs and oxidative stress biomarkers, with a 95% confidence interval and an 80% power. There were 85 cases.

The research protocol was approved by the Institutional Research Protocol Review Committee (Comité Institucional de Revisión de Protocolos de Investigación, CIRPI) of Hospital de Niños Sor María Ludovica, La Plata, Argentina. Parents or legal guardians participating in the study signed an informed consent form in the presence of a witness. Results were reported to parents. Children whose BLLs were above 5 μg/dL were referred to the Department of Toxicology for follow-up.

Data collection instruments and techniques

Blood samples were collected in all children by venipuncture and divided into 2 heparin tubes: 1 mL in a tube used to measure BLLs and the rest in a tube that was immediately centrifuged to obtain plasma and packed red blood cells, then stored at -70 °C until processing. Plasma was used for the measurement of thiobarbituric acid reactive substances (TBARS) and packed red blood cells for the measurement of the enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx).

Biochemical testing

For BLL measurement, heparinized samples were diluted 1:10 in 15% nitric acid (Merck, Argentina) and centrifuged. The resulting supernatant was analyzed by atomic absorption spectrometry at a wavelength of 283.3 nm (equipment: Varian AA 240 Z®, 120 programmable sample dispenser, Zeeman background correction, Mulgrave, Australia). The internal quality control (IQC) process used the Lyphochek® whole blood metals control from BIO-RAD Laboratories, 8.03 μg/dL, with a percent coefficient of variation (CV%) of 6.8% between runs. The materials for IQC prepared at the laboratory included whole blood with added lead nitrate –$Pb(NO_3)_2$– (Merck, Argentina) at an average level of 5.1 μg/dL and a CV% of 8.8% between runs. It is worth noting that the laboratory has been part of an external quality control (EQC) called German External Quality Assessment Scheme (G-EQUAS). The percent relative error at the level reported by our laboratory, compared to the consensus value, was at a range of 7.3%-11.0%, i.e. within the acceptability range of the EQC scheme. The limit of detection (LOD) with this method was 0.8 μg/dL, and the limit of quantification (LOQ) was 2.7 μg/dL.

Enzyme activity and lipid peroxidation levels were measured using a Shimadzu UV-1800® spectrometer. The lipid peroxidation index was established using the method described by Ohkawa et al.[15] According to this method, malondialdehyde (MDA) and thiobarbituric acid (TBA) react at a high temperature (90-100 °C) in acid mean to form the TBA/MDA adduct, measured at 532 nm, and the result was expressed as nmol MDA/mL of plasma.

Erythrocyte CAT activity was measured with red blood cells that were first hemolyzed using distilled water (1/20 dilution) and

centrifuged at 10 000 *g* for 10 min; the resulting hemolyzed sample was diluted once again (1/100 dilution) using a phosphate buffer with a pH of 7.0. Enzyme activity was measured based on the method described by Aebi,[16] by reducing substrate absorbance (H_2O_2) for 5 min at 240 nm (e240 ¼ 0.0394 per mmol/cm). Results were described as unit (U), corresponding to the consumption of 1 μmol of H_2O_2 per minute per gram of hemoglobin (Hb).

SOD activity was measured using a commercial kit (Ransod®; Randox Labs, Argentina). This method uses xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-Iodophenyl)-3-(4-nitrophenol)-phenyltetrazolium chloride (INT) and form red formazan dye. SOD activity was measured based on the extent of inhibition from this reaction. A U is defined as the one that causes 50% of inhibition in INT reduction rate in assay conditions. Values referred to Hb levels.

GPx activity was measured using red blood cells hemolyzed using distilled water (1/5 dilution) and centrifuged at 10 000 *g* for 10 min. The resulting supernatant was used to measure enzyme activity according to the method proposed by Paglia and Valentine,[17] which determines NADPH consumption rate per minute during 10 min at 340 nm. A U is defined as the consumption of 1 μmol of NADPH per minute and expressed as U/g Hb.

Characteristics of the population

An unmet basic need (UBN) indicator was developed using the method described by the National Statistics and Censuses Institute of Argentina to establish the characteristics of the population. The maternal level of education was also recorded.

Statistical analysis

The statistical analysis of data was done using the R software, version 3.5.1. The normality of all variables was determined using the Kolmogorov-Smirnov test. For the initial analysis, BLLs were considered a continuous variable and expressed as mean with a 95% confidence interval, considering the variable distribution as a normal log. If BLLs were below the LOD, the mean value was adjusted using the extrapolation method based on a linear regression considering the characteristics of value distribution above the LOD to estimate values below the LOD, which have smaller error rates than all the standard replacement techniques.[18] Thus, BLLs were classified into 3 groups: < 2.7 μg/dL (LOQ), between 2.7 and 4.9 μg/dL, and ≥ 5.0 μg/dL.

Redox parameters did not show a normal distribution and were expressed as median and interquartile range (IQR). However, the enzyme GPx showed a normal distribution, so its values were expressed as geometric mean ± standard deviation (SD).

Spearman's test was used to assess the correlation between levels of lead and oxidative stress indicators. The Mann Whitney (MW) or Kruskal Wallis (KW) tests were used to compare TBARS, enzyme activity, and BLLs above or below the LOQ (2.7 μg/dL). This value was used because it is the minimum level as of which BLLs can be measured.

In all cases, a value of $p < 0.05$ was considered significant.

RESULTS

A total of 131 children aged 1 to 6 years old participated in the study (43.5% were females). Their mean age was 2.33 years (IQR: 1.51-3.68). In relation to social variables, 53.2% of families lived in households with UBNs and 78% of mothers had completed more than 7 years of formal education.

The geometric mean of BLLs was 1.90 μg/dL (1.71-2.10). BLLs were below the LOQ (2.7 μg/dL) in 67.9% (n = 89) of children; 29.0% (n = 38) of measurements were between the LOQ and 5 μg/dL. Only 4 children (3.1%) had values above 5 μg/dL, the limit value established by the United States Centers for Disease Control and Prevention.

Table 1 describes antioxidant enzyme activity and levels of lipid peroxidation products.

Table 2 shows oxidative stress biomarker levels based on BLL categories.

The statistical analysis of the correlation between BLLs and each oxidative stress biomarker found a statistical difference in terms of TBARS (r = 0.24; $p = 0.012$).

DISCUSSION

Environmental exposure to lead is a known public health problem because this metal is still present in urban areas and industrial sites across countries in this region.[19] In this regard, large studies developed in countries like the United States contrast with the few data available about BLLs in children with environmental exposure in different regions of Latin America.

This study reveals non-industrial environmental exposure given that 97% of children showed BLLs < 5 μg/dL and this is supported by the lower BLLs observed here compared to previous studies. In our first article,[20] mean BLLs reported in children younger than 5 years was 4.3 μg/dL. Consistent with this, a critical reduction in BLLs was also observed in the pediatric population in the city of Córdoba, Argentina,[21] where BLLs were 2.58 ± 0.30 μg/dL, a significantly lower value than what had been previously reported (7.70 ± 1.10 μg/dL).[22] However, in both populations, BLLs were mildly higher than those reported in United States children: 0.86 μg/dL.[23]

Therefore, studies like this one are fundamental for the implementation of public policies or to test the success of such policies in regions like Latin America, where large surveys and regular lead tracing studies are uncommon.[2,3] In addition, most studies have a cross-sectional approach, which may minimize the permanent changes resulting from a prolonged contact with lead, even at low levels of exposure.

Another confounding factor that should be taken into consideration is the inclusion of children living near specific lead sources, which may imply antenatal exposure not only to lead, but also to other toxic substances. In addition, industrial areas are, in general, inhabited by populations with socioeconomic disadvantages and nutritional deficiency, which may conduct to a greater absorption of several neurotoxics, including lead.

Also, it is worth noting that routine biomonitoring to determine lead exposure is usually limited to measuring lead levels in different biological matrices and delta-aminolevulinic acid dehydratase (δALAd) and ferrochelatase activity; all these parameters are changed to BLLs that are higher than those reported here.[24] For these reasons, the exploration of an association between BLLs and oxidative stress biomarkers should be further investigated. In line with this, epidemiological studies conducted in children[25-27] have failed to provide conclusive evidence in relation to antioxidant enzymes and lipid peroxidation levels.[10,11,25-27]

TABLE 1. *Oxidative stress biomarker levels*

Biomarker	Median (IQR)
CAT (KU/g Hb)	131.6 (108.2-157.9)
SOD (U/g Hb)	1262.9 (1135.7-1415.3)
GPx (U/g Hb) *	24.4 ± 2.7
TBARS (nmol MDA/mL of plasma)	10.7 (8.1-14.0)

*Mean ± SD.
CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; TBARS: thiobarbituric acid reactive substances; IQR: interquartile range; SD: standard deviation.

TABLE 2. *Comparison of oxidative stress biomarker levels based on blood lead levels*

Biomarker	BLL ≤ LOQ (n = 89)	BLL > LOQ (n = 42)	*p* value
CAT (KU/g Hb)	123.2 (105.0-156.5)	146.3 (116.9-162.6)	0.07
SOD (U/g Hb)	1277.0 (1129.0-1444.0)	1256.0 (1151.0-1346.0)	0.76
GPx* (U/g Hb)	24.3 ± 2.7	24.6 ± 2.7	0.52
TBARS (nmol/mL)	10.0 (8.0-12.5)	12.0 (9.5-15.5)	0.02

*All enzymes are expressed as median and IQR between parentheses, except for GPx enzyme activity, which is expressed as mean ± SD; LOQ: BLL = 2.7 μg/dL.
CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; TBARS: thiobarbituric acid reactive substances; LOQ: limit of quantification; IQR: interquartile range; SD: standard deviation.

Thus, although this study did not find differences in CAT, SOD or GPx levels, lipid peroxidation levels were related to BLLs above the LOQ, and this is consistent with most studies carried out in children that reported a higher lipid peroxidation with a direct correlation to high BLLs.[11]

Therefore, based on our results, there is evidence of the implication of lipid peroxidation in adverse events caused by environmental exposure to lead, a biomarker that may be proposed as a complement to other tools for the early diagnosis of environmental exposure to lead.

To sum up, in Argentina, public health research in relation to pediatric population exposure to environmental contaminants is an emerging field of study. Further studies like this one will help to improve knowledge about the problem of lead exposure in children and provide a mechanistic approach to lead toxicity.

CONCLUSIONS

BLLs observed in children aged 1 to 6 years suggest a low exposure to lead. Still, with BLLs below those recommended by international agencies, lead may cause cell damage, resulting in permanent alterations that may lead to a higher vulnerability to challenging events later in life.

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