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Declining blood lead and zinc protoporphyrin levels in Ecuadorian Andean children

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ABSTRACT

Objectives: To investigate current lead (Pb) exposure in children living in Andean Ecuadorian communities. Blood Pb (PbB) and zinc protoporphyrin (ZPP) levels were used respectively as biomarkers of acute and chronic Pb poisoning. The current PbB–ZPP levels were compared with previous pediatric PbB–ZPP levels recorded over years in the study area.

Design and methods: Samples of whole blood were collected from 22 Andean children of Quechua and Mestizo backgrounds and measured for PbB concentrations by graphite furnace atomic absorption spectroscopy. ZPP/heme ratio and ZPP whole blood (ZPP WB) levels were measured with a hematofluorometer.

Results: The mean PbB level for children in the current study group was 14.5 μg/dL, which was significantly lower than the mean PbB level of 41.1 μg/dL found in the same study area in the 1996–2000 test period, and lower than the 22.2 μg/dL mean level found in the 2003–2007 period. The current mean ZPP/heme ratio was 102.1 μmol/mol, and the mean ZPP WB level was 46.3 μg/dL, both lower than values previously found in children in the study area.

Conclusion: While the current pediatric PbB–ZPP levels in the study area remain elevated in some children, the overall levels indicate a decline relative to levels observed in the same Pb-contaminated area in the period between 1996 and 2007. The elevated ZPP levels suggest a history of chronic Pb exposure, and potential iron deficiency in some children. The overall reduction in PbB–ZPP levels suggests a positive outcome of a Pb-exposure education and prevention program, and the therapeutic intervention of succimer chelation therapy.

Introduction

Pediatric lead (Pb) poisoning continues to be a global health burden, particularly in developing countries where Pb is used in occupational activities (such as, Pb smelting, battery recycling, and Pb glazing of ceramics) in which children participate directly, or are exposed from living in close proximity to the Pb-contaminated sites [1–6]. Pb is highly neurotoxic, with deleterious effects on the nervous system, particularly the developing nervous system. Pediatric Pb exposure, even at low exposure levels has been associated with neurocognitive impairment, including adverse effects on intellectual performance [7–9]. The main route of Pb poisoning in children is via pica or the ingestion of Pb-contaminated substances. In children, approximately 40–50% of ingested Pb is absorbed through the gastrointestinal tract and distributed to the soft tissues, including brain, liver, and kidneys, and more than 70% of absorbed Pb is stored in the bone and teeth [10].

The conventional and most reliable biomarker for acute or recent pediatric Pb exposure is the concentration of Pb in whole blood (PbB). One of the targets of Pb poisoning in children is the hematologic system, where Pb inhibits the activities of enzymes responsible for heme biosynthesis [10]. Following exposure, Pb in the blood is concentrated primarily in erythrocytes, where it binds to delta-aminolevulinic acid dehydratase (ALAD) [10–12]. Pb inhibits the enzymes ALAD and ferrochelatase, which is necessary for the chelation of iron (Fe) by protoporphyrin [10–12]. The resulting accumulation of protoporphyrin in the absence of Fe attracts zinc as a replacement, forming zinc protoporphyrin (ZPP). In cases of prolonged or chronic Pb exposure, Fe in

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hemoglobin (Hb) is essentially replaced by zinc. Elevated ZPP levels indicate Pb-induced inhibition of heme biosynthesis [10]. Whereas, PbB level is a measure of recent exposure, the ZPP/heme ratio may be useful as a biomarker for prolonged Pb exposure, since elevated ZPP levels lag elevated PbB levels by weeks to months, and may reflect chronic Pb exposure for up to two years [13–16]. Elevated ZPP, a biomarker of Pb toxicity, may also be an indication of Fe deficiency in children, although this is not invariably the case, since the ZPP measure has been shown to have a high false-positive rate for indication of Fe deficiency [16].

Children living in rural Andean communities of Ecuador where Pb glazing of ceramics is a local backyard industry have been found to have high PbB and ZPP levels [17–21]. These elevated PbB levels have been significantly associated with abnormal neurocognitive performance in the children living in the study area [22–24]. In addition, elevated levels of Pb found in the milk of breast-feeding mothers and in the blood of mother–infant pairs living in the same study area suggest that some children are already exposed to Pb during the prenatal and breast-feeding periods [25].

Following our initial investigations, a Pb-exposure education and prevention program was initiated in the study area, and subsequently, intervention with succimer (DMSA) medical treatment was provided for children living in the study area who were found to have elevated PbB levels [18,26]. Case studies of children in the study area have shown improvement in neurocognitive performance of some children as the PbB levels declined [27]. As part of our on-going Pb-exposure education and prevention efforts, we have continued to monitor PbB levels in the study area. The purpose of the present study was to further investigate the Pb exposure levels in a cohort of children currently living in the study area, and to compare the findings with earlier PbB levels obtained on cohorts of children living in the same communities between 1996 and 2007.

Materials and methods

Participants and location

In 2012, 61 inhabitants of the Pb-contaminated study area, of which 22 were children, were examined for PbB and ZPP levels. The present study focuses exclusively on the 22 children living in the study area. The PbB–ZPP levels for the 39 adults are presented elsewhere. The participants consisted of 12 females and 10 males ranging in age from 1.5 years to 16 years who were available for testing. The mean age for the current group of children was 9.3 years (SD: 4.1; median: 9.5) living in villages around La Victoria in the Cotopaxi region of Ecuador at an altitude of approximately 2850 m. In addition to La Victoria Centro, the children who were tested resided in the communities of La Victoria, including El Tejar, Mulinlivi and El Paraiso. The source of Pb exposure in the study area is discarded Pb-acid automobile storage batteries from which adults and children extract Pb for use in the glazing of ceramics, particularly roof tiles produced in a local Pb-glazing cottage industry. Hand-to-mouth ingestion of Pb-contaminated food, dust and soil, and the inhalation of small air-borne particulates from the heavily Pb-laden smoke produced by the glazing kilns are the primary routes of Pb exposure in the children [18,19]. All participants were examined at the local Subcentro de Salud in La Victoria, Ecuador.

Informed consent was obtained from the parents/guardians of the children prior to testing. This study was approved by the Human Studies Committee (Comité de Bioética) of the Universidad San Francisco de Quito. The study was conducted under the auspices of Universidad San Francisco de Quito Colegio Ciencias de la Salud, Escuela de Medicina in Quito, Ecuador. The results of this investigation were presented to the parents/guardians of the children who participated in the study. The participants and their families were counseled regarding their Pb exposure, and were referred to local health officials for medical intervention where appropriate.

Blood tests

Samples of 2–4 mL of whole blood were drawn by venipuncture from the participants following thorough cleaning of the skin using swabs containing isopropanol. The samples were collected from the antecubital vein and kept in 4-mLVacutainer collection tubes with Li-heparin. All whole blood samples were stored in a refrigerated container. The blood samples were later analyzed for Pb concentration by graphite furnace atomic absorption spectroscopy with Zeeman background correction (Perkins Elmer 5000 Zeeman HGA-500 spectrophotometer, Norwalk, Connecticut). ZPP/heme was measured directly using a hematofluorometer (ProtoFluor-z, Helena Laboratories, Inc., Beaumont, TX), which presents results in μmol/mol heme. Control samples (Kaulson Laboratories, West Caldwell, NJ) were run at low, medium and high levels. ZPP WB values are expressed in μg/dL with an assumed hematocrit of 35%. The normal reference ranges used were 30–70 μmol ZPP/mol heme, and 15–36 μg ZPP/dL whole blood. All blood tests were performed at the Boston Children's Hospital Department of Laboratory Medicine.

Statistical analysis

The mean, standard deviation, range, and median were calculated for PbB concentration, ZPP/heme ratio and ZPP WB level obtained on each participant in the study. Because some of the PbB and ZPP data were skewed, nonparametric statistics were used in the data analysis. Differences between means were analyzed using the Mann–Whitney *U* test. The association between PbB level and ZPP/heme ratio was analyzed by Spearman *rho* correlation analysis. All Z and p values reported for the Mann Whitney *U* test and the Spearman correlation coefficient are tied values. An alpha level of ≤ 0.05 was accepted as an indication of statistical significance.

Results

To determine the Pb exposure levels over time, the results for the children in the current study were compared to Pb exposure data pooled over 5-year intervals from cohorts of children tested previously in the same Pb-contaminated study area. The aggregate data are illustrated in the box plots of Fig. 1, which show the distribution of PbB levels of the children in the study area for three different cohorts tested in 5-year intervals: 1996–2000 ($n = 274$), 2003–2007 ($n = 329$), and 2012 ($n = 22$). The mean PbB level obtained for the children during the 2012 test period (current study) was 14.5 μg/dL (SD: 7.9; median: 14.0; range: 4.0–37.0), and significantly lower than the mean PbB level of 41.1 μg/dL (SD: 24.6; median: 37.6; range: 6.1–128.2) found in the same study area in the 1996–2000 test period (Mann–Whitney *U*: $Z = -5.570$, $p = <0.0001$). The mean PbB level of 14.5 μg/dL for the current participants was also lower than the mean PbB level of the children tested in the 2003–2007 examination period (mean: 22.2 μg/dL; SD: 19.8; median: 15.0; range: 2.1–107.0), but the difference did not reach statistical significance (Mann–Whitney *U*: $Z = -1.056$, $p = 0.291$). Overall, the data presented in Fig. 1 illustrate group reductions in PbB levels over time, indicating declining Pb exposure in the study area.

The Pb exposure levels of the children in the study area were further probed by comparing the PbB levels at yearly intervals to examine more closely the decline in Pb exposure. Fig. 2 shows the PbB levels by year of cohorts of children tested in the study area from 1996 to the current group tested in 2012. Similar to the box plots of Fig. 1, the line graph shown in Fig. 2 illustrates an overall decline in PbB levels over the years, beginning in 1996. The mean PbB level of 14.5 μg/dL obtained in the current study was significantly lower than the mean PbB level of 44.6 μg/dL found in the 1996 cohort (Mann–Whitney U: $Z = -5.061$, $p = <0.0001$). An exception to this declining trend was the mean PbB level of 45.8 μg/dL for a group of children