



Blood Lead Levels and Their Association with Iron Deficiency and Anemia in Children

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Received: 30 October 2023 / Accepted: 26 March 2024

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Abstract

Lead is a ubiquitous and neurotoxic heavy metal particularly harmful to children, who are more susceptible than adults to its toxic effects. The prevalence of lead poisoning and iron deficiency (ID) is high in socioeconomically vulnerable child populations, negatively affecting neurocognitive development in children. Although numerous studies have shown the association between blood lead levels (BLL) and ID, the issue remains controversial. Here, we aimed to identify the association between BLL and iron nutritional status in children. We conducted an analytical cross-sectional study of healthy children aged 1–6 y attending periodic health checks in primary healthcare units from La Plata and its surroundings, Buenos Aires, Argentina, between 2012 and 2017. We performed anthropometric evaluations and determined BLL, hemoglobin (Hb) and serum ferritin levels. Blood lead levels ≥ 5 $\mu\text{g}/\text{dL}$ were defined as elevated BLL; ferritin levels < 12 ng/ml were considered ID; Hb levels < 11 g/dL (< 11.5 g/dL for children ≥ 5 y) were defined as anemia. Data were analysed using Mann Whitney test, Student's t-test, chi-square test and logistic regression. The R package (v. 4.2.2.) was used for the statistical analysis of data. The sample included 392 children (mean age, 2.4 ± 1.4 y; 44.6% females). The prevalence of elevated BLL, ID and anemia was 8.7%, 26.3% and 31.8%, respectively. We found a significant association between elevated BLL and ID (odds ratio [OR], 95% confidence interval [CI]: 3.16 (1.50, 6.63)). The prevalence of elevated BLL was 16.2% and 5.8% in children with and without ID, respectively ($p=0.003$). We also found association between elevated BLL and anemia (OR 95% CI: 3.03 (1.49, 6.29)). In conclusion, blood lead levels ≥ 5 $\mu\text{g}/\text{dL}$ were significantly associated with ID and anemia in children aged 1–6 years.

Keywords Lead · Iron Deficiency · Anemia · Children

Introduction

Lead is a toxic metal widely distributed in the Earth's crust. Currently, children are exposed to lead through particles dispersed in the air, food, water, dust and soil [1]. Blood lead

levels (BLL) have markedly decreased in the last 20 years, not only in population groups from developed countries but also from developing countries, including Argentina [2–4]. However, such BLL are still worrying, considering there is no safe blood lead threshold value below which lead has no adverse/toxic health effects [5].

Iron deficiency (ID) is the most common nutritional disorder worldwide and the main cause of anemia in infants, having a high prevalence in developing countries [6]. Lead poisoning and ID are highly prevalent in socioeconomically vulnerable child populations, negatively affecting the neurocognitive development of children [7–11]. Additionally, very low lead concentrations have been associated with negative developmental outcomes in children [12].

The epidemiological association between iron deficiency and lead poisoning has been examined by Wong in a review [13]. Divalent metal transporter 1 (DMT1) is one of the mechanisms believed to be involved in lead absorption in the small intestine, as well as the primary iron transporter.

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In the state of ID, the expression of DMT1 increases. This positive regulation during deficiency could explain the increased lead absorption by intestinal cells and the higher lead levels in individuals with ID [14].

Differences in BLL between children with and without ID living in contaminated environments are well-documented [15, 16]. However, while some studies observe such an association [17–20], others do not when lead concentrations are low [21, 22].

In order to gain insight into the issue of lead exposure and iron status in children from Argentina exposed to lowly contaminated environments, we analysed the relationship between BLL and iron nutritional status in children aged 1 to 6 years.

Materials and Methods

We conducted an analytical cross-sectional study of healthy children aged 1–6 y performing periodic health checks at the Health Observatory of the Instituto de Desarrollo e Investigaciones Pediátricas (IDIP), La Plata Children Hospital, and in primary healthcare units from La Plata and its surroundings, Buenos Aires, Argentina, from March 2012 to March 2017. Subjects who met the following criteria were excluded: children with chronic disease diagnosis, acute and/or infectious disease at the moment of the study, genetic syndrome, neurological background, moderate or acute malnutrition, or whose parents or tutors refused to participate in this study. We used convenience sampling. Sample size was calculated to detect a difference of 1.2 $\mu\text{g}/\text{dL}$ between children with and without ID, assuming a 95% confidence interval (CI), an 80% power of the test, and a standard deviation (SD) of 2.4 $\mu\text{g}/\text{dL}$ according to a previous study [3]. The estimated minimum sample size was 126 children ($n=63$ each group). Nevertheless, considering that around 33% of children have ID, at least 192 children were included to ensure the number of children with ID.

Blood was collected by venipuncture to determine BLL, serum ferritin, C-reactive protein (CRP) and hemoglobin (Hb) levels. To measure BLL, heparanized blood was diluted (1:5) in 15% nitric acid (Merck, Argentina) and then centrifuged. The supernatant was analysed by atomic absorption spectroscopy (283.3 nm wave length) in a Varian Agilent 240Z AA 120 Programmable Sample Dispenser with Zeeman Effect background correction (Mulgrave, Victoria, Australia). The limits of detection (LOD) and quantification (LOQ) were 0.8 and 2.7 $\mu\text{g}/\text{dL}$, respectively. Blood lead levels ≥ 5.0 $\mu\text{g}/\text{dL}$ were defined as elevated BLL [23]. Ferritin was determined by chemiluminescence in an Access Beckman Coulter analyser, considering ferritin levels < 12 ng/ml as ID. In case of elevated CRP (≥ 5 mg/L), the

threshold value for ID was ferritin < 30 ng/mL [24]. Hemoglobin determinations were performed using a hematological counter (ABX Pentra 60, Montpellier, France). Values of Hb < 11 and < 11.5 g/dL for children < 5 and ≥ 5 years, respectively, were defined as anemia [25]. Iron deficiency anemia (IDA) was considered when Hb < 11 and < 11.5 g/dL for children < 5 and ≥ 5 years, respectively, and ferritin levels < 12 ng/ml.

Weight and height were assessed using standard methods. Weight was measured with a Tanita UM-061 digital electronic scale (0.1 g precision) (Tanita Corporation of America Inc., Illinois, USA) and height was measured with a portable SECA stadiometer (0.5 cm precision; SECA, United Kingdom). Weight-for-age (W/A), height-for-age (H/A) and body mass index (BMI) were determined and evaluated using the World Health Organization (WHO) Child Growth Standards [26].

Sources of lead exposure in children were collected using a questionnaire administered by trained staff. Sociodemographic information such as the child's age and sex, the geographic location of the family house, family composition, parental employment status, parental level of education, housing conditions (building material, floor type, presence and/or location of a bathroom, number of rooms), access to water and health services were recorded. Based on these data, we developed the unmet basic needs (UBNs) indicator according to the methodology described by the National Statistics and Censuses Institute of Argentina (Instituto Nacional de Estadísticas y Censos, INDEC) [27]. Determinants of external lead exposure included presence of gas station (fuel distribution), car repair and/or paint shop, smithy, bus stops, high traffic roads, dirt roads, garbage dumps or polluted streams less than 100 m away.

We used the Kolmogorov-Smirnov test to analyse the normality of variables. Ferritin and BLL concentrations were expressed as geometric means (GM) and 95% CI considering their log normal distribution. In case of $\text{BLL} < \text{LOD}$, mean values were adjusted with the extrapolation method based on linear regression [28]. The rest of the variables were expressed as means \pm standard deviations (SD) if they had a normal distribution, and as medians (P25; P75) in case of non-parametric data. Children were divided into two categories according to their age (< 2 and ≥ 2 y) taking into account the higher prevalence of ID in younger children and the increased lead exposure due to physiological, biological and behavioral patterns such as exploring the environment during the oral stage (hand-to-mouth), pica habits and frequent contact with the ground. Student's *t*-test or Mann-Whitney test were used to compare quantitative variables by age and sex, and chi-square test was used to study the association between $\text{BLL} \geq 5$ $\mu\text{g}/\text{dL}$ and ID or anemia. Odds ratios (OR, 95% CI) were determined using logistic

regression. The multivariate logistic regression model was used to adjust for confounding variables (sex and age of children < 2 y). The R package (v. 4.2.2) was used for the statistical analysis of data. In all cases, $p < 0.05$ was considered statistically significant.

Results

A total of 392 children (mean age, 2.4 ± 1.4 y; 44.6% female) were enrolled. The sociodemographic and anthropometric characteristics of the study sample are detailed in Table 1. From the total sample, 79.6% reported having at least one source of external lead exposure: living < 100 m from car repair/paint shops (38.1%), bus stops (35.2%), high traffic roads (31.6%), polluted streams (31%) and garbage dumps (26.2%).

Biochemical parameters are detailed in Table 2. From the total number of children, 8.7% had elevated BLL, 26.3% had ID and 31.8% were anemic.

Table 3 depicts BLL, ferritin and Hb mean values and the prevalence of elevated BLL, ID and anemia according to the age of children (< and ≥ 2 y). Whereas ferritin and Hb levels were lower in children < 2 y, the prevalence of elevated BLL, ID and anemia was higher as compared with children ≥ 2 y. Mean BLL levels did not differ between groups. The analysis of the variables by sex did not result in significant differences (data not shown).

Results of the comparison of ferritin and Hb according to BLL < or ≥ 5 $\mu\text{g/dL}$ are shown in Fig. 1. Mean ferritin levels were lower in children with elevated BLL (95% CI): 12.41 ng/mL (9.37, 16.43) compared with BLL < 5 $\mu\text{g/dL}$ (18.24 ng/mL (17.07, 19.48), $p = 0.010$). Likewise, median Hb values (reported as median (P25, P75)) were lower in children with elevated BLL (10.9 g/dL (10.6, 11.6) vs. 11.5 g/dL (10.9, 12.0); $p = 0.001$).

Results of the adjusted and unadjusted logistic regression models used to determine the association between elevated BLL, ID and anemia are presented in Table 4. We found a significant association between elevated BLL and ID

Table 1 Characteristics of the study sample ($n = 392$)

Children	n (%)	Mean \pm SD
Age (y)		2.4 \pm 1.4
Age range		
1–1.9	225 (57.4)	
2–2.9	53 (13.5)	
3–3.9	49 (12.5)	
4–4.9	33 (8.4)	
5–5.9	32 (8.2)	
Sex		
Male	217 (55.4)	
Female	175 (44.6)	
Anthropometric measurements		
W/A Z-score ($n = 379$)		0.18 \pm 1.11
Low weight (W/A Z-score < -2)	6 (2)	
H/A Z-score ($n = 375$)		-0.42 \pm 1.35
Chronic growth retardation (H/A Z-score < -2)	30 (8)	
MI Z-score		0.63 \pm 1.16
Overweight/Obesity (BMI Z-score > 2)	42 (11)	
Families		
Maternal age (years)		26.6 \pm 6.2
Maternal education (years) *		10 (8;12)
Paternal education (years) *		8 (7;12)
Maternal employment status	230 (58.6)	
Housewife	150 (38.3)	
Working outside the home	12 (3.1)	
Unemployed	369 (96.7)	
Paternal employment status	13 (3.3)	
Active		
Unemployment		
Homes with UBNs	181 (46.2)	
Overcrowding	99 (25.3)	
Inadequate housing conditions	159 (40.5)	

W/A, weight-for-age; H/A, height-for-age; BMI, body mass index; * Median (Q1; Q3) UBN: unmet basic needs

Table 2 Biochemical parameters

BLL ($\mu\text{g/dL}$)*		1.96 (1.83, 2.11)
< LOD	58 (14.8)	
\geq LOD and < LOQ	194 (49.5)	
\geq LOQ and < 5 $\mu\text{g/dL}$	106 (27)	
\geq 5 $\mu\text{g/dL}$	34 (8.7)	
Serum ferritin (ng/mL)*		17.65 (16.541, 18.84)
ID **	99 (26.3)	
Hemoglobin (g/L) ($n = 389$)		11.39 \pm 0.90
Anemic	123 (31.8)	
IDA	49 (12.5)	

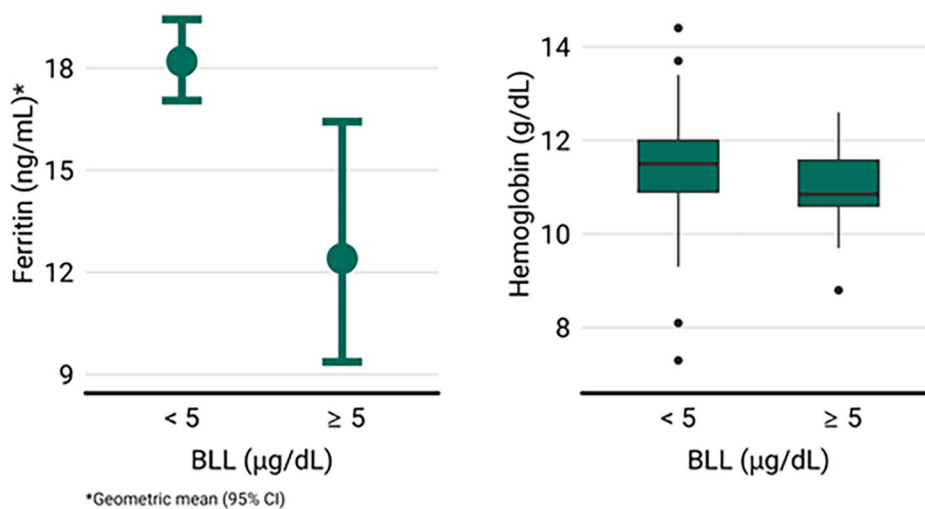
BLL, blood lead level; LOD, limit of detection (0.8 $\mu\text{g/dL}$); LOQ, limit of quantification (2.7 $\mu\text{g/dL}$); ID, iron deficiency. IDA, iron deficiency anemia. *Geometric mean (95% CI). ** of these, 12 children had CRP level > 5 $\mu\text{g/mL}$ and 12 $\text{ng/mL} \leq$ ferritin < 30 ng/mL

Table 3 Blood lead, ferritin and hemoglobin levels by age of the children

	< 2 y $n = 225$	≥ 2 y $n = 167$	p-value
BLL* ($\mu\text{g/dL}$)	2.01 (1.81, 2.23)	1.91 (1.74, 2.10)	0.503
BLL ≥ 5 $\mu\text{g/dL}$ (n, %)	27 (12)	7 (4.2)	0.001
Ferritin* (ng/mL)	14.72 (13.47, 16.09)	22.63 (20.86, 24.54)	< 0.001
ID (n, %)	79 (37.6)	20 (12)	< 0.001
Hb (g/dL)†	11.14 \pm 0.90	11.72 \pm 0.80	< 0.001
Anemia (n, %)	89 (40.5)	34 (20.4)	< 0.001

*Geometric mean (95% CI). †Mean \pm SD.

BLL: blood lead level, ID: iron deficiency, Hb: Hemoglobin

Fig. 1 Ferritin and Hb levels according to BLL < or ≥ 5 $\mu\text{g/dL}$ **Table 4** Adjusted and unadjusted logistic regression model results of the association between elevated BLL, ID and anemia

		Elevated BLL n (%)	OR (95% CI) unadjusted	OR (95% CI) adjusted*
ID	Yes	16 (16.2)	3.16 (1.50, 6.63)	2.47 (1.14, 5.36)
	No	16 (5.8)	reference	reference
Anemia	Yes	19 (15.4)	3.03 (1.49, 6.29)	2.52 (1.21, 5.30)
	No	15 (5.7)	reference	reference

*Adjusted values by sex and age < 2 y

($p=0.003$). The prevalence of elevated BLL was 16.2% in children with ID and 5.8% in children without ID. Similarly, bivariate analysis showed a significant association between elevated BLL and anemia ($p=0.003$).

Finally, the multivariate logistic regression model for elevated BLL adjusted by sex and age of the children confirmed the obtained associations (Table 4).

Discussion

In the present study, a direct association between elevated BLL ($\geq 5 \mu\text{g/dL}$) and ID was found in children aged 1–6 y exposed to low environmental lead poisoning. These associations remained even when data were adjusted by sex and age < 2 y.

Numerous reports have analysed the currently studied associations in varying child age-ranges, levels of environmental lead exposure, thresholds for elevated BLL and parameters to evaluate iron nutritional status. For instance, in agreement with the present findings, a study in Chinese children aged 0–5 y with low levels of lead exposure showed that blood lead concentrations were inversely associated with iron status [29]. Another report of children aged 1 through 12 y found that the prevalence of lead poisoning was higher in children with than without ID (3.4% vs. 1.1%) [30]. A longitudinal study of 1,275 children followed-up from 9 to 42 months of age determined that children with ID were at higher risk of developing lead poisoning (OR: 4.12, 95% CI: 1.96; 8.65) than children with normal/adequate iron status [17]. On the other hand, and as opposed to the present results, a recent study of children between 0.54 and 14.87 y did not find a significant association between BLL and trace elements such as iron, probably due to the relatively low lead levels and the adequate iron status of the children analysed [31].

In addition to the higher proportion of children with elevated BLL and ID, our data revealed that ferritin and Hb levels were markedly lower in children with elevated BLL, which coincided with studies performed in Brazil [19], Pakistan [20], and India [32]. In line with our findings, a recent systematic review of 58 studies in pediatric populations (0–15 y) from 1974 to 2021 concluded that elevated BLL could be related to low iron deposits and higher risk of anemia [33]. Similarly, a meta-analysis demonstrated that the risk of developing IDA was higher among children with BLL $> 10 \mu\text{g/dL}$ than in children with lower BLL (OR: 2.75 (95% CI, 1.10–6.85 $\mu\text{g/L}$; $p=0.0303$) [34].

This study is one among few carried out in Argentina evaluating the association between BLL and iron status in a child population without evident clinical signs or symptoms of lead poisoning, performing periodic health checks.

Such association is particularly relevant in younger children undergoing nervous system development, considering that most research has found association of ID and lead poisoning with child cognitive development and behaviour [35, 36].

Nevertheless, the current study has some limitations. First, results correspond to a sample of children performing health checks in the public health system of a region of the province of Buenos Aires, Argentina, which cannot be extrapolated to the general population. Secondly, since this is a cross-sectional study, causality cannot be inferred. Third, although the indicators used to define ID are readily accessible, they may prove insufficient. Finally, we did not evaluate dietary lead intake as an important source of lead in children, as it has been reported in other studies [37, 38].

Bearing in mind that there is no safe blood lead threshold value below which lead has no adverse effects on neurodevelopment, prevention strategies should not be discontinued, particularly in vulnerable populations. Furthermore, considering the high ID prevalence currently found, the individual risk of lead poisoning once the iron status of children has been determined should be evaluated, just like the presence of anemia during health checks to prevent the combined effects of ID and lead poisoning on child cognitive development and behavior.

Conclusion

BLL $\geq 5 \mu\text{g/dL}$ was significantly associated with ID and anemia in children aged 1–6 years.

Author Contributions L.D. and A.V. contributed to the first draft, the study design, investigation and final writing. N.M. and M.S. participated in the investigation and the final writing of the manuscript. M.V.F. participated in study design, statistical analysis and final writing. H.F.G. was in charge of supervising and administering the project and participated in the final writing of the manuscript. All authors read and approved the final manuscript.

Funding This study received funding from IDIP.

Declarations

Ethics Approval The study protocol was approved by the Institutional Research Review Board of IDIP. The study results were communicated to parents/tutors. Children whose BLL were $\geq 5 \mu\text{g/dL}$ were referred to the Toxicology Service for follow-up.

Consent to Participate Written informed consent was signed by all the participating parents and/or tutors and attested by a witness.

Conflict of Interest The authors declare they have nothing to disclose.

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