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Lead, hemoglobin, zinc protoporphyrin and ferritin concentrations in children

Concentrações de chumbo, hemoglobina, zinco protoporfirina e ferritina em crianças

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Keywords

Lead Poisoning. Anemia. Hemoglobin. Zinc. Protoporphyrin. Ferritin. Cross-sectional studies.

Abstract

Objective

To assess the relationship of blood lead and hemoglobin, zinc protoporphyrin, and ferritin concentrations in children.

Methods

A cross-sectional study was carried out in 136 anemic and non-anemic children from two rural villages near a lead smelter in Adrianópolis, Southern Brazil, from July to September 2001. Hemoglobin electrophoresis was performed to exclude children with hemoglobin variants and thalassemia syndromes associated with anemia. Lead was determined by atomic absorption spectrophotometry; hemoglobin by automated cell counting; zinc protoporphyrin by hematofluorometry; ferritin by chemiluminescence. Student's t-test, Mann-Whitney test, and the χ^2 test were used to assess the significance of the differences between the variables investigated in anemic and non-anemic children. Stepwise multivariate linear regression analysis was performed using two models for anemic and non-anemic children respectively.

Results

Lead was negatively associated to hemoglobin ($p < 0.017$) in the first model, and in the second model lead was positively associated to zinc protoporphyrin ($p < 0.004$) after controlling for ferritin, age, sex, and per capita income. There was an inverse association between hemoglobin and blood lead in anemic children. It was not possible to confirm if anemic children had iron deficiency anemia or subclinical infection, considering that the majority (90.4%) had normal ferritin.

Conclusions

The study detected a relationship between anemia and elevated blood lead concentrations. Further epidemiological studies are necessary to investigate the impact of iron nutritional interventions as an attempt to decrease blood lead in children.

Descritores

Intoxicação por chumbo. Anemia. Hemoglobina. Zinco. Protoporfirina. Ferritina. Estudos transversais

Resumo

Objetivo

Avaliar a relação entre as concentrações sanguíneas de chumbo, hemoglobina, zinco protoporfirina e ferritina em crianças.

Métodos

Estudo transversal realizado com 136 crianças anêmicas e não anêmicas residentes em duas vilas da região rural de Adrianópolis, Estado do Paraná, próximas a uma

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usina de beneficiamento de chumbo, de julho a setembro de 2001. A eletroforese de hemoglobina foi realizada para excluir as crianças com anemia associada a hemoglobinas variantes e síndromes talassêmicas. O chumbo foi determinado por meio de espectrofotometria de absorção atômica; a hemoglobina por contador celular automático; a zinco protoporfirina por hematofluorimetria, e a ferritina por quimioluminescência. Os testes t de student, Mann-Whitney e χ^2 foram utilizados para verificar a significância das diferenças entre as variáveis. Foi realizada uma análise de regressão linear multivariada stepwise, utilizando dois modelos, para crianças anêmicas e não anêmicas respectivamente.

Resultados

No primeiro modelo o chumbo estava negativamente associado com a hemoglobina ($p < 0,017$), e no segundo modelo, estava positivamente associado com a zinco protoporfirina ($p < 0,004$), controlando-se por ferritina, idade, sexo e renda per capita. Detectou-se uma associação inversa entre as concentrações sanguíneas de hemoglobina e chumbo, em crianças anêmicas. Não foi possível confirmar se as crianças anêmicas tiveram anemia ferropriva ou infecção subclínica, considerando-se que a maioria delas (90,4%) apresentou concentrações normais de ferritina.

Conclusões

Detectou-se uma relação entre anemia e concentrações sanguíneas elevadas de chumbo. É necessário o desenvolvimento de futuros estudos epidemiológicos investigando o impacto de intervenções nutricionais com ferro na tentativa de diminuição dos níveis sanguíneos de chumbo em crianças.

INTRODUCTION

Lead (Pb) poisoning in early life may have disastrous effects on child growth and mental development such as behavior and cognitive problems and poor school performance.^{13,15} Recent research has indicated that even blood Pb concentrations below 10 $\mu\text{g/dL}$ (0.48 $\mu\text{mol/L}$) may be associated with negative outcomes in children.¹⁵ Iron deficiency is the most common cause of anemia worldwide, being associated with lower cognitive function, impaired motor abilities, behavioral problems, and decreased social interaction.⁴

Determination of lead in blood or serum is considered a good indicator of acute lead poisoning, widely used in epidemiological studies.³ Hemoglobin (Hb) is the World Health Organization (WHO) accepted indicator of anemia,¹⁶ and serum ferritin is the most used indicator of iron deficiency.¹¹ Zinc protoporphyrin (ZPP) is a biological indicator of both Pb poisoning and iron deficiency.¹¹

The prevalences of iron deficiency anemia and Pb poisoning remains unusually high especially in developing countries with the highest prevalences among the poor and young children.²³

According to previous studies carried out in Brazil, there is a region located in the Ribeira river valley where lead contamination has been assessed and seems to constitute a public health problem.⁷ The only study¹⁷ evaluating human exposure in the region and nearby

showed that children who were living in two rural villages of the municipality of Adrianópolis, State of Paraná, Southern Brazil, in the vicinity of a lead smelter, had the highest concentrations of lead in blood. Treatment for Pb poisoning with chelators is extremely expensive and not much effective if the population is continuously exposed to the heavy metal.

Some studies have shown that iron deficiency predisposes to Pb poisoning by increasing gastrointestinal Pb absorption.^{1,8} Although the association between iron deficiency and Pb poisoning has been investigated in a number of studies, results are still inconsistent. According to a large review on the interactions between iron deficiency anemia and Pb poisoning, some studies show a positive association^{2,22} whereas others show no association.^{9,19}

The aim of the present study was to assess the relationship between blood lead and hemoglobin, zinc protoporphyrin, and ferritin concentrations in children.

METHODS

A cross-sectional study was carried out in two rural villages near a Pb smelter, 25 km from the center of the municipality of Adrianópolis, State of Paraná, Southern Brazil, from July to December 2001. The villages were chosen for their vicinity to the Pb smelter which had been in activity for more than 50 years until its closure by the end of 1995. Besides, a previous study¹⁷ had confirmed Pb contamination in the area by the discharge of fumes containing Pb oxides

over the region. The Pb smelter was situated half way (1.5 to 2.0 km) between the local two schools. All 140 children aged 2-11 years who were attending the schools participated in the study. Four children with chronic diseases were excluded from the study.

Children's parents or guardians responded to a questionnaire on demographic and socioeconomic information, morbidity and area of residence. Fasting venous blood samples were collected from the children into EDTA and trace metal-free heparin vacuum tubes. Complete blood counts, including Hb determination, were performed on the samples within 4-6 hours of collection using a CellDyn 3000 CD Abbott Laboratory automated cell counter. The samples were then stored at 2-4°C, and tested within seven days for Hb subtype quantification according to the following procedures: 1) microscopic red blood cell morphological quantification; 2) cellulose acetate electrophoresis at alkaline pH using saponin hemolysate for diagnosing Hb variants and thalassemia syndromes; 3) red blood cell osmotic fragility test;²⁰ 4) citrate agar electrophoresis at pH 6.3 to confirm the pattern obtained in the Hb electrophoresis at alkaline pH. Eight children presented sickle cell trait but they were not excluded from the study, considering that this hemoglobin variant is not associated to anemia.¹⁸

The samples for Pb determination were properly stored in a refrigerator at 6°C and analyzed in duplicate within 15 to 30 days after collection. Blood Pb was assayed using graphite furnace atomic absorption spectrophotometry with Zeeman background correction (Model SIMAA 6000; Perkin-Elmer). The samples were diluted 1:10 with 1% Triton X-100 in 0.1% nitric acid, and a mixture of ammonium dihydrogen phosphate and magnesium nitrate was used as chemical modifier. Quantification limit obtained for Pb was 0.01 µmol/L (0.2 µg/dL) in 1:10 blood dilution, corresponding to 0.10 µmol/L in total blood. For determining the quantification limit a

blood sample was obtained from a non-exposed person, Pb concentrations were determined in 10 preparations, and the calculation was made according to the International Union of Pure and Applied Chemistry (IUPAC) recommendations.⁶ The blood samples which showed Pb levels below the quantification limit were given a value corresponding to 0.048 µmol/L (half of the limit value of the method quantification). For determining the method accuracy, Pb reference material in bovine blood was used (NIST 955b, level 2), obtaining 96% recovery. ZPP was determined using an AVIV hematofluorometer (AVIV Associates, Lakewood, NJ) and serum ferritin by chemiluminescence (Diagnostic Products Corporation - DPC).

Children were considered Pb contaminated if their blood Pb concentrations were equal or greater than 0.48 µmol/L (10 µg/dL).³ The cut-off points adopted for the diagnosis of anemia were Hb below 110 g/L for children aged less than 6 years and below 120 g/L for those children aged 6 years or more.¹⁸ Ferritin values above 10 µg/L were considered normal.¹⁴

Student's t-test, Mann-Whitney test, and the χ^2 test were used to assess the significance of the differences between the variables investigated in anemic and non-anemic children. Correlations between Pb and Hb, ferritin and ZPP concentrations were measured by linear regression analysis using Spearman's correlation coefficient. In the stepwise multivariate linear regression analysis, using two models for anemic and non-anemic children, Pb concentrations were considered as the dependent variable and age, sex, per capita income (Brazilian minimum-wage [BMW] = approximately US\$60), and Hb, ZPP and ferritin as independent variables. The Kolmogorov-Smirnov normality test was applied to evaluate normality of the residues. All tests were performed in the Stata statistical software.

The university's Ethics Review Board approved the study protocol. Children's parents/guardians signed informed consents before blood collection.

Table 1 - Age, sex, per capita income, and blood concentrations of lead (Pb), zinc protoporphyrin (ZPP), ferritin and hemoglobin (Hb) in children. Southern Brazil, 2001.

Variables	Anemic* (N=34)		Non anemic (N=102)		p-value for group comparison**
	Mean (\pm SD) or % (N)	Minimum/maximum	Mean (\pm SD) or % (n)	Minimum/maximum	
Age (years)	6.85 (2.5)	2.0 / 10.0	6.69 (2.52)	2.0 / 11.0	0.74***
Sex (male)	32.4 (11)		45.1 (46)		0.20****
Per capita income (BMW)	0.44 (0.32)	0.05 / 1.67	0.49 (0.70)	0.05 / 3.05	0.46
Pb (µmol/L)	0.50 (0.33)	0.04 / 1.47	0.47 (0.28)	0.03 / 1.58	0.98
ZPP (µg/dL)	68.82 (53.93)	28.0 / 277.0	55.78 (25.04)	20.0 / 214.0	0.74
Ferritin (µg/L)	26.09 (12.29)	1.5 / 48.5	32.66 (16.86)	2.00 / 81.3	0.06
Hb (g/L)	108.7 (11.3)	76 / 119	129.8 (9.6)	110 / 159	<0.001**

SD: Standard deviation; BMW: Brazilian minimum wage (approx. US\$ 60)

*Anemia: Hb<110 g/L for children <6 years of age and <120g/L for children \geq 6 years

**p-value for the Mann-Whitney test

***p-value for the t-test

****p-value for the χ^2 test (to evaluate the association between anemia and sex)

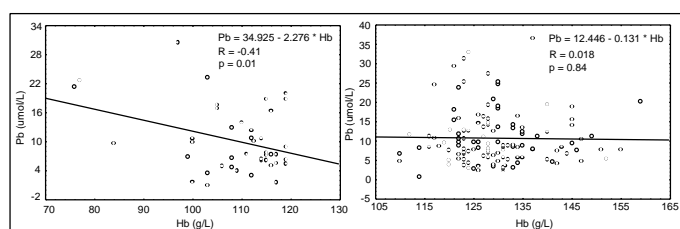


Figure - Correlations between blood lead (Pb) and hemoglobin (Hb) concentrations in children. Southern Brazil, 2001.

RESULTS

Table 1 shows there were no statistically significant differences between age (6.85 ± 2.5 vs 6.69 ± 2.52 years), sex (32.4% vs 45.1% males), *per capita* income (0.44 ± 0.32 vs 0.49 ± 0.70 Brazilian minimum-wage), Pb (0.50 ± 0.33 vs 0.47 ± 0.28 $\mu\text{mol/L}$), ZPP (68.82 ± 53.93 vs 55.78 ± 25.04 $\mu\text{g/dL}$), and ferritin (26.09 ± 12.29 vs 32.66 ± 16.86 $\mu\text{g/L}$) concentrations in anemic ($n=34$) and non-anemic ($n=102$) children, respectively. There was a statistically significant correlation between Pb and Hb ($r=-0.41$) for anemic children, but no significant correlation for non-anemic children (Figure). Both anemic and non-anemic children showed statistically significant correlations ($p \leq 0.04$) between Pb and ZPP ($r=0.35$; $r=0.28$ respectively with Spearman's correlation coefficient). Stepwise multivariate linear regression analysis, using separate models for anemic and non-anemic children, showed that, in the first model, Pb was associated to Hb ($p=0.017$), and in the second model Pb was associated to ZPP ($p=0.004$) (Table 2).

DISCUSSION

The relationship between iron deficiency and elevated blood Pb concentrations has been reported to be strongest in children because they have more hand-to-mouth activity and absorb more Pb than adults.³ A review¹² on the association between iron deficiency

and Pb toxicity found it in seven of 15 epidemiological studies investigated, including a longitudinal study.²² A study by Watson²¹ found that subjects with low serum ferritin concentrations had increased absorption of Pb and iron in contrast to iron-replete subjects. However, Serwint et al¹⁹ did not find a correlation between blood Pb and ferritin levels for children with low and moderate Pb exposure, and Choi⁵ only found an influence of ferritin on Pb, once serum iron declined. In the present study there were negative correlations between Pb and Hb, and Pb and ferritin concentrations for anemic children, but only the former was significant. These children had lower mean values of ferritin than non-anemic ones, but not significant ($p=0.06$). Even anemic children had normal mean values of ferritin.

As ZPP concentrations are affected by both iron deficiency anemia and Pb exposure, the relationship between blood Pb and ZPP in anemic and non-anemic children was assessed separately. The correlation between ZPP and Pb was not as high as expected. A study carried out by Hershko et al¹⁰ found positive correlations between ZPP and Pb concentrations, higher for a group of non-anemic children who had blood Pb concentrations equal or greater than $30 \mu\text{g/dL}$ ($\geq 1.44 \mu\text{mol/L}$; $r=0.53$) than for a group of non-anemic children with Pb concentrations below $30 \mu\text{g/dL}$ ($r=0.37$). The correlations between ZPP and Pb found were lower than in the study by Hershko et al,¹⁰ probably as a result of the lower blood Pb concentrations seen in these children.

The present study detected an inverse association between Hb and blood Pb concentrations in anemic children. However, it was not possible to confirm if the anemic children studied had iron deficiency anemia, considering that the majority (90.4%) presented normal concentrations of ferritin. This acute

Table 2 - Multivariate linear regression analysis on blood lead and age, sex, per capita income, hemoglobin (Hb), ferritin and zinc protoporphyrin (ZPP) concentrations as independent variables. Southern Brazil, 2001.

1 st model (anemic children)			
Dependent variable = blood lead			
Variables	B	Unstandardized coefficient Standard error	p-value
Intercept	37.201	10.763	0.002
Hb	-2.483	0.991	0.017
$R^2=0.164$			
Residual analysis: Kolmogorov-Smirnov normality test ($p>0.20$)			
2 nd model (non-anemic children)			
Dependent variable = blood lead			
Variables	B	Unstandardized coefficient Standard error	p-value
Intercept	6.075	1.384	0.000
ZPP	6.622E-02	0.023	0.004
$R^2=0.072$			
Residual analysis: Kolmogorov-Smirnov normality test ($p>0.15$)			

phase protein is not a good indicator of iron deficiency in some poor areas where subclinical infection is a problem.¹⁴ The only longitudinal study²² which evaluated the association between iron deficiency anemia assessed by Hb, mean corpuscular volume, and red cell distribution width showed that iron deficiency precedes Pb poisoning.

The logistic regression models utilized here explained 16.4% and 7.2% (r^2), respectively, variation in the concentrations of Pb, showing that there are probably other independent variables associated to Pb in blood, or that the determination of Pb in blood was not a good parameter to evaluate Pb poisoning in this population.

Blood Pb concentrations reflect recent exposure to Pb, and Pb in bone reflects chronic exposure to this heavy metal. The accumulation of Pb in bone can start in fetal life and continue into old age.³ It would be interesting to compare blood Pb measurements of the children studied with biomonitoring for lead body

burden such as 24-hour urine lead following mobilizing test by chelator or in vivo bone x-ray fluorescence analysis, considering that these children have been probably chronically exposed to Pb.

In conclusion, further epidemiological studies are needed to investigate the relationship among Pb, Hb, ZPP and ferritin, and the impact of iron nutritional interventions as an attempt to decrease blood lead in children. The relationship among Pb, Hb, ZPP and ferritin could be better studied in a larger population, considering that the studied sample size was too small to detect interactions among the independent variables. In regions where children are chronically exposed to Pb, other tests, apart from Pb in blood, should be used.

While the results of these studies are not available, it would be important to recommend an iron-rich diet for children at risk for lead poisoning, as has been advocated in the US by the Centers for Disease Control and Prevention.³

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