Original article

Synergistic effects of iron deficiency and lead exposure on blood lead levels in children

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Background: **Lead poisoning is a well recognized environmental health problem in children. Independent association of iron deficiency and lead exposure with elevated blood lead level (BLL) has been reported. Whether iron deficiency in combination with chronic lead exposure increases BLL and susceptibility to its harmful effects in children needs to be elucidated.**

Methods: **In this case-control study, 246 children were randomly recruited. They comprised 123 children of lead smelters/battery recycle plant workers living close to the industries at Wah/Gujranwala, Pakistan (lead exposed group) and 123 children living 30 km away from the industrial area (controls). Blood lead analysis was carried out on the anodic stripping voltammeter lead analyzer 3010B. Blood counting was done on a Sysmex hematological analyzer and serum ferritin was determined by kit method on Immulite-1000.**

Results: **Of the 123 children in each group, 42 (34%) were iron deficient in the exposed group while 35 (28%) in the controls. The children's median age was 4 years (69 males and 54 females in each group). Lead exposed iron deficient children had significantly higher BLL median (quartile) 13.1 μg/dL (10.1-16.8) as compared with 9.6 μg/dL (7.6-10.3) in the iron deficient controls (P<0.05). Elevated BLL level was found in 31% of the lead exposed children and in 11% of the controls. Lead exposed children revealed a stronger negative correlation (r= -0.54; P=0.001) between BLL and serum ferritin than the controls (r=-0.36; P=0.01).**

Conclusion: **Iron deficiency in combination with lead exposure synergistically elevates blood lead levels and susceptibility to its harmful effects in children.**

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doi: 10.1007/s12519-011-0257-9

World J Pediatr 2011;7(2):150-154

Key words: **blood lead levels; children; iron deficiency; lead exposure; serum ferritin**

Introduction

Lead toxicity is a prevalent child health problem in the large industrial cities of South Asia.[1] Kadir and co-workers[2] reported that nearly half of the Pakistani children in the industrial cities are exposed to lead. Iron deficiency (ID) is also a common nutritional problem seen during early childhood in the developing countries.[3] Children are more vulnerable to develop ID because of their increased iron requirements and the fact that they do not take enough iron in their daily diet.[4] Both ID and lead poisoning can adversely lead to impairment of neurocognitive development in children.

Lead toxicity is reported to be the most common pediatric disease in the United States due to occupational and environmental lead exposures.[5] Continued high lead exposure is affecting the health of children and the economic growth of Pakistan, therefore a comprehensive strategy is needed to reduce children's exposure to lead.[2] An effort is being made to study the prevalence of elevated blood lead levels (BLLs) in lead exposed industrial workers and their children living in close proximity to the lead-related industries.[6]

Several epidemiological studies have revealed a significant association between ID and lead poisoning.[7,8] Similarly, a longitudinal study on 1-4 year old children showed a four to five fold increase in BLLs in association with ID.[9] Schell et al[10] reported an inverse association between dietary iron and BLL. An association of ID with elevated BLL has been reported by other researchers in a cross-sectional study.[11] Children with ID had significantly higher BLLs than healthy children within varying strata of environmental lead exposure.[12]

An independent association of ID and lead exposure with elevated BLL in children has been reported. Whether iron deficiency in combination with lead exposure can affect BLL is not yet well established

in children. Our study aims to find out the synergistic effects of iron deficiency and chronic lead exposure on BLLs resulting in greater susceptibility to its harmful effects in children of lead based occupational workers. Identifying a causal relationship of iron deficiency with elevated BLL can devise an effective means of screening and iron supplementation in the high-risk children from lower socioeconomic strata in the developing countries.

Methods

The case-control study was carried out in the children of lead smeltering and battery recycle plant workers living close to the industries at Wah and Gujranwala, Pakistan after approval from the institutional review committee of Army Medical College, National University of Science and Technology (NUST) Islamabad, Pakistan.

Subjects

A total of 125 lead exposed children (exposed group) aged 1-6 years were randomly recruited from the offspring of lead smeltering and battery recycle plant workers living in the industrial areas after informed consent was obtained. Two children were unwilling to participate in the study. An equal number of children (n=123) living more than 30 km away from the industries (control group) were included. Children with any acute or chronic illness or on drugs were excluded. Demographic data of the children were collected. The children underwent a thorough physical examination with weight and height being noted.

Blood and biochemical analysis

5 ml venous blood was collected in lead free EDTA and plain tubes. Blood complete counts were measured on a Sysmex automated hematology analyzer KX-21 (Sysmex, Milton Keynes, UK). Serum ferritin is a highly sensitive and specific indicator of iron deficiency with or without anemia.[8] Serum ferritin was measured using immunoassay on immulite 1000 (Siemens LA, California, USA). The children were defined as being iron deficient if serum ferritin was ≤12 μg/L.

Blood lead level was determined by the anodic stripping voltammetry method[13] on a 3010B ESA Lead Analyzer using kits of the same manufacturer with 3-level control materials. The method has an operating range of 1 to100 μg/dL and a detection limit of 1 μg/dL. CV of the method was 4.6%.

We used BLL 10 μg/dL as cut-off value according to the guidelines of the Centers for Disease Control and Prevention (CDC).[14] Furthermore, the children were living close to lead contaminated area with their parents who also worked in the lead based industries. House dust samples were collected from the floors, walls, and windowsills by the method described by Reynolds and co-workers.[15] Dust analysis was done on graphite furnace atomic absorption analyst 800 (Perkin Elmer, USA).

Statistical analysis

Statistical analysis was performed using SPSS software version-17 (SPSS Inc, Chicago, Illinois, USA). Kolmogorov-Smirnov test was applied on data which revealed non-Gaussian distribution for all the variables (P<0.05). Mean, SD, median, and inter quartile range (IQR) were calculated for descriptive statistics. Children of both lead exposed and control groups were further grouped into iron deficient children (IDC; serum ferritin <12 μg/L) and non iron deficient children (non-IDC; serum ferritin ≥12 μg/L). The Kruskall-Wallis test and Mann-Whitney U test were applied among the four groups of the children. Spearman's rank correlation coefficient analysis between BLLs and serum ferritin was made. A P<0.05 was considered statistically significant.

Results

Altogether 246 children participated in the study, and they comprised equal numbers from both lead exposed and control groups. The children's mean age was 4 years, and each group consisted of 69 (56%) boys and 54 girls (44%). Baseline characteristic data of the children are shown in Table 1. As shown in the Fig., the lead exposed children had significantly higher BLLs [median (range): 8.1 (1-20.9) μg/dL] than controls [6.7 (1.4-13.3) μg/dL (P<0.05)]. Of the 123 exposed children of occupational workers, 38 (31%) had BLL higher than CDC permissible limit (>10 μg/dL) as compared with 14 (11%) in the controls (P<0.05). To further elaborate the synergistic effect of iron deficiency and lead exposure on BLLs, the iron deficient children were identified in both the exposed and control groups. Of the 123 children in each group, 42 (34%) were found iron deficient in the lead exposed group while 35 (28%) in the controls based on their serum ferritin level of less than 12 μg/L. Lead exposed children and controls were further sub-grouped into IDC and Non-IDC (Table 2). Blood lead level, serum ferritin and hematological parameters among the four subgroups were compared using the Kruskal-Wallis test. Iron deficient children in the lead exposed group had significantly high BLL as compared to non-IDC in the exposed group as well as IDC in the control group (P<0.05). Lead levels were significantly higher in the house dust samples collected from the exposed area as compared with the control area. Hematological parameters revealed a decrease in hemoglobin concentration, mean corpuscular

Table 1. Baseline characteristics of the study population (n=246)

Parameters	Exposed group (n=123) mean±SD	Control group (n=123) mean±SD
Age (y)	4.07±1.59	4.28±1.54
Gender (M/F)	69/54	69/54
Weight (kg)	21.5±8.29	20.47±4.77
Height (cm)	112.6±27.68	117.85±17.99
BLL (μg/dL)	9.03±4.49*	6.48±2.71
Ferritin (μg/L)	20.75±12.39	22.38±12.29
Hemoglobin (g/dL)	11.93±1.22*	12.23±0.96
MCV (fl)	79.10±9.44*	81.74±9.01
MCH (pg)	25.06±2.94	24.39±3.91
MCHC (g/dL)	29.93±2.98	30.41±3.31

*: P<0.05. BLL: blood lead level; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

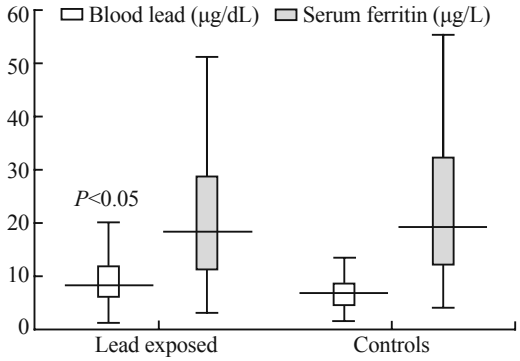

Fig. Box plots showing the blood lead level and serum ferritin in lead exposed children (n=123) and controls (n=123).

Table 2. Comparison of BLL, serum ferritin and hematological parameters in IDC with Non-IDC of lead exposed and control groups (n=246)

Parameter	Lead exposed group		Control group	
	IDC (n=42) median (quartile)	Non-IDC (n=81) median (quartile)	IDC (n=35) median (quartile)	Non-IDC (n=88) median (quartile)
BLL (μg/dL)	13.1 (10.1-16.8)*†	7.0 (4.8-8.2)	9.6 (7.6-10.3)*†	5.6 (3.8-7.0)
Serum ferritin (μg/L)	9.0 (7.0-11.0)*	23.0 (18.5-36.2)	10.6 (7.9-12.0)*	24.0 (18.5-35.0)
Hemoglobin (g/dL)	11.2 (10.3-11.6)*	12.6 (11.9-13.1)	11.7 (10.0-12.5)*	12.4 (12.1-12.9)
MCV (fL)	69.5 (65.5-78.8)*	81.4 (78.3-89.7)	76.1 (69.7-82.4)*	82.6 (78.3-90.0)
MCH (pg)	23.9 (21.5-25.0)*	26.7 (24.4-27.6)	20.4 (17.4-26.6)*	26.4 (24.3-27.5)
MCHC (g/dL)	28.9 (26.5-31.2)*	30.1 (28.9-32.8)	27.0 (26.0-29.0)*	33.0 (29.0-33.9)

*: P<0.05, IDC in the exposed group and controls compared with Non-IDC in the respective groups (Kruskal Wallis test); †: P<0.01, IDC of the lead exposed group compared with that of the controls (across groups). BLL: blood lead level; IDC: iron deficient children; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) with increased blood lead levels in the iron deficient subgroup in contrast to the non iron deficient subgroup in each of the two major groups (Table 2). Hemoglobin concentration levels were significantly lower in the lead exposed IDC sub-groups as compared to the rest three sub-groups (P<0.05).

Blood lead levels revealed a negative correlation with serum ferritin levels (r=-0.54; P=0.001) and hemoglobin concentrations (r=-0.51; P=0.001) in the lead exposed group. Weak negative correlation was also observed between BLL and serum ferritin (r=-0.36; P=0.001) in the control group. The correlation between iron deficiency and BLL was relatively more evident in the lead exposed children as compared with the controls.

Discussion

It is generally accepted that BLL is the best index for monitoring the actual exposure of individuals to lead. We found a significantly large number (31%) of children who had BLL greater than the CDC recommended levels due to exposure to the lead from the industries. Dust lead concentration was significantly higher in the exposed children's homes as compared to the controls. Children can swallow and breathe lead in dust while they play on the floor.

We also demonstrated that IDC had higher BLL compared with non-IDC in both the exposed and the control groups. However, BLLs were higher in the IDC of the lead exposed group than in those of the controls. This finding was consistent with the study which clearly reported an association of elevated BLLs with low iron levels.[16] Similarly, another study from California on 1 to 6 year old children states that iron deficiency is associated with higher blood lead level.[8] A longitudinal analysis of children followed up by an urban primary clinic showed that there is an association of iron deficiency with low level lead poisoning.[7] Several studies have found an association between iron deficiency and lead toxicity.[17] While observing the causes of elevated blood lead levels among children living in rural Philippines, an inverse gradient was found between hemoglobin concentration and BLL, representing another aspect of iron deficiency as a cause of elevated BLL.[18] The results of a study conducted in Turkey to observe the interaction between anemia

and BLL in children were also in agreement with the results of the present study.[19] While identifying the risk factors for elevated BLL in 3 to 4 year old children, iron deficiency was identified as a valid risk factor.[20]

The present study demonstrates a low correlation between serum ferritin and BLLs in the lead exposed children and controls. A Brazilian study on children found a relationship between anemia and elevated BLL.[21] Although most studies are in favor of this association there are certain studies which negate any such link. In one study no significant correlation was found between blood lead level and serum ferritin level.[22] Similarly, a study on Costa Rican children failed to find any significant association between ID and elevated BLL,[23] but the failure of this study may be attributed to the small sample size.

A possible explanation for the association of iron deficiency with elevated BLL could be the fact that lead utilizes iron's absorptive pathway which is the divalent metal transporter-1 (DMT1) to enter the gastrointestinal tract.[24] Hence, when there is deficiency of iron, the absorption of lead increases through this receptor, making iron deficient children more prone to lead toxicity. Another likely explanation for this association can be that iron deficiency sharply increases the expression of DMT-1 in the duodenum, leading to increased absorption of lead.[25]

Hematological parameters showed a negative correlation with elevated BLL in children. Rondó and co-workers[21] reported that anemic children had elevated BLL. Others have used some of these parameters like MCV[7] or hemoglobin to assess iron deficiency and to see their correlation with elevated BLL. Thus, keeping this in view iron deficiency can also be used as a screening procedure for elevated BLL in children chronically exposed to lead.

The strength of the present study is that we included iron deficient children in the study, not only those children who were frankly anemic. This is particularly important because WHO reports that 40% to 50% of children in developing countries are iron deficient but not anemic.[4] The prevalence of iron deficiency in Karachi and Peshawar Pakistan has been reported to be 45% and 63% respectively.[25,26] Moreover, in our study we included 1-6 year old children who were at highest risk for lead poisoning and iron deficiency.[27] We have also studied the synergistic effect of iron deficiency and lead exposure on blood lead levels, considering the greater susceptibility of harmful effects of lead toxicity on the lead exposed children. The study presents a better overview of the condition compared to most studies on either iron deficiency[7] or lead contaminated environment as a cause of elevated blood lead level.[28] In our study we observed the synergistic effects of both iron deficiency and lead exposure on blood lead level in a much more effective manner using a control group for comparative purposes.

Iron fortification may help reduce BLL among most children.[29] This method may be better than chelation therapy as it is effective even at a lower blood lead level and is far cheaper and safer than chelation therapy. Several studies have been conducted to see the effects of iron fortification on blood lead level whereby some have shown favorable results[11] while others have negated any benefit of such measures.[30] By far not a single study has been conducted in Pakistan to see the effect of iron fortification on BLL, thus the present study has opened venues for researchers to make iron fortification a measure of secondary prevention of lead poisoning in the high-risk children in the developing countries.

Nevertheless, our study had a few limitations. We targeted children belonging to a low socioeconomic group and the findings of the study cannot be generalized. As a case control study, our study was difficult to assess the temporal pattern of exposure and co-existing factors leading to elevated BLL. Since both ID and lead poisoning occurred in the low socioeconomic group their association may be due to common environmental factors. Therefore the role of environmental factors and a dose dependent relationship between iron deficiency and lead toxicity may be confirmed by future studies.

In conclusion, our study demonstrates iron deficiency in combination with lead exposure synergistically elevates blood lead level and susceptibility to its harmful effects in Pakistani children living close to lead related industries. This has led to an important public health perspective of prevention of lead exposure and introducing iron fortification as an effective measure to combat lead poisoning in the children residing in lead contaminated environments.

Acknowledgements

We would like to thank all colleagues and technical staff who contributed to this study.

Funding: This study was supported by a grant from the Higher Education Commission, Pakistan (20-808/RND/07).
Ethical approval: The study was approved by the institutional review committee of Army Medical College, NUST Islamabad, Pakistan.
Competing interest: None.
Contributors: Khan DA proposed the concept and designed the study, analyzed and interpreted the data, critically revised and drafted the main body of the article. Ansari WM collected the samples and analyzed them, analyzed the data and did further drafting. Khan FA provided advice on data interpretation, medical aspects and critically revised the manuscript.

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Received December 11, 2009
Accepted after revision January 21, 2010