Relationship between Blood Lead Levels and Hematological Parameters in Children from Al-Fallujah City in Iraq

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Abstract

Environmental exposures to lead remain a serious problem in the developing and industrializing countries. Children are the highest risk aged-group for lead poisoning. This study was designed to assess lead exposure in Al-Fallujah city by analyzing blood lead levels in children and adults and to explain the relationship between blood lead levels, hematological parameters and ferritin levels in the children. The study was performed on-(90) subjects, (65children and 25 adults).Venous blood samples were taken for estimation of hematological parameters, serum ferritin levels and blood lead levels. The children group was subdivided into four groups as: group (A) (low ferritin, low Hb), group (B) (low ferritin, normal Hb), group (C) (normal ferritin, low Hb) and healthy control group (D) (normal ferritin, normal Hb).The results of this study demonstrating that all children groups: group (A) (n=14), group (B) (n=7), group(C) (n=17) and group (D) (n=27) had blood lead levels above the acceptable level 10µg/dl. There was significant increase in blood lead levels (10%) in the control group of children compared to adult group ($P<0.05$). In addition showed a significant increase in blood lead levels(52%)in group (A) and (29%) in group(C) compared with control group($P<0.001$,$P<0.01$) respectively, while no significant increase in blood lead levels was shown in group (B). Thus the current study showed that elevated mean blood lead level above the acceptable limit of (10µg/dl) in all children groups, suggesting that iron deficiency anemia may amplify the effect of lead contamination in the environment.

Key words: Blood lead levels, Hematological parameters, Ferritin, Iron deficiency anemia.

العلاقة بين تركيز الرصاص والتغيرات الحاصلة بدلائل الدم لاطفال العراق في مدينة الفلوجة

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الخلاصة

التعرض البيئي للرصاص مازال يمثل مشكلة جدية في البلدان النامية و البلدان الصناعية و أن الأطفال هم الفئة العمرية الأكثرتعرضاً للتسمم بالرصاص.الهدف من هذه الدراسة هوتقييم التعرض للرصاص في الأطفال و البالغين في مدينة الفلوجة من خلال تحليل مستويات الرصاص في الدم .وكذلك توضيح العلاقة بين مستويات الرصاص في الدم ،معالم مكونات الدم و مستويات الفريتين في الأطفال.وقد أجريت الدراسة على(٩٠) شخص بينهم(٦٥طفل و ٢٥ بالغ). تم أخذ عينات دم من الأشخاص الذين شملتهم الدراسة لتقدير معلمات تكوين الدم, ومستويات الفريتين في مصل الدم, و مستويات الرصاص في الدم. تم تقسيم مجموعة الأطفال التي خضعت للدراسة الى اربع مجاميع : مجموعة (A)(الفيريتين منخفض,خضاب الدم منخفض)، مجموعة(B) (الفيريتين منخفض,خضاب الدم طبيعي), مجموعة (C) (الفيريتين طبيعي,خضاب الدم منخفض), و مجموعة السيطرة(Control) (الفيريتين وخضاب الدم طبيعي).مستويات الرصاص في كل مجاميع الأطفال الذين شملتهم الدراسة: مجموعة (A) (العدد=١٤),مجموعة(B)(العدد=٧)، مجموعة(C) (العدد=١٧) ومجموعة السيطرة(Control) (العدد=٢٧) بلغت اكثر من الحد المقبول (10µg/dl).وأن هناك زيادة معنوية (١٠٠%)في معدل مستوى الرصاص لدى الأطفال في مجموعة السيطرة بالمقارنة مع مجموعة البالغين التي خضعت للدراسة ($P<0.05$) . أن مستويات الرصاص في الدم ازدادت بصورة معنوية (٥٢%) في مجموعة (A) و(٢٩%) في مجموعة (C) بالمقارنة مع مجموعة السيطرة($P<0.01$،$p<0.001$) على التوالي في حين لم تشهد الدراسة زيادة معنوية في مجموعة (B).نتائج هذه الدراسة تشير الى ارتفاع معدل الرصاص الى مستوى اعلى من الحد المقبول (10µg/dl)في أطفال مدينة الفلوجة. والنتائج التي توصلنا اليها تقترح أن فقر الدم بسبب نقص الحديد يكبّرتأثيرالتلوث البيئي بالرصاص.

الكلمات المفتاحية: غليسيريل بهينيت ، الدهون الصلبة المجهرية ، جسيمات الثيوفليين النانوية .

Introduction

Lead is a non- essential metal, its presence in the body at any level could be considered as contamination [(1)]. It is a divalent cation (Pb^{+2}), exists in both organic and inorganic forms [(2)].It occurs naturally in the Earth's crust . It is ubiquitous in the environment of industrialized cities because it comes mainly from human activity [(3)].The elimination half–time of lead in blood is approximately 30-35 days[(4)]; therefore blood lead concentrations relatively reflect the exposure history of the previous few months [(5)].

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Lead is stored in bone, with elimination half – time estimates of 20-30 years [6].The mechanisms of lead toxicity include the ability of lead to mimic or compete with calcium, and to interact with proteins by binding with every available functional group [7].The most critical effects are those on; heme biosynthesis, the nervous system and kidney[8].Lead interferes with heme biosynthesis by altering the activity of several enzymes ,one of the most sensitive hematological effects is inhibition of the enzyme delta-amino levulinic acid dehydratase (ALAD) resulting in accumulation of 6-amino levulinic acid (ALA) in blood, urine, and soft tissues, including brain[9]. In addition, ALA undergoes auto oxidation, generating free radicals that may contribute to toxicity, and promotes oxyhemoglobin oxidation [10]. The mitochondrial enzyme ferrochelatase is the second enzyme in the heme biosynthetic pathway inhibited by lead. Ferrochelatase catalyzes the transfer of iron from ferritin into protoporphyrin to form heme. Inhibition of this enzyme results in increase of the substrates erythrocyte porphyrin (EP), and zinc protoporphyrin (ZPP) [11].

At relatively high levels of lead exposure, anemia may occur due to the interference with heme synthesis and also to red cell destruction .Lead- induced anemia occur in children at blood lead levels of 40µg/dl [12].

Lead impair the activity of erythrocyte pyrimidine -5'-nucleotidase ,resulting in increase in pyrimidine nucleotides in red blood cells ;which lead to a deficiency in maturing erythroid elements, if sufficiently severe, result in induction of basophilic stippling and premature erythrocyte hemolysis; thus decreased red blood cells counts and eventually anemia[13].

Anemia is a common condition worldwide, although the burden is highest in the developing countries where nutrient deficiencies and chronic infections are revalent[14].

Iron deficiency anemia is the main cause of anemia in children and pregnancy [15].The most accurate initial diagnostic test for iron deficiency anemia is the serum ferritin measurement [16].

The aim of this study was to assess the magnitude of lead exposure in children living in Al-Fallujah city and explain the relationship between blood lead levels, hematological parameters and serum ferritin levels in children.

Materials and Methods

This study was carried out on a total of 90 subjects living in Al-Fallujah city grouped as follows: children group (n=65), age range (4-11years) (7.26±2.38, mean±SD) and adults group (n=25), age range (20-45years) (30.08±7.43, mean±SD). The children group was subdivided into four groups as shown in (Table1). A venous blood sample (8ml) was taken from each child (after obtaining their parents' consent) and (from each adult a venous blood sample of (5ml) was taken for estimation of blood lead levels). Blood samples from each child divided into three tubes.

Table (1): Children groups.

Group (A) n=14)	Low ferritin, low Hb.
Group (B) (n=7)	Low ferritin, normal Hb.
Group (C) (n=17)	normal ferritin, low Hb.
ControlGroup (D) (n=27)	Normal ferritin, normal Hb.

The first tube (2ml in plain tube) for separation of serum to estimate serum ferritin by Immunoradiometric assay method using ready – made kit for this purpose and Automated Gama Counter. The second tube (1 ml in EDTA tube) used for estimation of hematological parameters using Automated Device. These parameters included hemoglobin (Hb), packed cell volume (PCV), and mean corpuscular volume (MCV). The third tube (5ml in EDTA tube) used for estimation of lead by using Flame Atomic Absorption Spectrophotometer (AAS)**.**

The cutoff values of the studied parameters are listed in (Table2).The results were expressed as the mean ± standard deviation (SD).The T-test was performed to assess the significance of the differences between the mean blood lead levels in adults and children group and the variables (Hb, MCV, PCV, and ferritin levels) investigated in children groups. P values of less than 0.05 were considered significant.

Table(2): The cutoff values of the studied parameters.

Studied parameters	Cutoff values
Blood lead levels	≥10µg/dl [18]
Ferritin levels	≤ 12ng/ml [19]
PCV	<33- 34% [19],[20]
MCV	<73 µm³[20]
Hb < 5years 5-11years	< 11g/dl < 11.5g/dl [19]

Results

The data presented in (Table 3) showed a significant increase in the mean blood lead level (10%)in control children group (17±3.17µg/dl;mean±SD) compared with adult group (15.36 ± 3.29 µg /dl ; mean ± SD), (P<0.05).(Table 4), and (Figure 1) showed a significant increase in blood lead levels (52%) in group (A) (25.85±1.75µg/dl), and (29%) in group(C) (22±5.67µg/dl) compared with control group, (P<0.001, P<0.01) respectively, while no significant differences was shown in group (B) compared to control group (P>0.05).

Table (3): Comparison of blood lead levels between adults and children groups.

Adult n=25	Control Children n=27
15.36±3.29*	17±3.17 Pb (µg/dl)

Data are presented as (mean±SD).
n=number of subjects.
* Show significance between adults and control children group (significance were evaluated by T-test), *P<0.05.
Control Children (normal ferritin, normal Hb) and Pb (lead).

Table (4): Comparison of blood lead levels among children groups.

	Control D	A (n=14)	B (n=7)	C (n=17)
Pb (µg/dl)	17±3.17	*** 25.85±1.75	19. 58±5.94	22±5.67

Data are presented as (mean±SD).
n=number of subjects.
* Show significance between control and other groups (significance between groups were evaluated by T-test), **P<0.01, *** P<0.001and #non- significant.
group (A) (low ferritin, low Hb), group (B) (low ferritin, normal Hb), group(C) (normal ferritin, low Hb), control group (normal ferritin, normal Hb) and Pb (lead).

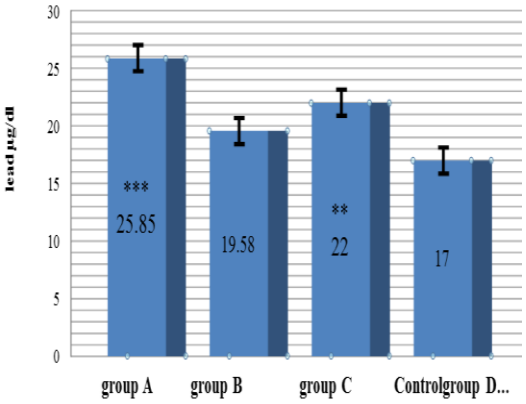

Figure (1): The blood lead levels in children groups

(Table 5) Showed a significant decrease in the Hb 10%), MCV (16%) , PCV (14%) and ferritin levels (87%) in group(A) (10.49±0.62g/dl,68.69 ±4.2µm³, 32.21±1.79%, 6.86±3.33ng/ml) respectively compared with control group (12.4±0.75g/dl, 81.77±5.05µm³, 37.47±2.03%, 52.83±19ng/ml), (P<0.001),while there was only statistically significant decrease in ferritin levels(85%) in group (B) (8.11±2.98ng/ml) compared to control group, (P<0.001).In group (C) (Table 5) showed a significant decrease in the levels of Hb (13%), MCV(16%), and PCV(9%) (10.83±0.66g/dl, 68.98±.30µm³, 34.0±1.89%) respectively compared with control group, (P<0.001, P<0.001, and P<0.01).

Table(5): Comparison of hematological parameters and serum ferritin level among children groups.

	Control D (n=27)	A (n=14)	B (n=7)	C (n=17)
Hb (g/dl)	12.4±0.75	10.49±0.62***	11.98±0.48#	10.83±0.66***
MCV(μ³m)	81.77±5.05	68.69±4.2***	79.25±8.12#	68.98±4.30***
PCV%	37.47±2.03	32.21±1.79***	37.1±2.06#	34.0±1.89**
Ferritin (ng/ml)	52.83±19	6.86±3.33***	8.11±2.98***	55.21±14.69#

Data are presented as mean ±SD.

n=number of subjects.

* Shows significance between control and other groups (significance between group were evaluated by T-test), *P<0.05, **P<0.01, *** P<0.001, and # non-significant.

group(A)(low ferritin, low Hb), group(B)(low ferritin, normal Hb) , group(C)(normal ferritin, low Hb), control(normal ferritin, normal Hb), Hb(hemoglobin), MCV(mean corpuscular volume), PCV(packed cell volume).

Discussion

There are considerable variations in the sources and pathways of lead exposure[18][20].In Al-Fallujah city it is attributable to miscellaneous sources; which may be more significant than universal exposure .There is a number of cement, fire brick, and ceramic plants at about 5 km to the east of Fallujah. ; The high traffic density[19]; presence of large numbers of gasoline electricity generators and also this city undergoes two major military operations and there is no doubt that war can leave a terrible legacy of pollution behind; bombs, missiles, shells, and bullets flood the environment with lead, nitrates, nitrites, hydrocarbons, phosphorous, radioactive debris, corrosive and toxic heavy metals [21],[22].

This study showed that blood lead level in the control children group was significantly (10%) more than adult group living in the same area (Table3). The results of this study indicated that children living in Al-Fallujah city exposed to high lead levels in their environment; where their mean blood lead level twice as high as the cutoff value of 10μg/dl[17]. In addition recent studies indicated that there was no safe threshold for the neurological adverse effects of lead in children, like impairment of cognitive functioning and academic achievement could occur at blood lead levels below 5μg/dl [23].

However the basis of the special concern for infants and children relates to certain structural, functional and behavioral differences between them and adults [3]. Children are at increased risk for lead exposure, as well as for adverse effects, because: children have behavioral characteristics (outdoor activity, less concern for hygienic conditions, hand –to –mouth activities or pica), which increase the risk of lead exposure; children eat and drink more per unit of body weight than adults, so that their relative lead intake is increased[24]; lead absorption in the G.I.T. is substantially higher in children, about 50% ,compared with about 10% in adults[25]; there is a greater prevalence of nutritional deficiencies (e.g. iron and vitamin D) among children , which enhance absorption of lead from the G.I.T. [26] ;the blood- brain barrier is not yet fully developed in young children and neurological effects of lead occur at lower thresholds than adults[27].The results of this study showed significant decrease in Hb, MCV, PCV and ferritin levels in group(A) (10%, 16%, 14% and 87%) respectively.(Table5), (p<0.001), indicated that children in this group suffered from iron deficiency anemia[16]; while there was a significant increase in mean blood lead level (52%) [(Table4), (Figure1)], (p<0.001) compared to control group. This result is in agreement with previous study in which iron deficiency anemia strongly associated with observed toxicities of lead[28],other studies showed a strong correlation between proportions of children with elevated blood lead levels and low iron levels[29],[30],while other studies showed that iron deficiency present even at low lead levels[31],[32].

The hematological findings showed that only mean ferritin level in group (B) was significantly decrease (85%) compared to control group (p<0.001) (Table5). This is due to the depletion of stored iron only[19]; while there was no significant difference in the mean blood lead levels in this group compared to control group [(Table4),(Figure1)].The results are in agreement with a previous study in which ferritin depletion alone has no influence on the blood lead levels in children [33].

The results of this study showed significant decrease in Hb, MCV and PCV in

group (C) (13%, 16%, and 9%), respectively (Table5), ($p<0.001$, $p< 0.001$, $p< 0.01$), with a significant increase in mean blood lead level (29%) [(Table 4), (Figure 1)], ($p<0.01$) compared to control group. There are other studies showed significant association between anemia and blood lead levels [34],[35] and this is consistent with the results of our study, while others showed poor correlation [36], or no correlation [37].

Our results could be explained by referring to iron homeostasis, which is regulated at the level of iron absorption, since increase iron absorption would be consequently increase lead absorption, because ferrous ion transported across the apical membrane by divalent metal transporter (DMT1) which is of broad specifity, including manganese, copper, zinc, cadmium, and lead cations [38]. Absorption of intestinal iron is regulated by three mechanisms .The first is influenced by recent dietary iron intake" dietary regulator". The second, iron absorption can be modulated in response to body iron stores "store regulator" it is capable of changing the amount of iron absorbed to a limited extent. The third, a signal communicates the state of bone marrow erythropoiesis (developing erythrocytes in the bone marrow) to the intestine "erythropoietic regulator"[39].When red-cell production in the bone marrow is accelerated, absorption of intestinal iron is increased due to stimulation of the "erythropoietic regulator" [40], which has the dominant function in the control of iron absorption, and it could produce an increase in intestinal iron absorption much greater than that which the" store regulator" could produce[41].This may explain our findings that only in group(A); there is stimulation of the "erythropoietic regulator"; which resulted in significant increase in blood lead levels, while in group(B) only the "store regulator" is stimulated and there was no significant increase in blood lead levels. This is not consistent with our results in group (C), because according to our hematological findings in this group anemia may be due to infection or inflammation, even when the illness was mild and brief [42], in which the usual flow of iron from reticulo-endothelial and parenchymal cells as well as iron absorption is decreased [39], there may be misclassification of iron status due to the coexistence of anemia of infection or inflammation and iron deficiency anemia ; when inflammatory disease and iron deficiency coexist, serum ferritin values may be within the normal range; because ferritin as an acute-phase protein may be elevated by infection or inflammation[43].

School children (the age range of our studied group) carry the heaviest burden of intestinal parasitic infestation [44], and there is a strong association of hookworm with increased prevalence of iron deficiency anemia [45], also mild inflammatory conditions such as upper-respiratory infections and otitis media, which remain common in children can be a major source of error in diagnosing iron deficiency [42], and under estimation of iron deficiency by using serum ferritin levels [15]. This may be one of the limitations in this study and the coexisting iron deficiency anemia in group(C) may be the predisposing factor in the significant increase in blood lead levels in this group. Therefore it is important for further study to include the measurement of serum transferrin receptor, to differentiate between iron deficiency anemia (IDA) and anemia of inflammation or infection, because it remains normal in the anemia of infection or inflammation, but elevated with IDA [46].

The results of this study showed that blood lead levels in children were below the levels that cause anemia (Figure 1), since it is uncommon for lead poisoning to produce anemia and microcytosis unless it is severe [47]. Therefore the hematologic findings in our study were the result of iron deficiency anemia and anemia of infection or inflammation.

Conclusion

The results of this study showed that children living in Al- Fallujah city are suffer from a high risk of environmental lead exposure indicated by the elevated mean blood lead level above the acceptable limit of (10µg/dl), while the adults from the same city had significantly lower blood lead levels than children. The results of this study suggest that iron deficiency anemia may amplify the effects of lead contamination in the environment by increasing the intestinal absorption of both iron and lead; consequently iron deficiency and lead intoxication are common companions.

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