RELATION BETWEEN ANEMIA AND BLOOD LEVELS OF LEAD, COPPER, ZINC AND IRON AMON CHILDREN

By

[**] Hegazy AA[*], Zaher MM.,

[***]Abd Elsalam M[**], Morsy A A[***] RAM S

From

[*]Department of Community and Occupational Medicine,[**]Department of Pediatric Medicine and [***]Department of Clinical Pathology, Faculty of Medicine for Girls,

Al-Azhar Univerisity.

Abstract:

Background: Anemia is a wide health problem among infants and children in many parts of the world, and is often associated with decreased some trace elements (iron, zinc, copper) and increased heavy metals as lead. Lead plumbing has contaminated drinking water for centuries and has contributed to elevated blood lead concentrations in children.

Objectives: This study was done to determine the association of blood lead level (BLL) above 10μg/dl to the increased risk of anemia of varying severity.

It also aimed at investigating the relationship between anemia and the changes in - blood iron, zinc and copper levels.

Measurement of lead level in drinking water was also considered being a known - source of exposure to lead.

Subjects and methods: The study is a cross-sectional performed on 60 children from the pediatric clinic in Al-Zhraa University hospital and special Pediatric clinic in a rural area. Venous blood samples were taken from the studied population for estimation of hematological parameters as well as iron and ferritin levels. The concentration of zinc, copper, and lead was measured in the serum using the atomic absorption spectrophotometer. According to the level of hemoglobin, the studied population was divided into an anemic and a control group at a level more than 11g/dl .The anemic group was further classified into mild (Hb level 10-10.9 g/dl), moderate (Hb level 8-9.9 g/dl) and severe (Hb level< 8g /dl) anemia. The studied subjects were also categorized into low ($\geq$10 μg/dl) and high ($\geq$10 μg/dl) blood lead level groups.

Results: Approximately 63.33% of the children had blood lead level more than and equal to 10μg/dl while only 36.67% of them had blood lead level less than 10μg/dl. At the blood lead level range of 10-20 μg/dl, significant association was obtained for mild and severe anemia. In children with anemia, the level of iron and ferritin were found to be significantly lower than those of the control groups (p value <0.001). However, other elements (zinc and copper) did not show any statistically significant difference between the anemic and the control groups. Environmental assessment of the lead level in drinking water revealed the presence of levels higher than the permissible limit determined by the World Health Organization (WHO).

Conclusion: Elevation of Lead level in drinking water might be one of the causes behind increased BLL in children with anemia. Active steps should therefore be taken towards the control of lead pollution thus reducing the prevalence of anemia among children.

Keywords: lead; copper; zinc; iron; children; anemia

Introduction

The deficiency of certain trace elements generally causes hypochromic microcytic anemia. Iron deficiency as well not only causes hypochromic microcytic anemia,but also increases the absorption of other elements such as lead (Pb) and cadmium (Cd). Therefore, in patients with hypochromic microcytic anemia the serum levels of these elements may increase causing deterioration of anemia. Generally, heavy exposure to (Pb and Cd) is known to cause hypochromic microcytic anemia[1]. Iron absorption occurs predominantly in the duodenum and jejunum. A number of dietary factors influence iron absorption, ascorbate and citrate increase iron uptake. Lead is a particularly pernicious element to iron metabolism. Lead is taken up by the iron absorption machinery, and secondarily blocks iron through competitive inhibition. Further, lead interferes with a number of important iron dependent metabolic steps such as heme biosynthesis[2].

Lead poisoning has been a significant public health problem for centuries. Lead poisoning in children was defined as blood lead level equal to or greater than 10μg /dl [3], being associated with adverse behavioral and developmental outcomes. Recently no level less than 10μg /dl is considered safe.[4]

Human exposure to lead occurs primarily through diet, air, drinking water and ingestion of paint chips where absorption is increased mainly in persons suffering from iron and calcium deficiency.[5]

Environmental lead exposure occurs from automobile exhaust in areas of the world where leaded gasoline is still being used. At home exposure among children may occur either because of ingestion of old leaded chips or because of pigments and glazes used in pottery[6].

For centuries, lead plumbing has helped in contamination of drinking water and contributed to elevated blood lead concentrations in children[7].

The mobilization of heavy metals in the environment due to industrial activities is of serious concern due to the toxicity of these metals in humans and other forms of life[8]. These metals tend to persist indefinitely, circulating and eventually accumulating throughout the food chain, becoming a serious threat to the environment. Among these toxic heavy metals, mercury, lead and cadmium have been called "the big three" due to their major impact on the environment[9].

Copper is an essential trace element present in the diet, it is needed to absorb and utilize iron[10]. Zinc is absorbed in small intestine; absorption is inhibited by the presence of phytates and fiber in the diet, as well as dietary iron and calcium[11].

Anemia in children is leads to increased morbidity and mortality [12]. Adverse health effects of anemia in children include impaired psychomotor development and renal tubular function, poor cognitive performance and mental retardation[13,14].

This study was therefore done to investigate the association of elevated blood lead level ≥ 10μg/dl and the higher risk of anemia of varying severity among children compared to levels less than 10μg/dl. The relationship between anemia and serum iron (Fe), copper (Cu) and zinc (Zn) was also determined. Lead level in drinking water was measured being considered a possible source of exposure to lead.

Subjects and Methods

Study population:

This research was performed on 60 children from the pediatric clinic in Al-Zhraa Univerisity hospital and special pediatric clinic in a rural area. The mothers of children were informed about the aim of the study and a consent was obtained. Data related to age, gender, residence, source of drinking water, degree of fathers and mothers education and their jobs as well as their socioeconomic condition were collected from the mothers.

According to the WHO criteria used to define anemia in children based on hemoglobin level less than 11g/dl the studied population was divided into an anemic group and a normal control group[15]. The anemic group was further classified into categories of mild (Hb level 10-10.9 g/dl), moderate (Hb level 8-9.9 g/dl) and severe (Hb level< 8g /dl) anemia.

The studied population was also classified into a group of less than 10μg /dl and another group of more than or equal 10μg /dl of lead level measured in the blood.

Laboratory investigations:

A venous blood sample was taken from each child and divided into three tubes. The first tube contained EDTA for estimation of the hematological parameters using Celttac autoanalyzer, these parameters included the red blood cell count (RBC),hemoglobin (Hb),hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW). The second tube contained heparin for lead, copper and zinc estimation by atomic absorption spectrophotometer[16] (Perkin Elmer HGA 460-Germany). The blood lead level (BLL) was determined by the graphite furnace atomic absorption spectrophotometer, but the serum concentration of Cu and Zn were measured with the flame atomic absorption spectrophotometer .The third tube was used for estimation of serum iron by Hitachi 911 autoanalyzer using Roche reagent kits and for estimation of serum ferritin by Elecsys 1010 –Japan.

Environmental assessment:

Samples of drinking water were taken from tap and hand pump waters for detection of lead level using the atomic absorption spectrophotometer (Graphite Furnace, Perkin Elmer HGA-600, USA). The lead level in piped water was found to be 2.9μg/dl from urban area and 3.6μg/dl from rural area. In hand pump water collected from 2 separate hand pumps, in a rural area, the lead levels were 3.1μg/dl and 2.3μg/dl.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) version 12. Chi-square test was performed to compare individual characteristics and the t-test was performed to compare the hematological parameters between the anemic and control groups. Results were expressed as the mean ± standard deviation (SD). P values of ≤ 0.05 and ≤ 0.001 were considered statistically significant and highly significant, respectively. A correlation was performed for the blood levels of lead, Fe, Cu and Zn versus the different hematological parameters.

Results

This study was done on 60 child with ages ranging from 2 to14 years with a mean value of 6.27± 3.40 years.

According to the blood lead level, approximately 63.33% (n=38) of children had blood lead ≥10μg/dl (high blood lead level group) and 36.67% (n=22) had blood lead level <10μg/dl (low blood lead level group), with range from 7 to 20 μg/dl.

The frequency distribution of individual characteristics was studied among the high and low blood lead level (table 1). Though no significant statistical difference was detained for all individual characteristics yet a high frequency of schoolchildren (≥6 years old) distribution and those consuming tap water was detected in the high blood lead level group. Also, high prevalence of illiterate mothers and fathers and those of low social standard was detected in high blood lead level group. High prevalence of high blood lead level was detected among unemployed mothers and employed fathers.

A significantly greater proportion of children with lead levels ≥ 10μg/dl (63.3%) had anemia compared to those children with lead levels <10μg/dl (36.7%) (table 2).

The prevalence of different categories of anemia among the high (≥10μg/dl) and low (≥10μg/dl) blood lead level groups was demonstrated in table 3. The difference in distribution was greater for mild and severe form of anemia (HBLL 28.9%; LBLL 4.5%) and (HBLL 21.1%; LBLL 4.5%), respectively.

The mean values and standard deviation of different hematological parameters and the levels of lead, iron, copper and zinc among the studied population, after being divided into an anemic and a control according to the hemoglobin level were studied (table 4). As regard hematological parameters, all values were highly significantly lower among the anemic than the control group except for the RDW which showed a highly significant elevation among the anemic group. As for the RBC count no statistically significant difference was detected between the groups.

Comparing the results of serum Cu, Fe and Zn levels between the anemic and control groups revealed a highly significant decrease in the serum level of Fe among the anemic than the control group ($p<0.001$). However no statistically significant difference was seen between both groups for serum Cu and Zn levels (fig 1). As for the serum Pb level between anemic and control groups, it was found that the level of Pb is highly significantly increased among the anemic group compared to control group ($p<0.001$; fig 2).

The table 5 represents the correlation between the hematological parameters and the serum levels of lead, Cu and Zn. A highly significant negative correlation between BLL and Hb, Hct, MCV, MCH, serum Fe, and ferritin ($r=-0.461$, $r= -0.484$, $r=-0.267$, $r=-0.381$, $r=-0.470$ and $r=-0.552$, respectively) ($p<0.001$). But there was a highly significant positive correlation between BLL and RDW ($r= 0.458$; $p=0.001$). Cu showed a positive significant correlation with RBC ($r=0.264$; $p<0.05$) and a negative significant correlation with ferritin ($r=-0.257$; $p<0.05$). In addition a positively significant correlation was detected between the Zn levels and Hb, Hct, MCH and ferritin ($r=0.324$, $r=0.305$, $r=0.308$ and $r=0.314$) but negatively significant correlation for RDW ($r=-0.266$) ($p<0.05$).

Table (1): Frequency distribution of individual characteristics among the low and high blood lead level group.

Characters of studied group	Blood lead level Low <10μg/dl (n=22)		High ≥10 μg/dl (n=38)	
	No	%	No	%
Age: - school children (≥6 years old)	9	40.9	20	52.6
- Pre-school children (<6 years old)	13	59.1	18	47.4
Gender:				
- Male	9	40.9	23	60.5
- Female	13	59.1	15	39.5
Residency:				
- Urban	12	54.5	20	52.6
- Rural	10	45.5	18	47.4
Mother education:				
- Education	3	13.6	4	10.5
- Illiterate	19	86.4	34	89.5
Father education:				
- Education	3	13.6	4	10.5
- Illiterate	19	86.4	34	89.5
Mother work:				
- Employed	3	13.6	4	10.5
-Unemployed	19	86.4	34	89.5
Father work				
- Employed	16	72.7	28	73.7
-Unemployed	6	27.3	10	26.3
Sources of drinking water:				
- Tap water	12	54.5	20	52.6
-Hand pump water	10	45.5	18	47.4
Social standard:				
- Middle	3	13.6	4	10.5
-low	19	86.4	34	89.5

Table 2: prevalence of anemia among the low and high blood lead level.

	Low blood Lead level (LBLL) <10μg/dl (n= 22)		High blood Lead level (HBLL) ≥10 μg/dl (n=38)	
	N	%	N	%
No anemia	16	72.7	14	36.8
Anemia	6	27.3	*63.2	24

* Significant chi-square test ($p < 0.05$).

Table 3: Frequency distribution of hemoglobin level among the low and high blood lead level groups.

Categories of anemia according to hemoglobin level	LBLL (10μg/dl (n= 22>		HBLL (μg/dl (n=38 10≤	
	No	%	No	%
No anemia (Hb level ≥ 11g/dl)	16	72.7	14	36.8
Mild anemia: (Hb level 10-10.9 g/dl)	1	4.5	11	28.9
Moderate anemia: (Hb level 8-9.9 g/dl)	4	18.3	5	13.2
Severe anemia: (Hb level< 8g/dl)	1	4.5	8	21.1

Table 4: The mean and standard deviation of the different hematological parameters, blood lead level and serum elements (Fe, Cu and Zn) among the anemic (n=30) and non-anemic control (n=30) groups.

	Anemic group (n=30) mean± SD	Control group (n=30) mean ± SD
RBC ($x10^6$ mm^3)	4.05±0.51	4.24±0.28
Hb (g/dl)	8.97±0.88	12.13±0.46**
Hct	28.59±2.26	36.56±2.01**
MCV (μ^3)	70.02±14.80	81.48±6.70**
MCH	21.74±3.21	27.08±1.23**
MCHC	31.38±1.98	33.54±1.91**
RDW	17.27±2.66	13.24±0.60**
Ferritin (ng/ml)	40.21±23.46	82.28±9.76**

** Highly significant t-test ($p<0.001$).

Table 5: Correlation of the different hematological parameters versus the blood lead level as well as the serum copper and zinc among the studied population (n=60).

Hematological parameters	Blood lead level r value	Copper r value	Zinc r value
RBC ($x10^6$ mm^3)	0.118	0.264*	0.015
Hb (g/dl)	-0.461**	-0.159	0.324*
Hct	-0.484**	-0.209	0.305*
MCV(μ^3)	-0.267*	-0.177	0.248
MCH	-0.381**	-0.156	0.308*
MCHC	-0.155	-0.020	0.120
RDW	0.458**	0.238	-0.266*
Ferritin (ng/dl)	-0.552**	-0.257*	0.314*
Fe (μg/dl)	-0.470**	-0.136	0.186

* significant r value ($p< 0.05$).

** highly significant r value ($p<0.001$).

Fig 1: the mean values of serum trace elements (Cu,Zn,Fe) among the anemic (n=30) and non-anemic control (n=30) groups.

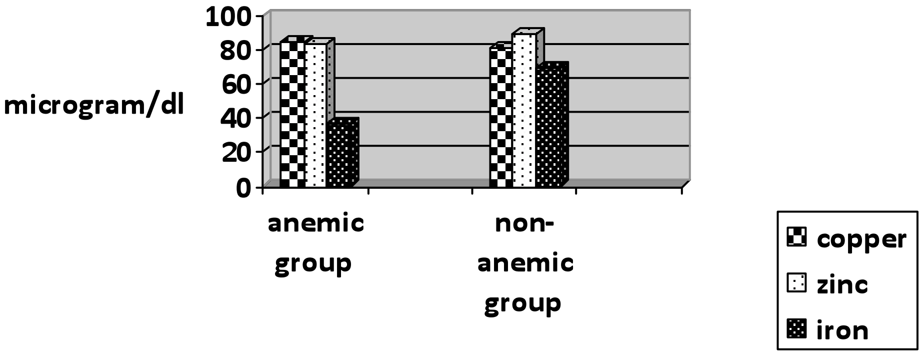
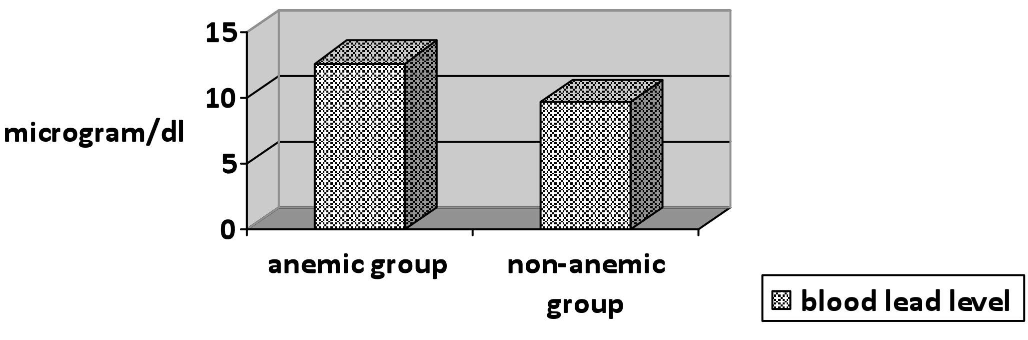


Fig 2: the mean values of blood lead level among the anemic (n=30) and non-anemic control (n=30) groups.



Discussion

More than the half of the children (63.33%) in our study had BLL ≥10μg/dl, similar to a study done by Jain et al[6]., also reported significant association of moderate and severe anemia with blood lead levels in the range of 10-19.9 μg/dl. while in this study reported significant association of mild and severe anemia with the range of 10-20 μg/dl blood lead levels, this difference may be due to large number of sample in their study. Also our study is similar to the estimates obtained for children in India[17,38]. The cutoff value of 10μg/dl is significant, defined by the Center for Disease Control and Prevention as a limit for an elevated blood lead level, primarily based on neurological toxicity[18]. But recently no level less than 10μg/dl is considered safe[4].

Schwartz et al.[19] reported that children living near primary lead smelter in the US of Idaho, had blood lead level near 25μg/dl and were associated with anemia in a dose-related manner. Drossos et al.[20] reported that children with BLL ≥30μg/dl had a linear decline in hemoglobin level. On the contrary, Froom et al.[21] suggested that hemoglobin level did not correlate well with BLL and suggested that anemia is not related to lead at low BLL. Other studies have reported a variable association[22-26].

Lead causes anemia by impairment of heme synthesis and an increased rate of red blood cell destruction[27]. On the other hand, it is also possible that iron deficiency, which is a proven cause of anemia, leads to increase in the absorption of lead in the body, resulting in high BLL[28,29]. Although a causal pathway cannot be determined, our findings clearly demonstrate an association between varying severity of anemia and elevated BLL.

High frequency of school children (≥6 years old) distribution was detected in the high blood lead level group. This means blood lead level associated with increasing age, may be due to usage of crayon in school children.

Fe, Cu and Zn are essential elements for the maintenance of life and health. Pb which is a heavy metal, can be harmful to human health. So, we determined the serum levels of these trace elements in children. Because of the presence of high BLL in drinking water as reported by the WHO, this study was carried out the relationships between high blood lead levels and trace elements as well as hematological parameters in children.

In this research the serum level of iron in anemic group was found significantly lower than the control as was expected, similar to the results obtained by Jain et al[4]. Iron deficiency is a World health problem, especially for infants and rapidly growing adolescents where it plays an essential role in many biological processes. Therefore, it is important to maintain iron concentration within its narrow normal range[30].

The serum Zn level of anemic group in this study is lower than the control group but is not statistically significant. There is an antagonism between Zn and Fe absorption from gastrointestinal as increased concentration of iron in the intestinal lumen may antagonize the uptake of Zn[31]. A study was done by Sebahat et al.[1] found that decline in serum Zn level and increase in serum Cu level in anemic group compared to control group.

Also in our study the serum Cu level in anemic group is higher than control group but this increase is not statistically significant. Cu has a role in the absorption of iron. The oxidation of ferrous iron into ferric state is carried by ceruloplasmin. This depletion of Cu could impair iron absorption[32].

We found that the serum level of Pb in anemic group was highly significantly increased than the control. Fe deficiency increased absorption of Pb from the intestine. In a study made in Canada, the BLL was shown to be high in babies with Fe deficiency [24]. It is notable that the studies with largest number of subjects for analysis all found significant associations between Fe deficiency and elevated BLL[29,33].

Our results showed that Hb, Hct, MCV, MCH and ferritin values of children with anemia were decreased and RDW level was increased in the anemic than the control subjects.

We showed that the serum level of lead was high in children with anemia. It may be that decreased iron level increase lead absorption and affect heme synthesis consequently negatively affected hematological parameters[27].

In this research, investigated water samples were considered suitable for drinking according to EMH[34], as the lead levels were lower than the permissible limit (5μg/dl), but according to WHO[35] they were considered higher than the permissible limit (1μg/dl). In Dakahlya-Egypt , lead level in drinking water was higher than the permissible limit according to WHO[36]. In Egypt the control of lead has not been efficient, so the level of lead in drinking water in some sporadic areas was still high levels.

In 2003-2004, some tap water in Washington, DC, was found to exceed Environmental Protection Agency (EPA) regulations. This was thought to be caused by a change in water disinfection procedures, which increased the water's ability to leach lead from connector pipes between the water mains and interior plumbing in old houses [37].

In developing countries such as India, control of lead pollution has been much slower and more sporadic. Some studies have estimated that more than half the children in India have blood lead levels ≥ 10μg/dl [38].

Conclusion

In this study, relatively low or high (≥ 10μg/dl) lead levels in children were associated with elevated risk of mild and severe anemia, decreasing iron absorption and negatively affecting the hematological parameters. Lead level in drinking water was high according to WHO, and this may be one of the leading causes for elevated BLL in children. Lead pollution might be controlled and steps should be taken to reduce the prevalence of childhood anemia.

References

1. Sebahat T, Aziz P, Murat I, Gunfer T, Gulten E, Mevlut B, Yasin KT and Osman G (2007). Interaction between anemia and blood levels of iron, zinc, copper, cadmium and lead in children. Indian J Pediatr; 74:827-30.
2. Goyer RA (1993). Lead toxicity: current concerns. Environ Health Prespect. 100:177-87.
3. Ellis MR and Kane KY (2000). Lightening the lead load in children. Am Fam Physician; 62:545-54, 559-60.
4. CDC (2005). Preventing lead poisoning in young children: a statement by the Center for Disease Control and Prevention. Atlanta, GA. Available at http:// www.cdc.gov/nceh/lead/publications/prevleadpoisoning.pdf.
5. Abdel-Maaboud RM, El-Attar MM, Mohamad NA, Ahmed SA and Medhat A (2005). Lead toxicity in some rural communities in Assiut Governorate. Ass Un Bull Environ Res; 8:57-66.
6. Jain NB, Laden F, Guller U, Shankar A, Kazani S and Garshick E (2005). Relation between blood lead levels and childhood anemia in India. Am J Epidemiol; 161:968-73.
7. Lanphear BP, Matte TD and Rogers J (1998). The contribution of lead contaminated house dust and residential soil to children's blood lead levels. A pooled analysis of 12 epidemiologic studies. Environ Res; 79:51-68.
8. Igwe JC and Abia AA (2007). Equilibrium sorption isotherm studies of Cd (II), Pb (II) and Zn (II) ions detoxification from waste water using unmodified and EDTA-modified maize husk. Electronic J Biotechnology; 10:536-48.
9. Volesky B and Holan ZR (1995). Biosorption of heavy metals. Biotechnology Progress; 11:235-50.
10. Jones AA, Disilveslro RA, Coleman M and Wanger TL (1997). Copper supplementation of adult man: effect on blood copper enzyme activities and indicators of cardiovascular disease risk. Metabolism; 46:1380-3.
11. Lonnerdal B (2000). Dietary factors influencing zinc absorption. J Nutr; 130:1378s.
12. Kapur D, Agarwal KN and Agarwal DK (2002). Nutritional anemia and its control. Indian J Pediatr; 69:607-16.
13. Lozoff B, Wolf AW and Jimenez E (1996). Iron-deficiency anemia and infant development: effect of extended oral iron therapy. J Pediatr; 129:382-9.
14. Ozcay F, Derbent M and Aldemir D (2003). Effect of iron deficiency anemia on renal tubular function in childhood. Pediatr Nephrol; 18:254-6.

15. World Health Organization. United Nations Children's Fund (2001). Iron deficiency anemia, assessment, prevention and control: a guide for programme managers. Geneva, Switzerland: WHO.
16. Fernandez FJ and Kahn L (1971). Graphite Atomic Absorption Spectrophotometry for metals, Atomic Absorption,New sletter;10:65.
17. Kaul B (1999). Lead exposure and iron deficiency among Jammu and New Delhi children. Indian J Pediatr; 66:27-35.
18. Franko EM, Palome JM, Brown MJ, Kennedy CM and Moore LV (2000). Blood lead levels in young children- United States and selected states,1996-1999., Morb Mortal Wkly Rep;49:1133-7.
19. Schwaetz J, Landrigan PJ and Baker EL (1990). Lead induced anemia: dose response relation and evidence for a threshold. Am J Public Health; 80:165-8.
20. Drossos CG, Mavroids KT and Papadopoulov-Dafotis Z (1982). Environmental lead pollution in Greece. Am Ind Hyg Assoc J; 43:796-8.
21. Froom P, Kristal-Bonch E and Benbassat J (1999). Lead exposure in battery- factors workers is not associated with anemia. J Occup Environ Med; 41:120-3.
22. Bashir R, Khan DA and Saleem M (1995). Blood lead levels and anemia in lead exposed workers. J Pak Med Assoc; 45:64-6.
23. Carvalho FM, Barreto ML and Silvany-Neto AM (1984). Multiple causes of anemia amongst children living near a lead smelter in Brazil. Sci Total Environ; 35:71-84.
24. Cohen AR, Trotzky MS and Pincus D (1981). Reassessment of the microcytic anemia of lead poisoning. Pediatr; 67:904-6.
25. Osterode W, Barnas U and Geissler K (1999). Dose dependent reduction of erythroid progenitor cells and inappropriate erythropoietin response in exposure to lead: new aspects of anemia induced by lead. Occup Environ Med; 56:106-9.
26. Willows ND, and Gray-Donald K (2002). Blood lead concentration and iron deficiency in Canadian aboriginal infants. Sci Total Environ; 289:255-60.
27. Goyer RA and Rhyne BC (1973). Pathological effects of lead. Int Rev Exp Pathol; 12:1-77.
28. Bradman A, Eskenazi B, Sutton P and Goldman LR (2001). Iron deficiency associated with higher blood lead level in children living in contaminated environments. Environ Health Perspect; 109:1079-84.
29. Wright RO, Tsaih SW and Schwartz J (2003). Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinc. J Pediatr; 142:9-14.
30. Andrews NC (1999). Disorders of iron metabolism. New Eng J Med; 341:1986-95.
31. Ece A, Uyanik BS, Iscan A, Ertan P and Yigitoglu MR (1997). Increased serum copper and decreased serum zinc level in children with iron deficiency anemia. Biol Trace Elem Res; 59:31-9.
32. Newhouse IJ, Clement DB and Lai C (1993). Effects of iron supplementation and discontinuation on serum copper, zinc, calcium and magnesium levels in women. Med Sci Sports Exerc; 25:562-71.
33. Kaufmann RB, Clouse TL, Olson DR and Matte TD (2000). Elevated blood lead levels and lead screening among US children aged one to five years:1988-1994. Pediatr; 106: E79.
34. Egyptian Ministry of Health (EMH) (1995). Standards for drinking water. Internal Report.
35. World Health Organization (WHO) (1997). Guideline for drinking water quality health criteria and other supporting information. Geneva, (2):254-66.
36. Ghanem AA, El-Azab SM, Mandour RA and El-Hamady MS (2008). Relationship between lead levels in drinking water and mother breast milk-Dakahlya-Egypt. The Internal J of Tox; 5:1559-3916.
37. American Academy of Pediatrics (2005). Lead exposure in children : prevention, detection and management. Pediatrics; 116:1036-46.
38. Patel AB, Williams SV and Frumkin H (2001). Blood lead in children and its determinants in Nagpur, India. Int J Occup Environ Health; 7:119-26.