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Lead (Pb), Cadmium (Cd) and Essential Element Levels and their Interactions: A Hospital Based Case Control Study in Anemic Children

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Abstract

The prevalence of anemia is still higher among the developing countries like India. In addition, a higher level of multiple micronutrient deficiency and exposure to heavy metals through environment priming the health issue. The etiology of anemia is multi-factorial, among physiological iron deficiency and hemopoietic toxicity caused by Lead (Pb) are considered as important. Current study aimed to unveil serum essential element (Fe, Zn, Cu, Mg and Ca), blood Pb and Cd levels among anemic (Group-A; n=77) and non-anemic children (Group-B, n=77). Blood Pb, Cd and serum elements were quantified by Graphite Furnace and Flame Atomic Absorption Spectroscopy, respectively. Hemoglobin, reticulocyte count and δ-Aminolevulenic Acid Dehydratase (δ-ALAD) activity were also assessed. Mean Hb, serum Fe and δ-ALAD activity was significantly ($p<0.01$) lower in Group-A compared to Group-B. Blood Cd levels were non-detectable, whereas mean Pb levels were higher among Group-A compared to Group-B but in prescribed safe limits (<10 μg/dL). Group-A children's serum Fe levels correlated positively with ALAD activity ($p<0.05$), serum Zn and Ca levels ($p<0.01$); whereas a significant ($p<0.01$) inverse correlation was noted between Hb levels and serum Zn levels. Elevation of δ-ALAD activity is presumed under IDA to meet the physiological requirement of Hb, but in the current study despite severe anemia in Group-A no such observation was noted. In conclusion, anemia among Group-A children may be iron deficient rather induced by hematopoietic toxicity. Further, Zn deficiency is more prevalent in children of both the groups, which need intervention strategies. Dual deficiency effect of Fe and Zn on physiological levels and function of δ-ALAD activity needs to be elucidated.

Keywords: Iron deficiency anemia; Blood Pb levels; δ-ALAD; Serum essential elements

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Introduction

Anemia is a condition in which the number of red blood cells is insufficient to meet the body's physiologic needs. It is a widespread public health problem associated with an increased risk of morbidity and mortality, especially in pregnant women and young children [1]. The etiology for anemia is a multi-factorial classified as nutritional such as vitamin and mineral deficiencies as well as non-nutritional such as infection and hemoglobinopathies. It is well documented that nutritional anemia is the most prevalent condition compared to non-nutritional anemia; further about 50% of the anemia is due to iron deficiency, and the remainder due to other causes [1]. The prevalence of anemia is particularly high in developing countries, where 39% of children under five years old, 48% of 5 to 14 year old children, 42% of all women and 52% of pregnant women are anemic [1].

The diagnostic procedures to determine anemia involves a number of tests such as physical examination of 'conjunctiva, tongue' and laboratory tests such as estimation of hemoglobin, reticulocyte count, complete blood picture, peripheral smear for shape and size of RBCs, serum iron and ferritin levels. A set of any of these tests may give an indication of type of anemia. A normal 'Mean Corpuscular Volume' (MCV) associated with an increased reticulocyte count suggest rapid RBC loss, as in hemolysis or acute blood volume loss [2]. Multiple micronutrient deficiencies often coexist in populations in developing countries. A deficiency of one micronutrient may influence the absorption, metabolism and/or excretion of another micronutrient [1]. Moreover, excess zinc ingestion is a cause of copper deficiency [3].

On the other hand, the heavy metals such as Lead (Pb), Cadmium (Cd), Mercury (Hg) and

Arsenic (As) are known as potential environmental contaminants and pose public health problem. Further, due to vast industrial applications of Pb, lack of enforcement strategies coupled with awareness, the population of developing countries are more prone to exposure [4]. The recent studies suggested a higher prevalence of blood lead levels (BLL) among the children residing in the industrial area compared to urban; whereas the children from rural background had no exposure to Pb. Similarly the accumulation of Cd has raised concern recently due to its vast industrial and agricultural practices [5]. It was proposed that Pb and Cd are similar to trace element, and may enter in to blood stream through 'Divalent Metal Ion Transporter-1' (DMT- 1). The recent studies suggest that iron deficiency further increases absorption *per se* of Pb [6]. Exposure to Pb affects many organs, primarily hemopoietic, renal and central nervous systems, especially in growing children and pregnant women. The recent reports suggest that subclinical Pb toxicity affects the heme synthesis by inhibiting the δ-Aminolevulenic Acid Dehydratase activity (δ-ALAD), which is one of the key enzymes in heme synthesis [7,8]. It is well established fact that 'inhibited δ-ALAD activity' has been considered as a hallmark of 'Pb toxicity', whereas in 'iron deficiency' this enzyme levels were also altered. Our studies on induction of 'iron deficiency' in laboratory animals through dietary restriction have shown the elevated levels of δ-ALAD activity to meet the physiological levels of hemoglobin [9].

In view of the given background, current study aimed to determine the serum essential element levels (Fe, Zn, Cu, Mg & Ca), blood Pb and Cd levels as well as their interactions, if any, in anemic children undergoing blood transfusion.

Methods

Study design and selection of subjects

A cross sectional case-control study was conducted among children from 'Pediatric division of Niloufer Hospital, Hyderabad, India'. The National Family Health Survey-4, India (NFHS-4: 2015-16) reported prevalence of moderate and severe anemia were 30% and 2% respectively among children. Therefore, considering total prevalence as 32%, an odds ratio 2.6 and power 90, a sample size of 154 was arrived.

Anemic children (Group-A; n=77) admitted in the pediatric in-patient ward of Niloufer Hospital for 'Blood Transfusion' were enrolled as study subjects. The children with moderate (Hb: 8.00% g to 9.99% g) to severe (Hb<8.00% g) anemia was included in the study (WHO Criteria). Children aged between 1.6 to 11.0 years, with no history of previous blood transfusion were included in the study. Similarly age and sex matched 'non-anemic' children (Group-B; n=77) admitted in the same in-patient ward suffering from 'other than anemia' were selected as control subjects. The children who have the history of blood transfusion, evidence of hemolysis/blood loss, hemotological malignancy and chronic diseases were excluded from the study.

The information on age, height, weight, 'clinical signs and symptoms' for anemia, heavy metal toxicity and nutritional deficiencies were collected using a pre-tested questionnaire. In addition the information on dietary habits and cooking practices was also collected from the attendants of subjects. The study and procedures were approved by Institutional Review Board (Institutional Ethics Committee for Human Studies) of the ICMR-National Institute of Nutrition, Hyderabad, India. As per the recommendations of IRB, written consent was obtained from the participants or their parents.

Sample collection

Approximately 3.0 mL of blood was collected by venipuncture from all the children. Two aliquots of blood samples were prepared using vacutainer tubes, one for whole blood and other aliquot was used for serum. The blood samples from Group-A children were collected before the 'blood transfusion'. Peripheral blood smear was prepared for examination of abnormalities in RBC, WBC and platelets. Reticount was performed to determine the percentage of reticulocytes.

Laboratory Analysis

Estimation of serum albumin and hemoglobin levels

Serum albumin levels were estimated as described by Doumas & Biggs. [10], based on increase in absorbance of Bromocresol Green (BCG) at 630 nm. Binding of the dye to serum albumin at pH 4.2 is directly proportional to the concentration of albumin estimated using Bovine Serum Albumin (BSA) as standard. The Hemoglobin (Hb%) levels were determined by cyanmethemoglobin method, briefly, 20 µL of blood was mixed with 5.0 mL of 'Drabkins reagent' (Potassium Ferric Cyanide Solution) and allowed to stand for 5 min to develop color. The color intensity was measured at 540 nm against known standard using Spectrophotometer (UNICAM-UV 300, Thermo, Cambridge, UK). Levels of Hb in each samples were then calculated based on [A540unk X Conc. of Hb Std. (g/dL)]/A540std.

Preparation of sample for metal/elemental estimations

To estimate the blood Pb, Cd levels and serum elemental levels, samples were subjected to 'closed microwave digestion" as explained by Reddy et al. [8]. Briefly, 1.0 mL of blood and 0.5 mL of serum were digested with 2.0 mL of ultrapure nitric acid (Merck, Mumbai, India) in a closed microwave (MARSXpress, CEM Corp., Matthews, USA) digestion system. The certified reference standard samples for Pb, Cd and serum essential elements were also processed along with the samples, as quality controls.

Estimation of blood Pb, Cd and serum elemental levels

The blood Pb and Cd levels were determined in microwave digested samples against standard plot obtained from Pb and Cd standards on Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS, Thermoelectron, USA). Certified blood Pb and Cd standards were used to ensure quality of estimates. Serum essential elements such as Fe, Zn, Cu, Mg and Ca levels were measured using Atomic Absorption Spectrophotometer (Varian 220, Pala Alto, CA, USA) in the closed microwave digested serum samples. Certified serum standards with two levels of essential elements were also analyzed simultaneously on the instrument to ensure quality of estimates.

δ-ALAD activity

Blood δ-Aminolevulenic Acid Dehydratase (δ-ALAD) activity was determined as explained by Granick et al. [11], method. In brief, aliquots of 50 µL heparinized whole blood were added to Saponin buffer with and without 'aminolevulenic acid' as substrate and each was incubated at 37°C for 1 h. After termination of the reaction by addition of 50 µL trichloroacetic acid-HgCl2 solution, the samples were centrifuged (1000 × g, 5 min, 25°C). The resulting supernatant was isolated and combined with an equal volume of freshly-prepared modified Ehrlich reagent. After 10 min at 25°C, the absorbance of the mixture was determined at 553 nm using the UV 300 spectrophotometer. ALAD activity (as nM/hr/mL blood) in each sample was calculated as = (OD553 × 1378).

Prevalence of deficiency/toxicity and interactions

The WHO criteria followed for categorization of anemia in to moderate (Hb: 8.00% g to 9.99% g) to severe (Hb: <8% g). The prevalence of micronutrient deficiencies was computed considering the cut-off values of serum Fe (<70 µg/dL), Zn (<70 µg/dL), Cu (<80 µg/dL), Mg (<1.8 mg/dL) and Ca (<8.0 mg/dL). The safe cut off limits for blood Pb and Cd levels were considered as <10.0 µg/dL and <0.12 µg/dL in children (CDC, Atlanta). The interactions/associations between essential elements and Pb, ALAD activity was assessed using statistical method of 'Pearson's correlation co-efficiency'.

Statistical analysis

Mean and Standard Deviation (SD) were calculated for all the variables. The student t-test was applied to assess the statistical difference in mean levels of various parameters between two groups. Relationships were identified by means of Pearson's bivariate moment correlation coefficient. The results were considered significant at p<0.05. The data were analyzed using SPSS 15.0 Window's version.

Results

Anthropometry

The information on anthropometry, dietary habits of Group-A and Group-B children were given in Table 1. The mean age of children from both the groups was comparable. Whereas mean height (p<0.05) and weight (p<0.01) of Group-A children was significantly low as compared to Group-B. However, mean Body Mass Index (BMI) was comparable between groups. The albumin levels were ranged from 1.18 g/dL to 4.69 g/dL in Group-A, whereas 1.65 g/dL to 4.54 g/dL in Group-B children (Table 1). Mean serum albumin levels in children from Group-A and Group-B were 3.1 ± 0.73 g/dL and 3.5 ± 0.47 g/dL, respectively.

Clinical profile

The information on 'signs and symptoms of anemia, metal toxicity and nutritional deficiency' were given in Table 2. Pale conjunctiva (99%) and fever (61%) was observed among the majority of children from Group-A, whereas no pale conjunctiva was reported in control Group-B. Bald tongue and anorexia was also high among the Group-A children compared to Group-B (Table 2). The signs and symptoms for nutritional deficiencies such as Bitot spot, keratomalacia etc. was not observed except conjunctival xerosis in 3% of Group-A children.

The mean serum albumin levels were significantly (p<0.01) low among Group-A children compared to Group-B (Table 1). Hemoglobin levels were ranged from 5.6 g/dL to 9.9 g/dL and 12.0 g/dL to 15.4 g/dL in Group-A and Group-B children, respectively. The mean Hb levels were significantly (p<0.01) low in Group-A compared to Group-B (Figure 1). A twofold higher reticulocyte count (above 1.0%) was noted in Group-A children than Group-B children. Approximately 40% of children from Group-A had 'microcytic hypochromic condition', whereas no such condition was observed among Group-B children (Table 3).

Table 1: Anthropometry and Dietary Habits of children.

S. No.	Parameter	Group–A (n=77)	Group–B (n=77)
1	Age (Y)	4.4 ± 2.73**	6.6 ± 2.62
2	Height (Cm)	90.2 ± 19.9**	115.4 ± 18.32
3	Weight (kg)	12.3 ± 5.86**	18.8 ± 7.02
4	BMI (Kg/m^2)	14.7 ± 2.66	13.9 ± 2.45
5	Albumin (g/dL)	3.1 ± 0.73**	3.5 ± 0.47
6	Vegetarian	27% (n=21)	5% (n=4)
	Non-Veg. (weekly once)	73% (n=56)	95% (n=73)

Values are mean ± SD; * Significantly different at P<0.05; ** Significantly different at P<0.01

Table 2: Signs & symptoms of anemia, Metal toxicity and Nutritional deficiency.

S. No.	Parameter	Group–A (n=77)	Group–B (n=77)
1	Anorexia	16% (n=12)	5% (n=4)
2	Dryness of throat	4% (n=3)	Nil
3	Metallic Taste	3% (n=2)	Nil
4	Nausea& Vomiting	12% (n=9)	18% (n=14)
5	Abdominal Discomfort	12% (n=9)	18% (n=14)
6	Convulsions	16% (n=12)	27% (n=21)
7	Irritability	3% (n=2)	Nil
8	Fever	60% (n=46)	64% (n=49)
9	Pica Eating	4% (n=3)	Nil
10	Pale conjunctiva	99% (n=76)	Nil
11	Bald Tongue	17% (n=13)	Nil
12	Skin Rashes	9% (n=7)	Nil
13	Conjunctival Xerosis	4% (n=3)	Nil

Values are Percentage of indication

Table 3: Peripheral smear examination and reticount.

S No	Peripheral picture	Group-A (n=77)	Group-B (n=77)
1	Microcytic hypochromic	39% (n=30)	Nil
2	Normocytic normochromic	61% (n=47)	100% (n=77)
	Reticount (%)		
1	Less than 1%	71% (n=55)	84% (n=65)
2	1–1.5%	24% (n=18)	16% (n=12)
3	More than 1.5%	5% (n=4)	Nil

Values are Percentage of indication

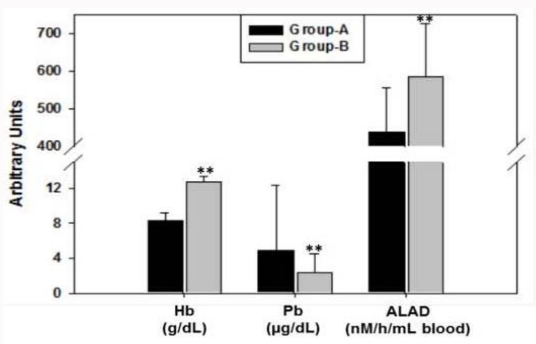

Figure 1: Blood hemoglobin, Pb and ALAD activity levels.

Serum elemental levels

Mean serum elemental levels of the study participant children were given in Figure 2. Serum Fe levels ranged from 10.5 µg/dL to 241.0 µg/dL and 16.0 µg/dL to 269.0 µg/dL in Group-A and Group-B children, respectively. The mean serum Fe levels were significantly (p<0.01) low among the children from Group-A as compared to Group-B (Figure 2). Serum Zn levels ranged from 28.0 µg/dL to 245.0 µg/dL in Group-A, whereas Group-B children had 32.0 µg/dL to 156.0 µg/dL. Mean serum Zn levels were comparable between the groups. Group-A children serum Cu levels were ranged from 36.5 µg/dL to 195.0 µg/dL, whereas Group-B children serum Cu levels were

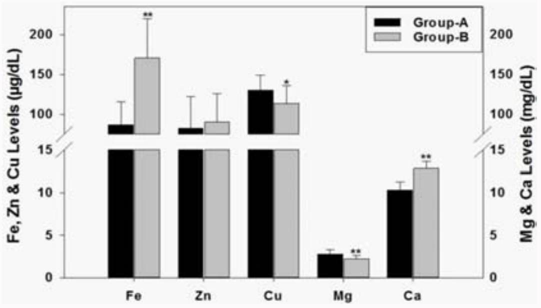

Figure 2: Serum essential element levels.

24.0 µg/dL to 195.0 µg/dL.

Mean serum Cu levels was significantly ($p<0.05$) higher in Group-A children than Group-B (Figure 2). Serum Mg levels were ranged from 1.57 mg/dL to 4.13 mg/dL and 1.19 mg/dL to 3.31 mg/dL in Group-A and Group-B children, respectively. Serum mean Mg levels were significantly ($p<0.01$) higher in Group-A children compared to their counterparts (Figure 2). Group-A children serum Ca levels ranged from 4.8 mg/dL to 15.3 mg/dL, whereas in Group-B it ranged from 9.6 mg/dL to 14.2 mg/dL. A significant ($p<0.01$) low levels of serum Ca was noted in Group-A children compared to their counterpart children (Figure 2).

Blood Pb, Cd levels and ALAD activity

Blood cadmium levels were below the detection limits (0.1 ppb) in children of both the groups. While, the Blood Lead Levels (BLL) varied widely among Group-A children. The BLLs were ranging from 0.15 µg/dL to 39.81 µg/dL in Group-A, whereas in Group-B children were 0.18 µg/dL to 8.14 µg/dL (Figure 1). The mean BLL were 4.9 ± 7.45 µg/dL and 2.4 ± 2.12 µg/dL in Group-A and Group-B children, respectively. Though the mean BLL of Group-A children were significantly ($p<0.05$) higher than Group-B, they were within the cut-off limits specified (<10 µg/dL) by CDC, Atlanta. The ALAD activity of the Group-A and Group-B children were ranged from 52.0 nM/mL to 841.0 nM/mL and 267 nM/mL to 909 nM/mL erythrocytes/hr, respectively. The mean ALAD activity of Group-A children (437.8 ± 215.93 nM/mL erythrocytes/h) was significantly ($p<0.01$) lower than counterparts (583.0 ± 142.07 nM/mL erythrocytes/h) (Figure 1).

Prevalence of deficiency and toxicity

Among Group-A children, moderate anemia was noted in 55% children, whereas, severe anemia in 45% suggesting the need to undergo the blood transfusion. Serum Fe levels were deficient in 42% of Group-A and 5% of Group-B children. However, 47% of children from Group-A and 39% of Group-B children had serum Zn deficiency. About 12% of the children from both the categories were deficient for serum Cu levels. The serum Mg levels were deficient in 4% and 18% of the children from Group-A and Group-B, respectively. Whereas, serum Ca levels were deficient in 7% of children from Group-A, but no such deficiency was observed in their counterpart children.

Ten percent of Group-A children had BLL above 10 µg/dL, the safe cut-off limits set by Centre for Disease Control and Prevention, Atlanta, USA. In addition, 7% of Group-A children had elevated (>20 µg/dL) blood Pb levels. In contrast, the BLL among the Group-B children were within the acceptable limit (<10 µg/dL) of CDC, Atlanta. However, 25% of Group-A and 16% of Group-B children had BLL above 5 µg/dL.

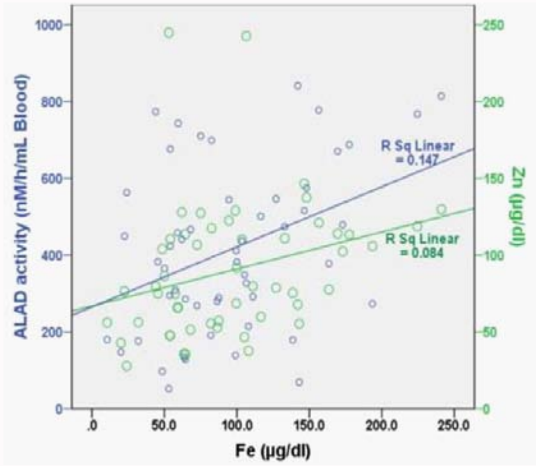

Figure 3: Correlations of Fe with ALAD activity and Zn levels - Group-A children.

Correlations among Pb, Hb and ALAD Activity with serum elements

The correlations among the blood Pb levels, Hb, ALAD activity with serum elemental levels was given in Table 4. Blood Pb levels didn't correlated either with Hb levels, ALAD activity and serum elemental levels except with serum Ca and albumin levels in Group-A. However, Group-A children's serum Fe levels correlated positively with ALAD activity ($p<0.05$), serum Zn and Ca levels ($p<0.01$) (Figure 3). Whereas, a significant ($p<0.01$) inverse correlations was noted between Hb levels and serum Zn levels among Group-A children (Table 4).

Blood Pb levels of Group-B children correlate inversely with serum Zn ($p<0.01$) and Mg ($p<0.05$) levels (Table 4). Nonetheless, the ALAD activity was correlated positively with serum Zn ($p<0.01$) and Mg ($p<0.05$) levels in Group-B children. Whereas, neither Hb nor serum Fe levels have shown correlations with either BLL or serum elemental levels among Group-B children.

Discussion

Iron Deficient Anemia (IDA) is still a devastating public health issue in the third world countries like India. It is well established that the etiology for anemia is a multi-factorial and classified as nutritional deficiencies such as vitamin and minerals, as well as non-nutritional such as infection and hemoglobinopathies. It is noteworthy to mention that the predominant cause of anemia is due to iron deficiency, which could be attributed to inadequate dietary intake or consumption of foods with low amounts of bio-available Fe along with presence of inhibitors such as phytic acid and low intake of animal foods [1,12].

Physicians relies on 'pale conjunctiva and bald tongue' as characteristic 'sign and symptom' of anemia. In the current study, Group-A children were moderate to severe anemic and were undergoing for blood transfusion. Majority of children from Group-A had characteristic feature of pale conjunctiva (>98%) and bald tongue. In addition, significantly lower levels of Hb, serum Fe and albumin levels among the anemic children suggests the magnitude of 'Iron Deficiency Anemia' among Group-A children. As per the WHO criteria of anemia categorization, severe anemia was noted among 45% of children and moderated anemia was 55% in the anemic children. Similarly, the National Family Health

Table 4: Correlations among Hb, ALAD activity, serum elements and Pb.

	Group-A Children									Group-B Children								
	Alb	**Hb**	**ALAD**	**Fe**	**Zn**	**Cu**	**Mg**	**Ca**	**Pb**	**Alb**	**Hb**	**ALAD**	**Fe**	**Zn**	**Cu**	**Mg**	**Ca**	**Pb**
Alb	1	0.038	0.188	0.198	0.109	0.251*	0.511**	0.729**	0.436**	1	-0.147	0.121	0.126	0.357*	0.425**	0.423**	0.854**	-0.071
Hb	0.038	1	-0.16	-0.044	-0.298**	0.196	0.039	-0.221	0.053	-0.147	1	0.255	-0.01	0.058	-0.272	0.104	-0.087	-0.103
ALAD	0.188	-0.16	1	0.369*	0.084	0.13	0.026	0.235	-0.174	0.121	0.255	1	0.03	0.532**	-0.084	0.402*	0.201	-0.065
Fe	0.198	-0.044	0.369*	1	0.340**	0.119	0.1	0.298**	0.019	0.126	-0.01	0.03	1	0.046	-0.099	-0.05	-0.077	0.083
Zn	0.109	-0.298**	0.084	0.340**	1	-0.043	0.112	0.383**	-0.054	0.357*	0.058	0.532**	0.046	1	0.035	0.734**	0.564**	-0.389**
Pb	0.436**	0.053	-0.174	0.019	-0.054	0.107	0.196	0.274*	1	-0.071	-0.103	-0.065	0.083	-0.389**	0.088	-0.325*	-0.151	1

Alb: Albumin; Hb: Haemoglobin; ALAD: Aminolevulenic Acid Dehydratase Activity; *significantly correlated at P<0.05; **Significantly Correlated at P<0.01

Survey of India (NFHS) also reported that 30% of children are moderately anemic and 2% are severe anemia (NFHS-4: 2015-16). This was further corroborated with a 2 fold increase in reticulocyte count (above 1.0%) and 'microcytic hypochromic condition' among anemic children compared to non-anemic children suggest a rapid turnover of the reticulocytes. It is well established fact that a high reticulocyte percentage or count reflects a marrow that is attempting to compensate for red cell destruction, or recovering from anemia [13]. It is noteworthy to mention that, neither the metal toxicity nor other nutritional deficiency 'signs and symptoms' were noted among children of either of the groups.

Recently the growing evidence suggests the association/ relationship exist between 'multiple micronutrients', further deficiency of one micronutrient may influence the levels of other micronutrients [8,14,15]. In the current study, the serum Fe levels were deficient in the anemic children; in addition to this, they were deficient for other essential elements such as serum Zn, Cu, Mg and Ca levels. A decade of studies conducted by ICMR-NIN, Hyderabad in different vulnerable groups such as children, pregnant/ postpartum women and livestock suggest an inter-relationship exist between Fe and Zn along with other essential elements [8,16,17]. Further, it is alarming to note that Zn deficiency is more rampant in this population as compared to Fe deficiency. Trace mineral deficiencies usually occur when dietary intake is inadequate or result from metabolic imbalances produced by antagonistic or synergistic interactions among metals [3].

Plethora of literature suggests that Pb exposure results in hemopoietic toxicity [6,8]. In the current study though the mean BLLs of anemic children were higher compared to non-anemic children, but within specified limits (<10 μg/dL) of CDC, Atlanta. So the anemia among Group-A children could be due to nutritional deficiency. Further, higher mean of BLL in anemic children compared to controls could be due to increased absorption *per se* of Pb under essential elemental deficiency, which could be mediated through Divalent Metal ion Transporter-1 (DMT-1) at the mucosal surface [1,8]. Our previous studies of dietary iron restriction followed by Pb exposure have shown significantly higher BLL suggesting the increased absorption through GI tract under Fe deficient condition [9].

The associations between BLLs, ALAD activity, Hb and serum essential element levels have been considered to illustrate the relationship between different functional parameters [6]. Serum albumin levels are long been considered as an index of health and disease status. In the current study anemic children had hypoalbuminism, which could be malnutrition/altered nutrition or low protein intake by the children. In addition, significant positive correlations were noted between serum albumin levels with serum Cu, Mg, Ca and Pb. It is well established fact that albumin acts as buffering agent and involved in transportation of a variety of substances (ligands) include endogenous substances such as bilirubin and fatty acids, metals and other ions, hormones, as well as exogenous substances such as folic acid and drugs [18,19]. The positive association between albumin and Pb could be to attributed to presence of '-thiol' group containing amino acids in albumin, which has higher affinity towards Pb, and deficiency of essential elementals might have increased the Pb absorption per se [8,14]. In addition, significant inverse relationship between Zn & Hb and positive association of Fe with ALAD and Zn suggests the importance of 'co-factors' for heme synthesis. Similarly, complex interactions were noted between different essential elements in both anemic and non-anemic children infers the interdependency of various elements to perform physiological function. Lack of data on serum ferritin/transferrin levels and MCV are the limitations of current study, whereas the strengths are quantification of various essential elements and heavy metals and assessing their interactions.

Conclusion

The results of present study conclude that anemia among the Group-A children may be iron deficient rather heavy metal induced hematopoietic toxicity. Further, Zn deficiency is more prevalent in children of both the groups, which need intervention strategies. Dual deficiency effect of Fe and Zn on physiological levels and function of δ-ALAD activity needs to be elucidated.

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