Evaluation of Status of Trace and Toxic Metals in Biological Samples (Scalp Hair, Blood, and Urine) of Normal and Anemic Children of Two Age Groups

Faheem Shah · Tasneem Gul Kazi · Hassan Imran Afridi · Naveed Kazi · Jameel Ahmed Baig · Abdul Qadir Shah · Sumaira Khan · Nida Fatima Kolachi · Sham Kumar Wadhwa

Abstract Anemia affects a substantial portion of the world's population, provoking severe health problems as well as important economic losses to the region in which this condition is found. This study was designed to compare the levels of essential trace and toxic elements in scalp hair, blood, and urine samples of anemic children (n = 132) with age range 1–5 and 6–10 years of both genders. For a comparative study, 134 non-anemic age- and sex-matched children as control subjects, residing in the same city, were selected. The metals in the biological samples were measured by flame atomic absorption spectrophotometry/electrothermal atomic absorption spectrometry prior to microwave-assisted acid digestion. The proposed method was validated using certified reference samples of hair, blood, and urine. The results indicated significantly lower levels of iron, copper, and zinc in the biological samples as compared to the control children of both genders (p = 0.01–0.008). The mean values of lead and cadmium were significantly high in all three biological samples of anemic children as compared to non-anemic children of both age groups

F. Shah · T. G. Kazi (✉) · H. I. Afridi · J. A. Baig · A. Q. Shah · S. Khan · N. F. Kolachi · S. K. Wadhwa
National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080, Pakistan
e-mail: tgkazi@yahoo.com

F. Shah
e-mail: shah_ceac@yahoo.com

H. I. Afridi
e-mail: hassanimranafridi@yahoo.com

J. A. Baig
e-mail: jab_mughal@yahoo.com

A. Q. Shah
e-mail: aqshah07@yahoo.com

N. Kazi
Liaquat University of Medical and Health Sciences, Jamshoro 76080, Pakistan
e-mail: tgkazi@yahoo.com

($p = 0.005–0.001$). The ratios of essential metal to toxic metals in the biological samples of anemic children of both age groups were significantly lower than that of controls. Deficiency of essential trace metals and high level of toxic metals may play a role in the development of anemia in the subjects under study.

Keywords Anemia · Essential and toxic metals · Biological samples · Children

Introduction

Anemia is one of the world's most widespread health problems, especially among children: approximately 40% of children are anemic across various African and Asian countries [1]. The World Health Organization (WHO) estimates that more than one third of the world population is anemic. It affects more than 50% of children, <5 years old, and has serious consequences since severe anemia [hemoglobin (Hb) level<5 μg/dL] is associated with an increased risk of death [2]. Although there are many other nutritional and infectious (gastrointestinal) causes of anemia, iron (Fe) deficiency is often a contributory factor in many of these cases [3]. Fe deficiency anemia leads to weakness [4], poor physical growth [5], and delayed psychomotor development [6]. In the past, several studies have shown that Fe deficiency anemia often leads to irreversible impairment of the child's learning ability and other behavioral abnormalities [7]. The neurochemical roles of Fe are not fully understood, but it is clear that low levels of Fe can have a significant adverse impact on brain function since the capacity of blood to deliver oxygen to neuron cells and tissues is thus reduced [8]. Recent macroeconomic estimates suggest that the average impact of Fe deficiency anemia, through both physical and cognitive channels, could be as large as 4% of GDP in less developed countries [9]. A recent study on the prevalence and etiology of nutritional anemia in early childhood in urban areas of Hyderabad, Pakistan (Fig. 1) indicated a high prevalence of anemia and Fe deficiency in 40% of children [10]. Zinc (Zn) deficiency in humans has been found in infants, preschool children, and schoolchildren [11]. Zinc deficiency associates with Fe deficiency anemia in areas where Fe deficiency is a problem [12].

It was investigated that anemia is one of the major manifestations of copper (Cu) deficiency both in animals and human beings [13]. It is essential to maintain Fe homeostasis in the human body; its deficiency leads to anemia and neutropenia [14]. Cu-assisted enzymes are necessary for the utilization of Fe to make hemoglobin, a main component of red blood cells [14]. Recent attention has been directed to the element chromium (Cr); it can improve insulin sensitivity and therefore may be involved in carbohydrate and lipid metabolism [15].

It has been reported that children are extensively vulnerable to the exposure of toxic metals, cadmium (Cd), and lead (Pb) because of their narrow tolerance to these nonessential elements, while nickel (Ni) is required in very trace quantity [16]. This is due to their higher energy requirements, fluid, air, and food intake per unit of body weight, and especially the brain of neonates may increase their Pb absorption [17]. It is biologically plausible that Fe deficiency could lead to higher Cd and Pb levels in children. Controlled animal studies consistently demonstrate higher Pb levels in Fe-deficient animals than in Fe-replete controls [18]. It is also possible that Fe deficiency modifies behavior, increasing pica or hand-to-mouth behavior in children and thereby increasing ingestion exposures to toxic metals in their environment [19]. Absorption of Pb appears to be higher in children who have low dietary iron or calcium (Ca) intakes; thus, dietary insufficiencies, which are

Fig. 1 Map of Pakistan showing the sampling city

not uncommon in lower socioeconomic children, may contribute to their Pb absorption [20, 21].

Cadmium also appears to interact with the process of Fe uptake on the surface of the mucosal cells as well as with the Fe-releasing process at the contra-luminal side [22]. The importance of adequate intake of Zn and Fe in diminishing the effects of Cd has long been recognized [23].

The determination of trace quantities of metals in biological samples requires the use of sensitive and selective techniques such as atomic absorption spectrometry. This technique has a need for solubilization of the analyte and complete or partial decomposition of the matrix using convective systems, microwave ovens, or dry ashing [24, 25]. The main advantage of microwave-assisted samples pretreatment is its requirement of a small amount of mineral acids and a reduction in the production of nitrous vapors [26, 27]. The main advantage of microwave-assisted samples pretreatment is its requirement of small amount of mineral acids and reduction in the production of nitrous vapors [28, 29].

In this study, we evaluate whether Fe, Cu, and Zn deficiency is related to increased level of toxic metals in biological samples (scalp hair, blood, and urine) of anemic and non-anemic children of both gender with age range 1–5 and 6–10 years. The biological samples were prepared by microwave-assisted acid digestion method. Fe, Cu, and Zn concentrations in all biological samples under study were assessed by flame atomic absorption spectrometry, while Cd, Cr, Ni, and Pb were analyzed by electrothermal atomic absorption spectrometry.

Material and Methods

Apparatus

A Pel domestic microwave oven (Osaka, Japan), programmable for time and microwave power from 100 to 900 W, was used for acid digestion of biological samples. A Perkin-Elmer model 700 (Norwalk, CT, USA) atomic absorption spectrometer, equipped with flame burner and graphite furnace HGA-400, pyrocoated graphite tube with integrated platform, an autosampler AS-800, and deuterium lamp was used as background correction system. The energy sources were hollow cathode lamps (Perkin-Elmer) operating at a recommended current. The instrumental parameters are shown in Table 1. About 10 µL of digests and standard solutions and 10 µL of chemical modifier were injected into a graphite tube. Argon (Ar, purity 99.996%) was used as purge gas. Ar flow rate was adjusted to 200 mL/min, while during the atomization step, Ar flow was stopped. The chemical modifier, employed for Cr, Ni, and Pb was 15 µg Mg $(NO_3)_2$, whilst for cadmium, 5 µg Pd as Pd $(NO_3)_2$ was added during each sampling measurement.

Reagents and Standard Solutions

Ultrapure water obtained from ELGA labwater system (ELGA, Bucks, UK) was used throughout the work. All chemicals used were of analytical reagent grade and were supplied by Merck (Darmstadt, Germany). Concentrated nitric acid (approx. 15.8 mol/L) and 30% H_2O_2 were used for all wet acid digestions. Working standard solutions of Cd, Ni, Cr, Fe, Zn, Cu, and Pb were prepared immediately prior to their use by stepwise dilution of certified standard solutions (1,000 ppm) obtained from Fluka Kamica (Buchs, Switzerland), with 0.2 mol/L HNO_3. All solutions were stored in polyethylene bottles at 4°C. For the accuracy of methodology, certified samples of human hair (BCR 397, Brussels®, Belgium), Clincheck control-lyophilized® human urine and human whole blood® (Recipe, Munich Germany) were used. All glassware and plastic materials used were previously soaked for 24 h in 5 mol/L nitric acid, washed with distilled water, and finally rinsed with ultrapure water, dried, and stored in a class 100 laminar flow hoods.

Sample Collection and Pretreatment

An epidemiological cross-sectional survey was conducted among 266 anemic and normal children with age range 1–5 and 6–10 years and living in an urban area of Hyderabad, Pakistan. We visited families personally to inform the parents of 315 anemic and normal children about the aims of the study and request permission for their child to participate at homes, clinics, and hospitals. Complete data were gathered from only 266 children, including 134 normal children and 132 anemic children of two age groups (1–5 and 6–10 years) of both genders (detail was reported in Table 2). A questionnaire was provided to the children's parents to collect information (age, gender, socioeconomic status, food habit). The biochemical tests were carried out in pathorogical laboratories by standard methods. Children selected for the study were randomly assigned to two groups (healthy and anemic) based on the following criteria: the anemic children with Hb concentration of <11.0 µg/dL and the healthy group of children with ≥11.0 µg/dL. The other physical parameters of both groups of anemic and healthy children were obtained by a standard method as shown in

Table 1 Measurement Conditions for Electrothermal Atomization/Flame Ionization AAS 700

For electrothermal atomization AAS

Parameters	Pb	Cd	Cr	Ni
Lamp current (mA)	8.0	6.0	7.5	3.5
Wavelength (nm)	283.3	228.8	357.9	232.0
Slit width (nm)	0.7	0.7	0.7	0.2
Background correction	D_2 lamp	D_2 lamp	D_2 lamp	D_2 lamp
Cuvette	Cup	Cup	Cup	Cup
Dry temperature (°C)	140	140	140	140
Dry time (ramp/hold) (s)	15/5	15/5	15/5	15/5
Ashing temperature (°C)	700	850	1400	1000
Ashing time (ramp/hold) (s)	10/20	10/20	10/20	10/20
Atomization temperature (°C)	1800	1650	2500	2300
Atomization time (ramp/hold) (s)	0/5.0	0/5.0	0/5.0	0/5.0
Cleaning temperature (°C)	2600	2600	2600	2600
Cleaning time (ramp/hold) (s)	1/3	1/3	1/3	1/3
Carrier gas (mL/min)	200	200	200	200
Sample volume (µL)	10	10	10	10

For flame ionization AAS

Elements	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Burner height (mm)	Oxidant (air, L/min)	Fuel (acetylene, L/min)
Zn	214.0	0.7	7.5	7.5	17.0	2.0
Fe	248.5	0.2	7.5	7.5	17.0	2.0
Cu	324.8	0.7	7.0	7.5	17.0	2.0

Table 2 Number of Subjects as Control and Anemic Patients

Age groups	Control groups		Anemic patients	
	Male	Female	Male	Female
1–5	25	22	28	22
6–10	42	38	39	40
Total	67	60	67	62

Table 2. The serum ferritin, hemoglobin, hematocrit, red blood count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were significantly higher in healthy children than in anemic children ($p < 0.01$), while there was no significant difference in the levels of white blood cells (WBC), neutophils, lymphocytes, eosinophils, monocytes, basophils, and platelets ($p < 0.05$). The level of δ-ALAD and ascorbic acid were elevated in anemic children (Table 2). The study protocols were in accordance with the Helsinki Declaration [30]. This research project was evaluated and approved by the Higher Education Commission, Pakistan.

Venous blood samples (3–5 mL) were collected using metal-free Safety Vacutainer blood collecting tubes (Becton Dickinson, Rutherford, NJ, USA) containing >1.5 µg ethylenediaminetetraacetic potassium salt (K_2EDTA/mL) and were stored at −20°C until analysis. All identified cases and controls were asked for a 10- to 100-mL urine spot sample in a wide-mouth jar previously washed with 10% nitric acid and rinsed with deionized water, then urine was transferred into acid-washed 100-mL polyethylene tubes (Kartell, Milan, Italy) with polypropylene lids which were decontaminated before handling. Urine samples were stored at −15°C until they were analyzed. Prior to sub-sampling for analysis, the samples were shaken vigorously for 1 min to ensure a homogeneous suspension. The samples of hair were obtained using stainless steel scissors from the nape of the neck. The scalp hair samples were washed as reported in our previous studies [31, 32]. After washing, hair samples were dried at 80°C for 6 h. Hair samples were put into separate plastic envelopes with an identification number for each participant.

Microwave-Assisted Acid Digestion

A microwave-assisted digestion procedure was carried out in order to achieve a shorter digestion time. Duplicate samples of scalp hair (200 mg) and 0.5 mL of blood and urine samples of each anemic child and the control individuals were directly placed into Teflon PFA flasks. Two milliliters of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, v/v) were added to each flask and kept for 10 min at room temperature, then the flasks were placed in a covered PTFE container. This was then heated following a one-stage digestion program at 80% of total power (900 W). Complete digestion of blood and urine samples required 2–4 min, while 5–8 min was necessary for scalp hair samples. After cooling the digestion flasks, digest samples were filtered through Whatman 42 filter paper, transferred into a 10-mL flask, and brought to volume with ultrapure water. Blank extractions were carried out through the complete procedure. Blanks, standards, and sample solutions were prepared in a similar acid matrix. The validity and efficiency of the microwave-assisted digestion method was checked

with certified values of certified reference materials (CRMs) of all three biological samples and with those obtained from conventional wet acid digestion method (CDM) [33].

The percentage recovery of all metals in CRM samples obtained by microwave-assisted acid digestion (MWD) was calculated according to the following equation:

$$\%Recovery = \frac{[Metals\ obtained\ by\ MWD]}{[Metals\ obtained\ by\ CDM]} \times 100.$$

The percent recoveries varied in the range of 99.1–99.9 for all four analytes under study. Non-significant differences were observed ($p > 0.05$) when comparing the values obtained by MDM and CDM (paired t test; Table 3). The overall recoveries of Cd, Pb, Cr, Ni, Fe, Cu, and Zn in certified biological samples using the microwave digestion method as compared to certified values are 98.10%, 99.18%, 97.98%, 98.27%, 97.64%, 98.70% and 97.77%; 99.07%, 98.9%, 98.2%, 98.6%, 95.2%, 98.65%, and 98.05%; and 98.87%, 97.8%, 98.12%, 99.3%, 98.40%, 97.60%, and 98.63% in CRM whole blood, CRM human urine, and CRM BCR human hair, respectively (Table 4). Mean values for all the metals differed <1–2% from the certified values.

Statistical Analysis

All statistical analyses were performed using computer program Excel X State (Microsoft Corp., Redmond, WA) and Minitab 13.2 (Minitab Inc., State College, PA). Student's t test was used to assess the significance of the differences between concentrations of metals recorded in the biological samples of both anemic and non-anemic children.

Results and Discussion

The results reported in Table 5 show that the concentrations of essential trace metals (Fe, Cu, and Zn) were altered in all three biological samples of anemic children as related to controls. Trace mineral patterns in biological samples provide fruitful data not only as a diagnostic procedure but also in providing answers pertaining to treatment.

The effects of micronutrient deficiencies on a population's health have received great attention in the last three decades [34]. Fe deficiency is probably the most frequent nutritional disorder in the world. A recent estimate based on the WHO criteria indicated that around 600–700 million people worldwide have a marked Fe deficiency anemia and about half the children in developing countries are affected [35]. The resulted data of the metals under study in biological samples (scalp hair, blood, and urine samples) of control and anemic children are shown in Table 5. The results are given as mean values with standard deviation (±SD) for each metal. A significant difference exists among values of each metal in anemic and control children of both groups (unpaired t test). The mean values of iron in the biological samples of anemic children of both age groups were significantly lower as compared to healthy children of matched age groups ($p = 0.01$–0.001). The same trend was observed in female children related to non-anemic females of both age groups. The results indicate the prevalence of Fe deficiency anemia in children of both genders. In the population of developing countries, the amount of Fe absorbed from the diet is not sufficient to meet many individuals' requirements. The consumption of a predominantly cereal-based diet, rich in phytate, oxalate, phosphate, fiber, and other inhibitors of Fe absorption, was the main cause of Fe deficiency diseases. Phytates strongly inhibit Fe absorption in a dose-dependent fashion, and even small amounts of phytates have a marked

Table 3 Clinical and Biochemical Characteristics of Healthy and Anemic Children

Parameters	Male		Female		Normal range
	Normal	Anemic	Normal	Anemic	
1–5 years					
Serum ferritin (µg/L)	32.3±1.7	21.1±2.01	34.2±3.1	19.9±2.5	<30
Hemoglobin (mg/dL)	11.6±0.5	7.7±1.3	12.2±0.6	8.2±0.3	11.5–16.5
Haematocrit (%)	42.7±3.2	26.6±2.2	39.5±3.2	28.7±3.5	35–55
δ-ALAD (µmol PBG/min/L erythrocytes)		23.5±3.6		35.6±5.2	
Ascorbic acid (mg/dL)		1.13±0.35		1.09±0.21	
Red blood count RBC (mm^3)	4.3±0.5	2.7±0.3	4.5±0.6	2.9±0.7	3.5–5.5
MCV (µm)	82.5±4.4	61.2±5.3	85.6±3.8	63.4±3.7	75–100
MCH (pg)	31.4±1.9	19.5±1.3	33.9±1.6	22.4±0.5	25–35
MCHC (g/dL)	34.9±2.3	22.3±1.6	36.6±1.1	21.2±2.6	31–38
WBC (mm^3)	6.8±1.9	8.3±0.3	6.5±2.5	8.1±0.2	3.5–10
Neutrophils (%)	55.2±6.5	63.3±8.2	61.2±9.3	69.2±2.8	35–80
Lymphocytes (%)	27.3±3.9	29.3±3.5	28.7±4.6	33.5±3.7	15–50
Eosinophils (%)	31.5±1.2	27.5±2.3	32.3±3..4	26.5±3.1	15–50
Monocytes (%)	12.9±2.3	11.3±3.2	12.5±2.2	12.1±1.2	2–15
Basophils (%)	13.2±1.3	12.5±2.1	13.6±2.3	12.4±1.5	2–15
Platelets (mm^3)	163.5±26.3	182.4±23.3	143.3±16.2	159.9±12.2	100–400
6–10 years					
Serum ferritin (µg/L)	35.3±3.3	16.5±2.9	37.3±4.4	17.6±6.2	<30
Hemoglobin (mg/dL)	12.3±1.2	7.3±2.3	12.9±1.5	6.3±2.3	11.5–16.5
Hematocrit (%)	45.6±5.6	26.9±3.4	47.7±2.9	24.5±4.1	35–55
δ-ALAD (µmol PBG/min/L erythrocytes)		22.6±3.4		37.6±1.98	
Ascorbic acid (mg/dL)		1.22±0.14		1.25±0.25	
Red blood count RBC (mm^3)	4.6±0.5	2.1±0.3	4.8±0.9	2.4±0.4	3.5–5.5
MCV (µm)	85.6±2.1	53.5±3.4	87.6±3.2	57.4±2.9	75–100
MCH (pg)	33.8±3.1	19.9±2.3	34.3±1.8	23.2±1.4	25–35
MCHC (g/dL)	36.8±1.9	23.4±2.1	37.2±2.3	24.5±2.9	31–38
WBC (mm^3)	7.4±1.8	7.6±1.3	7.1±0.5	7.5±0.7	3.5–10
Neutrophils (%)	57.3±3.4	59.2±5.8	56.9±2.2	62.3±6.6	35–80
Lymphocytes (%)	31.3±2.9	30.5±2.7	34.4±4.8	33.9±3.9	15–50
Eosinophils (%)	36.9±1.5	38.2±2.9	35.2±3.5	37.7±5.3	15–50
Monocytes (%)	12.5±1.4	11.9±2.3	12.3±2.0	12.5±2.1	2–15
Basophils (%)	12.8±1.8	12.3±1.5	12.6±1.7	12.6±1.9	2–15
Platelets (mm^3)	235.2±22.5	258.7±41.5	227.7±30.5	264.5±33.9	100–400

effect [36]. This is especially likely to be true during infancy and childhood when physiological Fe requirements are highest. If the amount of absorbable Fe in the diet cannot be immediately improved, Fe supplement must be included in the diet to control Fe deficiency anemia [37].

Table 4 Determination of Cd, Cr, Ni, Fe, Cu, Zn, and Pb in Certified Samples by CDM and MWD ($N = 10$)

Elements	Conventional digestion method CDM	Microwave digestion method MWD	T value[a]	% recovery[d]	Certified values
Certified sample of whole blood (µg/L)					
Cd	1.21±0.07 (5.78)	1.187±0.09 (7.58)	0.0912	98.10	1.2±0.4
Pb	105.67±8.2 (7.76)	104.8±7.3 (6.96)	0.0012	99.18	105±24
Ni	7.52±0.62 (8.24)	7.39±0.49 (6.63)	0.041	98.27	7.5±1.8
Cr	1.98±0.11 (5.55)	1.94±0.09 (4.64)	0.0039	97.98	2.0±0.5
Fe[e]	14.4±0.98 (6.80)	14.06±1.02 (7.25)	0.037	97.64	14.2±3.24
Cu	13.87±1.02 (7.35)	13.69±0.89 (6.5)	0.72	98.70	13.9±2.7
Zn[e]	2.25±0.09 (4.00)	2.20±0.08 (3.64)	0.091	97.77	2.27±0.06
Certified sample of urine (µg/L)					
Cd	11.83±0.91 (7.69)	11.72±0.85 (7.25)	0.44027	99.07	11.8±2.5
Pb	41.2±1.96 (4.75)	40.77±2.86 (7.01)	0.0038	98.9	41.0±9.9
Ni	11.88±0.87 (7.32)	11.72±0.69 (5.89)	0.0173	98.6	11.9±3.0
Cr	14.57±0.78 (5.35)	14.31±0.93 (6.49)	0.00044	98.2	14.6±3.0
Fe[e]	0.0355±0.0006 (1.69)	0.0338±0.0004 (1.18)	0.997	95.2	0.039±0.01
Cu	54.00±0.7 (1.29)	53.27±0.6 (1.13)	0.41	98.65	56±10
Zn[e]	0.205±0.006 (2.93)	0.201±0.007 (3.48)	0.47	98.05	0.210±0.05
Certified sample of human hair (µg/g)					
Cd	0.53±0.025 (4.72)	0.524±0.024 (4.58)	0.2256	98.87	0.52±0.024
Pb	33.29±1.21 (3.63)	32.56±1.18 (3.62)	0.096	97.8	33±1.2
Ni	46.07±1.41 (3.06)	45.75±1.38 (3.02)	0.9242	99.3	46.0±1.4[c]
Cr	90.94±5.99 (6.59)	89.23±6.53 (7.32)	0.0023	98.12	91.0±10[b]
Fe	575.0±22.0 (3.8)	565.8±24.0 (4.2)	0.00112	98.40	580.0±10[b]
Cu	108.4±6.7 (6.1)	105.8±7.2 (7.3)	0.00049	97.60	110±5[c]
Zn	197.2±12.8 (6.2)	194.5±11.3 (5.7)	0.000034	98.63	199±5

Values in parentheses are RSD

[a] Paired t test between CDM and MWD $df = 9$, T (critical) at 95% C.L. = 2.262, $p<0.50$

[b] Informative value

[c] Indicative value

[d] Percent recovery was calculated according to: $\frac{[MDM]}{CDM} \times 100$

[e] Indicated in milligrams per liter

The concentrations of Zn in anemic children of both age groups and genders were found to be significantly lower in all three biological samples ($p = 0.005$–0.001) as compared to healthy children (Table 5). Zn deficiency may be a contributing factor in anemia [38]. Zn deficiency in developing countries is due to low consumption of meat and fish along with food rich in phytate. Food rich in phytate significantly reduce the absorption of Zn, increasing the chance of Zn deficiency. Socioeconomic status of children was strongly associated with levels of Zn in blood and scalp hair samples, and the lowest concentration of Zn was found in children from the families with low incomes and in children who rarely consumed meat [39, 40].

The low level of Cu was observed in biological samples (blood and scalp hair) of anemic children ($p = 0.002$–0.001), while higher values of Cu were recorded in urine samples as compared to those values of Cu in healthy children of the same age groups. Copper is

Table 5 Trace Element Concentrations in Biological Samples (Scalp Hair, Blood, and Urine Samples) of Normal and Anemic Subjects

Age groups	Male (n = 134)				Female (n = 132)			
	Normal	Anemic	T value *(t_{Exp})	df	Normal	Anemic	T value *(t_{Exp})	df
Iron								
Scalp hair (μg/g)								
1–5	18.9±3.2	13.5±3.0	6.32	51	16.6±2.2	13.8±2.7	3.77	42
Range[a]	13.2–22.5	10.6–16.1			12.5–19.3	11.3–17.5		
6–10	26.2±2.1	18.5±2.3	15.7	79	24.2±3.4	16.6±3.2	10.2	77
Range[a]	24.6–28.3	14.1–18.8			21.2–27.2	13.4–19.7		
Blood (mg/l)								
1–5	496.5±47.2	258.8±52.5	17.3	53	474.9±45.2	267.0±54.4	13.8	42
Range[a]	343–509	287–451			321–476	214–316		
6–10	789.9±65.5	546.0±51.8	18.8	79	709.6±106	574.3±94.3	5.95	76
Range[a]	725–840	495–597			647–814	476–665		
Urine (mg/L)								
1–5	1.6±0.3	0.9±0.3	8.48	52	1.4±0.6	0.95±0.5	3.6	42
Range[a]	1.1–2.2	0.6–1.3			0.9–2.2	0.6–1.4		
6–10	1.9±0.5	0.9±0.37	10.3	77	1.9±0.5	0.9±0.44	9.36	76
Range[a]	1.4–2.4	0.5–1.2			1.4–2.4	0.6–1.4		
Zinc								
Scalp hair (μg/g)								
1–5	189.3±14.5	153.8±15.8	8.53	53	178.4±19.8	149.7±16.5	5.22	42
Range[a]	179–206	126–173			159–201	122–163		
6–10	225.1±11.4	184.0±8.9	18.2	79	230.0±8.9	190.5±7.9	20.7	76
Range[a]	214–236	176–193			224–242	182–199		
Blood (mg/L)								
1–5	19.9±2.06	13.2±1.95	12.1	52	20.9±2.2	13.7±1.73	12.1	42

Range[a]	16.7–23.2	10.2–16.5			17.8–24.8	10.4–15.7		
6–10	11.3±3.1	6.7±2.5	7.37	79	12.6±2.5	7.3±2.23	9.86	76
Range[a]	8.7–13.9	5.3–8.1			10.2–15.1	5.9–8.8		
Urine (mg/L)								
1–5	0.9±0.28	1.8±0.35	10.4	52	0.9±0.33	1.8±0.4	8.14	42
Range[a]	0.7–1.2	1.6–2.2			0.8–1.3	1.7–2.1		
6–10	1.1±0.24	1.7±0.28	10.3	77	0.8±0.5	1.6±0.6	6.41	77
Range[a]	0.7–1.0	1.4–2.0			0.5–1.3	1.2–2.2		
Chromium								
Scalp hair (μg/g)								
1–5	3.5±0.7	2.2±0.69	6.79	52	3.7±1.17	2.3±0.96	4.34	42
Range[a]	3.4–4.1	2.6–3.0			3.7–4.3	2.7–3.2		
6–10	3.8±0.62	2.5±0.71	8.08	78	3.8±0.72	2.6±0.67	7.61	77
Range[a]	3.5–4.2	1.4–1.8			3.01–4.56	2.0–3.2		
Blood (μg/L)								
1–5	88.9±9.4	56.2±10.4	12	53	86.32±9.9	54.2±8.9	11.3	0
Range[a]	72.2–101	45.8–63.2			75.1–91.1	45.9–62.6		
6–10	91.6±8.2	61.5±6.5	18.4	79	86.1±7.2	58.5±6.4	17.9	76
Range[a]	83.9–109.3	55.9–67.2			79.0–93.2	49.1–65.9		
Urine (μg/L)								
1–5	21.6±2.7	14.3±2.8	9.66	53	19.5±1.75	13.6±2.1	10.1	42
Range[a]	18.5–25.6	12.4–16.2			16.5–21.4	10.6–16.4		
6–10	16.4±3.98	9.5±2.9	8.96	77	14.9±3.2	12.7±2.92	3.17	76
Range[a]	12.5–20.3	6.7–12.4			11.7–18.2	9.9–15.5		
Copper								
Scalp hair (μg/g)								
1–5	15.3±3.0	12.3±3.2			14.9±2.64	11.2±3.2		
Range[a]	11.1–18.3	9.9–15.3			12.2–16.8	9.5–12.9		

Table 5 (continued)

Age groups	Male (n = 134)				Female (n = 132)			
	Normal	Anemic	T value *(t_{Exp})	df	Normal	Anemic	T value *(t_{Exp})	df
6–10	17.3±1.15	13.4±0.9	17.1	79	17.28±1.39	12.0±1.4	16.7	78
Range[a]	15.9–18.7	12.5–14.6			15.9–18.67	10.6–13.45		
Blood (mg/L)								
1–5	1.3±0.17	1.02±0.2	5.51	53	1.3±0.46	0.98±0.37	3.58	42
Range[a]	1.11–1.57	0.62–1.08			0.9–1.66	0.69–1.36		
6–10	1.5±0.25	0.83±0.2	13.4	79	1.2±0.23	0.93±0.21	5.41	76
Range[a]	1.29–1.81	0.62–1.038			0.9–1.54	0.83–1.036		
Urine (mg/L)								
1–5	0.2±0.037	0.23±0.048	6.83	52	0.14±0.031	0.24±0.026	11.6	43
Range[a]	0.16–0.225	0.135–0.36			0.11–0.20	0.18–0.29		
6–10	0.15±0.066	0.26±0.078	6.83	77	0.16±0.042	0.24±0.051	7.58	76
Range[a]	0.13–0.17	0.185–0.36			0.12–0.22	0.18–0.33		
Nickel								
Scalp hair (µg/g)								
1–5	4.6±1.6	3.4±2.1	3.59	52	4.2±1.32	3.2±1.57	3.59	43
Range[a]	3.02–6.4	1.72–5.3			2.9–6.7	2.6–4.2		
6–10	6.9±1.25	5.9±1.0	3.99	79	6.9±2.02	4.9±1.86	4.54	77
Range[a]	5.4–8.5	4.2–6.8			4.9–9.0	2.6–6.2		
Blood (µg/L)								
1–5	0.9±0.38	0.72±0.11	3.86	52	0.88±0.35	0.73±0.4	3.62	42
Range[a]	0.8–1.4	0.7–1.1			0.7–1.2	0.65–1.0		
6–10	1.4±0.5	1.1±0.4	4.29	79	1.3±0.3	1.0±0.33	3.77	76
Range[a]	0.9–1.9	0.7–1.2			0.8–1.7	0.7–1.4		

Urine (μg/L)								
1–5	3.4±0.62	3.0±0.78	3.69	52	2.9±0.73	2.3±0.87	3.55	43
Range[a]	2.5–4.5	2.28–3.5			2.1–3.7	1.7–3.1		
6–10	3.6±0.53	3.1±0.39	6.8	77	3.2±0.53	2.95±0.48	6.1	76
Range[a]	3.1–4.4	2.6–4.2			2.7–3.8	2.3–3.4		
Cadmium								
Scalp hair (μg/g)								
1–5	1.11±0.62	1.74±0.57	3.84	51	1.3±0.5	1.9±0.6	3.6	42
Range[a]	0.5–1.5	1.5–2.3			1.0–2.1	1.7–2.4		
6–10	2.1±0.65	2.7±0.52	4.6	79	1.5±0.7	2.1±0.65	3.92	77
Range[a]	1.9–2.2	2.4–2.9			0.9–2.5	1.7–2.34		
Blood (μg/L)								
1–5	3.3±0.79	4.2±0.85	3.99	53	2.7±0.54	3.7±0.66	5.5	42
Range[a]	2.5–4.3	3.7–5.1			2.1–3.5	3.0–4.4		
6–10	3.9±0.77	4.6±0.85	3.87	79	3.5±0.75	4.1±0.92	4.01	76
Range[a]	3.2–4.7	3.8–5.6			2.8–4.2	3.22–5.1		
Urine (μg/L)								
1–5	1.2±0.56	2.2±0.73	5.63	52	0.8±0.35	1.6±0.42	6.86	43
Range[a]	0.5–1.8	2.0–3.0			0.4–1.2	1.2–2.1		
6–10	1.4±0.6	2.5±0.7	7.57	77	1.2±0.4	1.8±0.5	5.87	76
Range[a]	0.8–2.1	1.9–3.2			0.9–1.7	1.3–2.5		
Lead								
Scalp hair (μg/g)								
1–5	4.6±0.83	6.8±0.78	9.91	51	4.2±1.12	6.5±0.89	7.54	42
Range[a]	3.9–5.72	5.9–7.72			3.1–5.33	5.36–7.41		
6–10	7.2±2.14	13.1±1.68	13.9	79	6.4±1.7	12.5±2.04	14.4	77
Range[a]	4.9–9.3	11.4–14.9			4.6–8.3	10.4–14.6		

Table 5 (continued)

Age groups	Male (n = 134)				Female (n = 132)			
	Normal	Anemic	T value *(t_{Exp})	df	Normal	Anemic	T value *(t_{Exp})	df
Blood (μg/L)								
1–5	132.3±23.5	152.6±25.4	3.56	53	123.2±21.2	149.8±25.6	3.75	42
Range[a]	120–166	128–178			112–159	110–164.2		
6–10	144.7±18.9	168.8±20.5	5.49	79	148.9±12.9	169.8±16.2	6.32	76
Range[a]	131–164	145–186			134–164	142–182		
Urine (μg/L)								
1–5	41.9±5.7	54.2±7.3	6.87	52	40.8±4.8	54.5±5.9	8.45	42
Range[a]	36.6–52.0	46.4–63.5			37.7–45.2	48.6–60.5		
6–10	48.2±6.5	62.4±7.7	8.93	76	45.3±5.1	57.4±6.2	9.43	76
Range[a]	41.9–54.7	54.8–71.2			40.5–50.2	50.9–63.8		

$t_{critical}$ at 95% confidence limit for corresponding degree of freedom at 42, 43, 51, 52, 53, 77, 78, and 79 are 2.018, 2.017, 2.008, 2.007, 2.006, 1.992, 1.991, and 1.991, respectively

df degree of freedom

[a] Minimum–maximum values

required for normal infant development, red and white blood cell maturation, Fe transport, bone strength, cholesterol metabolism, myocardial contractility, glucose metabolism, brain development, and immune function [41]. A deficiency of either Fe or Cu will result in anemia, namely, Fe deficiency anemia or Cu deficiency anemia. Copper is essential for the functioning of many Cu-dependent enzymes [42] such as ceruloplasmin (responsible for antioxidant protection, Fe metabolism, and Cu transport), and it was established that the anemia appears to be related to defects in Fe mobilization due to the combined defect of both red ceruloplasmin ferroxidase activity and intracellular utilization [43]. Cu is a major component of catalytic centers of different redox enzymes, and thus, its presence is essential for normal physiologic function such as cellular respiration, free radical defense, synthesis of melanin pigment, connective tissue biosynthesis, and cellular Fe metabolism [44]. Infants fed a high Fe formula absorbed less Cu than infants fed a low Fe formula, suggesting that high Fe intakes may interfere with Cu absorption in infants [45]. Starvation and muscle catabolism increase Zn and Cu losses in urine, which seems to be especially sensitive to low Cu and ceruloplasmin levels and anemia. About 95% of the Cu in the blood is bound to ceruloplasmin. These enzymes play a role in the regulation of Fe metabolism. In Fe deficiency anemia, the liver excretes excess Cu into the bile, and it is removed from the body in the feces and urine [46].

The concentrations of Cr in scalp hair, blood, and urine were found to be significantly lower in anemic patients of both genders when compared to control subjects of matched age groups ($p = 0.003–0.01$; Table 5). Chromium deficiency results in impaired glucose tolerance, hyperglycemia, and glycosuria that cannot be controlled with insulin [47]. Studies on humans have established that premature infants, and those with evidence of intrauterine growth retardation, have significantly lower hair and blood Cr status as compared to infants born full term. These findings indicate that Cr is indeed an essential trace element during children's growth and development [48, 49]. Malnourished children responded promptly to Cr supplementation as reported in a previous study [50].

The mean concentration of Ni was found to be lower in biological samples of anemic patients of both sexes and age groups as compared to healthy children (Table 5), but the difference was not significant ($p > 0.05$). Deficiency in Ni leads to health problems such as anemia and deformation of leg bones [51]. It was reported that the degree of Fe deficiency was relatively moderate, but a more generalized anemia occurred in Fe deficiency in the absence of nickel chloride (NiCl). Nickel Chloride perhaps due to its ability to change Fe absorption, required to maintain bone marrow metabolism and to increase ceruloplasmin activity, inhibited the alteration on hemoglobin synthesis. Furthermore, NiCl possibly for its action on Cu content and Cu–Zn superoxide dismutase activity, inhibits the shortening of red cells life span caused by superoxide radicals [52].

The mean levels of Cd and Pb in the biological samples of non-anemic male children from the age groups 1–5 and 6–10 years were found to be significantly lower than those recorded in anemic children ($p = 0.003–0.001$ and $p = 0.002–0.001$, respectively). In females, the same pattern was recorded. It was observed that the levels of Cd and Pb were higher in older children of both genders because they are of school age and subsequently were involved in other outdoor activities, so the environmental exposures have increased. Literature survey shows that Cd may interact with Fe metabolism [53]. It was further shown in rats that a divalent metal transporter in the duodenum has affinity both for Fe and for Cd, thus allowing for competition between the essential and the toxic elements [54]. Thus, after the experimental depletion of Fe to rats, retention of Cd [55] and the Cd content in the liver [56] have increased. An in situ experiment also proved that Cd

retention in the intestine of rats increased after a period of Fe deficiency [57], and the Cd-induced inhibition of Fe absorption was also observed in an experiment with perfused mouse duodenum [58].

It is biologically plausible that Fe deficiency could lead to higher Pb levels in children. Controlled animal studies consistently demonstrate higher lead levels in Fe-deficient animals than in Fe-replete controls [18]. The mechanism for enhanced absorption is likely to be the substitution of Fe^{+2} with Pb^{+2} and increased active transport into the body [59]. Similarly, it is possible that Pb^{+2} may occupy vacant Fe^{+2} sites in the hematopoietic system, thereby reducing lead excretion. Clinical studies of chelation therapy suggest that Fe-deficient children may retain more lead in their bodies [60]. It is also possible that Fe deficiency modifies behavior, increasing pica or hand-to-mouth behavior in children and thereby increasing ingestion exposures to lead in their environment [19].

The population-based probabilities at 95% confidence intervals for anemic and healthy children of both age groups and genders were calculated at different probabilities ($p=0.05$, 0.01, and 0.001). The unpaired Student's t test revealed 42, 43, 51, 52, 53, 77, 78, and 79 probabilities for understudy children based on age, sex, and elemental contents. Our calculated t value exceeds these, so the difference between our means of normal and anemic children for different elemental content showed significant differences ($p = 0.01$–0.001).

Fe/Pb and Fe/Cd ratio were lowered in the scalp hair, blood, and urine samples of anemic children as compared to the normal subjects of both genders in age groups 1–5 and 6–10 years (Table 6). In non-anemic children, the correlation between Fe/Pb and Fe/Cd was better compared to the correlation values obtained in anemic children. Experimental studies intended to show that the low Fe concentrations were demonstrated in the hair and blood of anemic children, and it was also reported that the concentration of Zn in anemic children was significantly decreased ($p>0.001$) when compared with those of age- and gender-matched controls.

Table 6 Mole Ratio in Between Fe/Cu, Fe/Pb, and Fe/Cd in Biological Samples of Normal vs. Anemic Children of Both Genders in Two Age Groups

Gender	Age group	Fe/Pb		Fe/Cd	
		Normal	Anemic	Normal	Anemic
Scalp hair	1–5	15.2	7.4	34.3	15.6
Male	6–10	13.5	4.6	25.1	12.2
Female	1–5	14.7	7.9	25.7	14.6
	6–10	14.0	4.9	32.5	15.9
Blood	1–5	13,923	6,292	302,795	124,010
Male	6–10	20,252	12,247	407,615	238,879
Female	1–5	14,301	5,405	353,982	112,756
	6–10	17,680	13,168	417,571	276,507
Urine	1–5	142	61.6	2683	823
Male	6–10	146.2	53.5	2731.3	724
Female	1–5	127	54.5	3522	1006
	6–10	156	58.2	3186	647

Conclusion

It was concluded that deficiency of essential trace metals and high level of toxic metals co-occurred with the anemic condition of children. The present study revealed that among other pathophysiological tests, regular monitoring of toxic metals in biological/clinical specimens of anemic children plays an important part in identifying possible sources of contamination/intoxication. Children are particularly susceptible to iron deficiency due to their increased Fe needs for rapid growth and the relatively low iron content of their diets due to poverty and cereal-based diet. It is necessary to add iron via food supplements. The results of this study provided guidance to clinicians and other professional investigating the deficiency of essential trace metals and excessive level of toxic metals in biological samples of healthy and anemic children.

References

1. Hall A, Miguel E (2001) Anemia in schoolchildren in eight countries in Africa and Asia. Public Health Nutr 4(3):749–756
2. Brabin BJ, Premji Z, Verhoeff F (2001) An analysis of anemia and child mortality. J Nutr 131(2S-2):636S–645S
3. Lanzkowsky P (1995) Pediatric hematology and oncology, 2nd edn. Churchill Livingstone, New York
4. Haas JD, Brownlie TT (2001) Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. J Nutr 131:676S–688S
5. Grantham-McGregor S, Ani C (2001) A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr 131:649S–666S
6. Sherriff A, Emond A, Bell JC, Golding J (2001) Should infants be screened for anaemia? A prospective study investigating the relation between haemoglobin at 8, 12, and 18 months and development at 18 months. Arch Dis Child 84:480–485
7. Booth IW, Aukett MA, Booth W, Aukett MA (1997) Iron deficiency anaemia in infancy and early childhood. Arch Dis Child 76(6):549–554
8. Halterman JS, Kaczorowski JM, Aligne CA, Auinger P, Szilagyi PG (2001) Iron deficiency and cognitive achievement among school-aged children and adolescents in the United States. Pediatrics 107:1381–1386
9. Horton S, Ross J (2003) The economics of iron deficiency. Food Policy 28(1):51–75
10. Afridi HI, Kazi TG, Kazi GH (2005) Evaluation of status of trace and toxic metals in scalp hair samples of normal and anemic human subjects. American Biot Lab 23(8):27
11. Castillo-Duran C, Garcia H, Venegas P (1994) Zinc supplementation increases growth velocity of male children and adolescents with short stature. Acta Paediatr 83:833
12. Singh S, Ravishanker R, Singhi P, Nath R (2003) Low plasma zinc and iron in pica. Indian J Pediatr 70(2):139–143
13. Percival SS (1995) Neutropenia caused by copper deficiency. Nutr Rev 53:59–66
14. Tammura H, Hirose S, Watanave O (1994) Anemia and neutropenia due to copper deficiency in enternal nutrition. J Parenter Enteral Nutr 18:185–189
15. Lukaski HC (1999) Chromium as a supplement. Annu Rev Nutr 19:279–302
16. Soylak M, Saraçoglu S, Tüzen M, Mendil D (2005) Determination of trace metals in mushroom samples from Kayseri, Turkey. Food Chem 92:649–652
17. Mendil D, Unal OF, Tuzen M, Soylak M (2010) Determination of trace metals in different fish species and sediments from the River Yesilirmak in Tokat, Turkey. Food Chem Toxicol 48:1383–1392
18. Eden AN, Mir MA (1997) Iron deficiency in 1- to 3-year old children. A pediatric failure? Arch Pediatr Adolesc Med 151:986–988
19. Federman DG, Kirsner RS, Federman GS (1997) Pica: are you hungry for the facts? Conn Med 61:207–209

20. Park JD, Cherrington NJ, Klaassen CD (2002) Intestinal absorption of cadmium is associated with divalent metal transporter 1 in rats. Toxicol Sci 68(2):288–294
21. Balajewicz AH, Pietrzik JJ, Schlegel-Zawadzka M, Piatkowska E, Zachwieja Z (2001) The influence of lead and cadmium environmental pollution on anthropometric health factors in children. Przegl Lek 58:315–324
22. Mahaffey KR, Annest JL (1986) Association of erythrocyte protoporphyrin with blood lead level and iron status in the second national health and nutrition examination survey, 1976–1980. Environ Res 41:327–338
23. Hammad TA, Sexton M, Langenberg P (1999) Relationship between blood lead and dietary iron intake in preschool children. A cross-section study. Ann Epidemiol 6(1):30–33
24. Soylak M, Karatepe AU, Elçi L, Dogan M (2003) Column preconcentration/separation and atomic absorption spectrometric determinations of some heavy metals in table salt samples using amberlite XAD-1180. Turk J Chem 27:235–242
25. Afridi HI, Kazi TG, Kazi N et al (2009) Determination of copper and iron in biological samples of viral hepatitis (A–E) female patients. Biol Trace Elem Res 129:78–87
26. Soylak M, Elci L, Dogan M (1999) Flame atomic absorption spectrometric determination of cadmium. Cobalt, copper, lead and nickel in chemical grade potassium salts after an enrichment and separation procedure. J Trace Microprobe Tech 17:149–156
27. Kazi TG, Memon AR, Afridi HI et al (2008) Determination of cadmium in whole blood and scalp hair samples of Pakistani male lung cancer patients by electro thermal atomic absorption spectrometer. Sci Total Environ 389:270–276
28. Kazi TG, Jalbani N, Baig JA et al (2009) Determination of toxic elements in infant formulae by using electrothermal atomic absorption spectrometer. Food Chem Toxicol 47:1425–1429
29. Afridi HI, Kazi TG, Kazi N et al (2009) Status of essential trace metals in biological samples of diabetic mother and their neonates. Arch Gynecol Obstet 280:415–423
30. Williams JR (2008) The Declaration of Helsinki and public health. Bull World Health Organ 86:650–651
31. Afridi HI, Kazi TG, Kazi GH et al (2006) Analysis of heavy metals in scalp hair samples of hypertensive patients by conventional and microwave digestion methods. Spectrosc Lett 39:203–214
32. Kazi TG, Afridi HI, Kazi GH et al (2006) Evaluation of essential and toxic metals by ultrasound-assisted acid leaching from scalp hair samples of children with macular degeneration patients. Clin Chim Acta 369(1):52–60
33. Kazi TG, Afridi HI, Kazi N et al (2008) Distribution of zinc, copper and iron in biological samples of Pakistani myocardial infarction (1st, 2nd and 3rd heart attack) patients and controls. Clin Chim Acta 389 (1–2):114–119
34. Gomber S, Kumar S, Rusia U, Gupta P, Agarwal KN, Sharma S (1998) Prevalence and etiology of nutritional anaemia in early childhood in an urban slum. Indian J Med Res 107:269
35. Oski FA (1993) Iron deficiency in infancy and childhood. N Engl J Med 329(3):190–193
36. Hallberg L, Brune M, Rossander L (1989) Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. Am J Clin Nutr 49:140–144
37. Lozoff B, Jimenez E, Wolf W (1991) Long-term developmental outcome of infants with iron deficiency. N Engl J Med 325:687–693
38. Sondstrom B (1990) Effect of inositol hexaphosphate on retention of zinc and calcium from the human colon. Eur J Clin Nutr 44:705–708
39. Brown KH, Wuehler SE, Peerson JM (2001) The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. Food Nutr Bull 22:113–125
40. Isbir T (1997) Mean zinc contents of serum, hair, erythrocytes, and urine of 32 children. Trace Elem Electrolytes 14(2):87–90
41. L'Abbe MR, Friel JK (1992) Copper status of very low birth weight infants during the first 12 months of infancy. Pediatr Res 32:183–188
42. Larsson S, Kallebring B, Wittung P, Malmstrom BG (1995) The Cu A center of cytochrome-c oxidase: electronic structure and spectra of models compared to the properties of Cu A domains. Proc Natl Acad Sci 92:7167–7171
43. Tapiero H, Townsend DM, Tew KD (2003) Trace elements in human physiology and pathology. Copper. Biomed Pharmacother 57(9):386–398
44. Gacheru N, Trackman PC, Shah MA (1990) Structural and catalytic properties of copper in lysyl oxidase. J Biol Chem 265(31):19022–19027
45. Linder MC, Hazegh-Azam M (1996) Copper biochemistry and molecular biology. Am J Clin Nutr 63 (5):797S–811S
46. Abdelrahim II, Mahgoub HM, Mohamed AA et al (2009) Anaemia, folate, zinc and copper deficiencies among adolescent schoolgirls in Eastern Sudan. Biol Trace Elem Res 132:60–66

47. Jeejeebhoy KN (1999) Chromium and parenteral nutrition. J Trace Elem Exp Med 12(2):85–89
48. Barnes B, Bradley SG (1994) Planning for a healthy baby: essential reading for all future parents. Vermillion, London
49. Payne DL, Adeleye B, Hunt DJ, Stoecker BJ (1996) Uptake and retention in suckling rats of chromium fed with human milk or infant formulas. Biol Trace Elem Res 53:1–6
50. WHO (1996) Trace elements in human nutrition and health. World Health Organization, Geneva
51. Nielson F (1996) Other trace elements. In: Ziegler EE, Filer LJ (eds) Present knowledge in nutrition. ILSI, Washington, pp 353–377
52. Arnich N, Cunat L (2004) Comparative in situ study of the intestinal absorption of aluminum, manganese, nickel, and lead in rats. Biol Trace Elem Res 99(1–3):157–171
53. Goyer RA (1997) Toxic and essential metal interactions. Annu Rev Nutr 17:37–50
54. Park JD, Cherrington NJ, Klassen CD (2002) Intestinal absorption of cadmium is associated with divalent metal transporter 1 in rats. Toxicol Sci 68:288–294
55. Schaefer SG, Schwegler U, Schuemann K (1990) Retention of cadmium in cadmium-naive normal and iron-deficient rats as well as in cadmium-induced iron-deficient animals. Ecotoxicol Environ Saf 20:71–81
56. Tandon SK, Khandelwal S, Jain VK, Matthur N (1994) Influence of dietary iron deficiency on nickel, lead and cadmium intoxication. Sci Total Environ 148:167–173
57. Ohta H, Cherian MG (1995) The influence of nutritional deficiencies on gastrointestinal uptake of cadmium and cadmium metalothionein in rats. Toxicology 97:71–80
58. Iturri S, Nunez MT (1998) Effect of copper, cadmium, mercury, manganese and lead on Fe^{2+} and Fe^{3+} absorption in perfused mouse intestine. Digestion 59:671–675
59. Sargent JD, Stuket TA, Dalton MA, Freeman JL, Brown MJ (1996) Iron deficiency in Massachusetts communities: socioeconomic and demographic risk factors among children. Am J Public Health 86:544–550
60. Ruff HA, Markowitz ME, Bijur PE, Rosen JF (1996) Relationships among blood lead levels, iron deficiency, and cognitive development in two-year-old children. Environ Health Perspect 104:180–185