Determination of heavy elements levels in blood of children with anemia living at Al- Najaf City.

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الملخص

تهدف هذه الدراسة إلى تقييم مستويات كل من Fe ،Pb, Mn , Cu Se, Zn في دم الأطفال المصابين بفقر الدم والذين يسكنون في مركز مدينة النجف، كمؤشر على التلوث البيئي في هذه المدينة.تضمنت الدراسة خمسة وستين طفل بمعدل عمر(7.8±0.5)سنة منهم 34 فتاة و31صبي مصابين بفقر الدم و29 فتاة و28 صبي بأعمار مقاربة من الأصحاء.لوحظ أنة 35 طفل (16 فتاة و19 صبي) مصابين بفقر الدم من نوع نقص الحديد (IDA) و30 طفل (15 فتاة ، 15صبي) مصابين بفقر الدم فقط.لقد وجد ان هنالك فرقا معنويا في نسبة الهيموكلوبين ومكداس الدم ومعدل حجم كريات الدم الحمراء (P<0.01) وكذلك الفرتين (P<0.001) في مجموعة IDA من الفتيات والصبيان عند مقارنتهم مع مجاميع السيطرة. تشير النتائج إلى أن هنالك أرتفاع معنوي في مستويات Pb , Mn ، Znالمصلية في مجموعة IDA في الفتيات والصبيان عند مقارنتهم مع مجاميع السيطرة الخاصة بهم. بينما مستويات Cu, Se فقد سجلت أنخفاضا معنويا في مجاميع IDA ، OA في الفتيات والصبيان وعند مقارنتهم مع مجاميع السيطرة الخاصه بهم. نستنتج من ذلك أن الأطفال المصابين بفقر الدم من نوع نقص الحديد كانوا أكثر تحسسا للتلوث من الأطفال الأصحاء. دمائهم كانت مؤشر لما يحويه جسمهم من المعادن الفعالة بيولوجيا عاكستا مدى التداخل بين التلوث الجوي والسكان.

Abstract

This study aimed to determine Fe ,Pb , Mn ,Se, Cu ,and Zn levels in the blood of children with anemia , living at Al- Najaf City ,as indicators of environmental pollution in this city. Sixty five children (mean age 7.8 ±0.5) including 34 girls , 28 boys having anemia and the ages matched healthy children 29 girls and 28 boys ,were studied .We observed iron deficiency anemia (IDA) in 35 child (16 girls ,19 boys) ,and only anemia (OA) in 30 child (15 girls ,15 boys). It was found that significant difference of Hb, PCV ,MCV (P<0.01) , and ferritin(P<0.001) values in IDA patients (both girls and boys)were found, when compared with healthy children. The results show that there are significantly higher levels of Pb , Mn , and Zn ,in the serum of children in (IDA) group(both girls and boys) in comparison with their control groups . While Se , and Cu levels were significantly lower in (OA) and(IDA) groups , girls and boys, as related to their control groups. In conclusion ,children with iron deficient anemia are more sensitive to the pollutants and more at risk as compared to healthy children . Their bloods are considered as an index of biologically active metals in the body, reflecting direct interaction of atmospheric pollution of a population.

Introduction

The increasing pollution with many heavy metals which are harmful for the growth of the living organisms has been the subject of considerable interest [1-6].The development of the industry and expansion of the chemical compounds used in different branches of industry are leading to the environmental spread of heavy metals[7]. To many people, heavy metal pollution is a problem associated with areas of intensive industry. However, roadways and automobiles now are considered to be one of the largest sources of heavy metals. Zinc, copper, and lead are three of the most common heavy metals released from road travel, accounting for at least 90 of the total metals in road runoff [8]. Lead concentrations, however, consistently have been decreasing since leaded gasoline was discontinued. Smaller amounts of many other metals, such as nickel and cadmium, are also found in road runoff and exhaust. About half of the zinc and copper contribution to the environment from urbanization is from automobiles. Brakes release copper, while tire wear releases zinc. Motor oil also tends to accumulate metals as it comes into contact with surrounding parts as the engine runs, so oil leaks become another pathway by which metals enter the environment. On the road surface, most heavy metals become bound to the surfaces of road dust or other particulates[9] . During precipitation, the bound metals will either become soluble (dissolved) or be swept off the roadway with the dust. In either case, the metals enter the soil or are channeled into a storm drain. Whether in the soil or aquatic environment, metals can be transported by several processes. These processes are governed by the chemical nature of metals, soil and sediment particles, and the pH of the surrounding environment. Most heavy metals are cations, meaning they carry a positive charge. Zinc and copper, for instance, both carry a 2+ charge. Soil particles and loose dust also carry charges. Most clay minerals have a net negative charge[10]. Soil organic matter tends to have a variety of charged sites on their surfaces, some positive and some negative. The negative charges of these various soil particles tend to attract and bind the metal cations and prevent them from becoming soluble and dissolved in water. The soluble form of metals is thought to be more dangerous because it easily is transported and more readily available to plants and animals [11]. By contrast, soil bound metals tend to stay in place. Metals level and concentration of other elements in organ and animal tissues are indicative of the overall body chemical and mineral status.

The homeostasis of a particular mineral involves different mechanisms depending on the organ involved ; with participation of body tissue fluids, subject to modulation and high mobilization .The study of biological fluids and other materials, such as blood plasma and fecal material ,is relevant as function indicative of underlying normal biochemical possesses, living conditions and potential diagnostic tool to

identify a disease [12] .The levels of metals in the blood is considered as an index of biologically active metal in the body , reflecting also the environmental exposure of population . It has been determined that humans need nearly 72 trace elements, including very low concentration of heavy metals ,such as Cu ,Sn ,Cr ,Mn, Mo and Co .Most metals are toxic at high concentration while other provoke deleterious effects at low concentrations [13]. Cd , Pb, and V are pollutants of biological interest due to their biotoxicity [14,15] . This study aimed to determine Fe ,Pb , Mn ,Se, Cu ,and Zn levels in the blood of children with anemia , living at Al- Najaf City ,as indicators of environmental pollution in this City.

Materials and Methods

The research was performed on 65 of children (mean age 7.8± 0.5 year) living at Al- Najaf City Center, including 34 girls ,31 boys having anemia ;57 and aged matched healthy children (29 girls , 28 boys). Approximately 5 ml blood samples were taken from each child. Using disposable syringes and needles and placed into clean plastic tube without anticoagulants. Information was recorded about each child through taking samples. The number of red blood cells (RBC),hemoglobin concentration (Hb) ,mean corpuscular volume (MCV), pack cell volume(PCV) and ferritin ,of whole blood samples were measured according to :

- **RBC number :by using the RBCs counter and diluted solution(Hymes´ fluid)[16].**
- **Hb determination: hemoglobin concentration was analyzed colorimetrically by the cyanomethmoglobin Methods [17].**
- **MCV: the volume(in femtoliters ,fL)of the average circulating red blood cell were calculated manually, as follows: MCV(fL)=10*PCV(percent)/RBC(million/µL).[18]**
- **Ferritin determination: ferritin enzyme immunoassay test kit catalog number BC-1025, BioCheck ,Inc. 837:Cowan Rd.Burlingame,CA 49010.USA.**
- **Packed cell volume Measurement: Capillary tubes, micro centrifuge, and hematocrit reader were used to determine PCV value [19].**

Then the serum was separated with in 15 min of sample collection by centrifuged at 3000 rpm for 10 min ,for Fe ,Pb , Mn ,Se, Cu ,and Zn determination ,were preserved in 20°C until the time of analysis.The studied heavy metals were measured on an atomic absorption spectrophotometer (PYE-UNICUM SP-9) [20].

Statistical Analysis

Mean values of all data, standard deviation of means, and differences between groups were analyzed by *t*-test [21].

Results

The study involved 65 patients (34 girls, 31 boys), their ages matched healthy child age, weight, duration of disease for all samples expressed as mean ± standard deviation. There were no statistical significances observed in number ,age between patients (girls and boys) and control group while there are significant differences(P<0.001) in weight in patient groups (girls and boys)and control group ,as in (Table 1). Number of red blood cells (RBC),hemoglobin concentration (Hb) ,mean corpuscular volume (MCV), pack cell volume(PCV) ,and ferritin, were measured in patients ,and healthy controls .According to ferritin and Hb values, patients groups(both girls and boys)were subdivided into iron deficiency anemia (IDA) ,when ferritin <0.01 µg/dL and only anemia(ferritin normal ,low Hb)[8] . It was found significant difference of Hb, PCV ,MCV (P<0.01)was found , and ferritin(P<0.001) values in IDA patients (both girls and boys) , when compared with healthy children . On the other hand , no significant variations (except Hb)were indicated in the levels of this parameters in the group of OA (both girls and boys) ,with respect to their control groups (Table 2) . The mean ± SD levels of Fe ,Pb , Mn ,Se, Cu ,and Zn in serum sample of all groups included in (Table 3). In the present study, we found significant elevation in the serum levels of Pb ,Zn(P<0.01), and Mn (P<0.05) in IDA group (both girls and boys) with respect to their control groups . Whereas Fe, Se, and Cu show significant decline (P<0.005) for Fe in girls ,(P<0.001)for boys. However, P value for Se in girls were< 0.01 , <0.05 for boys . And (<0.05) for Cu in girls and boys. The data (Table 3) show that Se was significantly(P<0.01for girls ,and P<0.05 for boys) decreased in OA groups with respect to their control groups. However ,any difference was not observed for serum Fe ,Pb , Mn , Cu, and Zn levels.

Table 1:Age and sex wise distribution in different blood samples .

Sex	Group	Number	Age (years) (Mean±SD)	Weight (Kg) (Mean±SD)	Duration of disease (years) (Mean±SD)
Girls	Patients	34	7.9± 0.23	21.5 ±1.8	2.4 ±0.45
	Control	29	7.8 ±0.25	27.4 ± 2.1	
P(value)			N.S.	P<0.001	
Boys	Patients	31	7.5 ± 0.41	25.9 ±1.69	2.7 ± 0.16
	Control	28	7.6 ±0.91	31.3 ±2.3	
P(value)			N.S.	P<0.001	

Table 2: Clinical Parameters of girls and boys.

	Girls				Boys		
	Groups	Number	(Mean±SD)	P(value)	Number	(Mean±SD)	P(value)
Hb (g/dL)	IDA	19	6.8 ±0.41	<0.01	16	7.1 ±0.41	<0.01*
	OA	15	7.0 ±0.22	<0.01	15	7.5 .±0.32	<0.01*
	Control	29	11.5 ±0.52		28	12.3 ±0.81	
PCV (%)	IDA	19	29.2 ±0.54	<0.01	16	31.3 ±0.84	<0.01*
	OA	15	36.1 ±1.9	N.S.	15	38.2 ±2.3	N.S.
	Control	29	41.3 ± 2.1		28	44.4 ±2.5	
RBCs (million /mm^3)	IDA	19	4.4 ± 0.8	N.S.	16	4.8 ±0.77	N.S.
	OA	15	4.6 ±0.81	N.S.	15	4.7 ±0.8	N.S.
	Control	29	5.1 ±0.81		28	5.3 ±0.5	
MCV Mm^3(fL)	IDA	19	6.64 ±0.68	<0.01	16	65.1 ±11.2	<0.01*
	OA	15	78.5 ± 2.4	N.S.	15	81.1 ±26. 8	N.S.
	Control	29	82.6 ± 0.77		28	83.8 ±5.2	
Ferritin (µg/dL)	IDA	19	(83 ±3) *10 -4	<0.001	16	(95 ± 2)*10 -4	<0.001*
	OA	15	0.98 ±0.19	N.S.	15	1.32 ±0.21	N.S.
	Control	29	1.12 ±0.23		28	1.95 ±0.42	

Note:*=significant N.S.=non significant.

Table 3: Comparative of Pb, Se , Mn ,Cu, Zn levels (µg/L) in blood samples of patient and controls .

	Girls			Boys	
	Groups	(Mean±SD)	P(value)	(Mean±SD)	P(value)
Fe	IDA	356 ±93	<0.005	464 ±115	<0.001
	OA	1059 ±95.6	N.S.	1309 ±117	N.S.
	Control	1100 ±105		1320 ±121	
Pb	IDA	109 ±4.11	<0.01	115 ±5.3	<0.01
	OA	91.3 ±2.9	N.S.	92.5 ±3.9	N.S.
	Control	87.1 ±2.3		90.4 ±3.1	
Mn	IDA	11.2 ±2.4	<0.05	13.3 ±3.6	<0.05
	OA	9.1 ±1.7	N.S.	9.7 ±1.9	N.S.
	Control	8.3 ±1.6		8.9 ±1.8	
Se	IDA	97.2 ±13.4	<0.01	103.4 ±14.6	<0.05
	OA	91.8 ±11.7	<0.01	107.9 ±14.9	<0.05
	Control	122.7 ±17.8		143.3 ±20.9	
Cu	IDA	38.9 ±3.2	<0.05	40.4 ±4.1	<0.05
	OA	120.3 ±108	N.S.	124.3 ±12.6	N.S.
	Control	123.1 ±12.9		128.8 ±13.4	
Zn	IDA	144.2 ±18.9	<0.01	157.3±19.5	<0.01
	OA	113.1 ±15.5	N.S.	118.2±16.4	N.S.
	Control	112.5 ±15.2		116.9 ±16.1	

Discussion

As the remarkable properties of vitamins have revealed themselves to investigators, so too have those of the various minerals in our food and water [22]. The seven macrominerals calcium, chloride, magnesium, phosphorus, potassium, sodium and sulphur--now share the research spotlight with a longer list of essential trace minerals. These are needed only in minute amounts, but their absence results in many disease conditions [23] . The number of trace minerals known to be essential to life now exceeds thirty, and some researchers believe that for optimum health we need to take in every substance found in the earth's crust. Along with familiar trace minerals, such as iron and iodine, the body also needs others less well known, like cobalt, germanium and boron[24]. As part of the hemoglobin molecule, iron is vital for healthy blood; iron also forms an essential part of many enzymes. Iron deficiency is associated with poor mental development and problems with the immune system. It is found in eggs, fish, liver, meat and green leafy vegetables. Iron from animal protein is more readily absorbed than iron from vegetable foods. The addition of fat-soluble vitamins found in butter and cod liver oil to the diet often results in an improvement in iron status. Recently, researchers have warned against inorganic iron used to supplement white flour. In this form, iron cannot be utilized by the body and its buildup in the blood and tissues is essentially a buildup of toxins. On the other side, children being more sensitive to the pollutants and more at risk as compared to the adult individuals [25,26] . Their blood is considered as an index of biologically active metal in the body[27].

In the interpretation of the results in (Table 1) ,several factors may interfere for example the nutrition status of the patients , which may be introduced as a serious factor .We expected that patients have come from poor families ,in which malnutrition is very common. Some investigators have believed that anemia is due to decrease the appetite hence causing decline weight in patients suffering from anemia [24].

The result of this study , show that hemoglobin, PCV ,mean corpuscular volume ,and ferritin values of children with IDA were decreased significantly versus control groups in girls and boys .This is due to defect in Iron absorption . Iron absorption is regulated through control of uptake, transport, and storage proteins. Iron in foods exists in two forms, heme and non-heme. Heme iron, the more bioavailable form found in foods of animal origin, is derived from hemoglobin, cytochromes, and myoglobin. Iron functions as a carrier of oxygen or electrons in many biochemical pathways, such as oxygen transport and respiration. Low iron status not only stimulates the expression of proteins that enhance iron uptake but also down regulates the expression of ferritin, a protein that sequesters iron. When iron status is replete, less heme iron enters intestinal cells. Of the iron that does enter, more binds to intestinal ferritin, and less exits to the circulation.

Loss of iron occurs as enterocytes are shed into the intestinal lumen [28,29]. Serum ferritin concentration can be used to estimate iron stores. Most of the iron in the body (60 to 73%) is incorporated into hemoglobin in circulation. Smaller percentages exist in functional compounds, such as iron-containing enzymes, or are stored in the liver, bone marrow, and spleen [30]. Iron deficiency disrupts the synthesis of heme and causes erythrocytes to become microcytic. Of the tests for iron status, serum ferritin concentration has the highest diagnostic power. Ferritin normally accounts for about two-thirds of the iron stored in the body. In healthy individuals, serum ferritin is usually proportional to body iron stores [28,30].

In this research, the serum iron level in IDA groups (girls and boys) was found significantly lower than the other groups as it is expected. Hence concentration of metal in the blood is a significant factor for child healthy deficiency or excess of trace element may cause disorders in the absorption, distribution, metabolism, and elimination of other trace elements, for example iron (Fe) deficiency can increase absorption of lead (Pb) and cadmium (Cd)from gastrointestinal tract [31] .The iron deficiency causes hypochromic micorcytic anemia and increases absorption of other elements such as Pb , and Cd .In the same way heavy exposure to these elements whatever the reasons are may cause hypochromic micorcytic anemia itself [32] .Also results in table 3 show that , there were significant higher level of Pb in serum of children in IDA groups (girls and boys) with respect to their control groups. The best explanation is, greaten prevalence of deficiency in children can increase absorption of Pb . On the other side that Pb compounds are absorbed by human and animal bodies through alimentary and respiratory systems, they can also be absorbed by skin .As has been estimated, non –nutritional sources of Pb intake (e.g. dust and soil) by children can account for 75%.Exposure to Pb in urban environments is much higher due to traffic combustions and dusts containing tetraethyl lead [33,34].Pb has an influence on energetic processes and the synthesis of body proteins It impairs tissue oxidation processes ,mitochondrial enzyme activity ,and decreases membrane ATP-ase activity [35]. Manganese absorption is increased among individuals who are iron deficient[36]. Also ,children can be exposed to excess manganese in soils through hand to mouth behaviors .As our result show that IDA have significant higher level of Mn than control group .In addition to the above , we noticed serum Se level significantly decreased in IDA ,OA groups compared to control group , this is may be due to malnutrition in IDA , OA groups. In fact, selenoproteins are involved in processes concerning everything from reproduction to thyroid activity, correct eye functioning, DNA synthesis, muscle function, and the efficient working of the heart. For example, it's essential for helping the body to maintain healthy thyroid hormone function, which is critical for regulating metabolism. It is probably best

known as an antioxidant because it's an essential component of glutathione peroxides (GPx). This enzyme works together with vitamin E to carry out tasks associated with removing free radicals (toxins caused as a by-product of metabolic functions, and as a result of pollution)[37-40]. Copper deficiency is accompanied by a microcytic, hypochromic anemia ,decreased concentrations of transferrin-bound iron in plasma, and an accumulation of iron specific to the liver, along with increases in heme oxygenase (which degrades heme) [41-42].Intravenous administration of ceruloplasmin immediately stimulates the "outflow" of iron from the liver into the blood, implicating copper (and seemingly ceruloplasmin) in this process. Probably, *a* reduction in iron flow is not the only defect or even the main defect in copper deficiency responsible for anemia,. Indeed, heme production perhaps appears to be impaired. The exact step involved has not been identified (it is not the 6-aminolevulinate synthetase, or ferro-chelatase) but could involve making Fe(II) available to the mitochondria[43-44]. In addition, a reduced activity of cytochrome *c* oxidase leading to a slower rate of electron flow, and perhaps to less ATP production for synthesis of hemoglobin by erythrocytes, could be factors. (Liver AlP production, however, is not suppressed.) A final possibility is that the life span of erythrocytes is reduced, because they are not as protected against oxidative damage in copper deficiency when ceruloplasmin concentrations are low, as has been observed. Concentrations of copper-saturated ceruloplasmin (fully active in radical scavenging) are the first to fall in copper deficiency and can drop rapidly. Copper-zinc SOD concentrations in red cells are also decreased in deficiency, so protective enzymes both inside and outside these cells are less available [45,46] . The serum Zn level of IDA groups in our study is higher than control groups in (girls and boys). There is an antagonism between Zn and Fe absorption from the gastrointestinal tract .The concentration of Fe in the intestinal lumen may antagonize the uptake of Zn [47,48].

In conclusion, children with iron deficiency anemia are more sensitive to the pollutants and more at risk as compared to healthy children. Their blood is considered as an index of biologically active metal in the body, reflecting direct interaction of atmospheric pollution of a population.

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