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Assessment of Iron Profile and Serum Hepcidin Levels in Children with Typical Iron Deficiency Anemia in Khartoum State-Sudan

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Abstract: Introduction: iron deficiency anemia among is a serious issue, as iron usually involves in many growth and metabolic process, it caused by decreased iron stores in the body, which can occur due malnutrition, malabsorbtion and chronic blood loss. Usually iron deficiency anemia can be observed though figures of complete blood count, hemoglobin concentration, packed cell volume and red cell indices, which are decreased comparing to the reference ranges, and confirmation with studying iron profile, serum iron, serum ferritin (low levels), total iron binding capacity (increased). Lately hepcidin has been involved in iron deficiency evaluation, as it's a hepatic hormone can block iron, it should be increased in iron deficiency anemia as well, but if there is infection accompanied the patients with symptoms and findings of CBC, it could be low. This study conducted in Khartoum state, to evaluate iron deficiency anemia, sung CBC as basic selection criteria, then iron profile tests and hepcidin to evaluate their status among iron deficient children.

Material and method: 150 of children were recruited to conduct complete blood count, to select IDA children to conduct assessment of iron profile and hepcidin. This study was approved by the ethical committee of Alneelain University-faculty of medical laboratory science; families of recruited children gave permission to be involved in the study. Laboratory work was conducted at Dr. Elgeiaily Khalid Musa medical laboratory-Omdurman.

Result: out of 150 of children, 87 (58%) were presented anemia, with low Hb level and somehow elevated TWBC found, but considering IDA criteria, CBC with normal TWBC count and low red blood cell count (RBC), Hb and indices, 55 subjects were selected having IDA, their plasma samples were tested for iron profile which included serum iron, total iron binding capacity (TIBC) and ferritin and hepcidin concentrations. Significant difference obtained for iron profile and hepcidin levels when compared with reference ranges.

Keywords: CBC, iron profile, hepcidin and iron deficiency anemia

I. INTRODUCTION

Iron is a vital element in many metabolic processes in the human body, but in excess it is toxic, with the result that both iron deficiency and overload have severe consequences, iron deficiency is the most prevalent nutritional problem in the world today, affecting an estimated 500 million people.

The majority (85%) of the iron in the is contained in functional iron compounds, mainly in circulating hemoglobin (approximately 30 mg/kg) and in myoglobin (approximately 5 rng/kg), which acts as a reservoir of oxygen in muscles. Only a small proportion (0,2%) of body iron is available in the circulation for immediate erythroid cell uptake and is bound to the transport protein transferrin, whilst that not immediately required is stored in the form of ferritin which can bind large numbers of iron atoms: Iron may exist in both reduced, Fe(lI), and oxidized, Fe(III), forms[1].

Iron deficiency is a state in which there is insufficient iron to maintain the normal physiological function of blood and tissues, such as the brain and muscles. The more severe stages of iron deficiency are associated with anemia. Iron-deficiency anemia occurs when the hemoglobin concentration is below two standard deviations (–2SD) of the distribution mean for hemoglobin in an otherwise normal population of the same sex and age[2]. Iron deficiency results when either dietary intake does not meet the body's requirement or when there is chronic blood loss [3-4].

The risk of iron deficiency anemia is high during the second year of life because of increased iron requirements related to rapid growth[5-6-7]. Premature and low birth weight infants and infants with history of prolonged stay in the neonatal unit are at particularly high risk of developing iron deficiency anemia before 1 year of age[8]. Iron deficiency is generally characterized by a hemoglobin

level of less than 110 g/L, plus a measure of poor iron status[9]. The prevalence of anemia and iron deficiency anemia remains high in late infancy and early childhood despite the increased breastfeeding rate, improvements in public health, and development of iron-fortified foods [10-11-12-13]. Typically to evaluate the cause of anemia includes a complete blood cell count, and serum iron indices. Iron deficiency anemia is characterized by microcytic, hypochromic erythrocytes and low iron stores. The mean corpuscular volume is the measure of the average red blood cell volume and mean corpuscular hemoglobin concentration is the measure of the concentration of hemoglobin in a given volume of packed red blood cells.

The normal reference ranges for mean corpuscular volume is 80–100 fL and means corpuscular hemoglobin concentration is 320–360 g/l. The patient's cells are said to be microcytic and hypochromic, respectively, when these values are less than the normal reference range[14]. Red cell distribution width (RDW) measures the degree of anisocitosis (size difference) of the population of red cells and its elevation is neither sensitive nor specific for iron deficiency [15]. Iron profile includes, serum iron; typically, it is very low in patients with iron deficiency anemia.

However, mildly low to low normal serum iron values can also be observed with anemia of inflammatory disease[16]. Total iron binding capacity, it is a measure of the plasma iron concentration that can be bound by plasma transferrin. Iron saturation reflects the amount of plasma iron bound the transferrin and is low (<20%) in cases of iron deficiency anemia [16]. Serum level of ferritin correlates well with body iron stores. It is decreased in iron deficiency anemia and increased in iron overload. Ferritin is also an acute phase protein, and hyperferritinemia can occur with underlying disease, such as inflammatory disease, neoplasia, liver diseases.

Nevertheless, low serum ferritin concentrations (<12ng/ml) can be helpful in differentiating iron deficiency anemia from anemia of inflammatory disease [16]. It has long been recognized that anemia arises in many chronic diseases, including chronic renal failure and inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and chronic osteomyelitis. Although loss of erythropoietin plays a role in advanced kidney diseases, in most other conditions the explanation for the anemia of chronic disease has remained unsatisfactory.

 However, the new peptide hormone, hepcidin, has, it has been found, the remarkable property of causing profound iron deficiency in otherwise healthy individuals[17]. This hormone is a small peptide, synthesized in the liver in response to inflammation anywhere in the body, and can profoundly limit the absorption of iron from the intestine.

Total body iron stores are of the order of 2–3 g, and are relatively stable in a healthy person [18]. Few data are available on the evaluation of hepcidin in iron deficiency anemia. Previous studies indicate that hepcidin increases with iron overload and decreases with IDA[19-20].

II.   MATERIAL AND METHOD

This cross sectional study, conducted among 150 of children with iron deficiency anemia, they were recruited for iron profile assessment after they were evaluation of complete blood count with Mindray BC3000 (China) device and reagents, to consider iron deficiency anemia, low hemoglobin, packed cell volume and low red cell indices. Iron profile, as serum iron, serum ferritin and total iron binding capacity were measured with chemical assessment method using Biosystem (Germany) reagents and device (BTS350) as well as hepcidin, which measured by means of enzyme linked sorbent immunoassay (ELISA) using Abbexa (UK) trade mark. Blood samples were collected in ethylin diamin tetra acetic acid (EDTA) for CBC and Lithium heparin for iron profile and hepcidin assessment. Laboratory work was conducted at Dr. Elgeiaily Khalid Musa medical laboratory-Omdurman. Data obtained was analyzed by means of statistical package of social science (SPSS) program version 21.

III.   RESULT

Out of 150 children tested for anemia using their EDTA added blood to conduct CBC, 87 (58%) were presented anemia, with low Hb level and somehow elevated TWBC found, but considering IDA criteria, CBC with normal TWBC count and low red blood cell count (RBC), Hb and indices, 55 subjects were selected having IDA they were 36.7% of all tested subjects and 63% of anemic subjects, their age (mean±SD) was (6.4±4.3) years; they were 27 (49%) males and 28 (51%) females. All plasma samples of iron deficiency anemia subjects (55) were later tested later for iron profile which included serum iron, total iron binding capacity (TIBC) and ferritin and hepcidin concentrations. Considering gender distribution among anemic males CBC parameters, Hb, PCV, MCH, MCHC, RBC, TWBC with differential count and platelet count comparing to the reference value. Decreased significant difference obtained for Hb, PCV, MCH, MCHC and MCV with P value 0.000 for each, RBC count with P value 0.001 and lymphocyte (LYM) with P value 0.00. Increased significant difference obtained for monocyte (MON) with P value 0.00. While TWBC, neutrophil (NEU) and platelet count did not bring significant difference, as in table 1.

Table 1: Complete blood count among males with IDA

Parameters	Mean±SD	Reference value	P-value
HB	9.64±2.12	17.5 (15-20)	0.000
PCV	31.00±5.62	47.5 (45-50)	0.000
MCH	24.30±11.13	29.5 (27-32)	0.002
MCHC	29.59±2.66	33 (31.5-34.5)	0.000
MCV	71.93±12.16	90.5 (81-100)	0.000
RBC	4.16±0.82	4.75 (4.5-5)	0.001
WBC	7.22±2.54	7 (4-10)	0.659
NEU	39.44±11.94	35 (25-45)	0.064
LYM	48.89±12.15	60 (45-75)	0.000
MON	9.85±4.35	6 (2-10)	0.000
PLATE	308.30±120.81	300 (150-450)	0.724

Significant difference p value < 0.05.

While iron profile among anemic males brought highly significant difference (decreased for serum iron and ferritin) with P value 0.00 for each, slightly increased significant difference brought by TIBC with P value 0.046, they were contestant to IDA. And hepcidin brought decreased significant difference with P value 0.000 as in table 2.

Table 2 Iron profile and hepcidin among IDA males

Parameters	Case Mean+SD	Reference value	P value
S. Iron	83.26±38.34	120 (65-175)	0.000
Ferritin	15.37±13.83	82.5 (15-150)	0.000
TIBC	391.67±134.41	337.5 (250-425)	0.046
hepcidin	20.81±12.86	70 (46-94)	0.000

Significant difference *P* value < 0.05.

CBC parameters for anemic females brought highly decreased significant difference comparing to the reference value, Hb, PCV, MCH, MCHC and RBC count with P value 0.000 for each. Lymphocyte brought decreased significant difference with P value 0.00 and increased significant difference for monocyte with P value 0.04. MCV has no significant difference as well as TWBC and platelet count as in table 3.

Table 3: Complete blood count among female with IDA.

parameters	Mean±SD	Reference value	*P-value*
HB	9.18±2.07	14.25 (13.5-15)	0.000
PCV	28.43±7.89	40.5 (38-43)	0.000
MCH	21.50±3.20	29.5 (27-32)	0.000
MCHC	30.21±2.02	33 (31.5-34.5)	0.000
MCV	80.50±57.83	90.5 (81-100)	0.368
RBC	4.20±0.58	40.5 (38-43)	0.000
WBC	7.31±1.90	7 (4-10)	0.389
NEU	39.89±10.96	35 (25-45)	0.026
LYM	50.71±11.93	70 (45-75)	0.000
MON	8.07±3.45	6 (2-10)	0.004
PLATE	339.21±144.72	300 (150-450)	0.163

Significant difference p value <0.05.

Iron profile parameters. Serum iron and serum ferritin each has decreased significant difference with P value 0.001 and 0.000 respectively when they compared to the reference value. TIBC brought increased significant difference with P value 0.041. While hepcidin had low level than reference value bringing significant difference with P value 0.000, as in table 4.

Table 4: Iron profile among females with IDA

Parameters	Case (Mean±SD)	Reference value	*P value*
S. Iron	80.79±42.04	110 (50-170)	0.001
Ferritin	11.12±11.61	187.5 (25-350)	0.000
TIBC	408.54±175.18	337.5 (250-425)	0.041
hepcidin	25.18±18.44	60 (46-74)	0.000

Significant difference p value < 0.05.

Pearson's correlation conducted using measured CBC parameters against age, positive correlation obtained for Hb, PCV and MCH, but negative obtained with the rest of CBC parameters and age of anemic subjects as in table 5. While serum iron and TIBC both have negative correlation but serum ferritin has positive correlation with age. Hepcidin has negative correlation as table 6 respectively.

Table 5: Correlation of complete blood parameters with age of IDA subjects.

Parameters	*R-value*	*P-value*
HB	0.041	0.773
PCV	0.062	0.665
MCH	0.192	0.173
MCHC	-0.236	0.092
MCV	-0.189	0.180
RBC	-0.172	0.229
WBC	-0.288[*]	0.038
NEU	0.313[*]	0.024
LUM	-0.251	0.073
MON	-0.112	0.431
PLATE	-0.248	0.076

Table 6: Correlation of iron profile and hepcidin with age of IDA subjects.

Parameter	R value	*P value*
s. iron	-0.165	0.242
Ferritin	0.058	0.685
TIBC	-0.011	0.937
hepcidin	-0.046	0.744

IV. DISCUSSION

Poverty, malnutrition, and famine are self-explanatory causes of anemia in the multitude of people living with iron deficiency in developing countries, especially children and pregnant women. In addition, a cereal-based diet decreases iron bioavailability because phytates in grains sequester iron in a poorly absorbable complex. Other common causes in developing countries include hookworm infections and schistosomiasis, which cause chronic blood loss[21]. Strict vegan and vegetarian diets, malabsorption, and chronic blood loss resulting from heavy menstrual losses are well-known causes of iron-deficiency anemia in developed countries [22]. Iron deficiency affects more than 2 billion people worldwide [23] and iron-deficiency anemia remains the top cause of anemia, as confirmed by the analysis of a large number of reports on the burden of disease in 187 countries between 1990 and 2010 [21]. In this study the main concern was evaluation iron deficiency anemia (IDA) in children in multiple areas in Khartoum state, as people are come from different directions of the country due to enterer immigration, which always due to war, enhancement of living status,

which can be with seeking of education and treatment. So incoming population can reflect health and life style they have been sequestrated into. In order to achieve that, different children were enrolled in this study, they were selected according to the findings of complete blood count (CBC), which usually helps in sorting of anemia by hemoglobin (Hb) and related parameters, such as packed cell volume, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration. Total white blood cell count (TWBC) was taken under consideration, as ferritin is acute phase reactant, in case of inflammation, it produced and then then it would mask the decreased level associate to iron deficiency anemia, so these children selected with low Hb and related parameters and normal or low TWBC to call them with typical iron deficiency anemia, they were 55 children selected out of 150 tested subjects according to CBC. Then they tested for iron profile (serum iron, serum ferritin and total iron binding capacity (TIBC)) and hepcidin concentrations. Iron profile showed regular findings related to IDA; low levels of serum iron and ferritin and increased TIBC, hepcidin presented low than reference value. , hepcidin with CBC parameters could be indicators or diagnostic tools for iron deficiency in children, who already showed with low Hb levels and red cell hemoglobin content (MCHC) and later low hepcidin level as well, this in agreement of finding of among rheumatoid arthritis (RA) subjects presented with IDA and others with ID, as RA is a chronic disease, it presented that hepcidin level was low among subjects with RA than those with ID, as hepcidin is an iron regulatory hormone, in physiological condition, when iron gets low concentration in the body, hepcidin should be produced to enhance more iron absorption and then correct the deficiency, realizing that fact and compare with the finding of our study and the RA with ID subjects, low hepcidin and Hb with red cell hemoglobin concentration match output found[24]. Canadian study involved children with age range between 6-36 months, they were detected for iron deficiency anemia by means of CBC as first line of investigation could be performed [25], that is just identical to what this thesis depended on to select children to be enrolled in the later laboratory work to ascertain of IDA. In this study out of 850 children tested for anemia using their EDTA added blood to conduct CBC, 87 (10.3%) were presented anemia, with low Hb level and PCV, but considering IDA typical criteria, CBC with normal TWBC count and low red blood cell count (RBC), Hb and indices, 55 subjects were selected having IDA they were 6.5% of all tested subjects and 63% of anemic subjects, they presented with low serum iron, ferritin and increased TIBC, this in agreement of An Ethiopian concern was directed toward children, a community based cross-sectional study was conducted in Jimma Town, Southwest Ethiopia from April to July 2013. A total of 616 school children aged 6 to 12 years were included in the study. They tested for child for hematological examinations. Anemia was defined as a hemoglobin level lower than 11.5 g/dl and 12 g/dl. Iron deficiency anemia was defined when serum iron and ferritin levels are below reference values. Moreover, fresh stool specimen was collected for diagnosis of intestinal parasitic infection. Stained thick and thin blood films were examined for detection of Plasmodium infection and study of red blood cell morphology. Overall, prevalence of anemia was 43.7%, and that of IDA was 37.4%, due to low families incomes and infection with intestinal parasites and malaria[26]. Low hepcidin level among iron deficient children involved in this study matches finding of a study aimed to determine hepcidin role in iron status, that study was conducted on model animals under certain conditions, anemia and hypoxia, among both hepcidin levels found decreased, that study concerned about liver hepcidin gene expression, which was reduced [27].

V. CONCLUSION

Iron deficiency anemia presented in children assessed accompanied with levels of hepcidin indicating of presence of infections.

VI. RECOMMENDATION

Hepcidin and other related testes should be included routinely to rule out infections, in order to right diagnosis of iron deficiency anemia and to avoid missing diagnosis as well.

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