

Role of Serum Hepcidin levels in the Diagnosis of Iron Deficiency Anemia in Children in Saudi Arabia

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ABSTRACT

Hepcidin, produced in the liver, is a key regulator of systemic iron metabolism. This study aimed to estimate the significance of hepcidin for diagnosis of iron deficiency anemia (IDA) among children and to determine whether routine hematological parameters and iron profile correlate with hepcidin levels in children in Saudi Arabia. Blood samples from children were analyzed for hematology parameters, iron profile and hepcidin-25. A total of 128 children were classified according to hemoglobin level and iron parameters as: IDA (N=97; mean age 5 ± 3.5 years) and normal control (N=31; mean age 5.5 ± 3.2 years). Significantly lower levels of hemoglobin ($p=0.003$), serum iron ($p=0.001$), serum ferritin ($p=0.001$), transferrin saturation ($p=0.002$), and hepcidin-25 ($p=0.002$) were obtained in children with IDA as compared to normal control group. Significantly high levels of total iron binding capacity ($p=0.002$) were noted in IDA group. Positive correlation was observed between hepcidin-25 and serum ferritin ($r=0.660$), serum iron ($r=0.374$), transferrin saturation ($r=0.317$) and hemoglobin levels ($r=0.246$). Low levels of serum hepcidin are significantly associated with lower iron parameters in children, and could be useful indicator of IDA.

Keywords: Hepcidin-25, Iron deficiency anemia, Iron parameters, Serum hepcidin

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ITRODUCTION

Iron deficiency (ID), and specifically iron deficiency anemia (IDA), is one of the most severe and important nutritional deficiencies in the world today.¹ IDA has been associated with cognitive, motor, and behavioral impairment in children.² IDA is also one of the commonest causes of anemia in Saudi Arabia especially in women of child-bearing age and children.³ The commonly used tests to determine iron status include serum iron levels; and ferritin, which is used as an indicator of iron stores; soluble transferrin receptor (sTfR) levels, which reflect tissue iron stores; and transferrin saturation level etc. However, these tests have limitations such that ferritin level may be elevated in patients with co-existing inflammation, sTfR levels may be influenced by erythropoetic activity, and transferrin saturation may be affected by inflammation and undergoes diurnal variation.⁴

Hepcidin, secreted by liver, is the key hormone that regulates systemic iron homeostasis. It inhibits the transport of iron across the gut mucosa, thereby preventing excess iron absorption and maintaining iron levels within normal limits in the body. It also inhibits transport of iron out of macrophages.⁵ The classic IDA in humans is also associated with low hepcidin expression to enhance iron absorption and recycling, which makes this hormone a potential marker for detection of iron deficiency anemia, anemia of chronic disease and coexisting of IDA with anemia of chronic disease. Estimation of hepcidin levels for diagnosis and prognosis of anemia may provide a more effective approach for treatment of IDA and prevention of toxicity associated with iron overload.⁶

The use of serum hepcidin level as an index for ID or IDA has been tested in adult populations.^{7,8} However, very few studies have investigated the effectiveness of serum hepcidin measurements in children. The present study was undertaken with the objective of evaluating the role of serum hepcidin levels and their significance in diagnosing of IDA in children. Also, it was thought that the development of reference hepcidin preparations would enable inter-lab comparisons of assays and the standardization of units and reference ranges facilitate clinical use of hepcidin index. To this end, the hemoglobin level, and the various indicators of iron levels were measured, and correlated with serum hepcidin levels.

MATERIALS AND METHODS

This was a cross-sectional, conducted in children visiting the Maternal and Children Hospital in Al-Madinah Al-Munawarah. Written informed consent was obtained from the parents/legally acceptable representatives of the children before performing any study-related procedure. Assent was obtained from children ≥ 7 years of age. A total of 128 children, boys and girls, visiting the hospital from April 2014 to November 2014 were enrolled in the study. They were included two groups: Group I included 97 children diagnosed with IDA based on criteria using cut off level < 11.0 g/dl for hemoglobin and cut-off ≤ 50 ng/mL for serum ferritin, whereas Group II included 31 non iron deficiency children of hemoglobin level ≥ 11.0 gm/dl, which served as control for the IDA cases.⁹⁻¹⁰ Children with anemia due to any other cause, or testing positive for hemoglobin A2 or C-reactive protein (CRP) were excluded. Blood collected in the EDTA vacutainers was used for the measurement of complete blood cell count (CBC) on SYSMEX automated hematology analyzer and hemoglobin electrophoresis was performed. Serum from the plain vacutainers was separated and CRP levels were determined turbidmetrically using calibrated COBAS INTEGRA 700/800 auto-analyzer. The expected normal values in children were < 0.16 mg/dL, Serum hepcidin was measured using DRG Hepcidin-25 ELSA Kit. and iron profile were estimated by calibrated ARCHITECT C colorimetric systems. SPSS version 14 was used for data analysis unpaired t-test and χ^2 chi-square test were used. Correlations between variables were calculated using Pearson's correlation analysis for numerical data. The level of significance was set at $p < 0.05$.

RESULTS

The characteristics of the 97 children who diagnosed to have IDA (Group I) are compared with 31 normal healthy children (Group II) (Table 1). There were no significant differences in the baseline characteristics between the study groups with respect to age and gender distribution ($p > 0.05$).

There were significantly low levels of hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and high levels of TIBC in Group I as compared to Group II. Additionally levels of

serum iron, ferritin and transferrin saturation were significantly lower in Group I. Serum hepcidin levels in Group I were significantly low as compared to Group II. RBC count, HbA2 and CRP levels did not significantly differ between study groups (Table 2).

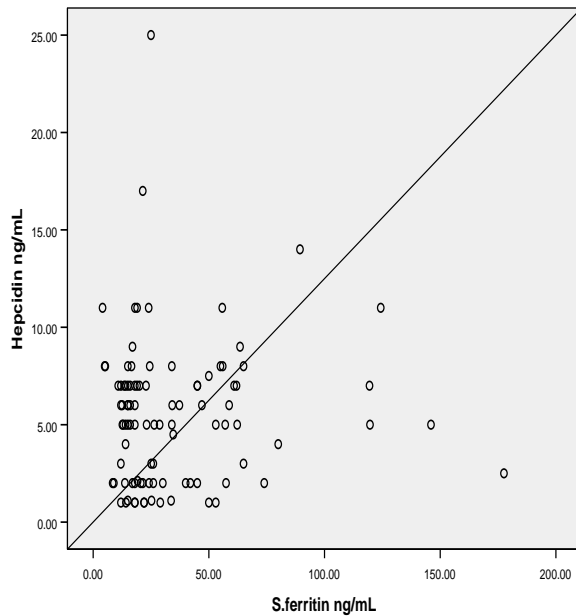
Serum hepcidin levels were significantly positively correlated with the levels of serum ferritin, serum iron, transferrin saturation and Hb levels (Fig 1).

Table 1. Baseline characteristics of study population

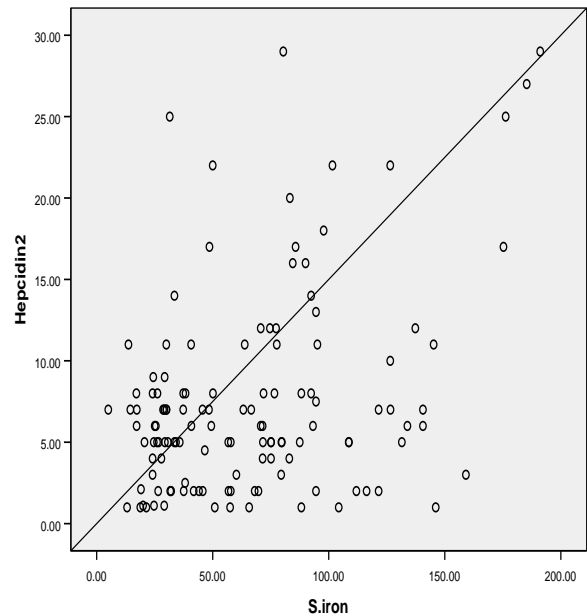
Subject Characteristics	Group I (IDA)	Group II (Normal)	p-value
Age, years (Mean \pm SD)	5 \pm 3.5	5.5 \pm 3.2	>0.05*
Male: Female	63:34	18:13	0.3625 [†]
SD: Standard deviation; p-value calculated using *unpaired t-test and [†] chi-square test.			

Table 2. Hematological data and iron parameters

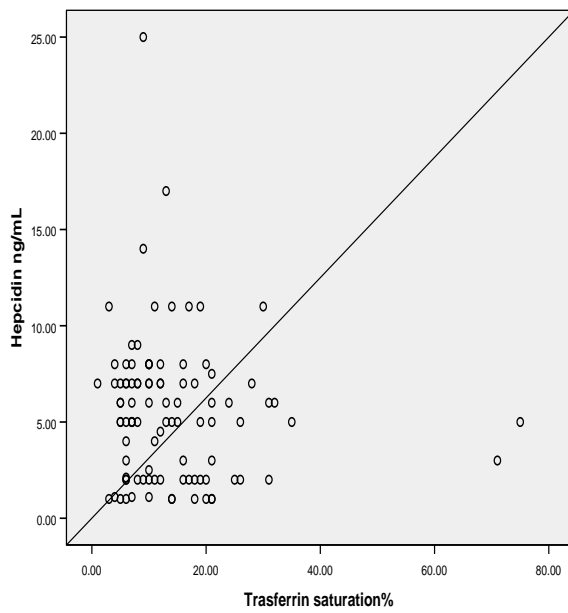
Subject Characteristics	Group I (IDA)	Group II (Normal)	p-value
Hb, g/dL	10.7 \pm 2.0	12.6 \pm 0.8	0.003
MCV, fL	66 \pm 7	80 \pm 3	0.001
MCH, pg	22 \pm 3	29 \pm 2	0.02
MCHC, g/dL	30 \pm 1.6	33 \pm 1.0	0.001
TIBC, μ g/dL	431 \pm 45	289 \pm 25	0.002
RBC, X10 ¹² /L	4.98 \pm 0.7	4.64 \pm 0.3	0.07
Hb A2, %	2.72 \pm 0.12	2.74 \pm 0.13	0.4
Serum iron, μ g/dL	53.8 \pm 35	104.3 \pm 37	0.001
Serum ferritin, ng/mL	35 \pm 15	85 \pm 10	0.001
Transferrin saturation, %	14 \pm 11	39 \pm 19	0.002
CRP, mg/dL	0.42 \pm 0.25	0.30 \pm 0.22	0.06
Hepcidin-25 ng/mL	5.47 \pm 3	13.80 \pm 7	0.002
All values are represented as Mean \pm SD; p-value calculated using unpaired t-test			



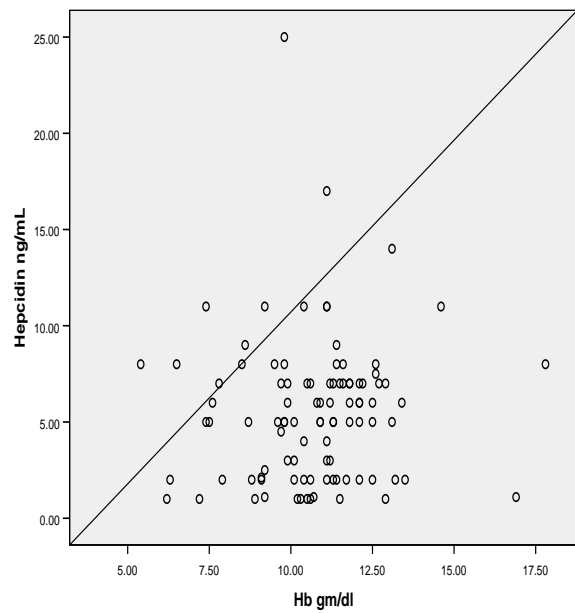
a) Serum hepcidin and serum ferritin
($r=0.660$)



b) Serum hepcidin and serum iron
($r=0.374$)



c) Serum hepcidin and transferrin saturation
($r=0.317$)



d) Serum hepcidin and hemoglobin levels
($r=0.246$)

Figure 1. Positive correlations between hepcidin with serum ferritin ($r=0.660$), serum iron($r=0.374$), transferrin saturation($r=0.317$) and hemoglobin($r=0.246$)

DISCUSSION

Iron deficiency anemia is one of the commonest nutritional disorders in children worldwide. The deficient children are at increased risk of impaired psychomotor and mental development. In spite of common use, the serum ferritin and other hematological parameters can be less sensitive in the diagnosis of IDA in presence of other systemic factors including inflammation. Ferritin is an indicator of storage iron, but its levels are elevated in patients with coexisting inflammation. Similarly transferrin saturation level may be affected by inflammation and undergoes diurnal variation.¹¹

Hepcidin The iron-regulatory hormone control the dietary absorption, storage, and tissue distribution of iron. Hepcidin causes ferroportin internalization and degradation, thereby decreasing iron absorption and recycling from macrophages. Hepcidin is regulated by iron concentrations in plasma and by erythropoietic demand for iron in a feedback manner.¹² Hepcidin levels are seen to be reduced in patients with iron deficiency. Therefore, measurement of serum hepcidin levels may be used as markers for iron deficiency.¹³ It has been use as an index for iron deficiency, in contrast very few studies have been estimated the significance of serum hepcidin measurements in children suffering from iron deficiency anemia.^{14 15} The study revealed that serum hepcidin levels were significantly lower in children with IDA as compared to children in the normal control group (P Value <0.05). Furthermore, serum hepcidin levels were correlated with serum iron indices and revealed positive correlations between serum hepcidin level and serum ferritin, serum iron, transferrin saturation, and hemoglobin levels. These findings are concordant with those of previous reports.¹⁶⁻¹⁷⁻¹⁸

The study revealed significant decreases (P Value <0.05) hemoglobin levels, red cells indices including mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) of study group I with hepcidin depleted compared to control group, reflecting microcytic and hypochromic type of iron deficiency anemia in group I compared to control.

Reduced serum hepcidin is an essential part of the physiological response to an iron deficiency anemia. Decreased hepcidin, serum ferritin; along with other indices signals that increased iron is needed. Hence combined evaluation of these indices may provide complementary clinical diagnostic IDA among children.

The levels of CRP are similar in both the study groups and children with signs of infection or inflammation were excluded from the present study. The levels of hemoglobin A2 were also estimated and showed insignificant differences between the case and control groups and children with signs of thalassemia were excluded. This finding is in agreement with previously reported studies.¹⁹

CONCLUSION

To conclude, serum hepcidin levels are significantly associated with iron status in children, and could be useful indices for diagnosis of IDA. There is a positive correlation between serum hepcidin and serum ferritin, serum iron, transferrin saturation and hemoglobin levels. Our study demonstrated that low levels of serum hepcidin are significantly associated with lower iron parameters in children, and could be useful indicator of IDA.

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DISCLOSURE OF CONFLICTS OF INTEREST

The authors declare no conflict of interest

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