

Evaluation of Urinary Hepcidin as a Marker for the Early Diagnosis of Iron Deficiency Anemia in Children

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Abstract

Background: Iron deficiency is an essential element, because nearly all living organism urine hepcidin levels could potentially be used in the determination of iron requirements and be an accurate indicator of physiological IDA. **Aim:** The present study aimed to evaluate the role of urinary hepcidin as an early diagnostic marker of iron deficiency anemia in children. **Methods:** This study included 45 children. Their age ranged from 6 to 16 years with hemoglobin levels ≤ 11.5 gm/dL and ferritin levels ≤ 20 ng/mL. The diagnostic performance of urinary hepcidin was estimated by analyzing the receiver operating characteristic curve. Diagnostic cut-off point with a high predictive value for iron deficiency was selected. **Results:** This study showed no statistically significant difference between the two studied groups regarding to demographic data. There were decrease in serum iron, ferritin, hemoglobin and Red blood cells (RBCs) indices in patient than the control group. There was non-significant decrease in urinary hepcidin in the study group compared to control group. The sensitivity and specificity of urinary hepcidin in diagnoses of iron deficiency anemia was 57.8 and 62.2 respectively with positive predictive value (63.3) in our study. There was positive correlation between urinary hepcidin and mean corpuscular volume (MCV), ($r = 0.221$), ($p = 0.036$), with the cut of value < 63.33 . **Conclusion:** We concluded that the decrease of urinary level of hepcidin was non-significant in iron deficiency anemia compare to control group and needed further researches.

Keywords

Hepcidin, Iron, Anemia, Pediatrics

1. Introduction

Iron is an essential element for nearly all living organisms. Iron is a key component of oxygen storage and transporting proteins, such as hemoglobin and myoglobin, as well as many enzymes that catalyze oxidation-reduction reactions necessary to generate energy and produce various metabolic intermediates for host defense [1]. Ferritin has been used as an indicator of iron stores but its levels are also elevated in patients with coexisting inflammation. Soluble transferrin receptor (STFR) levels are seen to reflect tissue iron deficiency, but they are also influenced by erythropoietin activity. In addition, transferrin saturation levels may be affected by inflammation and undergo diurnal variation [2].

Hepcidin-25, a 25-amino acid peptide hormone produced in the liver is a central regulator of systemic iron metabolism [3]. Hepcidin levels are reduced in patients with iron deficiency anemia (IDA). Therefore, blood or urine hepcidin levels could potentially be used in the determination of iron requirements and be an accurate indicator of IDA [4].

The aim of this study was to evaluate the role of urinary hepcidin as an early diagnostic marker of iron deficiency anemia in children.

2. Patients and Methods

This was a case control comparative study carried out in the outpatient clinics of general hospital in AL-Dakahlia governorate from 24th February 2019 to 2nd January 2020, included 45 children, their age ranged from 6 to 16 years and with hemoglobin levels ≤ 11.5 gm/dL and ferritin levels ≤ 20 ng/mL [5], 45 healthy children age and sex matched were included as a control group and were exclude conditions that might affect serum ferritin as (acute infection and acute inflammation) , patients suffering from chronic diseases or chronic infection, other forms of nutritional deficiency anemia (e.g. folic acid, B12), any form of hemolytic anemia and children who received iron therapy or blood transfusion the 3 months prior to the study.

Approach of the ethical committee of Al-Azhar faculty of medicine. A written informed consent was taken from all participant after proper explanation of the study, the steps of the study, the aim, the potential benefits and hazards, all were discussed with the parents of the studied groups. The included children groups had the right to withdraw from the study at any time without giving any reasons.

All children subjected to detailed medical history (personal, family, past history and history of present illness), general examination, abdominal examination and anthropometric measurements were done for all children (patients, control)

2.1. Laboratory Investigation

4 ml blood was withdrawn, 2 ml was collected on EDTA tube for complete blood count that were done immediately, 2 ml was collected in tube and centrifuged for serum iron and ferritin, early morning urinary sample was collected in sterile bottle and preserved in refreg (-20°C) till the time of assay, estimation of uri-

nary hepcidin by ELISA as assays were carried out in urine by a sandwich enzyme-linked immunosorbent assay (ELISA) technique using reagents provided by SinoGeneclon Co. Ltd., mindray MR-96A. The theoretical sensitivity or the minimum detectable dose (MDD) of urinary hepcidin ranged from 7.5 µg/L to 150 µg/L. The mean MDD was 1.2 µg/L (Catalog No: SG-10182, Human hepcidin kit).

2.2. Statistical Analysis

The collected data were coded and verified prior to data entry; all statistical analysis was carried out using the statistical package for Social Sciences (SPSS/Windows version 17.0.0. SPSS Inc., an IBM Company), Microsoft excel was used for drawing figures. 1) Descriptive statistics: Contains variables were presented as mean \pm SD (standard deviation) or number (percentage) as appropriate. 2) Analytical statistics: statistical analysis was carried out using the Student (t) test, and P value ($P < 0.05$) was considered as a significant, P value (>0.05) was no-significant and P value (<0.001) was highly significant.

2.3. Ethical Considerations

This study was discussed with the children and their parents. A written informed consent was taken after an explanation of the study to them and before the initiation of the research study: An informed written consent was obtained from all parents' patients and control groups before getting them involved in the study, Approach of the ethical committee of Al-Azhar faculty of medicine, The steps of the study, the aim, the potential benefits and hazards, all were discussed with the parents of the studied groups, Confidentially of all data were ensured, The patients and the control groups had the right to withdraw from the study at any time without giving any reasons.

3. Results

Table 1 shows no significant difference between the studied groups regarding to age and sex, there was significant decrease in mean of hemoglobin concentration (Hb), RBCs indices and serum iron, ferritin in patients group compared to control group.

Non-significant decrease in urinary hepcidin in patient group compared to the control.

Table 2 Correlation study between urinary hepcidin, other clinical and laboratory parameters was conducted in the whole study population and showed positive correlation between urinary hepcidin and MCV.

Figure 1 The ROC curve showed Sensitivity and specificity of urinary hepcidin in diagnoses of iron deficiency anemia that was 57.8 and 62.2 respectively with positive predictive value.

4. Discussion

This study included forty-five patients with iron deficiency anemia with age

range from 6 to 16 years and forty-five healthy children with matched age and sex. As regard to demographic data of studied cases, the mean of age of patients group was 8.78 ± 1.54 , however for control group was 9.31 ± 1.54 years with no significant difference between the two groups. Regarding gender, the results were presented as males were 21 cases represented (46.7%) in patients group and 28 cases (62.2%) in control group; However, females were 24 cases (53.3%) in patients group and 17 cases (37.8%) in control group with no significant difference between the two groups.

Table 1. Comparison between patient and control groups regarding demographic data, clinical and laboratory data.

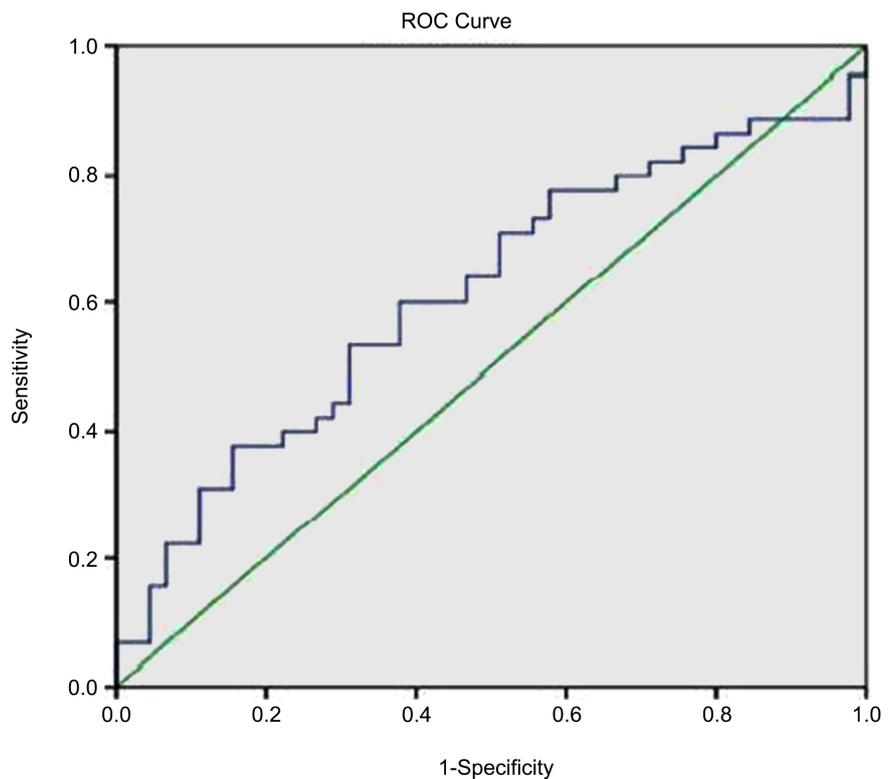
Parameter	Groups		Test of Significance
	Patients Group N = 45	Control Group N = 45	
Age (year)	8.78 ± 1.54	9.31 ± 1.54	t = 1.642 P = 0.103
Gender	Male	21 (46.7%)	X ² = 2.195 P = 1.38
	Female	24 (53.3%)	
Weight z score (Kg)	-0.25 ± 1.22	-0.19 ± 0.82	t = 2.014 P = 0.044
Height z score (cm)	-0.19 ± 0.98	-0.11 ± 0.99	t = 1.461 P = 0.148
Serum iron (mcg/dl)	47.9 (20 - 89.1)	64 (36 - 87)	z = -3.209 P = 0.001**
Serum Ferritin (ng/ml)	37.15 (4 - 77.8)	46 (28 - 78)	z = -2.787 P = 0.005*
Hemoglobin (g/dl)	9.76 ± 0.84	12.27 ± 0.58	t = -16.587 P < 0.001**
RBCs ($10^6/\mu\text{l}$)	4.03 ± 0.60	4.91 ± 0.58	t = -7.110 P < 0.001**
MCV (fI)	70.06 ± 5.86	82.55 ± 4.95	t = -10.927 P < 0.001**
MCH (p.g)	22.31 ± 2.36	29.05 ± 8.31	t = -5.233 P < 0.001**
MCHC (g/dl)	31.88 ± 1.69	32.49 ± 1.06	t = -2.075 P = 0.041*
PLTs ($10^3/\mu\text{l}$)	255 (82 - 482)	234 (145 - 442)	z = -1.283 P = 0.199
Urinary Hepcidin (ng/ml)	50.65 (26.53 - 126.95)	71.93 (25.49 - 132.92)	z = -1.969 P = 0.058

*Significant (P < 0.05); **Highly Significant (P < 0.001).

Table 2. Correlation study between urinary hepcidin, other clinical and laboratory parameters in the whole study population.

Parameter	URINARY HEPCIDIN	
	r	p
Age (year)	0.037	0.730
Height (cm)	-0.054	0.613
Weight (kg)	-0.089	0.407
Serum Iron (mcg/dl)	-0.044	0.683
Serum Ferritin (ng/ml)	0.011	0.918
Hemoglobin (g/dl)	0.136	0.201
RBCs (106/ μ l)	0.184	0.082
MCV (fI)	0.221	0.036*
MCH (p.g)	0.185	0.081
MCHC (g/dl)	0.058	0.589
PLTs (103/ μ l)	10.091	0.339

*Significant ($P < 0.05$); **Highly Significant ($P < 0.001$).

**Figure 1.** ROC Curve of urinary hepcidin in diagnosis of iron deficiency anemia.

The mean hemoglobin concentration, mean corpuscular volume, mean corpuscular Hemoglobin, red blood cells of the studied patients were significantly lower than those of the controls ($P > 0.01$) for each except mean corpuscular hemoglobin concentration ($P = 0.041$). The same results were obtained by Sanad and Gharib, Koch *et al.* and Elsayed [6] [7] [8].

Sanad and Gharib (2011), Przybyszewska *et al.* (2013) and Mupfudze (2014) [6] [9] [10] reported that MCV, MCH and MCHC were decreased significantly with the presence of iron deficiency anemia and this may be due that iron deficiency anemia is one of the microcytic anemia's in which $MCV > 70$ (fl/red cell) causing small red blood cells.

Lolascon (2009) and Sanghvi *et al.* (2010) [11] [12] stated that Iron deficiency anemia (IDA) is defined as a disease of the reduced erythrocyte production with low content of hemoglobin, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of hemoglobin (Hypochromic).

The means serum iron and serum ferritin levels of the studied patients were significantly lower than those of the controls with (P value = 0.001) for serum iron and ($P = 0.005$) for serum ferritin. The same results were obtained by (Sanghvi 2010), (Sanad and Gharib 2011), (Elsounesy, 2015), (Rasheed *et al.*, 2013), and (Elsayed, 2017) [6] [8] [12] [13], who study the relation between iron concentration and anemia and they found that the major cause of anemia is the poverty of iron.

We observed decrease in urinary hepcidin but with no significant value while the studies done by (Cherian *et al.*, 2008), (Sanad and Gharib, 2011), (Al-Mazahi *et al.* 2015), and (Elsayed, 2017) [6] [8] [14] [15] found that urinary hepcidin levels were significantly lower in iron deficiency anemia (IDA), (Ganz *et al.*, 2008) [16], stated that hepcidin is homeostatically regulated by iron and erythropoietic activity and suppressed in ID, allowing increase of absorption of dietary iron and replenishment of iron stores. The feedback loop between iron and hepcidin ensures stability of plasma iron concentrations [17], hepcidin production is also regulated the process which consumes most iron erythropoiesis [18], and our findings could be attributed to the small number of the included children.

Increased erythropoietic activity suppresses hepcidin production which allows the release of stored iron from macrophages and hepatocytes, and increased iron absorption, all resulting greater supply of iron for hemoglobin synthesis [19].

In the present study, hepcidin level had no correlation with age, and these findings were similar to the finding observed by (Cherian *et al.*, 2008) [14] who found that there were no significant correlation between urinary hepcidin and age. Our study also revealed an insignificant correlation between hemoglobin level and urinary hepcidin concentration (P value = 0.201). These results were in line with those of (Schulze *et al.*, 2008) [20] who characterized the role of hepcidin in anemia and found no correlation between Hb level, and urinary hepcidin concentration, while our results were in contrary with those of (Choi *et al.*, 2012) and (A El-Kelany *et al.*, 2019) [21] [22] who detected the urinary hepcidin level

asan early prediction of iron deficiency and found that there was significant positive correlation between Hb level and urinary hepcidin concentration.

The present study also revealed a significant positive correlation between urinary hepcidin and mean corpuscular volume (MCV), (P value = 0.036), and there was no correlation between hepcidin and MCHC (P = 0.589), there were no correlation between urinary hepcidin and MCH.

In agreement with our study, the study done by (Mohamed *et al.*, 2014) [23], to assess the correlation between hepcidin biochemical parameters and hematological indices among iron deficient anemic children aged (6 - 12) years in Gaza city, they found that there was no correlation between MCH and urinary hepcidin concentrations, and also did not agree with (Cherian *et al.*, 2008) and (Duru *et al.*, 2013) [14] [24] who detected the role of urinary hepcidin level in early prediction of iron deficiency in children and found that there was significant positive correlation between MCH and urinary hepcidin.

Our results showed no correlation between urinary hepcidin level and serum iron (r = 0.044), (P = 0.689), these results agreed with (Mohamed *et al.*, 2014) [23] who study the correlation between urinary hepcidin and hematological indices concentration among iron deficient anemic children and they found no correlation between urinary hepcidin and serum iron and this were in contrary with (Prentice *et al.*, 2012) [25] who found significant positive correlation between hepcidin and serum iron. The results of the correlation between ferritin and urinary hepcidin showed no significance (P = 0.918) and these results were in agreement with (Choi *et al.*, 2012) [21] and (A El-Kelany *et al.*, 2019) [22] who observed weak correlations between serum ferritin and hepcidin concentration, and in contrary with (Sanad and Gharib, 2011) [6] who found significant positive correlation between serum ferritin and urinary hepcidin concentration.

Our result shows that no correlation between urinary hepcidin and weight, height of the IDA patient height (P = 0.613); Weight (P = 0.407), and there was no correlation between urinary hepcidin and platelets (r = 10.091), (P = 0.339)

The Sensitivity and specificity of urinary hepcidin in diagnoses of iron deficiency anemia was (57.8) and (62.2) respectively with positive predictive value (63.3) in our study and Positive correlation between urinary hepcidin and MCV.

5. Conclusion

We concluded that urinary hepcidin decreased in patients with iron deficiency anemia but did not reach a significant level.

Recommendation

We recommend further studies are needed to evaluate if it can be used as early marker in diagnoses of iron deficiency anemia.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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