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Study of Hepcidin Level in Samples of Iraqi Patients With Iron Overload and Iron Deficiency Disorders

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Abstract:

Hepcidin, a regulator of iron recycling and absorption, is a small peptide created by the hepatocytes when there is an increase in body iron and inflammation. It is regarded as a master regulator for the metabolism of iron. Hepcidin decreases iron absorption from food by decreasing the transportation of iron across the enterocytes in the gut mucosa. Hepcidin decreases iron escape from macrophages, which are considered as the main site for storage of iron and decreases iron escape from the liver.

The aim of the study is to determine the properties of hepcidin as a diagnostic test of iron deficiency and to focus on its properties as a diagnostic test in iron overload.

Thirty-four patients with iron deficiency anemia (IDA) and thirty patients with beta - thalassemia major were collected randomly from the National center of Hematology and from thalassemia center in Al-Karama Hospital respectively. Together with thirty age and sex matched healthy volunteers were collected as a control.History was taken and physical examination was done. Blood samples were taken; hematological parameters were estimated for all three groups.

High hepcidin level was detected in 30 out of 34 patients with iron deficiency anemia with a mean range of (205.3 ng/ml). High hepcidin level is due to inflammatory conditions. In four patients with iron deficiency anemia, hepcidin levels were low and this is due to anemia and hypoxia. These stimulations decrease hepcidin production. In 30 patients with iron overload, hepcidin concentration was of low level with a mean range of (6.7 ng/ml) and this is due to stress erythropoiesis.

These results suggest that serum hepcidin in iron deficiency anemia patients is high in spite of that the patients were not receiving iron supplement while in iron overload (IOL) patients serum hepcidin level is low mainly because of stress erythropoiesis due to their poor management.

Keywords: *Thalassemia, Hepcidin, Iron, Iron Deficiency Anemia, Anemia, Iron Overload.*

دراسة مستوى الهيبسيدين في عينات من المرضى العراقيين المصابين بحالات نقص الحديد وإضطرابات زيادة الحديد في الدم

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الخلاصة:

تم معاينة **أ**ربعة وثلاثون مريضاً مصاباً بفقر دم نقص الحديد من المركز الوطني لبحوث وعلاج **أمراض الدم** اضافة الى ثلاثين مريض مصاباً بالثلاسيميا من مركز الثلاسيميا في مستشفى الكرامة بشكل عشوائي مع ثلاثين شخصاً من المتطوعين الاصحاء.

الهدف **من** هذه الدراسة هو تحديد خصائص الهيبسيدين كأختبار تشخيصي لنقص الحديد واضطرابات زيادة الحديد . تم اخذ المعلومات من المرضى والمتطوعين، **فضلاً عن إجراء الفحص البدني وأ**خذ عينات الدم لجميع الفئات **الثلاثة**.

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أظهرت نتائج **الدراسة إنخفاضاً** في مستوى الفيريتين في ثلاثين مريضاً مصاباً بفقر دم نقص الحديد وبمعدل (14.3) و زيادة في مستوى الهيبسيدين المصلي في ثلاثين مريضاً مصاباً بفقر دم نقص الحديد وبمعدل (205.3) **وتعود** هذه الزيادة الى وجود الإلتهابات، كما أظهرت النتائج **في** أربعة مرضى مصابين بفقر دم نقص الحديد **إنخفاضاً بمستوى** الهيبسيدين ويعود هذا الإ**نخفاض الى فقر الدم ونقص الأكسجة**. كان تركيز الفيريتين عالياً في ثلاثين مريض مصاباً بأضطرابات زيادة الحديد وبمعدل (4876.06) وتركيز الهيبسيدين المصلي منخفضا وبمعدل (6.7) **وذلك بسبب إنتاج** كريات الدم الحمراء تحت الإجهاد.

كلمات المفتاحية: فقر الدم، ثلاسيميا، هيبسيدين، زيادة الحديد، فقر دم نقص الحديد.

Introduction:

Hepcidin plays a central role in regulating dietary iron absorption and body iron distribution[1]. There are four main active regulation pathways (erythroid, iron store, inflammatory and hypoxia-mediated regulation) that control hepcidin production through different signaling pathways[2]. Increased erythropoietic activity and reduced tissue oxygen delivery suppress hepcidin production, thereby stimulating iron absorption/mobilization, whereas increased iron stores and inflammation act in the opposite way. In cases of iron deficiency anemia, anemia and hypoxia affect iron metabolism. These stimuli would be expected to decrease hepcidin production and remove the inhibitory effect on iron absorption and iron release from macrophages so that more iron is available for compensatory erythropoiesis. When IDA is coexisting with infections, chronic inflammatory disorders, and cancers, hepcidin level would be high. In cases of stress erythropoiesis, such as in β-thalassemia, hepcidin is suppressed, due to the dominant effect of the erythroid regulator [3].

Materials and Methods:

This study was conducted during the period from 1st October 2014 to 10th April 2015. The study was approved by the ethical committee of the Department of Pathology, College of Medicine, Al-Mustansiriyah University conforming to Hilsenki Declaration. Patients' oral consent was obtained.

Sixty four patients with iron deficiency anemia and iron overload disorders were collected randomly from The National Center of Hematology and Thalassemia center in Al-Karama teaching hospital. Thirty patients with β-Thalassemia major were randomly selected from Thalassemia center in Al-Karama teaching hospital. Their serum ferritin was >1000 ng/mL with a history of receiving >10 blood transfusions (Blood transfusion frequency is every 10-21 days).

Thirty four patients with iron deficiency anemia attributed to different diseases (Cancer, uterine bleeding, surgical operation, nutritional deficiencies and gastro intestinal bleeding) were included in this study. Those patients attended the National Center of Hematology. The criteria of inclusion of the patients are: History of symptoms of anemia confirmed by laboratory investigations. They were randomly selected and all are not on iron supplements and didn't receive blood transfusion in the last three months. The Hb concentration in children was less than 11g/dl and in males and females was less than 12 g/dl.

History was taken and physical examination was performed. Blood samples were collected by veni-puncture using Ethylene diamine tetra acetic acid (EDTA) tubes to perform complete blood picture and Hb-electrophoresis (to rule out concomitant hemoglobinopathy in IDA patients and controls) and by using clean disposable plain tube serum obtained by centrifugation of clotted blood for measurement of iron and total iron binding capacity (TIBC) by the direct colorimetric assay using automated analyzer, ferritin by enzyme immunoassay using automated immunoanalyzer, hepcidin by Enzyme linked immunosorbentassay (ELISA) using standard enzyme reader. Tests were performed at laboratory departments of

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National Center of Hematology and Al-Yarmook Teaching Hospital.

Thirty healthy subjects (15 males, and 15 females); age and sex matched, were also collected as a control. The same parameters were estimated for them and compared with reference range developed for normal subject. Mean, standard deviation were estimated, statistical analysis was done using the SPSS and Microsoft Excel program, p value <0.05 is considered as statistically significant.

Results:

The age of the IDA patients collected ranged from (13- 65 years) with a mean and standard deviation of (32.8 ± 14.44). All of them were not taking any iron supplements. The age of IOL patients range from (10-30 years) with a mean and standard deviation of (17.9±4.77). All of them were on chronic blood transfusion regimen. The age of the control ranged from (5-48years) with a mean and standard deviation of (22.6±12.33). Of the studied IDA patients, 30 of them had low ferritin level with a mean range and standard deviation of (4876.0667±1728.24567) as shown in (table-1) and (Figure-1).

Of the studied IDA patients, 30 of them (88.2%) had high hepcidin level; in four of them (11.7%) there was low hepcidin level. Of the studied IOL patients, all of them (100%) had low hepcidin level. All of the affected IO Lpatients are irregularly transfused either because of large family size with more than one member of the family being affected with thalassemia major and poor financial support or because of non-availability of the blood when needed. Mean serum hepcidin level was (205.3 ng/ml), (6.76 ng/ml) and (48.7 ng/ml) in IDA, IOL patients and controlsrespectivelyas shown in (table-1) (Figure-2) being statistically highly significant ($p < 0.05$).

It was found that high serum ferritin and low serum hepcidin in IOL group of patients has an obvious statistically significant negative correlation as expressed in (Figure-3).

It has been noticed that low serum ferritin and both of high serum hepcidin and high serum transferrin receptor (Trf) in IDA group of patients has an obvious statistically significant negative correlation as in (Figure-4).

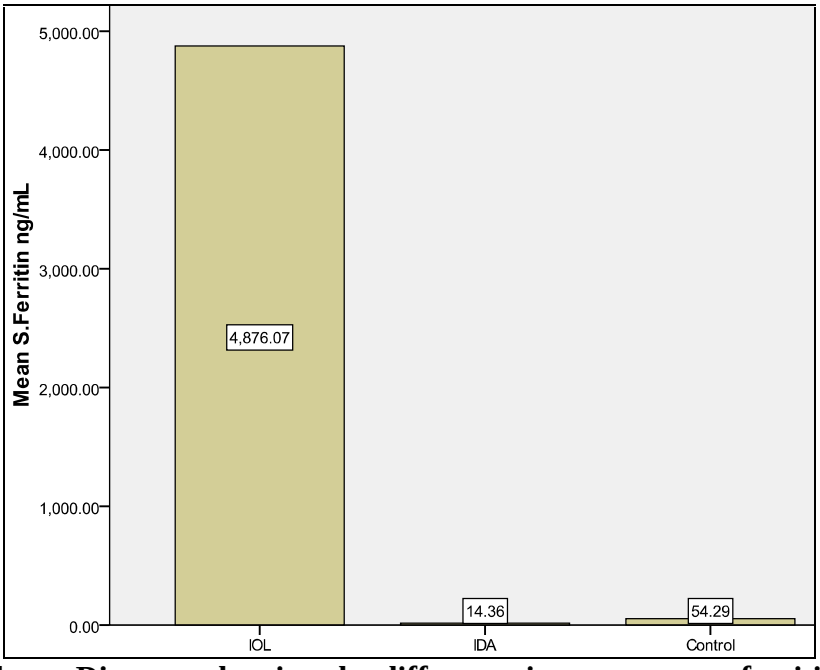

Figure-1: Column Diagram showing the difference in mean serum ferritin level among IOL patients, IDA patients and control group.

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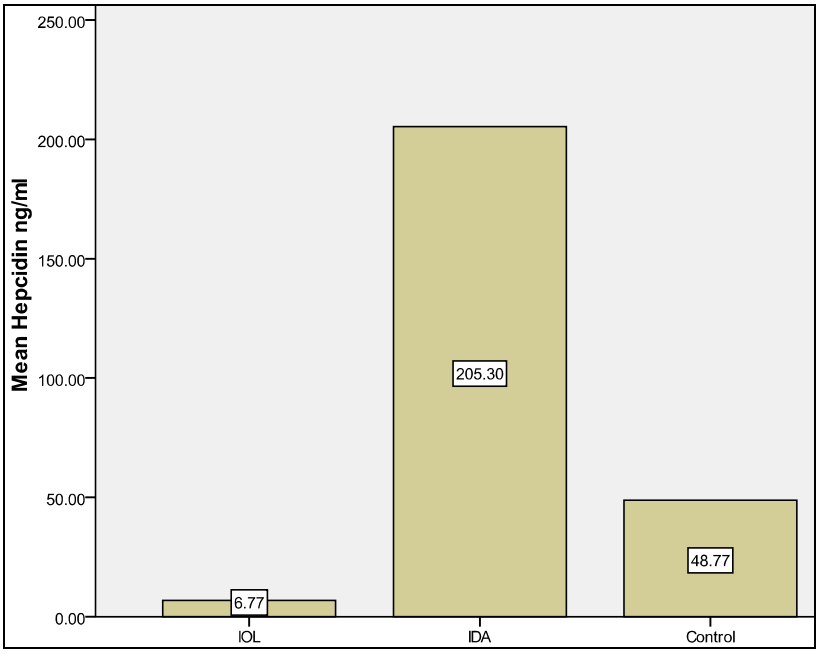

Figure-2: Column Diagram showing the difference in mean serum hepcidin level among IOL patients, IDA patients and control group.

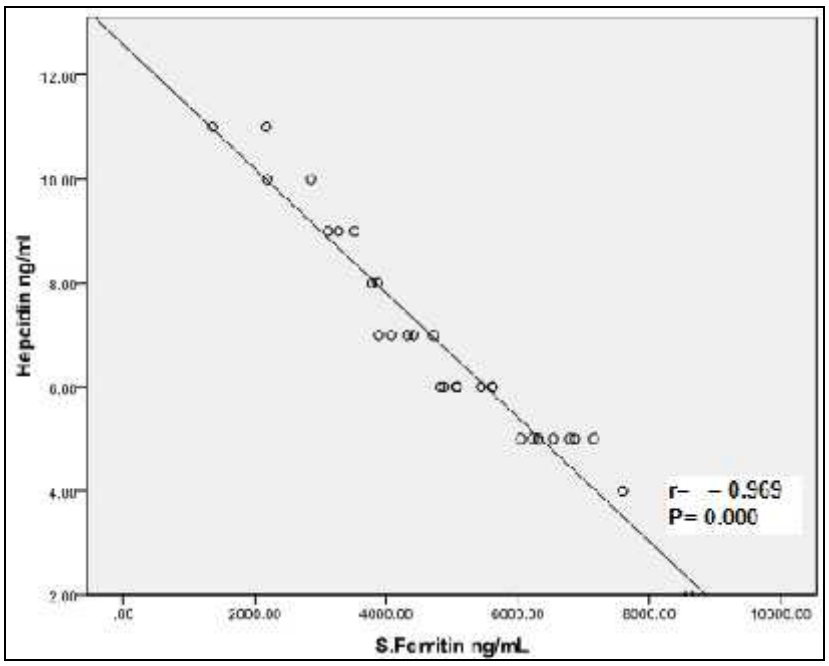

Figure-3: The Correlation between Hepcidin and Serum Ferritin levels in IOL Patients.

Table-1: Mean, standard deviation of hepcidin and transferrin receptor parameters in the patients and the control.

Parameter	Control	IDA	IOL	P value
Hepcidinng/ml	48.7±8.72	205.30±113.33	6.7±2.128	0.000*
Ferritin ng/ml	54.28±16.95	14.35±4.28	4876.06±1728.24	0.000*

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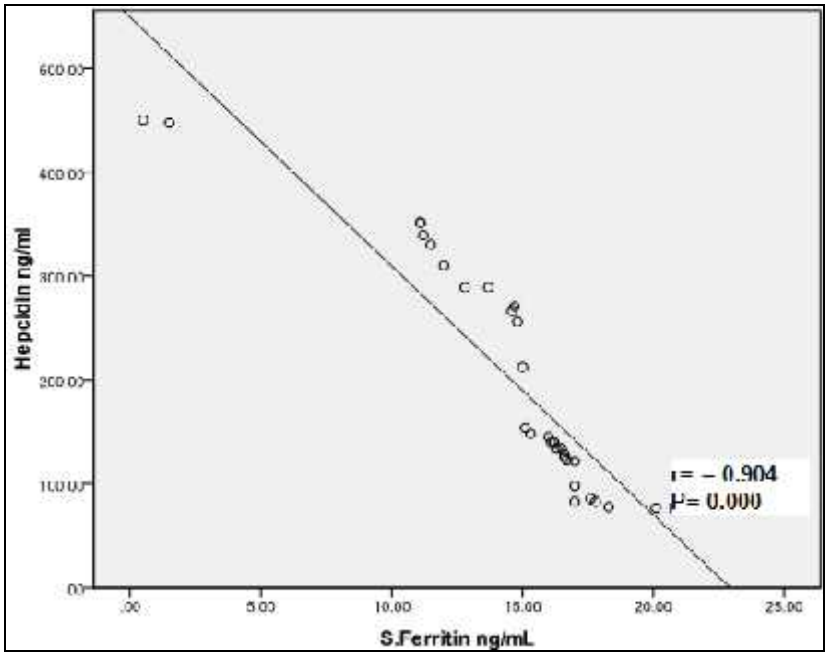

Figure-4: The Correlation between Hepcidin and Serum Ferritin levels in IDA Patients.

Discussion:

Ferritin level in 30 patients with IDA was of low level. The mean range was (14.3 ng/ml). Low levels of ferritin are seen in iron deficiency. Without enough iron, the body cannot produce sufficient levels of Hb. Iron deficiency anemia is the result. These results came in agreement with Hallberg et al.[4] who determined the serum ferritin concentration of 203 women aged 38 years. They concluded that a value of serum ferritin is the best predictor for iron deficiency and noted that the threshold was similar to one derived from earlier population surveys and studies of clinical cases[4].

Also Zanella et al. [5] study showed results similar to the present study results. They examined the sensitivity and predictive value of serum ferritin concentration to identify iron deficiency. The overall sensitivity and specificity of diagnosis were 82% and 95% for serum ferritin respectively. However the sensitivity was over 80% for ferritin in cases of severe anemia, in the absence of anemia the sensitivity dropped to 70% for ferritin. In a systematic review of the diagnostic value of various laboratory tests to diagnose iron deficiency it was concluded that serum ferritin was the most powerful test for simple iron deficiency in both populations and hospital patients[5].

In 30 patients with IOL, serum ferritin level was high with a mean range of (4876 ng/mL). These results reflect inadequate chelation and vulnerability to develop iron overload related compli-cations.

This finding agrees with Pootrakul, P. et al., who found that serum ferritin levels in beta-thalassemia patients were higher than normal[6]. The same result found by Mishra, A. and Tiwari, A. et al., who found that the serum ferritin levels in multi-transfused thalassemia major patients in India – Bhopal hospital are higher than that of the control group [7].

Easa Z. et al. also studied seventy patients with β – thalassemia including β – thalassemia major and thalassemia intermediaat time of their attendance to Thalassemia Center in Karbala Teaching Hospital. The result was also high ferritin level for all the β–thalassemia major patients included in that study[8].

Hepcidin level in 30 out of 34 patients with IDA was of high level. The

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mean range was (205.3 ng/ml) which is similar to the finding in Sharma S, Nemeth E, Chen YH, et al who found that patients have high hepcidin level in cases of infections, chronic inflammatory disorders, and cancers coexisting with IDA "anemia of chronic disease/inflammation" [9]. Also the results in this study came in agreement to Ganz et.al [10] who studied the role of hepcidin in inflammatory hypoferremia and how IL-6 and supernatants of lipopolysaccharide stimulated macrophages readily induce hepcidin in human hepatocytes and hepatic cell lines[10]. Four of IDA patients have cancer, three have chronic kidney disease, two have history of inflammatory bowel disease, seven have history of diabetes mellitus, two other patients have systemic lupus erythromatosis (SLE) and one case with history of Hashimoto's thyroiditis. These patients have autoimmune diseases with high hepcidin level and hyposideremia. This was similar to the finding in Ganz, T. and Nemeth, E. et al, who found that inappropriately elevated hepcidin and the resulting hypoferremia are also thought to play a pathogenic role in the development of the common forms of iron – restricted anemia[10], those associated with infections and inflammatory disorders including autoimmune diseases and some cancers. In these conditions, multiple cytokines are implicated in increasing hepcidin synthesis, including interleukin IL-6, IL-1, IL-22, and several members of the transforming growth factor β super-family. Inflammatory regulation of hepcidin probably evolved as a host defense mechanism to limit iron availability to microbes, but the same process may be maladaptive in noninfectious inflammatory disorders where it causes anemia. Five other patients with IDA are elderly patients with high hepcidin and hyposideremia. This was similar to the findings of Wendy P.J. den Elzen et al who found that aging is often associated with a low-grade pro-inflammatory state[11]. This mild pro-inflammatory state is thought to elicit a chronic elevation of circulating hepcidin. Four patients only out of thirty four patients with IDA have low hepcidin levels. This agrees with Weinstein et al and Nicolas et al who found that anemia and hypoxia affect iron metabolism[12, 13].

Hepcidin concentration in 30 patients with iron overload was of low level. The mean range was (6.7 ng/ml).Nearly the same reported by Kautz L, Jung G et al. [14], who found that in stress erythropoiesis, such as in β-thalassemia, hepcidin is suppressed, due to the dominant effect of the erythroid regulator.

Conclusion:

Only four patients out of thirty four cases of IDA have pure and simple IDA. The other thirty patients have mixed anemia with inflammation, chronic disease or cancer that overwhelmed the effect of anemia leading to an increase in hepcidin levels. In thirty patients with IOL, hepcidin mean level was low mainly because of stress erythropoiesis due to their poor management.

There is a critical need to support the chelation treatment and to start awareness about the outcomes of iron overload in patients. The study demonstrated elevated levels of serum ferritin in beta thalassemia major patients which give a general dreary view.

References:

1 - Kroot, J.; Tjalsma, H.; Fleming, R. and Swinkels, DW. Hepcidin in Human Iron Disorder: Diagnostic implica-tions. Clin Chem. 2011 Dec; 57(12): 1650-69.

2 - Piperno, A.; Mariani, R.; Trombini, P. and Girelli, D. Hepcidin Modulation in Human Diseases: From research to clinic. World J Gastroenterol. 2009 Feb 7; 15(5): 538–551.

3 - Zhao N, Enns CA, and Zhang A. Iron Regulation by Hepcidin. J Clin Invest. 2013 Jun; 123(6):2337-43.

Date of acceptance: 11-11-2015

4 - Hallberg, L. Screening for Iron Deficiency: An Analysis Based on Bone-Marrow Examinations and Serum Ferritin Determinations in a Population Sample of Women. BJH. 1993; 85(4): 787–798.
5 - Zanella, A.; Gridelli, L.; Berzuini, A.; Colotti, MT.; Mozzi, F. and Milani, S. Sensitivity and Predictive Value of Serum Ferritin and Free Erythrocyte Protoporphyrin for Iron Deficiency. J Lab Clin Med. 1989 Jan; 113(1): 73-8.
6 - Pootrakul, P.; Vongsmasa, V.; La-ongpanich, P. and Wasi, P. Serum Ferritin Levels in Thalassemias and the Effect of Splenectomy. Acta. Haematol. 1981; 66(4): 244-50.
7 - Mishra, A. and Tiwari, A. Iron Overload in Beta Thalassemia Major and Intermedia Patients. Maedica (Buchar). 2013 Sep; 8(4): 328–332.
8 - Easa, Z. Complications of High Serum Ferritin Level after Splenectomy in β Thalassemic Patients. Kufa Med. Journal. 2009; 12(1): 138-142.
9 - Sharma, S.; Nemeth, E. and Chen, YH. Involvement of Hepcidin in the Anemia of Multiple Myeloma. Clin Cancer Res J. 2008 June; 14(11): 3262-7.
10 - Ganz, T. and Nemeth, E. Hepcidin and Disorders of Iron Metabolism. Annu Rev Med. 2011; 62: 347-360.
11 - Elzen, WP.; Willems, JM.; Westendorp, RG.; deCraen. AJ.; Assendelft, WJ. and Gussekloo, J. Effect of Anemia and Comorbidity on Functional Status and Mortality in Old Age. CMAJ. 2009; 181(3-4): 151-7.
12 - Weinstein, DA.; Roy, CN.; Fleming, MD.; Loda, MF.; Wolfsdorf, JI. and Andrews, NC. Inappropriate Expression of Hepcidin is associated with Iron Refractory Anemia: Implications for the Anemia of Chronic Disease. Blood. 2002 Nov 15; 100(10): 3776-378.
13 - Nicolas, G.; Chauvet, C.; Viatte, L.; Danan, JL.; Bigard, X.; Devaux, I.; Beaumont, C.; Kahn, A. and Vaulont, S. The Gene Encoding the Iron Regulatory Peptide Hepcidin is regulated by Anemia, Hypoxia, and Inflammation. J Clin Invest. 2002 Oct; 110(7): 1037-1044.
14 - Kautz, L.; Jung, G.; Valore, EV.; Rivella, S.; Nemeth, E. and Ganz, T. Identification of Erythroferrone as an Erythroid Regulator of Iron Metabolism. Nat Genet. 2014; 46(7): 678-684.