Study of Hepcidin Level in Samples of Iraqi elderly anemic patients

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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 this study is to explore the diagnostic value of hepcidin in elderly patients with iron deficiency anemia and anemia of chronic disease. Anemia was defined according to criteria of the World Health Organization (hemoglobin level <13 g/dL for men and hemoglobin <12 g/dL for women).

Thirty elderly patients (Age 65 years and above) with anemia were collected randomly from the National center of Hematology and from Al-Karama Hospital. 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 the two groups.

We found strong correlation between serum hepcidin and body iron status. High hepcidin level was detected in elderly patients with anemia of inflammation.

Keywords: *Hepcidin, Iron, Iron Deficiency Anemia, Anemia, ferritin.*

دراسة مستوى الهيبسيدين في عينات من المرضى العراقيين المسنين المصابين بحالات فقر الدم

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الخلاصة

تم معاينة ثلاثون مريضا مسنا (بعمر 65 سنة فما فوق) مصاب بفقر الدم من المركز الوطني لبحوث وعلاج امراض الدم و مستشفى الكرامة بشكل عشوائي مع ثلاثين شخصا من المتطوعين الاصحاء.

الهدف من هذه الدراسة هو تحديد خصائص الهيبسيدين كأختبار تشخيصي في المرضى المسنين المصابين بفقر الدم بسبب نقص الحديد او بسبب الأمراض المزمنة. تم اخذ المعلومات من المرضى والمتطوعين , فضلا عن اجراء الفحص البدني واخذ عينات الدم للفئتين .

اظهرت نتائج الدراسة زيادة في مستوى الهيبسيدين المصلي في ثلاثين مريضا مسنا مصاب بفقر الدم . تعود هذه الزيادة الى وجود الالتهابات .

الكلمات المفتاحية : *فقر الدم , هيبسيدين , فقر الدم نقص الحديد , فيريتين.*

Introduction

In elderlies, anemia is considered as one of the common disorders [1]. It has been found that about one fifth of anemic old persons are considered to have anemia of inflammation [2] previously known as anemia of chronic disease, that is usually found in patients with infections or inflammatory disorders, such as cancer, chronic kidney disease and rheumatoid arthritis [3,4]. The pathophysiological mechanism of this condition is obscure; it is likely to be mediated by immune-driven processes [3,4]**.**

Hepcidin, a 25 amino-acid peptide hormone produced by the liver, it plays a central role in regulating dietary iron absorption and body iron distribution [5]. There are four main active regulation pathways (erythroid, iron store, inflammatory and hypoxia – mediated regulation) that control hepcidin production through different signaling pathways [6]. 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 iron deficiency anemia is coexisting with infections, chronic inflammatory disorders and cancers, hepcidin level would be high [7].

"Inflammaging", is a term used to describe the phenomenon of low – grade inflammation that is associated with aging. [8] This mild pro-inflammatory condition is assume to evoke a chronic elevation of circulating hepcidin which will lead to an impairment in the availability of plasma iron, limitation in hemoglobin synthesis and development of anemia of inflammation [10].

There was no data available on levels of serum hepcidin in elderlies with anemia in the general population but fortunately now more reliable serum hepcidin assays have become

available, [5] leading us to investigate the relation between serum hepcidin levels and anemia of inflammation in older persons in the general population.

Materials and Methods

This study was conducted during the period from 1st August 2017 to 10 th February 2018. Patient's oral consent was obtained. Thirty elderly patients with anemia attributed to different diseases (Cancer, uterine bleeding, surgical operation, nutritional deficiencies and gastrointestinal bleeding) were included in this study. Those patients attended the National center of Hematology and Al-Karama hospital. The criteria of inclusion of the patients are: History of symptoms of anemia confirmed by laboratory investigations. They were randomly selected. The hemoglobin level was <13 g/dL for men and <12 g/dL for women.

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 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 (Commercially available kit VIDAS Ferritin, 30 411, bioMerieux , France) , hepcidin by Enzyme linked immunosorbentassay (ELISA) using standard enzyme reader (Commercially available kit Human Hepcidin ELISA kit , CSB-E14239h ,CUSABIO, China).

Tests were performed at laboratory departments of AL-Yarmook teaching hospital. Thirty healthy subjects, 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 Statistical Analysis System- SAS (2012) program and Microsoft Excel program, p value <0.01 is considered as statistically significant.

Results

Of the studied elderly anemic patients, all of them (100 %) had high serum hepcidin level. Mean serum hepcidin level was (214.10 ng/ml), and (50.17 ng/ml) in elderly patients and controls respectively as shown in (table-1) (Figure-1) being statistically
highly significant ($p < 0.01$). It has been noticed that there is a low serum ferritin and high serum hepcidin level in

elderly anemic patients group with an obvious statistically significant negative correlation as in (Figure-2).

Table (1): Mean, standard deviation of hepcidin and serum ferritin parameters in the patients and the control

Group	S. Ferritin ng/ml	S. Hepcidin ng/ml
Control	54.73 ± 3.01	50.17 ± 1.43
Patients	15.34 ± 0.40	214.10 ± 20.86
P-value	0.0001	0.0001

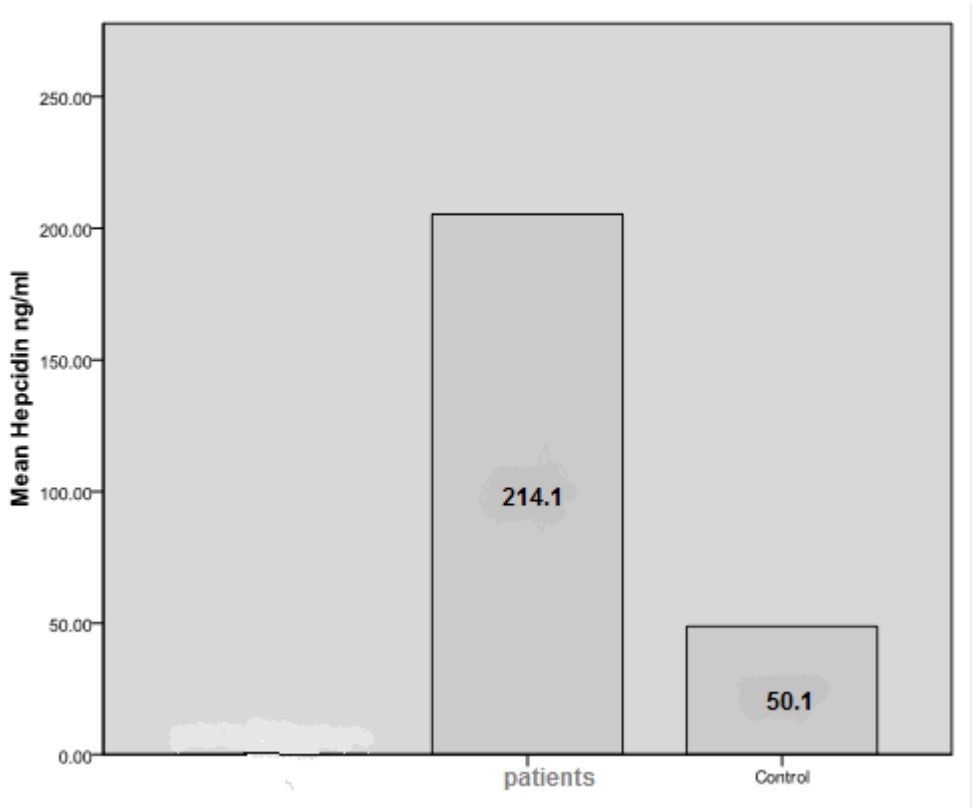

Figure (1): Column Diagram showing the difference in mean serum hepcidin level among elderly anemic patients and control group

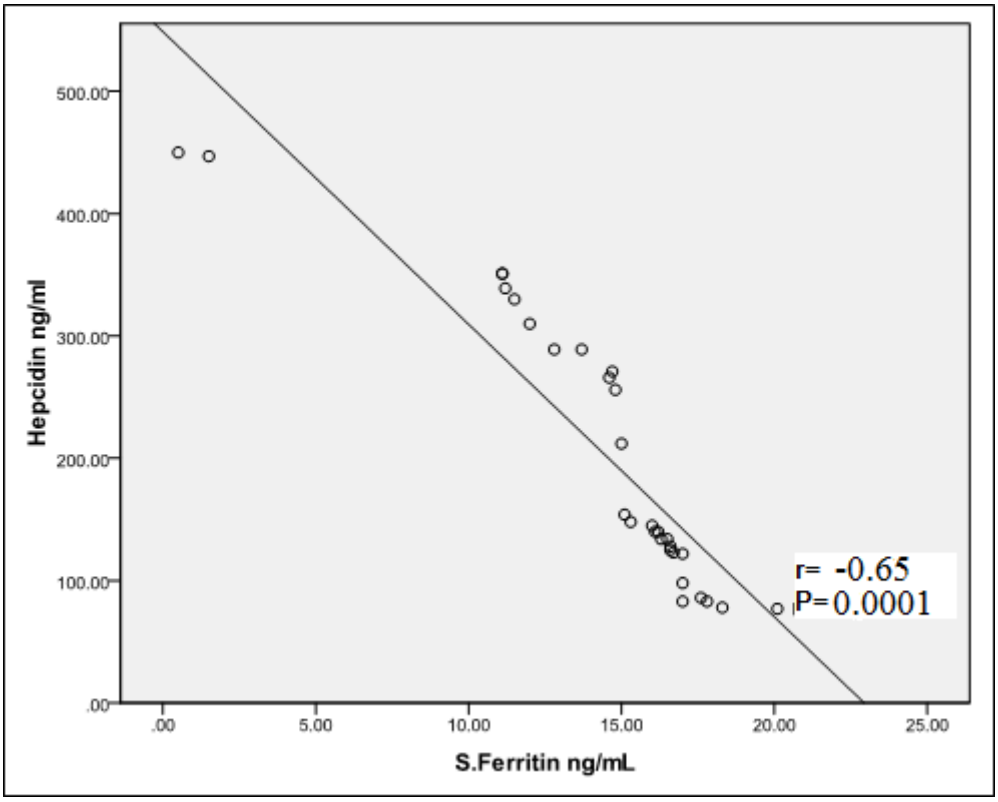

Figure (2): The Correlation between Serum Hepcidin and Serum Ferritin levels in elderly anemic Patients

Discussion

Hepcidin level in 30 elderly anemic patients was of high level. The mean range was (214.10 ng/ml) which is similar to the findings in Sharma et al. (2008) who found that patients have high hepcidin level in cases of infections, chronic inflammatory disorders and cancers coexisting with iron deficiency anemia " anemia of chronic disease/inflammation " [9]. Also the results in this study came in agreement with Ganz et al. (2010) [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]. Also elderly patients with iron deficiency anemia and high hepcidin this was similar to the findings of Wendy P.J. den Elzen et al. (2009) who found that aging is often associated with a low grade pro-inflammatory state [1]. This mild pro-inflammatory state is thought to elicit a chronic elevation of hepcidin.

The highest hepcidin levels were found in patients with anemia of chronic kidney disease. This is due to the restriction of the excretion and degradation of hepcidin by the kidney. [13].

Although in previous studies patients with chronic kidney disease were mostly found to have increased plasma hepcidin levels, the association between the glomerular filtration rate and hepcidin levels in such patients has not yet been precisely examined. [5]

Five of elderly anemic patients have iron deficiency anemia with infection, twelve have cancer, six have chronic kidney disease, two have history of inflammatory bowel disease, five have history of diabetes mellitus.

These patients have autoimmune diseases with high hepcidin level and hyposideremia. This was similar to the finding in Ganz et al. (2010), who found that inappropriately elevated hepcidin and the resulting hypoferremia are also thought to play a pathogenic role in the developmentof 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. All of the patients involved in this study were elderly (> 65 years old) with high hepcidin and hyposideremia. This was similar to the findings of Wendy P.J. den Elzen et al. (2009) who found that aging is often associated with a low-grade proinflammatory state [1]**.**

This mild proinflammatory state is thought to elicit a chronic elevation of circulating hepcidin. Ferritin level in 30 elderly anemic patients was of low level. The mean range was (15.34 ng/ml). Low levels of ferritin are seen in iron deficiency. Without enough iron, the body cannot produce sufficient levels of hemoglobin. Iron deficiency anemia is the result. These results came in agreement with Hallberg et al. (1993) [11] who determined the serum ferritin concentration of 203 women. 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 [11]. Also Zanella et al. (1999) [12] study showed similar results 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 [12].

Conclusion

Thirty elderly anemic patients have mixed anemia with inflammation, chronic disease, infection or cancer that overwhelmed the effect of anemia leading to an increase in hepcidin levels.

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