Original Article

Hepcidin Level in Sera of Patients with Chronic Hepatitis B Virus in Babylon Province

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Abstract

Background: The most prevalent disease in the world, is chronic hepatitis B virus (CHB). It has several causes like, drugs, alcohol consumption, toxicity and auto-immune disease, but in most cases it is caused by hepatitis viruses (viral hepatitis). Hepcidin regulates the body iron negatively by binding to the cellular iron exporter ferroportin in target cells, like macrophages, enterocytes and making its internalization and deprivation, thus reducing iron efflux into plasma causing decrease serum iron level and an elevated in intracellular iron. **Materials and Methods:** One hundred subjects; 50 diagnosed with chronic hepatitis B virus and 50 healthy subjects were enrolled in this study. Age ranged between (20–60) years (for patients and control). Hepcidin and ferritin level in serum were estimated by enzyme- linked immunosorbent assay (ELISA) technique, while serum iron concentration was calculated by the Siemens Dimension by Direct method (Ferene). **Results:** In chronic hepatitis B virus group serum hepcidin, ferritin and iron levels significantly elevated compared with control group ($P < 0.01$). On the other hand, the current study observed significant (p value <0.05) positive correlation for hepcidin with ferritin and iron in (CHB) patients. **Conclusion:** Among chronic hepatitis B patients in Babylon province, increase the level of some iron regulatory parameters; indicate the significant linked with the progression of chronic hepatitis B virus.

Keywords: Chronic hepatitis B virus, ferritin, hepcidin

Introduction

An inflammation of the liver organ cells (like hepatocyte infection or dysfunction) known as Hepatitis. Some patients display no signs of hepatitis, while further patients have jaundice (yellow-colored skin), diarrhea, vomiting, headache, reduced appetite, and nausea. If the hepatitis recurs within a half-year period is called Acute hepatitis or else, if the hepatitis continues for more than 6 months is called chronic hepatitis.[1]

The most prevalent disease in the world, is Hepatitis. It have several causes like, drugs, alcohol consumption, toxicity and auto-immune disease, but in most cases it is caused by hepatitis viruses (viral hepatitis). *Hepatovirus A*, *B*, *C*, *D*, and *E* is the most common causes for Hepatitis. Cytomegalovirus, *Epstein–Barr* virus, and yellow fever virus can cause liver inflammation.[2]

Around 2.57 billion subjects are infected with hepatitis B virus (HBV), and about 750000 deaths every year. Between them, more than 250 million are chronically infected and amplified the danger of emerging HBV-related liver diseases, like liver cirrhosis (LC) and hepatocellular carcinoma (HCC).[3] On the other hand, the incidence of Chronic hepatitis B virus (HBV) was 0.7% in Babylon governorate.[4]

The World Health Organization find motivated strategy to reduce viral hepatitis by 2030, leading to 90% reduction in new infections and a 65% reduction in mortality. Still, HBV patients aware of their infection represent only 10%.[5]

The risk factors for HBV viruses includes working in a healthcare setting, blood transfusion or organ

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transplantation without HBV screening, acupuncture, dialysis, tattooing, extended overseas travel, infusion bags, multiple-use of medication vials, intravenous drug use (IDU) and improperly sterilized surgical equipment.[6]

A 25-amino acid peptide in humans also known as liver-expressed antimicrobial peptide-1 (LEAP-1) is Hepcidin. It is encoded by the *HAMP* gene, it first exposed and categorized as antimicrobial with a highly disulfide-bonded peptide.[7]

Hepcidin regulates the body iron negatively by binding to the cellular iron exporter ferroportin in target cells, like macrophages, enterocytes and making its internalization and deprivation,[8] thus reducing iron efflux into plasma causing decrease serum iron level and an elevated in intracellular iron.[9]

Ferritin named and discovered in 1937, it is highly symmetrical and persistent iron-containing protein that was crystallized. Since a lot of iron can store in a huge cavity, it was called the main iron storage protein.[10]

Ferritin is abundant and has numerous functions. Commonly ferritin is present in the cytoplasm, and present in the mammalian mitochondria, nucleus, plasma, plant plastids and insect endoplasmic reticulum (ER).[11]

Ferritin as a cytosolic protein is found in most tissues, but small quantities are secreted into the serum, as an iron carrier. Ferritin in the plasma can be used as an indirect marker of the iron stored in the body.[12]

Serum ferritin levels elevated significantly in about one third of chronic viral Hepatitis patients. The most probable cause is increased the leakage of iron from damaged hepatocyte. Because elevated serum ferritin level associated with the degree of hepatic injury.[13]

Alterations in pathophysiological mechanisms made by HBV clarify the variation in hepcidin levels in these infections,[14] serum ferritin levels is a simple test used is to determine iron homeostasis of patients with liver diseases.[15]

Materials and Methods

Study design

A case-control study was designated.

Patients and control

One hundred subjects were enrolled in the present study. Fifty patients with CHB virus, for all patients complete history was taken, which include: body mass index,age and gender. Also, fifty apparently healthy subjects as a control group.

Patients with renal failure, autoimmune diseases (like Rheumatoid Arthritis), Obesity and Patients with cirrhosis and hepatocellular carcinoma, were excluded from the study. The patients and control age from 20 to 60 years. The statistical analysis was done by SPSS version 20. Less than 0.05, P-values considered significant.

Chemicals and methods

1 The sandwich-ELIZA kit as the method were used to determined serum hepcidin and ferritin concentration. In this kit, an antibody specific to hepcidin and ferritin were pre-coated to the micro- ELIZA plate (Bioassay Technology Laboratory, ELIZA kit).
2 Serum iron concentration was calculated by the Siemens Dimension device with Direct method (Ferene).

Ethical approval

These study was agreed dependent on the ethical principles originated in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 4 in 06/07/2022 to get this approval.

Results

This study comprise of 100 adults entitle in two groups:

1 Patients with chronic hepatitis B virus (n = 50)
2 Apparently healthy subject as a control group (n = 50)

Age

The difference in age (as mean) represented non-significant changed in CHB patients compared with the control group [Table 1]. According to age, the frequency distribution of patients with CHB was as following: 34 (68%) cases from 20–40 years and 16 (32%) cases between 41–60 years.

Gender

To avoid sex genome interference with the activity of liver enzymes interested in this study, the gender of CHB patients and control group was designated to be equal for both males and females, the percentage of males was 50% and percentage of females was 50% for each patients and control, [Table 1].

Body mass index (BMI)

Results in [Table 1] shows that patients with a normal body mass index were selected in this study.

Association of hepcidin level with ferritin and iron of chronic hepatitis B patients.

[Table 1] shows increase concentration of hepcidin, ferritin and iron with significant mean differences in CHB patients compared to the control group. On the other hand, the current study observed significant (p value <0.05) positive

Table 1: The study samples characteristics

Parameters	Mean ± SD		P-value
	CHB patients group (n=50) 25M/25F	Control group (n=50) 25M/25F	
Age (Years)	38.72 ± 10.79	35.52 ± 11.92	>0.05
BMI	23.08 ± 1.69	22.66 ± 1.95	> 0.05
Hpcidin (pg/ml)	165.56 ± 31.45	95.56 ± 32.47	< 0.05
Ferritin (ng/ml)	345.64 ± 29.06	310.1 ± 31.5	< 0.05
Iron (µmol/L)	24.94 ± 3.82	17.93 ± 3.07	< 0.05

SD= standard deviation; p< 0.05: significant; CHB= Chronic Hepatitis B; BMI: Body Mass Index; M=male; F= female

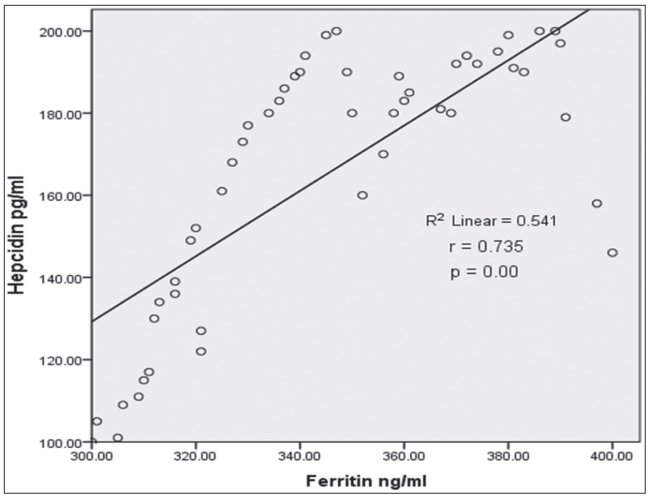

Figure 1: Correlation between Hepcidin and Ferritin in CHB patients

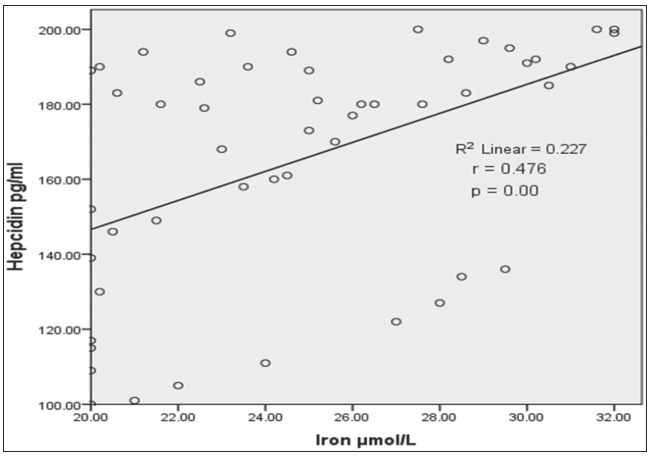

Figure 2: Correlation between Hepcidin and Iron in CHB patients

correlation for hepcidin with ferritin and iron in (CHB) patients, as in [Figures 1 and 2] respectively.

Discussion

The liver shows an essential role in reutilizing iron as the organ synthesizes transferrin (the major transporting protein) and ferritin (the main storage protein). Iron homeostasis might be disturbed by hepatic injury and hepatic dysfunction. Excessive iron deposition leads to promote injuries in the liver causing inflammation, hepatocellular necrosis, fibrosis, and carcinoma.[16] In this study, serum iron concentration were detected in both groups, serum iron elevated significantly in chronic HBV patients compared with healthy control, as in [Table 1].

This finding was observed by Maoa W *et al.,* 2015[17] and Wang J *et al.,* 2016,[18] they proposed that changed the level of serum iron were linked with CHB infection. our study is not in agreement with the finding of Wei Y *et al.*, 2018,[19] who attributed decreased serum iron levels in patients with HBV, as a risk factor for the prediction of HBV-related HCC.

Ferritin is the member of an acute phase proteins, iron overload and systemic inflammation caused by elevated ferritin level. The main iron storage protein in the liver is ferritin, where further iron in the body is deposited, and its appearance in the liver is prompted in primary or secondary iron overload disorders, causing elevated ferritin levels in the liver and circulation.[15]

Our study agrees with Gao Y H *et al.,* 2018[20] and Abdalwahed I B *et al.*, 2018,[12] they reported elevated serum ferritin concentration in patients with HBV compared to healthy control. Furthermore, Some studies have indicated that serum ferritin levels might be used to calculate the degree of hepatocyte damage in CHB.

Stores of iron, hypoxia, erythropoietic activity and inflammation are the major elements that disturb hepcidin levels.[21] In addition, hepcidin synthesis is stimulated by bacterial or viral infection.[18]

Generally, our study revealed an increase in hepcidin level in CHB patients comparative to control group [Table 1]. These finding are in harmony with Wang J *et al.*, 2016,[18] who stated that hepcidin in serum was elevated in CHB patients. While another revisions revealed that hepcidin in serum is decreased in CHB compared to controls.[14,18]

Also, hepcidin production is stimulated by improved tissue and systemic iron concentration. Hepcidin is capable to respond to discrepancies in body iron needed and its level changes accordingly.[22]

The current study observed significant positive correlation for Hepcidin with Ferritin (r = 0.735, P < 0.05), this is in accordance with the results of Wang J *et al.,* 2016[18]

and El-Lehleha A M *et al.,* 2017,[23] described positive relationships between hepcidin and ferritin in serum of CHB patients. But Nagashima M *et al.,*2006,[24] reported negatively correlated between them.

Associations between hepcidin and iron are frequently detected with chronic viral hepatitis. In the current study, there was a significant positive association between serum hepcidin and serum iron (r = 0.476, p <0.05). Similarly, El Wakil R *et al.*,2012,[25] established positive association between serum hepcidin and serum iron.

Another studies, with hepcidin, institute no association with iron in CHB patients or a negative relationship.[26,27]

Conclusion

Among chronic hepatitis B patients in Babylon province, increase the level of some iron regulatory parameters; indicate the significant linked with the progression of chronic hepatitis B virus.

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Conflicts of interest

There are no conflicts of interest.

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