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STUDY OF HEPCIDIN LEVEL IN EGYPTIAN HCV INFECTED PATIENTS AND ITS CORRELATION TO FERRITIN

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Abstract

The main objective of this study is to elucidate the correlation between Hepcidin and ferritin as a diagnostic biomarkers in HCV infected patients. Infection with hepatitis c virus is a major cause of choronic liver disease, many experimental and clinical studies suggest that excessive iron in CHC is a cofactor promoting the progression of liver damage and increasing the risk for fibrosis. This study contain two groups; Group I (healthy subjects): This group included fifteen healthy persons with ages ranged (33-63) years, they had no history of liver disease which may interfere with the studied parameters, This group represents 30%. Group II : This group included thirty five patients infected with hepatitis C virus, their ages range from 41 to 76 years, This group represents 70%.All clinical individuals in this study were collected from Outpatient Clinics of Zagazig University Hospitals. The following parameters ; Complete blood pictures , Liver functions tests , Kidney functions tests, Hepcidin levels and Ferritin levels were performed for all groups. The obtained result revealed a significant decrease of hepcidin in HCV infected patients compared to the control group. Also, a significant increase in ferritin level in infected patients compared to control group .

Keywords: Hepcidin, HCV, Ferritin, liver, fibrosis, cirrhosis.

Introduction

The liver is an essential organ and is the largest gland in the human body (Sherlock and Dooley, 2002). The liver receives approximately 25% of cardiac output (Schiff *et al*., 2007) .From the functions of the liver is the detoxification of various metabolites, synthesizing of proteins, and the production of biochemical that is needed and necessary for digestion. In humans, it is located in the upper right hand portion of the abdomen below the diaphragm .Moreover, It also plays a vital role in metabolism, regulation of glycogen storage, decomposition of red blood cells and hormone production (Abdel-Misih *et al*., 2010) On the other hand the Diseased liver includes hepatic fibrosis which refers to the accumulation of extracellular matrix or scar tissue in response to acute or chronic liver injury and hepatic cirrhosis which resulted from a wound healing response to injury. The dual blood supply, size of the organ, and abundant availability of nutritional material are factors favoring tumor growth like Malignant lymphomas in addition to primary liver tumors that can be developed readily within this organ (Kemeny and Schneider, 1989).

Hepatitis C virus belongs to the flavivirus that was defined as non-A, non-B hepatitis. The other major risk factor for transmission is intravenous drug use. Vertical transmission from mother to infant occurs in 3% to 5% of cases, and more in HIV-coinfected mothers. Sexual transmission may occur but is far less efficient than with other blood-borne viruses such as HIV or hepatitis B virus (HBV). Nosocomial transmission resulting from the use of non sterilized equipment for large scale immunization or parenteral therapeutic programs appears to be in part responsible for the high prevalence of HCV in some regions of the world. Infection with Hepatitis C virus is from the main causes of chronic liver diseases, like fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma, which affects approximately 170 million people worldwide (Flamm *et al*., 2003). Egypt is considered from the highest HCV prevalence in the world. 14.7% is the percentage of HCV in Egypt among general population in the year 2008 (El-Zanaty and Way, 2008).

Hepcidin is the major regulator of body iron homoeostasis. The HAMP (hepcidin antimicrobial peptide) gene is expressed predominantly in the liver and its mRNA encodes an 84 amino acid pre-pro-peptide which undergoes cellular cleavage (Valore and Ganz, 2008) to release the active 25 amino acid peptide into the circulation (Park *et al*.). The mature peptide contains eight cysteine residues that yield four disulphide bonds originally thought to confer a distorted hairpin-like structure. However, more recent analysis has produced an updated structure for hepcidin, comprising a stable sheet together with a hairpin loop (Wallace and Subramaniam, 2007). Hepcidin expression is also associated with the regulation of body iron status in both health and disease. Further mutations in the hepcidin gene have also been identified which alter the structure and function of the mature peptide (Jordan *et al*., 2009). Hepcidin is thought to exert its effects on iron metabolism by inhibiting iron efflux through the ferroportin transporter. This in turn impairs the release of iron from its target cells (namely, the reticulo-endothelial macrophages and the duodenal enterocytes) into the circulation. While both *in vivo* and *in vitro* studies support this rapid mode of action in macrophages (Chaston *et al*., 2008) (Mena *et al*., 2008). Patients with HCV infection have a relative deficiency of hepcidin compared to uninfected controls (Oates *et al*., 2000). Leading to unrestricted duodenal iron absorption and iron release from macrophages via the iron transporter ferroportin (Frazer *et al*., 2003).

Ferritin, composed of 24 polypeptide subunits, with an overall molecular weight of approximately 500 kDa that can store up to 4,500 ferric iron atoms (Harrison and Arosio, 1996). The iron stored in ferritin is available for utilization by other functional proteins and can be mobilized following lysosomal degradation of the ferritin complex (Bomford, 1988).

Materials and Methods

All clinical individuals in this study were collected from Outpatient Clinics of Zagazig University Hospitals.

Subjects

Fifty individuals were included in this study. Group I (healthy control), This group included fifty healthy persons with ages ranged (33-63) years, they had no history of liver disease, malignant tumors or any other diseases which may interfere with the studied parameters, This group represents 30% and the second group, included thirty five patients infected with hepatitis C virus, their ages range from 41 to 76 years, This group represents 70%.

Methods

All groups were subjected to Complete blood pictures (CBC), Liver functions tests (L.F.T), Kidney functions tests (L.F.T), Hepcidin and Ferritin levels. Complete blood count (CBC)Was done on automated cell counter, [XS 500i (Sysmex, Japan)], Liver and kidney functions were done by using biomed. reagents, hepcidin was determined using Human Hepc25 (Hepcidin 25) ELISA Kit supplied by Elab science Biotechnology Inc and The quantitative determination of Ferritin by means of particle-enhanced turbidimetric immunoassay using Spectrum Kit supplied by Egyptian Company for Biotechnology (S.A.E).

Results

Our results showed that the mean values of HB (11.9±1.0), RBCs(4.21±0.56), WBCs(5.9±1.9) and Platelet (313.51±81.53) in HCV infected patients compared to control group (13.1±0.7), (4.72±0.30), (4.9±0.7) and (365.78±46.09) as shown in table (1).

Table 1: Complete Blood picture**:**

Variables	Case	Control
	Mean± SD	Mean ±SD
HB	11.9±1.0 (10.1-13.6)	13.1±0.7 (12.0-14.3)
RBCs	4.21±0.56 (3.30-5.30)	4.72±0.30 (4.30-5.30)
WBCs	5.9±1.9 (3.7-10.0)	4.9±0.7 (4.1-6.3)
Platelet	313.51±81.53 (169.0-434.0)	365.78±46.09 (276.0-420.0)

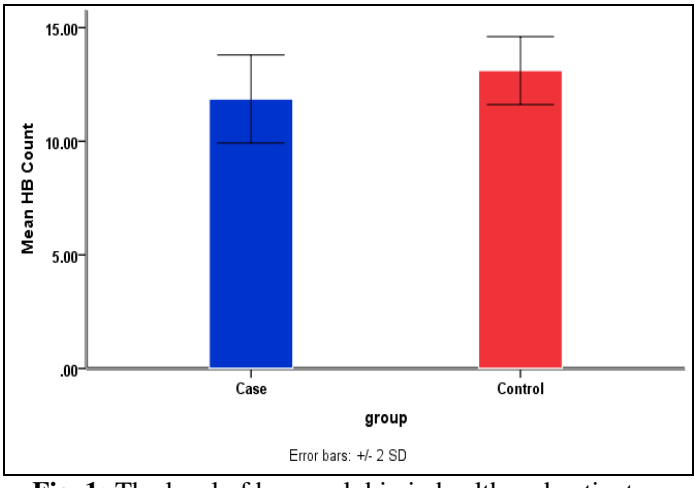

Fig. 1: The level of haemoglobin in health and patients.

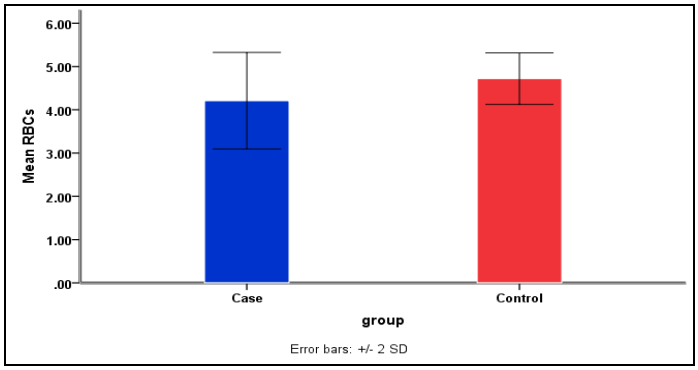

Fig. 2 : RBCs in health and patients.

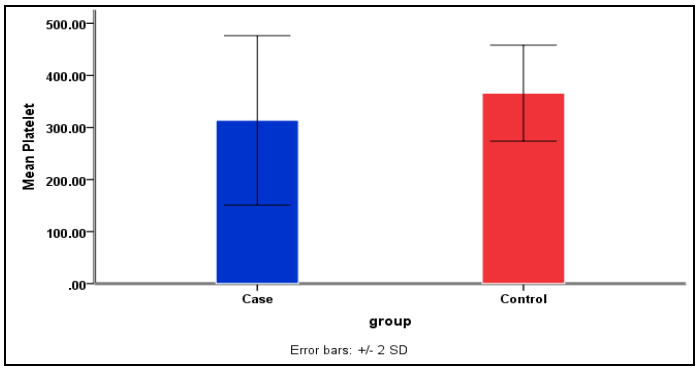

Fig. 3 : The level of Platelets in health and patient.

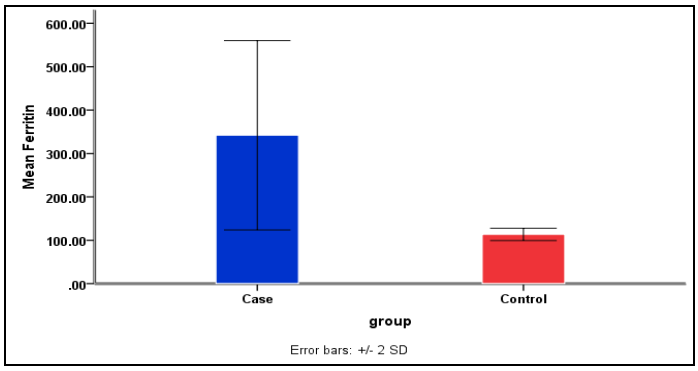

Fig. 4 : The level of ferritin in health and patients.

Our results showed that the mean values of ALT (52.0±4.9), AST (65.0±11.9), Albumin (2.47±0.24), Total bilirubin (0.69±0.22), Creatinine (0.78±0.17) and Bl.Urea (22.83±6.50) in HCV infected patients compared to control group ALT (24.3±6.3), AST (27.2±5.2), Albumin (3.86±0.26), Total bilirubin (0.67±0.18), Creatinine (0.78±0.15) and Bl. Urea (25.73±6.05) as shown in tables 2 and 3 respectively.

Table 2: kidney function:

Variables	Case	Control
	Mean± SD	Mean± SD
Creatinine	0.78±0.17 (0.55-1.17)	0.78±0.15 (0.60-1.10)
B Urea	22.83±6.50 (12.0-40.0)	25.73±6.05 (17.0-37.0)

Table 3: Liver functions:

Variables	Case	Control
	Mean± SD	Mean± SD
Total bilirubin	0.69±0.22 (0.30-1.20)	0.67±0.18 (0.35-1.0)
Albumin	2.47±0.24 (2.00-2.80)	3.86±0.26 (3.50-4.30)
AST	65.0±11.9 (47.0-72.0)	27.2±5.2 (17.0-38.0)
ALT	52.0±4.9 (40.0-61.0)	24.3±6.3 (14.0-35.0)

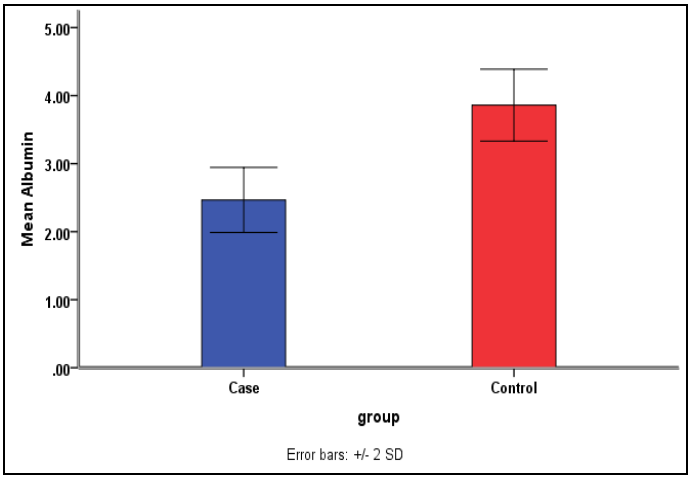

Fig. 5 : Albumin concentration in health and patients .

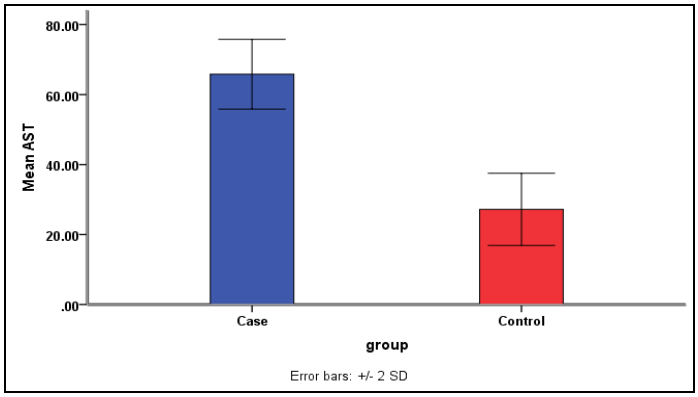

Fig. 6 : AST concentration in health and patients.

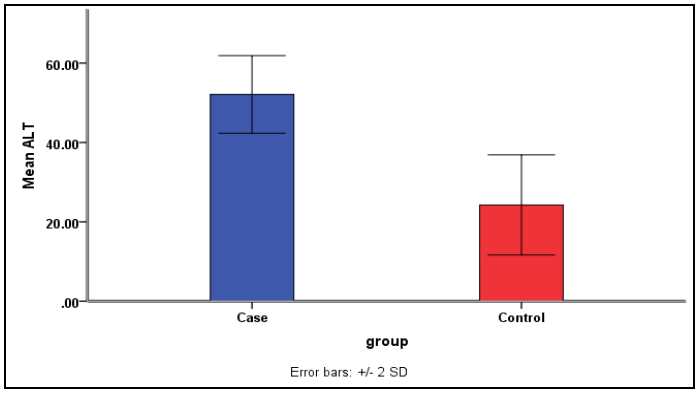

Fig. 7 : Albumin concentration in health and patients.

Our results showed a significant increase in the value of Ferririn as the mean values of Ferritin (342.9±109.1) and a significant decrease in the value of Hepcidin as the mean value of Hepcidin (30.2±6.2) in HCV infected patients compared to control group Ferritin (113.5±7.1) and Hepcidin (81.4±4.1).

Table 4: Ferritin, Hepcidin and HCV PCR:

Variables	Case	Control	Test*	P value
	Mean± SD	Mean ±SD		
Hepcidin	30.2±6.2 (20.8-43.8)	81.4±4.1 (74.5-87.5)	-29.28	<0.001 (S)
Ferritin	342.9±109.1 (139.0-594.0)	113.5±7.1 (101.2-126.0)	8.058	<0.001 (S)
HCV RNA by PCR	1116942.94±460033.03 (330802.0-1983356.0)	14.09±1.45 (12.0-16.0)	9.348	<0.001 (S)

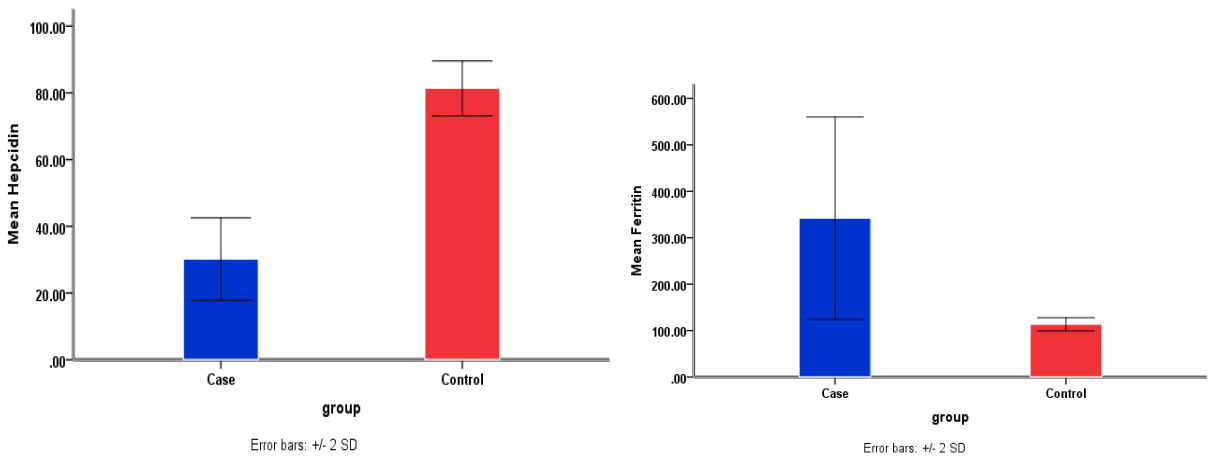

Fig. 8 : Serum ferritin in HCV infected patients compared to the control group .

Table 5: Correlation between hepcidin and other numeric variables.

Variable	r**	P
Age	-0.307	0.030 (S)
Total bilirubin	-0.050	0.732 (NS)
Albumin	0.380*	0.007 (S)
AST	-0.372	0.008 (S)
ALT	-0.134*	0.353 (NS)
B Urea	0.243	0.089 (NS)
Creatinine	0.010	0.943 (NS)
Platelet	0.289*	0.042 (S)
WBCS	-0.231*	0.106 (NS)
RBCS	-0.328*	0.020 (S)
HB	0.535	<0.001 (S)
Ferritin	-0.828	<0.001 (S)
HCV RNA by PCR	-0.857	<0.001 (S)

(**) Pearson correlation coefficient
(*) spearman Rho correlation coefficient

Discussion

Hepatitis C virus infection is one of the main causes of chronic liver disease worldwide. The long term hepatic impact of HCV infection is highly variable, from minimal changes to chronic hepatitis, fibrosis, and cirrhosis with or without hepatocellular carcinoma. The number of chronically infected persons worldwide may be approximately 177 million. CHC appears to be associated with disturbances in iron homeostasis, with serum ferritin and hepatic iron stores being elevated in approximately 50% of patients.

Hepcidin acts as a systemic iron regulatory hormone as it controls iron transport from iron exporting tissues into plasma. Our study was conducted to estimate the level of serum hepcidin and ferritin in hepatitis C infected patient.

The current study, demonstrated that HCV patients had a highly significantly lower hepcidin concentrations than those of matched controls. Hepcidin down regulation is likely to contribute to liver iron accumulation in this condition, and HCV infection may directly modulate hepcidin expression as it induces reactive oxygen species (ROS) through increased histone deacetylase activity. This is in agreement with the findings of Nagashima *et al.* (Nagashima *et al*., 2006), Fujita *et al*. (Fujita *et al*., 2008), Nishina *et al.* (Nishina *et al*., 2008) and Girelli *et al.* (Girelli *et al*., 2009), who stated that serum hepcidin was significantly lower in CHC patients than in controls. They attributed this to the suppressive effect of ROS, which is induced by HCV on hepcidin production. However, our study is not in agreement with the findings of Wrighting and Andrews (Wrighting and Andrews, 2006) and Trinder *et al.* (Trinder *et al*., 2008), who attributed that to the upregulation of hepcidin production by proinflammatory cytokines, particularly interleukin-6 that counteracts ROS-induced hepcidin suppression. Tsochatzis *et al.* (Tsochatzis *et al.*, 2010) had a midway view seeing that HCV infection down regulates serum hepcidin, whereas increasing inflammation and/or fibrosis tend to restore its levels. As a consequence, hepcidin down regulation could be indirectly documented only after normalization of ferritin values by means of the so-called hepcidin/ferritin ratio. In the current study, serum ferritin was significantly higher in the patients group than in control group, with a mean ± SD of 246.28 ± 15.12 and 101.21 ± 11.66, respectively. This is in agreement with the findings of Fujita *et al*. (Fujita *et al*., 2008), who found that serum ferritin was significantly higher in the CHC group than in the control group (P< 0.001); Sugimoto *et al.* (Sugimoto *et al*., 2009), who found that serum ferritin was significantly higher in the CHC group than in the control group (P< 0.001) (mean ± SD: 433±210 and 54±30, respectively); El-Wakil *et al*. (El-Wakil *et al*., 2012), who found that serum ferritin was significantly higher in the CHC group than in the control group (P < 0.05) (mean ± SD: 165.9 ± 120.9 and Marzouk *et al*. (Marzouk *et al*., 2013), who found that serum iron was decreased in CLD patients compared to control group.

However, our results were not in agreement with El-Wakil *et al*. (El-Wakil *et al*., 2012), who reported significantly increased serum prohepcidin values in CHC patients compared to the control subjects. Also, our results were not in agreement with Girelli *et al* (Girelli *et al*., 2009) who made study on 81 untreated CHC patients and 57 controls and concluded that serum hepcidin was reduced in patients with CHC. This may be due to low number of CHC patients involved in our study (GI=20 patients).

In the present study, We found that the mean value ± SD of the haemoglobin level in the patient group was 11.9±1.0gm/dl and the range was 10.1-13.6gm/dl compared to control group 13.1±0.7gm/dl and the range was 12.0-14.3gm/dl. In our study, the mean value ± SD of the RBCS count in the patient group was $4.21\pm0.56 \times 10^6$ /L and the range was $3.30\text{-}5.30\times 10^6$ /L compared to control group $4.72\pm0.30\times 10^6$ /L and the range was $4.30\text{-}5.30 \times 10^6$ /L.

In our study, the mean value ± SD of the WBCS count in the patient group was $5.9\pm1.9\times 10^3$ /L and the range was $3.7\text{-}10.0 \times 10^3$ /L compared to control group $4.9\pm0.7\times 10^3$ /L and the range was $4.1\text{-}6.3 \times 10^3$ /L .

In our study, the mean value ± SD of the PLT count in the patient group was $313.51\pm81.53\times 10^3$ /L and the range was $169.0\text{-}434.0 \times 10^3$ /L compared to control group $365.78\pm46.09\times 10^3$ /L and the range was $276.0\text{-}420.0 \times 10^3$ /L .

In our study, the mean value ± SD of the Total bilirubin level in the patient group was 0.69±0.22 mg/dl and the range was 0.30-1.20mg/dl compared to control group 0.67±0.18 mg/dl and the range was 0.35-1.0mg/dl.

In our study, the mean value ± SD of the Albumin level in the patient group was 2.47±0.24 mg/dl and the range was 2.0- 2.80 mg/dl compared to control group 3.86±0.26mg/dl and the range was 3.50-4.30mg/dl.

In our study, the mean value ± SD of the ALT level in the patient group was 52.0±4.9 U/L and the range was 40.0-

61.0 U/L compared to control group 24.3±6.3 U/L and the range was 14.0-35.0 U/L.

In our study, the mean value ± SD of the AST level in the patient group was 65.0±11.9 U/L and the range was 47.0-72.0 U/L compared to control group 27.2±5.2 U/L and the range was 17.0-38.0 U/L.

In our study, the mean value ± SD of the Creatinine level in the patient group was 0.78±0.17 mg/dl and the range was 0.55-1.17 mg/dl compared to control group 0.78±0.15 mg/dl and the range was 0.60-1.10 mg/dl.

In our study, the mean value ± SD of the B Urea level in the patient group was 22.83±6.50 mg/dl and the range was 12.0-40.0 mg/dl compared to control group 25.73±6.05 mg/dl and the range was 17.0-37.0 mg/dl.

Conclusion

The Level of serum ferritin was highly significant increased in HCV infected patients when compared to healthy individuals. However, The Level of serum hepcidin was highly significant decreased HCV infected patients when compared to healthy individuals.

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