STUDY OF HEPCIDIN LEVEL IN EGYPTIAN HCV INFECTED PATIENTS AND ITS CORRELATION TO IRON AND TOTAL IRON BINDING CAPACITY

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Abstract

Hepcidin is the central regulator of systemic iron homeostasis. Dysregulation of hepcidin production results in a variety of iron disorders. Hepcidin deficiency is the cause of iron overload in hereditary hemochromatosis, iron-loading anemias, and hepatitis C. Hepcidin excess is associated with anemia of inflammation, chronic kidney disease and iron-refractory iron deficiency anemia. The main objective of this study is to elucidate the correlation between Hepcidin and Iron 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 Iron 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 Iron level in infected patients compared to control group .
Keywords : Hepcidin, HCV, Iron, liver, fibrosis, cirrhosis .

Introduction

Hepatitis C virus (HCV) belongs to genus Hepacivirus, family Flaviridae, and it is a small positive strand RNA, enveloped virus. HCV is one of the main etiological agents of progressive liver diseases resulting in liver cirrhosis and hepatocellular carcinoma. Infection by HCV is the main cause of chronic liver diseases, mainly fibrosis, cirrhosis and liver failure as well as hepatocellular carcinoma that affect about 170 million people all over the world (Flamm *et al.*, 2003). In Egypt, the prevalence of HCV is the highest worldwide (Frank *et al.*, 2000) with an estimated prevalence of 14.7% among the general population during 2008 (El-Zanaty and Way, 2009).

Furthermore, HCV infection induces various extrahepatic manifestations leading to several manifestations of health significant which affect about 74% of patients. These manifestations include metabolic, endocrine, cardiovascular, renal, lympho-proliferative, and central nervous system disorders, that significantly responsible for HCV-related mortalities (Cacoub *et al.*, 2014). Annually, 700,000 deaths related to HCV are estimated all over the world (Dubuisson and Cosset, 2014).

Iron is an essential element important for all living organisms because it is responsible for regulation of many metabolic processes such as synthesis of DNA, transportation of oxygen, and production of energy. Homeostasis of iron, HCV life cycle and antiviral drugs efficacy, which act via viral replication inhibition and immune system (interferon) modulation, are linked together, but understanding these relationships has not yet been known (Sikorska, 2016). Intracellular iron enhances the translation of HCV, promotion or inhibition of its replication. According to the experimental studies, iron loading has been proposed to be a part of the antiviral defense mechanism resulting in controlling of HCV multiplication in chronic hepatitis C (CHC) (Fillebeen and

Pantopoulos, 2010). More than forty percent of patients with CHC have iron overload symptoms which result in an increased liver damage, exacerbated inflammatory activities, failure of treatment with interferon, and a high risk of hepato-carcinogenesis (Shan *et al.*, 2005). Furthermore, excessive iron is harmful because it evokes the inflammatory cytokine, reactive oxygen species (ROS), liver fibrosis, and hepatio-carcinogenesis (She *et al.*, 2002).

Accumulation of iron in liver is common in HCV infection results in liver fibrosis and increased risk of hepatocellular carcinoma (Lambrecht *et al.*, 2011). HCV Patients have a relative low hepcidin level compared to uninfected peoples (Fujita *et al.*, 2007; Girelli *et al.*, 2009) resulting in unrestricted duodenal iron absorption and iron release from macrophages through the iron transporter ferroportin (Nemeth and Ganz, 2009).

Hepcidin, a recently discovered hormone, is secreted mainly by hepatocytes and plays an important role in the homeostasis of iron. It was first discovered in the ultra-filtrate of human blood and urine samples and originally identified as a liver-expressed antimicrobial peptide (LEAP1) with direct antimicrobial activities against many species of bacteria and fungi (Krause *et al.*, 2000; Pigeon *et al.*, 2001). The name 'hepcidin' is related to the site of synthesis in hepatocytes (hepcidin) and its antimicrobial activities (hepcidin) (Politou and Papanikolaou, 2004).

The human hepcidin gene (HAMP) encodes a precursor of hepcidin–preprohepcidin that is 84 amino acid proteins. Preprohepcidin is cleaved to give 60 aa prohepcidin that gives rise to hepcidin. Hepcidin: 25 aa, 22 aa and 20 aa peptide are the three forms of hepcidin. These three forms can be detected in urine, but hepcidin-25 and hepcidin-20 only present in human serum (Leong and Lönnerdal, 2004; Rossi, 2005; Ganz and Nemeth, 2006; Kemna *et al.*, 2008). The major form of hepcidin is hepcidin-25, which contains 8

cysteine residues conjugated by disulfide bonds (Krause *et al.*, 2000).

The expression of hepcidin in hepatocyte is stimulated by increased stored iron as well as the inflammatory stimuli (Nemeth *et al.*, 2004; De Domenico *et al.*, 2007). Conversely, the expression of hepcidin is inhibited by anemia, hypoxia, decreased stored iron and increased erythropoiesis (Nicolas *et al.*, 2002; Semenza *et al.*, 2007).

So, hepcidin is considered as the master regulator of iron homeostasis. Generally, it decreases the level of iron in serum. This mechanism depends mainly on the interactions between hepcidin and ferroportin. Ferroportin, the only known mammalian cellular iron exporter, is expressed on the surface of hepatocytes, reticulo-endothelial macrophages, placenta cells and duodenal enterocytes. When Hepcidin binds to ferroportin it causes its internalization and degradation in endo-lysosomes, and blocks the transport of iron via ferroportin. In duodenal enterocytes, high hepcidin prevents the movement of dietary iron through ferroportin into blood circulation. In hepatocytes and macrophages, high levels of hepcidin prevent the movement of stored iron into blood circulation (Knutson *et al.*, 2005; Atanasiu *et al.*, 2007).

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 Iron 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 Elabscience Biotechnology Inc and The quantitative determination of Iron by means of particle-enhanced turbidimetric immunoassay using Spectrum Kit supplied by Egyptian Company for Biotechnology (S.A.E).

Results

Table 1 : Statistical analysis of complete blood count (CBC) in control and diseased samples.

CBC	Control (n=15)	Diseased (n=35)
Platelets ($\times 10^6$/L)	362.47 ± 11.57	301.69[**] ± 11.84
WBCs ($\times 10^9$/L)	5.37 ± 1.05	6.03 ± 1.05
RBCs ($\times 10^{12}$/L)	4.67 ± 1.01	4.15[**] ± 1.02
Hemoglobin (g/dL)	13.08 ± 0.21	11.98[**] ± 0.16

n: Number of examined samples.
Means with ** labels means that HCV group having are highly significant different than control means (P < 0.01)
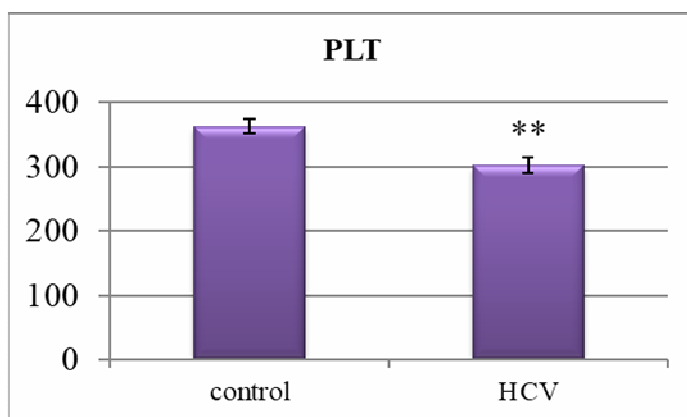** Highly significant

Fig. 1 : Platelets ($\times 10^6$/L) count in control and diseased samples.
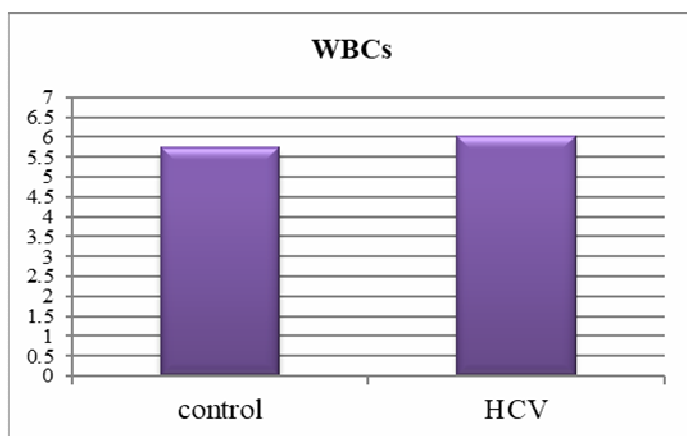

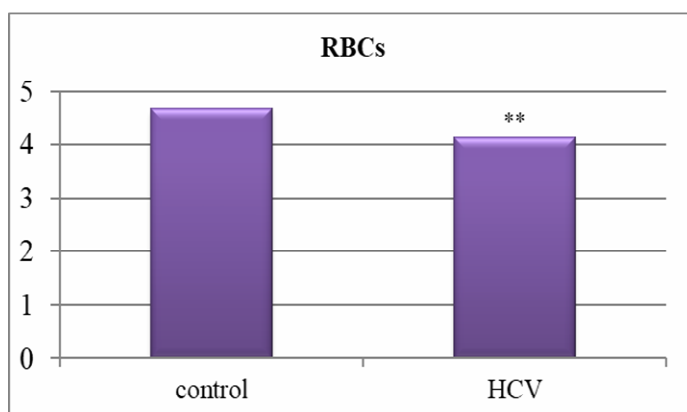**Fig. 2 :** WBCs count ($\times 10^9$/L) in control and diseased samples.

Fig. 3 : RBCS count ($\times 10^{12}$/L) in control and diseased samples.
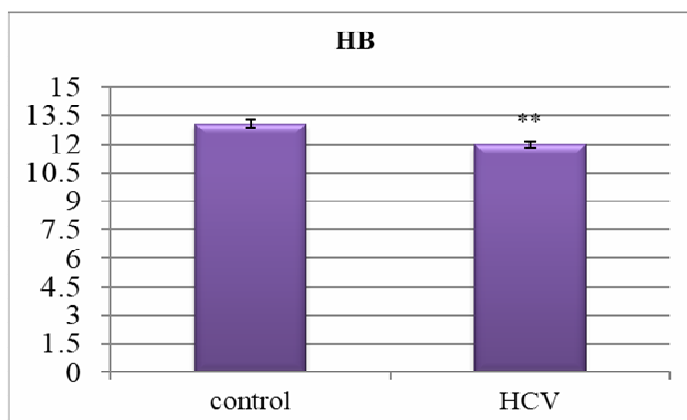

Fig. 4 : Hemoglobin (g/dL)in control and diseased samples.

Table 2 : Statistical analysis of liver function tests in control and diseased samples

Liver function tests	Control (n=15)	Diseased (n=35)
BIL T (mg/dL)	0.76 ± 0.04	0.79 ± 0.04
ALB (g/dL)	Median = 3.8 IQR= 0.40	Median = 3.5[**] IQR= 0.50
AST (U/L)	27.67 ± 1.33	36.66[**] ± 1.95
ALT(U/L)	23.44 ± 3.55	28.84 ± 1.07

n: Number of examined samples.

Means with ** labels means that HCV group having are highly significant different than control means (P < 0.01)
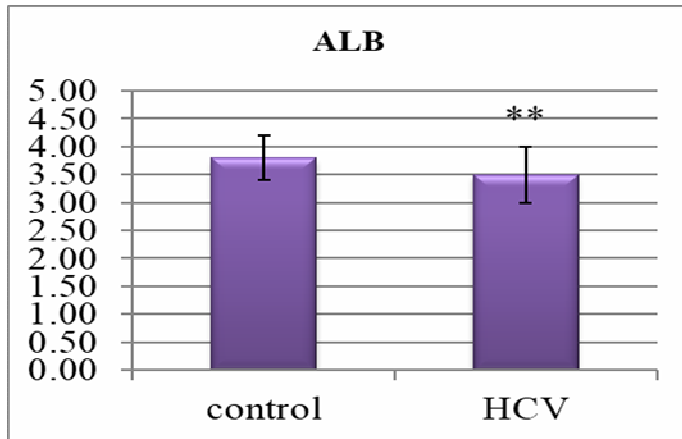
** Highly significant
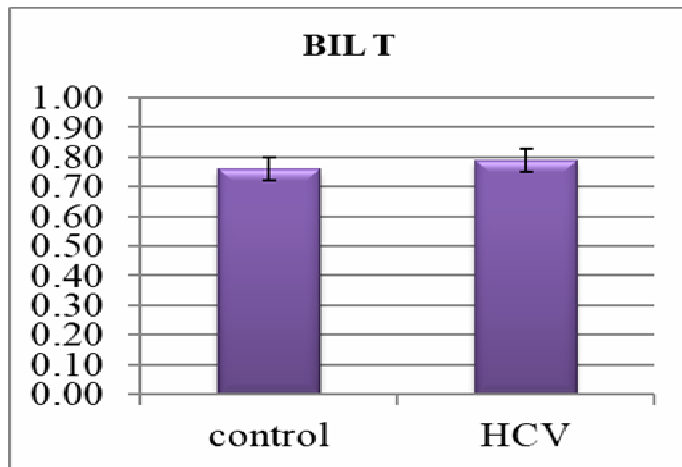

Fig. 5: Total bilirubin (BIL T)

Fig. 6: Albumin (ALB) (g/dL) in control and (mg/dL) in control and diseased samples. diseased samples
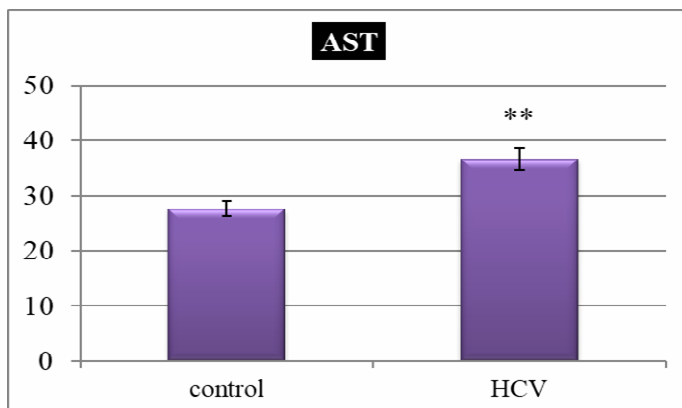

Fig. 7 : Aspartate aminotransferase (AST) (U/L) in control and diseased samples
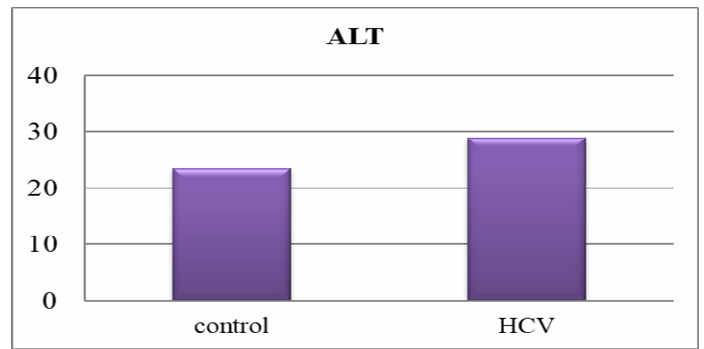

Fig. 8 : Alanine aminotransferase (ALT) (U/ L) in control and diseased samples.

Table 3 : Statistical analysis of kidney function tests in control and diseased samples.

kidney function tests	Control (n=15)	Diseased (n=35)
Blood urea (mg/dL)	26.33 ± 1.45	24.53 ± 1.55
Creatinine (mg/dL)	0.77 ± 0.02	0.83± 0.01

n: Number of examined samples.
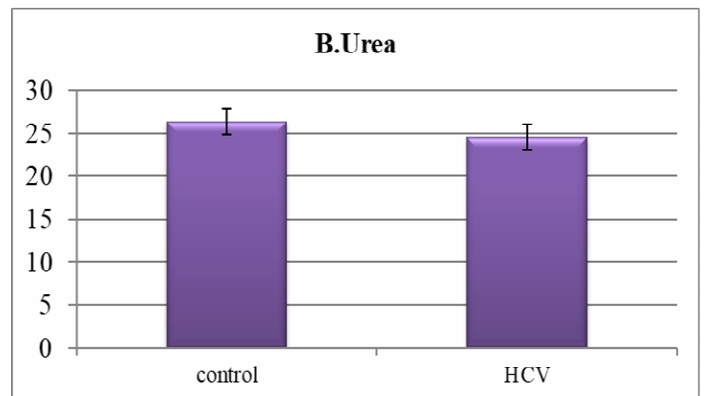

Fig. 9 : Blood urea (mg/dL) in control and diseased sample
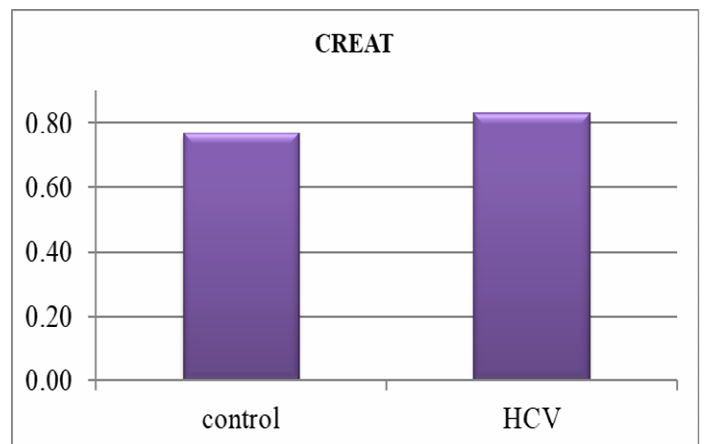
Fig. 10 : Creatinine (mg/dL) in control and diseased samples.

Table 4 : Statistical analysis of iron and total iron binding capacity in control and diseased samples.

tests	Control (n=15)	Diseased (n=35)
Iron (Ug/dL)	66.47 ± 2.13	119.43[**] ± 2.12
TIBC (Ug/dL)	379.78 ± 2.68	295.74[**] ± 1.47

n: Number of examined samples.

Means with ** labels means that HCV group having are highly significant difference than control means (P < 0.01)

** Highly significant

Fig. 11 : Iron (Ug/dL) in control and diseased samples

Fig. 12 : Total iron binding capacity (Ug/dL) in control and diseased samples.

Table 5 : Statistical analysis of hepcidin level in control and diseased samples

Test	Control (n=15)	Diseased (n=35)
Hepcidin (ng/mL)	81.49 ± 0.96	29.98** ± 1.03

n: Number of examined samples.
Means with ** labels means that HCV group having are highly significant difference than control means (P < 0.01)
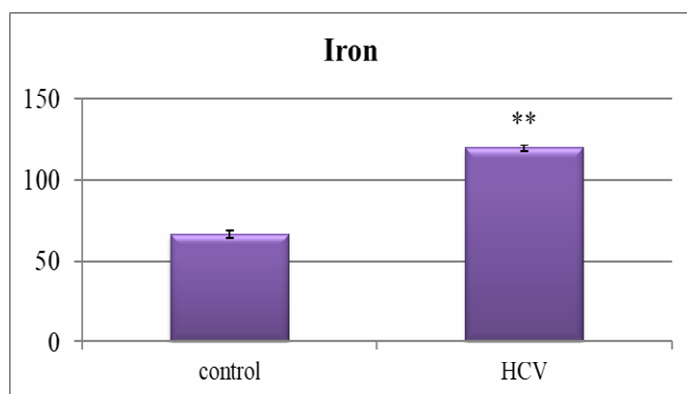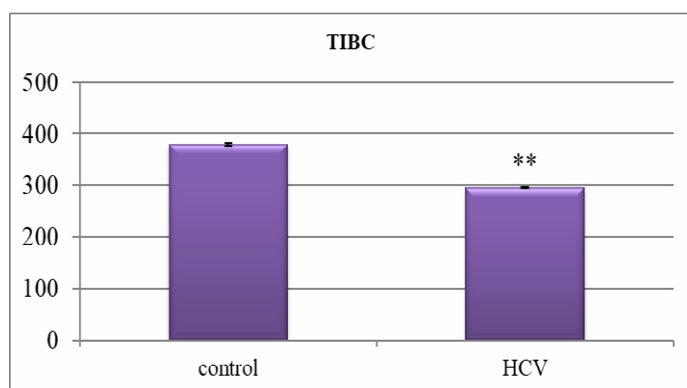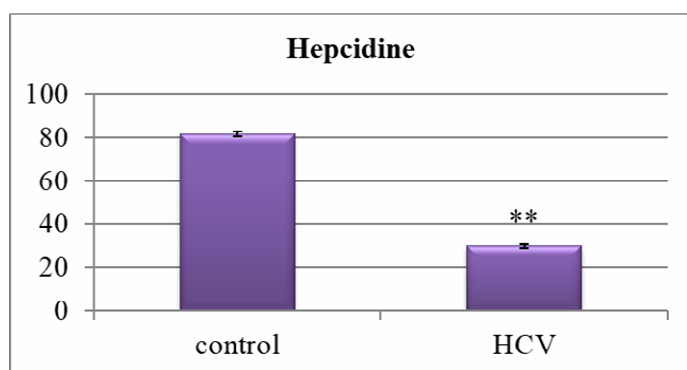** Highly significant

Fig. 13 : Hepcidin level (ng/mL) in control and diseased samples.

Table 6 : Correlation coefficient of relations between hepcidin and liver function test, kidney function test, CBC, iron, total iron binding capacity and HCV.

	Hepcidin	
	r	P-value
Age	-0.05	0.796[NS]
BIL T	-0.09	0.590[NS]
ALB	-0.07	0.656[NS]
AST	-0.40	0.005**
ALT	-0.24	0.094[NS]
B.Urea	0.17	0.241[NS]

CRET	-0.11	0.459[NS]
PLT	0.42	0.002**
WBCs	-0.26	0.073[NS]
RBCs	0.39	0.005**
HB	0.48	0.000**
Iron	-0.87	0.000**
TIBC	0.94	0.000**
HCV RNA by PCR	-0.65	0.000**

r: correlation coefficient
0 to 0.2 weak relationship
0.3 to 0.6 moderate relationship
0.7 to1 strong relationship
** Highly significant, NS non-significant

Discussion

Hepatitis C virus (HCV) is considered as a major health problem worldwide because HCV patients are at risk of progressive liver disease that favors the generation of long term complications such as cirrhosis, end stage liver disease and hepatocellular carcinoma (Spengler and Nattermann, 2007). Egypt has one of the highest prevalence of hepatitis C in the world. HCV genotype 4 is the most common strain in Egypt followed by HCV genotype 1 (90% and 10% respectively) (Bazeed *et al*., 2016)

The liver is the main iron storage organ and it plays a fundamental role in iron metabolism. The iron transport protein, transferrin, and the major iron storage protein, ferritin, are both synthesized in the liver. Iron homeostasis is critical for human because iron is an essential element necessary for many basic biological processes; however, excess iron may also be highly cytotoxic. Elevated serum levels of iron represent early markers for the severity of liver disease.

Hepcidin is synthesized in the liver and it is thought to be a key regulator for iron homeostasis. It is induced mainly by infection and inflammation. Hepcidin expression levels in chronic liver diseases were strongly correlated with either the serum ferritin concentration or degree of iron deposits in the liver (Farid *et al*., 2012).

The aim of the present study is evaluation of hepcidin level and its relation with iron state in HCV infected patients. Serum samples were collected from control (non HCV) individuals and diseases (HCV infected patients) for determination of liver function test (Total bilirubin, Albumin, ALT and AST), kidney function test (Blood urea and creatinine), CBC (Platelets, WBCs, RBCs and Hb), iron and iron binding capacity as well as determination of hepcidin level and its relations with the previous parameters.

In this study, the samples were collected from 8 (53.3%) males and 7 (46.7%) females as control samples (non HCV), and 11 (31.4%) males and 24 (68.6%) females as HCV infected patients (Table 4 and Fig. 13). The average age of the samples was 39.00 ± 3.55 and 43.54 ± 2.36 years for control and diseased samples, respectively (Table 4 and Fig. 14). The HCV viral load in each sample was measured by real-time polymerase chain reaction method, the quantitative determination of HCV RNA by PCR in diseased samples was 1106832.86 ± 71803.04 (IU/mL) (Table 4 and Fig. 15).

Bilirubin is a yellowish substance resulted from the breakdown of hemoglobin, which is the major component of RBCs. Chemical analysis of liver function tests revealed that total bilirubin level in control samples was 0.76 ± 0.04

mg/dL, while in HCV patients; it was 0.79 ± 0.04 mg/dL (Table 5 and Fig. 16).

Naturally, when the RBCs age, they are broken down in the body and bilirubin is released and passed on to the liver, which releases the bilirubin in the form of bile. If the liver is not functioning correctly especially in HCV infection, the bilirubin will not be properly released and it will elevate than expected. High level of bilirubin is the main cause of jaundice (yellow skin and eyes, darker urine).

This result was in line with many authors; Wahib *et al.* (2006) who studied twenty HCV/PCR-RNA positive patients and found that total bilirubin increased in 7 patients (35%) and de Souza (2017) who reported that hyperbilirubinemia has been associated with HCV

Albumin is produced only by the liver and it is the major protein, which circulates in the blood. It is important to maintain the oncotic pressure in the vascular system. Any decrease in the oncotic pressure as a result of low albumin, fluid leak from the interstitial spaces into the peritoneal cavity, causing ascites (Nagao and Sata, 2010). Albumin is also very important in the transportation of many molecules; such as free fatty acids, bilirubin, hormones and drugs (Quinlan *et al.*, 2005).

As illustrated in Table 5 and Fig. 17, serum albumin was 3.8 in control samples, while it was 3.5 in HCV patients. HCV patients were highly significant different than control samples (P < 0.01). This result was in accordance with Nagao and Sata (2010) who reported low albumin level (<4.0 g/dL). A low serum albumin concentration is an indicator on poor liver function.

Data found in Table 5 and Fig. 18, showed that the level of aspartate aminotransferase (AST) was 27.67 ± 1.33 in control samples, while this level elevated in HCV patients (36.66 ± 1.95), therefore, HCV patients were highly significant different than control samples (P < 0.01). This result agreed with Wahib (2006) who reported that AST increased in15% of HCV patients and Bazeed *et al.* (2016) who reported an extremely significant increased AST in HCV patients (42.7 U/L).

The level of alanine aminotransferase (ALT) was 23.44 ± 3.55 and 28.84 ± 1.07 in control and HCV patients, respectively (Table 5 and Fig. 19). This result was in line with Akkaya *et al.* (2007) who concluded that in HCV patients, alterations in the liver tissue is reflected by ALT elevation and mainly associated with periportal bridging/necrosis, viral load and duration of disease. Roshan and Guzman (2014) reported an elevation in ALT in HCV patients (56 U/L), as well as Bazeed *et al.* (2016) who reported an extremely significant increased ALT in HCV patients (50 U/L) and Hajarizadeh *et al.* (2016) who demonstrated that during recent HCV infection, higher ALT levels were detected.

This result could be attributed to when parenchymal liver cells are damaged, aminotransferases leak from the liver into the blood, resulting in elevated levels of these enzymes in the bloodstream (Akkaya *et al.*, 2007).

Results illustrated in Table 6 and Fig. 20, revealed that the level of blood urea was 26.33 ± 1.45 mg/dL in control samples and this level decreased in HCV patients (24.53 ± 1.55 mg/dL), while, creatinine level was 0.77 ± 0.02 and 0.83 ± 0.01 mg/dL in control samples and HCV patients, respectively (Table 6 and Fig. 21).

These findings were in line with Noureddine *et al.* (2010) who announced that HCV infection increases the rate of progression of CKD in patients with glomerulonephritis with an increase in serum creatinine (1.3 mg/dl).

Complete blood count (CBC) is one of the most commonly performed blood tests because it reveals the peripheral blood changes. It is routinely performed in health examinations, even in asymptomatic patients. The data present in Table 7 and Fig. 22, showed that platelets count was $362.47 \pm 11.57 \times 10^6$/L in control samples, meanwhile this level decreased in HCV patients to be $301.69 \pm 11.84 \times 10^6$/L. The HCV patients were highly significant different than control samples (P < 0.01).

This result was agreed with what had been reported by Li *et al.* (1999), Streiff *et al.* (2002), Kauf *et al.* (2012) and Tsai *et al.* (2015) who illustrated that thrombocytopenia occurs in HCV infection and in liver cirrhosis. This result could be attributed to; HCV patients have low levels of serum thrombopoietin (Giannini *et al.*, 2003), which is mostly produced by the liver tissue before its release into the bloodstream and it is the main regulator of platelet production (Kawasaki *et al.*, 1999).

The WBCs count was $5.37 \pm 1.05 \times 10^9$/L in control samples, while this count increased in HCV patients ($6.03 \pm 1.05 \times 10^9$/L) (Table 7 and Fig. 23). This result agreed with Streiff *et al.* (2002) and Tsai *et al.* (2015) who reported that HCV infected group showed significantly higher WBC, lymphocyte, and monocyte counts compared with control group.

As illustrated in Table 7 and Fig. 24, the RBCs count was $4.67 \pm 1.01 \times 10^{12}$/L and $4.15 \pm 1.02 \times 10^{12}$/L in control and HCV patients, respectively. There was a highly significant different between HCV patients and control samples (P < 0.01). This result disagreed with Tsai *et al.* (2015) who reported that HCV infected group showed significantly higher RBC count ($4.9 \pm 0.7 \times 10^6$ μl) compared with control group ($4.5 \pm 0.5 \times 10^6$ μl).

Hemoglobin level in control samples was 13.08 ± 0.21 g/dL but HCV patients had a lower level of Hb (11.98 ± 0.16 g/dL) (Table 7 and Fig. 25). A highly significant different was detected between HCV patients and control samples (P < 0.01).

This result agreed with Streiff *et al.* (2002) who reported that HCV is associated with low levels of Hb compared with control group but disagreed with Tsai *et al.* (2015) who reported that HCV infected group showed significantly higher Hb levels (14.7 ± 1.5 g/dl) compared with control group (13.4 ± 1.8 g/dl)

The liver is the main iron storage organ because a third of the total iron of the body is deposited in hepatocytes, portal tracts, sinusoidal mesenchymal cells, and reticuloendothelial cells (Franchini *et al.*, 2008). Increased serum iron level and the decreased level of TIBC were correlated with progressive hepatic parenchymal disease.

The data found in Table 8 and Fig. 26&27 revealed that the level of iron was 66.47 ± 2.13 Ug/dL in control samples, while in HCV patients, this level increased to 119.43 ± 2.12 Ug/dL. A highly significant difference was detected between HCV patients and control samples (P < 0.01). Meanwhile, the total iron binding capacity (TIBC) was 379.78 ± 2.68 Ug/dL in control samples, but in HCV patients, this level significantly decreased to 295.74 ± 1.47 Ug/dL.

This result could be attributed to iron accumulation within the liver in patients with CHC infection (Metwally *et*

al., 2004). These results were in a line with Fujita *et al.* (2007) and Guyader *et al.* (2007), also, Vagu *et al.* (2013) who found significant elevated levels of serum iron (P < 0.05) and ferritin (P < 0.001), associated with lower levels of TIBC (P < 0.05) in HCV patients with control group. Several studies reported that HCV patients have mild to moderate hepatic iron accumulation, which significantly worsens clinical outcomes, leading to an increased risk of hepatocellular carcinoma (Buyukasik *et al.*, 2011 and Liu *et al.*, 2012). Also CHC is frequently associated with hepatic iron overload (Hézode *et al.*, 1999 and Mohamed *et al.*, 2019).

Hepcidin hormone is mainly produced by the liver and released in circulation. When it reaches the circulation, it regulates the metabolism of iron by controlling iron transport to the duodenal enterocytes and iron export from the macrophages (Georgopoulou *et al.*, 2014). So, it is the key regulator of iron metabolism and is a significant biomarker for systemic inflammatory states. Hepcidin acts by binding to iron carriers, causing internalization and lysosomal degradation (Nemeth, 2010).

As illustrated in Table 9 and Fig. 28, the level of hepcidin hormone in control samples was 81.49±0.96 ng/mL, while in HCV patients, this level significantly decreased (P < 0.01) compared with control group to 29.98±1.03 ng/mL. The decreased level of hepcidin in HCV patients could be attributed to the state of chronic inflammation and elevated iron stores in chronic liver infection, which leads to the reduction of hepcidin concentrations.

This result agreed with Fujita *et al.* (2007), Farid *et al.* (2012) who reported that hepcidin is significantly lower in HCV patient.

The obtained result in this study was in accordance with Girelli *et al.* (2009), Piperno (2009), and Tsochatzis *et al.* (2010) who reported that CHC patients have low serum hepcidin levels because of necroinflammation and fibrosis. Also, Mohamed *et al.* (2019) illustrated that CHC patients showed decreased serum hepcidin concentration and hepcidin expression.

The results found in Table 10 illustrated the correlation coefficient of relations between hepcidin and liver function test, kidney function test, CBC, iron, total iron binding capacity and HCV. A highly significant correlation was detected between hepcidin and HCV, AST, platelets count, RBCs count, Hb, Iron and TIBC (P < 0.05). These correlations were positive between hepcidin and platelets count, RBCs count, Hb and TIBC (0.42, 0.39, 0.48 and 0.94, respectively), while negative correlations were detected between hepcidin and HCV, AST and iron (-0.65, -0.40 and -0.87). Meanwhile, non-significant correlations were detected between hepcidin and BIL T, ALB, ALT, blood urea, creatinine and WBCs.

These results agreed with Aoki *et al.* (2005), Girelli *et al.* (2009) and Terrence *et al.* (2012) but disagreed with Del Giudice *et al.* (2009), Tsochatzis *et al.* (2010) and Farid *et al.* (2012) who observed inverse correlations. These variations could be attributed to the differences in disease characteristics, patients' demographics and ethnicity (Mohamed *et al.*, 2019).

Conclusion

Infection by HCV is a major health problem, threats many peoples all over the world. The liver is the main detoxifying organ of many metabolites; also it plays an important role in synthesis of proteins and production of biochemicals essential for digestion and growth in addition to its role in iron storage and iron metabolism. For iron homeostasis, hepcidin is synthesized in the liver and it is the key regulator for iron homeostasis.

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