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SERUM PROHEPCIDIN, IRON AND HEPATIC IRON STATUS IN CHRONIC HEPATITIS C IN EGYPTIAN PATIENTS

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ABSTRACT

Background/Aims: Patients with chronic hepatitis C (CHC) often have increased liver iron. Hepcidin has recently emerged as a key regulator for iron homeostasis. Therefore, we aimed to study the relationship between serum prohepcidin, serum iron indices, hepatic necro-inflammation, fibrosis and hepatic iron density and to determine the predictors of advanced fibrosis in these patients.

Subjects and methods: Fifty CHC treatment naïve patients and 20 healthy controls were enrolled in this study. Complete blood count, liver function tests, serum iron indices and serum prohepcidin were assayed. Liver biopsy was performed for all patients for assessment of necro-inflammatory activity, fibrosis and liver iron density.

Results: Thirty-four patients (68%) had mild fibrosis (stage 0, 1, 2) and sixteen (32%) had advanced fibrosis (stage 3, 4). All cases were positive for liver iron stain (68% mild, 32% advanced). Mean serum prohepcidin level was significantly lower in CHC patients than healthy controls. In univariate analysis, prohepcidin was significantly associated with necro-inflammatory activity ($P<0.05$) and advanced fibrosis ($P<0.05$). Multivariate analysis revealed that necro-inflammatory activity and liver iron density are independently associated with stage of fibrosis. No significant correlations were found between prohepcidin and serum iron indices or liver iron score.

Conclusions: Serum prohepcidin is reduced in CHC which may be one -not the only- factor leading to iron overload in these patients. Histological grading and hepatic iron density are independent predictors of advanced fibrosis. Further studies are needed to clarify the role of viral and host genetic factors in hepatic iron deposition.

Keywords: *chronic hepatitis C, serum iron indices, hepatic iron, prohepcidin.*

INTRODUCTION

Increased hepatic iron concentration (HIC) is present in patients with chronic hepatitis C (CHC) and hepatic iron overload is more common among patients with end stage liver disease due to hepatitis C [(1)]. Increased liver iron is a cofactor promoting the progression of liver damage and increasing the risk of fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Among other potential links between iron metabolism and CHC, the possibility of enhancing hepatitis C virus (HCV) replication by serum iron has been postulated in experimental settings [(2)]. HIC has been inversely associated with the response to antiviral therapy [(3)]. Iron removal by phlebotomy improves liver function tests [(4)], histology [(5)], increases the probability of sustained HCV eradication with antiviral therapy [(6)], and decreases HCC development in CHC patients [(7)]. Elucidating the mechanism of iron accumulation in CHC may thus provide new tools for the management of CHC or for the prevention of its complications, or both [(8)].

Our understanding of iron metabolism has advanced dramatically in the past few years, mainly as a result of the discovery of hepcidin, a key regulator of whole-body iron homeostasis. The main physiological role of hepcidin in healthy individuals is to lower blood iron levels [(9)]. Hepcidin, a 25 amino acid (a.a.) peptide, and its inactive prohormone "prohepcidin", an 84 a.a. protein is synthesized in hepatocytes, secreted, detected in serum and excreted through the kidneys [(10, 11)]. Hepcidin becomes functional by cleavage of the N-terminal amino acids of prohepcidin [(12)]. Recent evidence showed that the proteolytic cleavage of prohepcidin to hepcidin is regulated by the hepatic prohormone convertase furin [(13)].

Hepcidin is an acute phase protein with antimicrobial activity that increases in response to infection and inflammation [(14)]. Hepcidin may be central regulator of intestinal iron absorption and iron recycling by macrophages [(11)]. Hepcidin binds to the trans-membrane iron exporter 'ferroportin' which is present on macrophages and the basolateral side of enterocytes inducing the internalization and degradation of ferroportin. By diminishing the effective number of iron exporters on the membrane of enterocytes or macrophages, hepcidin suppresses iron uptake and release respectively [(9)].

Hepcidin synthesis in the liver is sensitive to body iron levels; increasing with iron overload and decreasing in the case of iron deficiency. Hepcidin has been shown to be regulated by iron, inflammation, oxidative stress, hypoxia, alcohol, hepatitis C and obesity [15]. Hepcidin gene is down-regulated by increased erythropoietic demand, hypoxia and anaemia [16]. It is up-regulated by increased body iron stores and infection or inflammation [14].

Growing evidence has suggested the potential occurrence of dysregulation of the hepcidin system in patients with chronic viral hepatitis. It was reported that serum prohepcidin levels were lower in chronic HCV and hepatitis B virus (HBV) infection, and were negatively associated with total iron scores [17]. In addition, a negative association between serum prohepcidin and the degree of liver dysfunction has been previously reported [18]. Hepcidin expression levels in chronic liver diseases were associated with either the serum ferritin concentration or the degree of iron deposits in the liver [19].

Therefore, our aim was to study the relationship between serum prohepcidin, serum iron indices, hepatic necro-inflammation, fibrosis and hepatic iron density and to determine the predictors of advanced fibrosis in Egyptian CHC patients.

SUBJECTS AND METHODS

This study included fifty-eight consecutive patients (46 males and 12 females) with CHC referred to the Department of Tropical Medicine and Gastroenterology, Sohag University Hospital (in the period from January 2008 to January 2009) for liver biopsy as a pretreatment assessment of chronic hepatitis C. All patients included were positive for HCV antibody in their sera by ELISA and HCV RNA was detected in their blood by PCR for at least 6 months. Before inclusion in the study, all participants gave an informed consent and the study was approved by the local ethics committee.

Exclusion criteria were: alcoholism, coexisting HBV or human immunodeficiency virus infection, decompensated cirrhosis, liver disorders other than HCV infection, current or previous antiviral therapy, evidence of renal disease, and conditions affecting the interpretation of iron parameters as hematological disorders, chronic inflammatory diseases and iron therapy. Three patients were excluded because of unsatisfactory serum samples and five patients were excluded because of unsatisfactory liver biopsy, therefore only fifty patients (42 males and 8 females) were included in the final statistical analysis. The mean age of patients was 43.06 ± 8.17 years and ranged from 19 to 58 years. Forty-two patients (84%) were males and 8 (16%) were females. The clinical presentation was easy fatigability in 25 (50%), anorexia in 13 (26%), bleeding tendency in 9 (18%), weight loss in 7 (14%), jaundice in 5 (10%), hepatomegaly in 6 (12%) and splenomegaly in 2 (4%). Three patients (6%) were diabetic.

A total of twenty adult healthy volunteers matched for age and sex were included as controls. They were all negative for both HBsAg and anti-HCV. They completed a questionnaire with specific items relevant to iron metabolism as history of blood donations or loss and iron supplementation.

All patients and controls were subjected to complete medical history and physical examination looking for signs of chronic liver disease. For all participants, abdominal ultrasound examination was performed for evaluation of liver size, echo-pattern, smoothness or irregularity of the surface, measurement of portal vein diameter and exclusion of presence of any focal lesion. Spleen size was also evaluated. Any renal abnormality was also excluded.

Laboratory investigations

Ten ml blood samples were drawn from all patients and controls. Laboratory investigations included complete blood count using Beckman Coulter. Citrated plasma was used for estimation of prothrombin time and concentration. Six ml blood samples were centrifuged and the resulting sera were divided in aliquots and used for liver function tests (serum bilirubin, serum albumin, ALT& AST) using Hitachi 911 autoanalyser. HBsAg and HCV antibody were tested by AXSYM system based on microparticle enzyme immunoassay technology. Other serum aliquots were stored at – 20 ºC until assayed for serum iron indices and serum prohepcidin.

Assessment of iron indices and prohepcidin in serum:

Serum iron and total iron binding capacity (TIBC) were determined by colorimetric assay using Stanbio Iron and TIBC kit, Stanbio Laboratories, Texas, USA. Transferrin percent saturation (TS %) was calculated as (S. iron/TIBC) X 100 [20]. Serum ferritin was measured by immuno-enzymometric assay using Accu-Bind ELISA kits, Monobind Inc., USA. Serum iron was considered elevated if it was more than 150 µg/dl in men or more than 145 µg/dl in women. Serum ferritin was considered elevated if it was above 300 ng/ml in men or above 200 ng/ml in women. TS%

was considered elevated if it was more than 50% [21].

Serum levels of hepcidin prohormone were determined also by a hepcidin prohormone solid phase enzyme-linked immunosorbant assay kit as described by the manufacturer (IBL International, Hamburg, Germany RE54051).

Histological assessment:

Fifty liver biopsy samples were taken for histological examination from the patients only. In general, a sample of 1.5 cm in length that is 1.2-2 mm in diameter and contains at least 6-8 portal triads is considered adequate. Each was routinely-processed, formalin-fixed, paraffin-embedded according to the usual schedule used in the laboratory. Five micron serial tissue sections were cut, mounted on poly-L-lysine coated slides and dried overnight at room temperature. Sections were de-paraffinized, rehydrated and stained with hematoxylin and eosin (H&E) to assess the degree of liver affection.

H&E stained sections were examined to evaluate necro-inflammatory activity (histological grade) and fibrosis (histological stage) according to Desmet et al. [22] by two pathologists who were blinded to the clinical conditions and laboratory results. The histological grade was also performed taking into account the necro-inflammation in the portal tracts, periportal and lobular areas: 0= no necro-inflammatory activity, 1= minimal activity, 2= mild activity, 3= moderate activity, 4= severe activity. Fibrosis was evaluated as: 0= no fibrosis, 1= portal fibrosis without septa, 2= few septa, 3= numerous septa delineating nodules without cirrhosis, 4= cirrhosis. For comparisons, necro-inflammation was grouped into mild (0, 1, 2) and advanced (3, 4). Fibrosis was grouped into mild and advanced in the same way.

Histochemical staining:

Perls' stain was used for histochemical demonstration of hepatic iron stores. The staining was performed on formalin-fixed, paraffin-embedded tissue sections according to the staining protocol described by the manufacturer (Diapath srl Via Savoldini, 71-24057 MARTINENGO-BG- Italy). The sections were treated with diluted HCL acid to release ferric ions from binding proteins. These ions then react with potassium ferrocyanide to produce an insoluble blue compound (the Prussian blue). Perls' iron stain highlights ferric iron on histological sections.

Evaluation of histochemical reaction of Perl's stain:

Hepatic iron or haemosiderin deposition appears as bright blue granular staining. Iron stain was scored on a scale from 0 to 4 by assessing the overall amount of iron deposit in all cell types (hepatocytes, Kupffer cells and portal macrophages) as: 0 = none, 1= minimal, 2 = mild, 3 = moderate, 4 = marked, as modified from LeSage et al. [23] and Torbenson [24]. Biopsy samples with iron scores (0, 1, and 2) were grouped as mild and those with iron scores (3 and 4) were grouped as advanced. Iron distribution in the liver lobule was assessed according to its hepatocyte zonal distribution gradient from zone (1) to zone (3) as described by Torbenson [24].

Statistical analysis:

Data entry and analysis were done using SPSS software V.17 (Chicago, USA). Continuous values were described by mean and standard deviation. Categorical values were described by counts and proportions. Univariate analysis for determining the association of various clinical and laboratory variables with the grade of necro-inflammation and the stage of fibrosis was performed using *Student's t*-test for continuous variables and x^2 test for categorical variables. Correlations among the studied variables were tested by Spearman's correlation coefficient. Differences were considered statistically significant if *P* value was less than 0.05. All significant variables in univariate analysis were studied using multivariate logistic regression analysis to identify independent predictors of advanced fibrosis.

RESULTS

Our results showed that patients with CHC have significantly higher haemoglobin level, serum iron indices (serum iron, ferritin, TIBC and TS %) and significantly lower WBC, platelet count and prohepcidin than healthy controls. The base line laboratory criteria of all patients and controls are demonstrated in table 1.

Laboratory results and histological grading and staging:

In CHC patients, abnormality in serum iron and/or serum ferritin was present in 20/50 (40%) of patients. Serum iron was elevated in 14/50 (28%) and serum ferritin was elevated in 8/50 patients (16%). Both serum iron and ferritin levels were elevated in two patients. However, the TS% was in the normal range in all patients.

Patients were grouped according to necro-inflammatory activity into 17 (34%) case with grade (1), 16 (32%) case with grade (2), 7 (14%) case with grade (3), 10 (20%) case with grade (4) activity. Patients were divided according to the stage of fibrosis into 23 (46%) case with stage (1), 11 (22%) case with stage (2), 15 (30%) case with stage (3), 1 (2%) case

with stage (4) fibrosis. Iron deposit was scored into 17 (34%) case with score (1), 17 (34%) case with score (2), 12 (24%) case with score (3), 4 (8%) case with score (4) iron staining. Histological findings of the studied patients were summarized in table 2.

Studying the necro-inflammatory activity (table 3) revealed that 33 patients had mild activity (grade 1and 2) and 17 had advanced activity (grade 3 and 4). Patients with advanced activity have significantly higher levels of ALT, AST than those with mild activity (P<0.05). On the other hand, serum albumin and prohepcidin were significantly lower in patients with advanced activity than in those with mild activity (P<0.05). Also, serum ferritin was significantly higher in patients with advanced activity than in those having mild activity (P<0.05). Advanced hepatic iron stain was highly significantly associated with higher grades of necro-inflammation (P<0.001)

Histological assessment of fibrosis in biopsy materials (table 4) showed that 34 patients have mild fibrosis (stage 1 and 2) and 16 patients have advanced fibrosis (stage 3 and 4). Patients with advanced fibrosis have significantly higher ALT and AST levels than those with mild fibrosis (P<0.05). Serum albumin and prohepcidin levels were significantly lower in patients with advanced fibrosis than in patients with mild fibrosis (P<0.05). Serum ferritin showed significantly higher levels in patients with advanced fibrosis than in those with mild fibrosis (P<0.05). Advanced iron stain was associated with advanced fibrosis (P<0.001).

Liver iron scores:

Figure (1. A-F) and Table (5) show the results of histological assessment of liver iron deposition. All biopsies included were positive for iron stain. For statistical reasons, patients with score 0, 1 and 2 were grouped together (n=34, 68%) and labeled as mild iron staining. Whereas, patients with score 3 and 4 were grouped together (n=16, 32%) as advanced iron staining.

Our study showed that cases with minimal and mild iron staining (17+17) exhibited sinusoidal pattern (Kupffer cells and portal macrophages) without zonal gradient. Cases with moderate and strong iron staining (12+4) showed mainly hepatocellular pattern of iron distribution with a zonal gradient in 11/16 of them. There was an increased iron gradient from zone 3 to zone 1 with maximum at zone 1 (near the inflamed and fibrotic portal areas). This pattern of iron distribution was found in 8/12 cases with moderate and 3/4 cases with strong iron staining. However in sections with well formed cirrhotic regenerating nodules, the zonal pattern of iron staining was not noticed but a homogenous iron stain was present in the examined sections.

We then stratified our data according to patient sex because women may have lower serum iron indices than men. However, we found no significant difference between both groups in laboratory data except in haemoglobin level which was highly significantly lower in women than men (P<0.001) red cell count and TIBC which were significantly lower in women than men (P<0.05 for each).

Multivariate analysis:

Results of multivariate analysis of factors predicting advanced fibrosis revealed that only necro-inflammatory activity and hepatic iron density are independent predictors of advanced fibrosis(P<0.05).

Table 1: Laboratory characteristics of patients and controls

	CHC patients (n=50)	Controls (n=20)
WBC ($x10^3/\mu l$)	5.43±1.48**	8.43±1.87
RBC ($x10^6/\mu l$)	5.04± 0.63	5± 0.21
Hb (g/dl)	14.57± 1.38*	13.63± 1.33
Platelets ($x10^3/\mu l$)	209.08± 66.6*	277.45± 68.5
Prothrombin time (sec)	12.97± 1.01	13 ±0.15
Prothrombin conc (%)	84.35± 9.94	95.4±4.8
ALT (IU/L)	45.12± 31.7	22..64± 6.3
AST (IU/L)	37.32± 20.3	22.82± 6.66
S. Total bilirubin (mg/dl)	1.19± 0.87	0.82± 0.11
S. Albumin (g/dl)	3.95±0.51	4.05±0.23
S. Iron (µg/dl)	131.94±27.03**	83.91±9.28
TIBC (µg/dl)	395.34±65.11*	327±46.09
S. Ferritin (ng/ml)	186.62±98.77*	106±44.06
S. prohepcidin (ng/ml)	62.38±34.14*	95.5±19.77
TS%	33.79±7.06*	26.45±6.54

Data are expressed as mean ± SD.

*Significant difference from control group at p<0.05 using student's *t*-test.

**Highly significant difference from control group at p<0.001 using student's *t*-test.

Table 2: Histological findings of all patients

Histological parameter	Total number = 50
Grading (Necro-inflammation)	
Grade 1	17 (34%)
Grade 2	16 (32%)
Grade 3	7 (14%)
Grade 4	10 (20%)
Staging (Fibrosis)	
Stage 1	23 (46%)
Stage 2	11 (22%)
Stage 3	15 (30%)
Stage 4	1 (2%)
Liver iron density	
Score 1	17 (34%)
Score 2	17 (34%)
Score 3	12 (24%)
Score 4	4 (8%)

Data are expressed as number and (%).

Table 3: Laboratory data of patients grouped according to the grade of necro-inflammatory activity

	Mild activity (n=33)	Advanced activity (n=17)
ALT (IU/L)	35.16 ± 15.05	64.46 ± 12.1*
AST (IU/L)	29.11 ± 11.4	53.25 ± 17.3*
S. Albumin (g/dl)	4.07 ± 0.42	3.73 ± 0.59*
S. Prohepcidin (ng/ml)	70.12 ± 26.6	47.35 ± 19.33*
S. Iron (µg/dl)	132.12 ± 28.81	131.59 ± 24.01
TIBC (µg/dl)	395.97 ± 66.24	394.12 ± 64.85
S. Ferritin (ng/ml)	160.94 ± 92.93	236.47 ± 92.76*
TS%	33.76±7.6	33.86±6.01
Liver Iron Staining		
Mild (n=34)	28/33 (84.8%)	6/ 17 (35.3%)##
Advanced (n=16)	5/ 33 (15.2%)	11/17 (64.7%)

Data are expressed as mean ± SD or number and (%).
*Significant difference between groups at p<0.05 using student's *t*- test.
Highly significant difference between groups at p <0.001 using x^2 test.

Table 4: Laboratory results of patients grouped according to the stage of fibrosis

	Mild fibrosis (n=34)	Advanced fibrosis (n=16)
ALT (IU/l)	36.16±11.9	64.18±12.2*
AST (IU/l)	29.93±12.2	53.02±19.2*
S. Albumin (g/dl)	4.06±0.42	3.73±0.6*
S. Prohepcidin (ng/ml)	69.68±27.15	46.88±19.86*
S. Iron (µg/dl)	130.94±29.19	134.06±22.45
TIBC (µg/dl)	390.18±73.46	406.31±42.30
S. Ferritin (ng/ml)	159±58.21	245.31±88.09*
TS%	34.12±7.80	33.09±5.28
Liver Iron Staining		
Mild (n=34)	29/34 (85.3%)	5/16 (31.2%) ##
Advanced (n=16)	5/34 (14.7%)	11/16 (68.8%)

Data are expressed as mean ± SD or number and (%).
*Significant difference between groups at p<0.05 using student's *t*- test.
Highly significant difference between groups at p <0.001 using x^2 test.

Table 5. Iron scoring system in the liver

No of cases	Iron Score	Description	Hepatocytes	Kupffer cells and portal tract macrophages
0	0	None	----	-
17	1	Minimal	----	+
17	2	Mild	----	+
12	3	Moderate	++++	+
4	4	Marked	++++	+

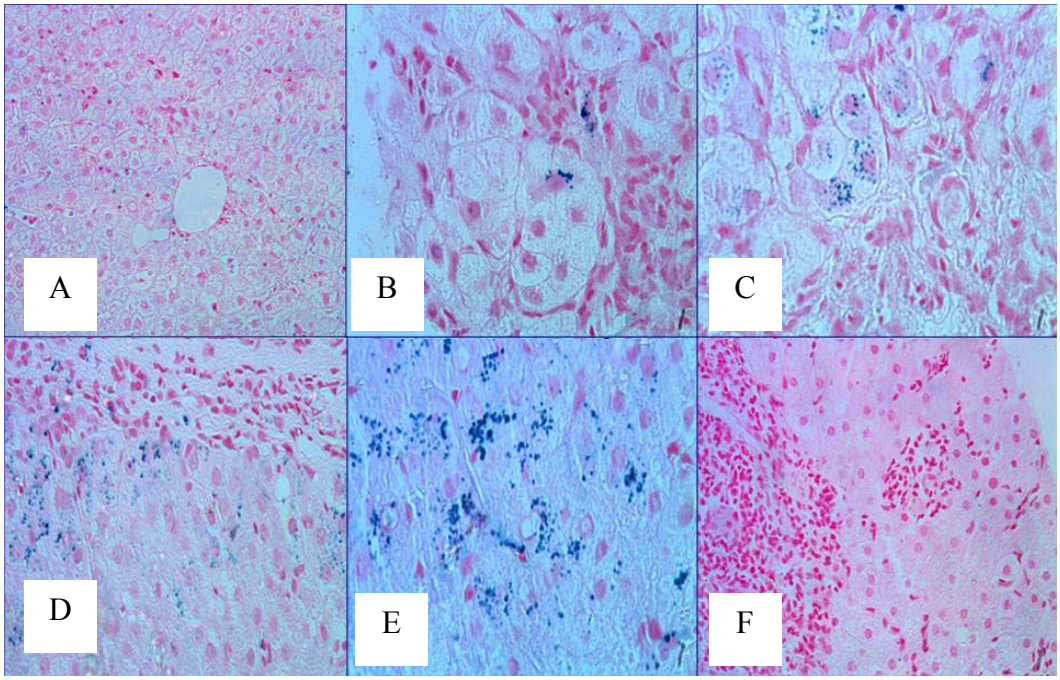

Figure 1: Liver needle biopsies, Perls' stain of iron; A- Minimal (Score I) iron staining in the hepatocytes and Kupffer cells X 200, B- Mild (Score II) iron staining both in the hepatocytes and portal macrophages X 400, C- Moderate (Score III) iron staining in the hepatocytes X 200, D- Moderate (Score III) iron staining both in the hepatocytes and portal macrophages X 100, E- Strong (Score IV) in the hepatocytes, sinusoidal and portal macrophages iron staining X 200, F- Negative control slide X 200.

DISCUSSION

Iron is normally stored in the liver in the form of ferritin and haemosiderin, predominantly in the hepatocytes [25]. Ferritin is water soluble and histochemically not stainable. Haemosiderin is normally a minor component of hepatic iron, but becomes the major form of storage iron with increasing iron overload. Iron in Kupffer cells is usually caused by accumulation of ferritin and haemosiderin released from damaged tissues or during cell turnover. Normal liver has no or only trace amount of stainable iron in hepatocytes [26].

Fabris et al. [27] reported that chronic viral hepatitis is not uncommonly accompanied by hepatic haemosiderin, which is generally mild and much less than would be expected for reasonably penetrant hereditary hemochromatosis. Iron excess in CHC is multifactorial. It may be due to associated hereditary hemochromatosis, haematological diseases, multiple transfusions, porphyria cutanea tarda and chronic alcohol abuse [28, 29, 30, 31]. Iron accumulation has also been linked with insulin resistance and liver steatosis in HCV-infected patients [32, 33]. However, if these factors are absent the mechanisms involved in iron overload remain unclear [34]. Diagnosis of hepatic siderosis is clinically important, especially in the care of patients with chronic viral hepatitis.

In the current study, all biopsies included in the statistical analysis were positive for iron stain. On the other hand, previous studies reported positive hepatic iron stain ranging from 12.5% to 63% of the examined CHC patients. It was 12.5% of CHC in Taiwan [35], 15.6% of CHC patients in Brazil [34], 19% in a study in Mayo Clinic [21]. Two studies in Italy [8, 32] found stainable liver iron in 38.8% and 47.4% respectively. Whereas, Haque et al.[36] found stainale liver iron in 63% of CHC in USA. The higher frequency of positivity of hepatic iron reported in our study may be related to the different HCV genotype prevalent among Egyptians (genotype 4). Other factors that might play a role are differences in gender, and the proportion of cirrhotics in the cohort [24]. Other possible factors are racial differences and host genetic factors as haemochromatosis gene mutations.

Our results showed that 68 % have mild (score 1 and 2) hepatic iron staining, while 32 % of patients have advanced (score 3 and 4) iron staining. This was in agreement with previous studies [15, 37, 38, 39] who reported that CHC patients frequently display mild to moderate elevation of HIC. In agreement with Fabris et al. [27], Brunt et al. [40], and Nash et al. [41]; who reported that the haemosiderin

deposits can be present in Kupffer cells, hepatocytes (with a zone 1–3 gradient), and/or portal areas; our data showed iron deposits primarily in Kupffer cells and macrophages and in the hepatocytes with a decreasing zonal gradient from periportal to centrilobular areas.

Although the iron-related oxidative stress may play a role in the pathogenesis of CHC, the association between serum iron markers, hepatic iron stores, hepatic necro-inflammatory activity and/or fibrosis remains controversial. Previous studies had evaluated the potential impact of hepatic iron store on CHC but they had produced discordant results [36, 42].

Our finding of a significant relation between hepatic iron stores and both the degree of necro-inflammation and fibrosis is in agreement with some previous reports. Haque et al. [36] reported an association between hepatic iron stores and histological activity index. Ikura et al. [43] reported that in CHC patients, iron is deposited in hepatocytes, sinusoidal cells, and portal mesenchymal cells, with the degree of portal mesenchymal iron deposition correlating with both hepatic inflammation and fibrosis. On the contrary, Boucher et al. [42] found no association between hepatic iron stores and histological activity.

Some studies [44, 45] reported no association between the presence of hepatic iron deposition and fibrosis score. Others reported that hepatic iron deposition was associated with severe hepatic fibrosis [21, 46, 47]. Furthermore, experimental data have shown that iron deposits may trigger hepatic stellate cell activation and thus induce liver fibrosis [44]. Casaril et al. [48] suggested that even a mild increase of serum iron values and hepatic iron deposition may worsen the course of chronic HCV infection and increase the progression of fibrosis. However, despite this association, they didn't find a significant correlation between the amount of hepatic iron store and fibrosis score. However, multivariate analysis of factors predicting hepatic fibrosis in the current series revealed that hepatic iron score is a significant independent predictor of advanced fibrosis. This association has been previously reported by Beinker et al. [46]

In the current series, our patients exhibited significantly elevated serum iron indices (S. Iron, TIBC, S. Ferritin, TS %). This was in agreement with Harrison-Findik [15]. We found that serum iron was elevated in 14 (28%) of patients and serum ferritin was elevated in 8 (16%) of patients. However, TS% was in the normal range in all patients. Consistent with these findings, it was reported that increased levels of serum iron, TS% and ferritinemia are encountered in 20-35% of patients with CHC and are associated with mild to moderate increase of hepatic iron load, predominant in sinusoidal location [15, 49].

Our finding of raised serum iron in 28% and raised serum ferritin in 16% of CHC patients in spite of positive hepatic iron in all patients could be explained by previous reports indicating that blood tests are of limited value in hepatic iron quantification because the results are unreliable indicators of hepatic and body iron stores [50, 51, 52].

Hepcidin levels influence the cellular distribution of excess iron, a potentially critical factor in its pathogenicity. Chronically low hepcidin as can occur in hepatitis C favours increased iron in hepatocyte stores and in plasma. Conversely, high hepcidin as in chronic inflammation favours iron depletion from blood but accumulation in reticuloendothelial cells [53].

To the best of our knowledge, this is the first study that evaluates the role of serum prohepcidin in hepatic iron deposition in a group of Egyptian patients with CHC. The significant reduction of serum prohepcidin in CHC patients in comparison to healthy controls in the current study is in agreement with previous reports [17]. Hepcidin “the product of prohepcidin" has also been reported to be reduced in CHC patients [8, 54] indicating that it is likely to be an important factor of liver iron accumulation in this condition. On the other hand, Olmez et al. [19] found no significant difference in prohepcidin level in CHC patients and controls. Other investigators [55, 56] reported significantly higher level of prohepcidin in CHC patients than in healthy controls.

In the present study, a significant reduction of serum prohepcidin with increasing grade of necro-inflammation was found. This was partially consistent with Olmez et al. [19] who reported a significant negative correlation between serum prohepcidin level and histological activity. On the other hand, Nagashima et al. [17] and Girelli et al. [8] didn't find such a correlation.

In agreement with Girelli et al. [8], we found that despite the significant reduction of serum prohepcidin in patients with advanced fibrosis than in those with mild fibrosis, no significant correlation was found between serum prohepcidin level and the stage of fibrosis. On the contrary, some previous studies [17, 19, 57] reported a significant negative correlation between serum prohepcidin and fibrosis stage.

A recent study, [58] indicated that in situations of liver function impairment, hepcidin synthesis as well as activity or

expression of converting enzymes might be altered and affect circulating prohepcidin concentration. This finding could also suggest that HCV interference with hepcidin synthesis is at the level of prohormone synthesis or maturation in the liver. These results suggest that, the progression of liver fibrosis affects synthesis of hepcidin and the inadequate hepcidin production can explain the majority of iron overload, which play a pivotal role in liver fibrosis. Multivariate analysis of factors predicting hepatic fibrosis in the current series revealed that hepatic iron score is a significant independent predictor of advanced fibrosis. This association has been previously reported by Beinker et al. [46].

Although this study showed that serum prohepcidin was significantly reduced compared to controls, serum prohepcidin didn't differ significantly with the score of hepatic iron deposition and it didn't correlate with any of the serum iron indices. Similarly, Olmez et al. [19] reported also chronically low prohepcidin in hepatitis C patients. Also, they didn't find an association between prohepcidin and serum iron parameters. Bekri et al. [53] and Fujita et al. [1] suggested that the chronically low hepcidin in hepatitis C patients favour the increased iron in hepatocyte stores. Nagashima et al. [17] reported a significant negative correlation between serum prohepcidin level in CHC patients on one hand and each of serum ferritin and total iron score in the liver.

In agreement with previous reports [59], our study showed that serum ferritin level may be a marker of hepatic inflammatory activity in HCV infected patients. This suggestion was supported by the decline in AST and ALT values after lowering serum ferritin by phlebotomy therapy [60].

As previously reported [21, 61], univariate analysis of our data showed a significant association between serum ferritin and the severity of hepatic fibrosis. On the contrary, Won et al. [62] reported no significant association between serum ferritin and hepatic fibrosis in Korean patients with CHC.

Hepcidin mRNA level in the liver was relatively low in chronic liver disease patients suggesting that it may play a pivotal role in the pathogenesis of iron overload in these patients [1, 57]. Correlation studies of hepatic mRNA revealed inconsistent results. While Fujita et al. [1] reported significant positive correlations with serum iron indices (serum iron, TS % and serum ferritin) and total iron score in the liver, the second study [57] reported that hepcidin mRNA was negatively correlated with parameters of iron stores as (serum ferritin and hepatic iron index) and stage of liver fibrosis in patients with CHC and those with hepatocellular carcinoma. The reason of these discordant results is mostly due to heterogeneity of the studied sample (inclusion of patients with various liver diseases as viral and non viral chronic liver diseases).

Sebastiani et al. [32] suggested that the mechanism of hepatic iron deposition in CHC is genotype dependent. In their study, they found that hepatic iron deposits were strongly associated with genotype 3 but they were not correlated to liver fibrosis in this group. While, in non-HCV-3 genotypes (1, 2, 4) hepatic iron deposits correlated with liver fibrosis. Izumi et al. [63] reported higher liver iron concentrations in HCV-1b compared with HCV-2-infected patients and suggested a direct influence on response to interferon based antiviral therapy.

We suggest that genotype 4 might be one of the factors promoting excess iron deposition in the liver of patients in the current study. However, this point requires further validation. Others [64] have suggested that excess hepatic iron deposition could be due to cytopathic effect of HCV on the hepatocytes and other cell types within the liver leading to alteration in their iron metabolism, and to iron accumulation. Alternatively, the excess hepatic iron deposition is simply a result of turnover from damaged hepatocytes as a result of chronic inflammatory activity associated with HCV [49]. Our results agree with the later explanation as advanced hepatic iron staining was associated with advanced necro-inflammation.

Our study has potential limitations. First, we didn't directly measure the active hormone "hepcidin" in serum since its specific ELISA kit was not available in Egypt nor was the immuno-histochemical staining kit. Second, the relatively small sample size especially of females didn't allow accurate comparison between both genders in their hepatic iron. Third, there were three samples negative for hepatic iron stain; unfortunately they were excluded from the study due to insufficient serum.

In conclusion, hepatic iron deposition is a common feature in Egyptian CHC patients. The significantly lower serum prohepcidin in Egyptian CHC patients might represent a biochemical correlate of necro-inflammation and fibrosis in those patients. Serum prohepcidin may not reflect the active form of hepcidin that influences iron metabolism. Chronic HCV infection may reduce serum prohepcidin which may be one –not the only- factor leading to iron overload in these patients. Histological grading and hepatic iron

density are independent predictors of advanced fibrosis. Further studies are needed to clarify the role of viral and host genetic factors in hepatic iron deposition.

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الملخص العربى

مؤشرات البروهبسدين والحديد فى المصل، وحالة الحديد فى الكبد فى مرضى الإلتهاب الكبدي الفيروسي س المزمن فى المصريين

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المرضى الذين يعانون من الإلتهاب الكبدى الوبائي المزمن غالبا ما تزيد فيهم نسبة الحديد في الكبد ، وقد برز الهبسدين فى الآونة الأخيرة كمنظم رئيسي لتوازن الحديد ، لذا يهدف هذا البحث إلى دراسة العلاقة بين البروهبسدين ومؤشـرات الحديـد فـى المصل والإلتهاب الكبدي النخري وتليف الكبد وكثافة الحديد فى الكبد وتحديد منبئات التليف المتقدم في هؤلاء المرضى. حيث تـم اختيار خمسين مريضا لم يسبق علاجهم وعشرين من الاصحاء وتم اجراء عد الدم الكامل ، اختبارات وظائف الكبد ، ومؤشـرات الحديد فى المصل ، والبروهبسدين فى المصل. وتم أخذ عينة من الكبد لجميع المرضى لتقييم نشاط الإلتهاب الكبدي النخري والتليف الكبدي وكثافة الحديد فى أنسجة الكبد.

وقد أظهرت النتائج ان أربعة وثلاثون مريضا (٦٨٪) يعانون من تليف كبدي خفيف (المرحلة ٢،١،٠) وستة عـشرة (٣٢٪) يعانون من تليف كبدي متقدم (المرحلة ٤،٣). وقد كانت جميع الحالات ايجابية لصبغة الحديد فى الكبـد (٦٨ ٪ معتدلـة ، ٣٢ ٪ متقدمة). وكان متوسط مستوى البروهبسدين فى المصل فى المرضى الذين يعانون من التهاب الكبد الوبائي المزمن (س) أقل بكثير منه فى الاصحاء. وفى تحليل احادي المتغيركان البروهبسدين مرتبط بشكل كبير بنشاط الإلتهاب الكبدي النخري والتليف الكبـدي المتقدم. وكشف تحليل المتغيرات المتعددة أن الإلتهاب الكبدي النخري وكثافة الحديد فى الكبد ترتبط بشكل مستقل مع مرحلة تليـف الكبد. ولم يتم العثور على ارتباط ذى دلاله احصائيه بين مستوى البروهبسدين فى المصل ومؤشرات الحديد فى المصل أو درجـة الحديد فى الكبد.

وقد استنتج البحث أن عدوى التهاب الكبد الوبائي المزمن (س) تؤدي الى انخفاض البروهبسدين فى المصل ويعتبر ذلك عاملا ولكنه ليس الوحيد الذي يؤدي إلى ارتفاع تركيز الحديد فى هؤلاء المرض و تعتبرالدرجات النسيجية وكثافة الحديد الكبدية عوامـل مستقلة تنبئ بالتليف الكبدي المتقدم وأن هناك حاجة إلى مزيد من الدراسات لتوضيح دورالعوامل الوراثية الفيروسـية والعوامـل الوراثية للعائل فى ترسب الحديد فى الكبد.