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Evaluation of Serum Hepcidin and Iron Levels in some Liver Diseases

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ABSTRACT

Background: Hepcidin regulates iron metabolism by triggering the degradation of ferroportin to regulate the release of iron into plasma from macrophages, hepatocytes and enterocytes. Moreover, there is a positive relationship between hepcidin levels and synthetic liver function suggesting that a uniform suppression of hepcidin may be linked to disease progression and development of HCC. **Aim of study:** The evaluation of serum hepcidin, Iron levels in hepatocellular carcinoma (HCC), cirrhosis and chronic hepatitis C (CHC) comparing these levels with serum alpha fetoprotein (AFP) levels in all participants. **Patients and Methods:** This study was conducted on a total number of 124 patients (49 with HCC, 49 with liver cirrhosis and 26 with CHC) in addition to 20 healthy individuals as control. Liver function tests, AFP, hemoglobin concentration and serum iron were analyzed for all participants. All HCC patients were newly diagnosed and none had received any form of anticancer therapy before collection of blood samples for biochemical analysis. Diagnosis of HCC was confirmed by imaging (computer tomography and ultrasound) and serum a-fetoprotein (AFP). Level of Hepcidin (pg/ml) was calculated by interpolation from a reference curve generated in the same assay with reference standards of known concentrations. **Results:** The mean level of AFP and hepcidin in HCC cases were 313.02, 40.71 ng/ml respectively. Hepcidin level was significantly decreased in HCC cases than liver cirrhosis (LC), CHC and control. There was a highly significant negative correlation between hepcidin level and each of AFP, AST, ALT and iron concentration among all study groups. Regarding to the data of ROC analysis for AFP, we found that, AFP at a cut-off value of 21 ng/ml achieved sensitivity of 66.7% and specificity of 72%, ($p<0.001$) for detection of HCC cases. ROC curve of Hepcidin showed, that the diagnostic ability of Hepcidin as amarker in discrimination diagnosis of HCC cases from other liver diseases at cutoff level of $\leq$ 42.7 ng/ml achieved 92 % sensitivity and 90% specificity, ($p<0.0002$) while ROC curve of Iron showed the best cut-off value $\leq$ 325 ng/ml had sensitivity of 90%, specificity of 89%, ($p<0.0001$). **Conclusion**: level of serum hepcidin was decreased in CHC, LC and more decreased in HCC patients when comparing with the control group suggesting that hepcidin hormone is responsive to disease progression and a marker for HCC.

Key words: Hepatocellular carcinoma, α-fetoprotein, Hepcidin

Introduction

Hepatitis C virus (HCV) infects approximately 170 million people worldwide (Lauer and Walker, 2001; Shepard *et al.,* 2005). It causes chronic liver diseases including chronic hepatitis, cirrhosis and hepatocellular carcinoma. HCV-associated chronic liver disease is the leading indication for liver transplantation (Charlton, 2001). Chronic inflammatory stress caused by hepatitis viruses B and C plays a major role in hepatocellular carcinoma (HCC) carcinogenesis (Metwelly *et al.,* 2004). Hepatocellular carcinoma (HCC, also called malignant hepatoma) is the most common type of liver cancer. Most cases of HCC are secondary to either a viral hepatitis infection (hepatitis B or C) or cirrhosis (alcoholism being the most common cause of hepatic cirrhosis). Hepcidin is synthesized by hepatocytes in response to both iron overload and inflammatory stimuli, an effect believed to be dependent on cytokine production (Kumar *et al.*, 2003). Before the recent discovery of hepcidin and its function in iron metabolism, anemia of chronic disease was seen as the result of a complex web of inflammatory changes. Over the last few years, many investigators have come to feel that hepcidin is the central actor in producing anemia of chronic inflammation (Nemeth and Ganz, 2006). The 25-amino acid peptide of hepcidin is secreted mainly by the liver and is considered the "master regulator" of iron metabolism. Hepcidin inhibits iron transport by binding to the iron export channel ferroportin. Inhibiting ferroportin prevents iron from being exported and the iron is sequestered in the cells (Rossi, 2005). Hepcidin activity is also partially

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responsible for iron sequestration seen in anemia of chronic inflammation such as Inflammatory Bowel Disease, Chronic Heart Failure, Carcinomas, Rheumatoid Arthritis and renal failure (Ashby *et al.*,2009) . Moreover, some studies demonstrated that expression of hepcidin mRNA was suppressed universally in HCC, irrespective of the degree of tumor differentiation (Ehab *et al.*,2013). Some studies indicate, there is a positive relationship between hepcidin levels and synthetic liver function suggesting that a uniform suppression of hepcidin may be linked to disease progression and development of HCC (Ehab *et al.,* 2013).

Kijima, *et al*., (2008)., reported that hepcidin is produced in patients with HCC, from noncancerous liver tissue, even though production is inhibited in cancerous tissue. Moreover, hepcidin mRNA expression was not related to the histological grade, vascular invasion, or recurrence of HCC (Elhamy *et al.,* 2009). In a more recent study by His-Huang *et al.,* (2009)., it was reported that hepcidin is down-regulated in HCC but still negatively correlated with hepatic iron stores. The altered expression of iron-regulatory genes accompanying HCC may disturb patient's iron balance leading to decreased hepcidin expression in these cases. This finding suggests that, hepcidin may play a role in defending the body against HCC development.

Methods:

Population samples:

The current study was conducted on 124 patients (73 males and 51 females , ages range 28- 73 years) admitted to Tropical Medicine Department in National Hepatology and Tropical Medicine Research Institute. Forty nine patients with liver cirrhosis (24 males and 25 females, ages range 34-70 with a mean of 53.24±9.78), forty nine patients with HCC (31 males and 18 females, ages range 40-73 with a mean of 57.78± 10.29) and twenty six patients with CHC (18 males and 8 females, ages range 28- 50 with a mean of 38.85 ± 8.1). This is in addition to twenty healthy control group (11 males and 9 females, ages range 18 – 67 with a mean of 41.4 ± 14.93) who were negative for serological markers of HBV and HCV. All HCC patients were newly diagnosed and none had received any form of anticancer therapy before collection of blood samples for biochemical analysis. Diagnosis of HCC was confirmed by imaging (computer tomography and ultrasound) and serum alpha-fetoprotein (AFP). The third group included 26 patients with CHC who are diagnosed according to laboratory investigations (presence of anti-HCV antibodies and detection of serum HCV RNA). Informed consent was obtained from all participants before enrollment in the study. The study was carried out in accordance with the principles of the Declaration of Helsinki and its appendices and local and national laws.

Ten ml of venous blood were withdrawn from each patient in dry sterile vacutainers. After centrifugation, the serum was tested for: Aspartate aminotransferase (AST), Alanine aminotransferase, Total bilirubin, direct bilirubin g-glutamyltranspeptidase (GGT), Iron and albumin concentrations were assayed using Beckman CX4 chemistry analyzer (USA, supplied by the Eastern Co. For Eng. & Trade-Giza, Egypt). AFP (ng/ml) was measured using Abbott, Axyam (USA, Supplied by al kamal company Cairo, Egypt). Hepcidin ELISA kit was purchased from DRG International Inc., USA, code (EIA-4705, Vers. 4.1). Level of Hepcidin (pg/ml) was calculated by interpolation from a reference curve generated in the same assay with reference standards of known concentrations. This assay was performed in duplicate according to the manufacturer's instructions. Abdominal ultrasound was performed for all the enrolled patients using a Toshiba (Japan) machine with a 3.5MHz convex probe; patients were examined after at least 8 h of fasting.

Histopathological investigations:

Liver biopsy specimens were formalin-fixed and paraffin embedded then sectioned and stained (hematoxylin and eosin) for routine histopathological examination.

Statistical analysis:

Continuous variables are expressed as mean and Standard error. Categorical variables are expressed as frequencies and percentage. Continuous variables were compared using ANOVA test and kruskal wallis test or the Mann–Whitney U-test. Categorical variables were compared using the chi-square test or fisher exact. Spearman correlation coefficient was used to assess the correlation between numerical variables. The ROC Curve (receiver operating characteristic) was used to evaluate the Sensitivity and specificity of AFP, Iron and hepcidin in diagnosis of HCC. A significance level of $P < 0.05$ was used in all tests. All statistical procedures were carried out using SPSS version 15 for Windows (SPSS Inc, Chicago, IL, USA).

Results:

The clinical data of 144 participants enrolled in this study were shown in Table 1; there was no significant difference between all study groups as regard sex distribution, smoking and alcohol consumption while

regarding symptoms and signs, a highly significant difference was found between all groups as regard Abdominal Pain, Weight loss, encephalopathy, bleeding and elevated BT.

Table 2 indicated that, there was no significant difference between patient groups as regard sex, smoking and alcohol consumption while Regarding clinical symptoms and signs, there was a highly significant difference was found regard encephalopathy, bleeding and elevated BT with HCC group showing the highest prevalence of symptoms and signs compared to other groups.

Table 1: Comparison between all studied groups regarding to clinical data.

		Groups								P*	Sig
		Control		Liver cirrhosis		HCC		CHC			
		N	%	N	%	N	%	N	%		
Sex	Male	11	55.0%	24	49.0%	31	63.3%	18	69.2%	.306*	NS
	Female	9	45.0%	25	51.0%	18	36.7%	8	30.8%		
Smoking	No	17	85.0%	30	61.2%	28	57.1%	14	53.8%	.126*	NS
	Yes	3	15.0%	19	38.8%	21	42.9%	12	46.2%		
Alcohol	No	19	95.0%	40	81.6%	45	91.8%	24	92.3%	.333**	NS
	Yes	1	5.0%	9	18.4%	4	8.2%	2	7.7%		
Abdominal pain	No	19	95.0%	26	53.1%	25	51.0%	13	50.0%	.004*	HS
	Yes	1	5.0%	23	46.9%	24	49.0%	13	50.0%		
Wt loss	No	20	100.0%	24	49.0%	28	57.1%	18	69.2%	.001*	HS
	Yes	0	.0%	25	51.0%	21	42.9%	8	30.8%		
Encephalopathy	No	20	100.0%	31	63.3%	25	51.0%	26	100.0%	.0001**	HS
	Mild	0	0.0%	8	16.3%	2	4.1%	0.0	00%		
	Moderate	0	0.0%	10	20.4%	22	44.9%	0.0	0.0%		
	Severe	0	0.0%	0.0	0.0%	0.0	0.0%	0.0	0.0%		
Bleeding	No	20	100.0%	29	59.2%	27	55.1%	26	100.0%	.0001*	HS
	Yes	0.0	0.0%	20	40.8%	22	44.9%	0.0	0.0%		
Elevated BT	No	19	95.0%	35	71.4%	29	59.2%	23	88.5%	.005*	HS
	Yes	1	5.0%	14	28.6%	20	40.8%	3	11.5%		

*Chi-Square Tests, **fisher exact test, P- value comparison was done between CHC, LC, HCC patients and healthy control, BT:Body Temperature..

Table 2: Comparison between patients groups regarding to clinical data.

		Type						P*	Sig
		Liver cirrhosis		HCC		CHC			
		N	%	N	%	N	%		
Sex	Male	24	49.0%	31	63.3%	18	69.2%	.172*	NS
	Female	25	51.0%	18	36.7%	8	30.8%		
Smoking	No	30	61.2%	28	57.1%	14	53.8%	.815*	NS
	Yes	19	38.8%	21	42.9%	12	46.2%		
Alcohol	No	40	81.6%	45	91.8%	24	92.3%	.223*	NS
	Yes	9	18.4%	4	8.2%	2	7.7%		
Abdominal pain	No	26	53.1%	25	51.0%	13	50.0%	.963*	NS
	Yes	23	46.9%	24	49.0%	13	50.0%		
Wt loss	No	24	49.0%	28	57.1%	18	69.2%	.241*	NS
	Yes	25	51.0%	21	42.9%	8	30.8%		
Encephalopathy	No	31	63.3%	25	51.0%	26	100.0%	.0001**	HS
	Mild	8	16.3%	2	4.1%	0	.0%		
	Moderate	10	20.4%	22	44.9%	0	.0%		
	Severe	0	.0%	0	.0%	0	.0%		
Bleeding	No	29	59.2%	27	55.1%	26	100.0%	.0001*	HS
	Yes	20	40.8%	22	44.9%	0	.0%		
Elevated BT	No	35	71.4%	29	59.2%	23	88.5%	.03*	S
	Yes	14	28.6%	20	40.8%	3	11.5%		

*Chi-Square Tests, **fisher exact test, p-value < 0.05 significant P- value comparison was done between CHC, LC and HCC patients.

Table 3 showed highly significant difference of AST, ALT, T.bili., D.bili., INR, and GGT levels in HCC, LC and CHC patients groups compared to healthy control ($p < 0.001$). AST, ALT, T.bili., D.bili., INR and GGT were highly significant increase in HCC group while regarding to Albumin, WBCs and platelets there was significant decrease in their levels in HCC group when compared to other groups.

Regarding the comparison between patients groups for radiological parameters, there was significant difference between all groups regarding to radiological parameters except PVT, hepatomegaly and hypertension which were shown no significant difference between all studied groups (table 4).

The mean levels of AFP and Iron in HCC cases were 313.02 and 295.2 ng/ml respectively while regarding to Hepcidin level it was 40.7ng/ml. The hepcidin level was significantly decreased in HCC cases than LC, CHC and control (Table 5).

Table 3: Comparison between studied groups regarding the laboratory findings.

Variable	Control N=20 Mean ± SD	LC N=49 Mean ± SD	HCC N=49 Mean ± SD	CHC N=26 Mean ± SD	P-value
ALT (IU/L)	30.0 ± 6.00	56.5 ± 15.44	66.2 ± 16.94	59.4 ± 18.93	<0.001*
AST (IU/L)	32.4 ± 9.06	63.0 ± 33.94	158.5 ± 70.27	61.4 ± 15.74	<0.001*
T.Bil (mg/dl)	0.74 ± 0.20	1.36 ± 0.701	2.81 ± 1.05	1.05 ± 0.38	<0.001*
D.Bil (mg/dl)	0.15 ± 0.06	0.31 ± 0.22	0.88 ± 0.44	0.24 ± 0.15	<0.001*
Albumin (g/dl)	3.88 ± 0.216	3.00 ± 0.535	2.69 ± 0.587	3.85 ± 0.399	<0.001*
GGT (IU/L)	34.2 ± 9.5	57.2± 19.2	269.9 ± 140.7	39.96 ± 16.05	<0.001*
INR	1.0 ± 0.079	1.07 ± 0.185	1.22 ± 0.137	1.15 ± 0.180	<0.001*
Hb (g/dl)	11.8 ± 1.73	10.9 ± 1.07	11.01 ± 1.04	11.5 ± 1.1	0.034*
WBCs ($\times 10^3$)/ul	6940.0 ± 2374.1	5651.7 ± 1543.1	5429.2 ± 1788.7	7880.0 ± 1458.3	<0.001*
Plts ($\times 10^3$)/ul	291.6 ± 88.9	176.5 ± 56.3	119.2 ± 31.2	301.0 ± 83.7	<0.001*

*p-value < 0.05 significant , Data present as mean ± SD; ALT, alanine aminotransferase; AST, aspartate aminotransferase; T.Bil, total bilirubin; D.Bil, direct bilirubin; GGT, γ-glutamyltransferase; INR, International Normalized Ratio; WBCs, white blood cells; Plts, platelets; Hb, hemoglobin; P- value comparison was done between CHC, LC, HCC patients and healthy control.

Table 4: Comparison between studied groups regarding the radiological examination (Sonar and Computed tomography).

Parameters	Control N (%)	LC N (%)	HCC N (%)	CHC N (%)	P=value
1-Liver					
Normal liver	20(100%)	0(0%)	0(0%)	20(40%)	P<0.001*
Bright liver	0(0%)	0(0%)	0(0%)	29 (60%)	
Coarse liver	0(0%)	49(100%)	49(100%)	0(0%)	
Focal lesion (HCC)	0(0%)	0(0%)	49(100%)	0(0%)	P<0.001*
2-Ascitis					
No	20(100%)	29(60%)	39(80%)	49(100%)	P=0.008*
Mild	0(0%)	11(23.3%)	4(6.7%)	0(0%)	
Mod	0(0%)	5(10%)	5(10%)	0(0%)	
Severe	0(0%)	4(6.7%)	1(3.3%)	0(0%)	
3-PVT					
Yes	0(0%)	5(10%)	5(10%)	0(0%)	P=0.19
No	20(100%)	44(90%)	44(90%)	49(100%)	
4-Splenomegaly					
Yes	0(0%)	5(10%)	11(23.3%)	0(0%)	P=0.01*
No	20(100%)	44(90%)	38(76.7%)	49(100%)	

*p-value < 0.05 significant, PVT, portal vein thromposis

Table 5: Comparison between AFP, Hepcidin and Iron in all studied groups.

		Mean	S.E	F*	P	Sig
AFP ng/ml	Control	5.86	.45	33.360	0.0001	HS
	CHC	10.50	3.41			
	LC	36.36	7.33			
	HCC	313.02	40.13			
Hepcidin pg/ml	Control	96.90	5.21	29.914	0.0001	HS
	CHC	57.96	5.65			
	LC	47.53	2.61			
	HCC	40.71	3.43			
Iron µg/dL	Control	105.3	33.2	25.713	0.01	S
	CHC	181.7	65.7			
	LC	295.2	95.1			
	HCC	413.02	131.6			

*ANOVA , p-value < 0.05 significant, AFP: Alpha feto protein

There was a highly significant negative correlation between Hepcidin level and each of AFP, Iron, AST and ALT while regarding to Albumin, there was a highly significant positive correlation (Table 6:8-Fig 1-2). Regarding to the data of ROC analysis for AFP in (Table-9) we found that, AFP at a cut-off value of 21 ng/ml had sensitivity of 66.7% and specificity of 72%, with (confidence interval (CI 95%): 0.67-0.80; p<0.001) (area under the curve=0.75)for detection of HCC cases.

ROC curve of Hepcidin showed, that the diagnostic ability of Hepcidin as amarker in discrimination diagnosis of HCC cases from other liver diseases at cutoff level of ≤ 42.7 ng/ml with 92% sensitivity and 90% specificity with (confidence interval (CI 95%): 0.870-0.966; p<0.0002) (area under the curve=0.912) for detection of HCC cases (Table-9; Fig-3). ROC curve of Iron showed the best cut-off value ≤ 325 ng/ml had sensitivity of 90%, specificity of 89%, with (confidence interval (CI 95%): 0.813-0.911; p<0.0001) for detection of HCC cases (area under the curve=0.879) (Table9-Fig4).

Table 6: Correlation between lron and hepcidin.

		Hepcidin
Iron	r	-0.502
	P	0.001
	Sig	HS

p-value < 0.05 significant

Table 7: Correlation between AFP and hepcidin.

		Hepcidin
AFP	r	-.424
	P	0.01
	Sig	S

p-value < 0.05 significant, AFP: Alpha feto protein

Table 8: Correlation between Hepcidin and laboratory findings.

	Hepcidin		
	r	P	Sig.
ALT	-0.48	<0.0001	HS
AST	-0.80	<0.0001	HS
Albumin	0.37	0.04	S
Bilirubin(total)	0.19	0.29	NS

p-value < 0.05 significant, Pearson correlation coefficient, ALT, alanine aminotransferase; AST, aspartate aminotransferase;

Table 9: ROC analysis of Hepcidin, Iron and AFP.

Variable	The best Cut off	Sensitivity	Specificity	Sig.	AUC	95% Confidence Interval	
Hepcidin	42.7	92%	90%	<0.0002	0.912	0.870	0.966
Iron	325	90%	89%	<0.0001	0.879	0.813	0.911
AFP	21	66.7%	72%	<0.001	0.75	0.670	0.800

p-value < 0.05 significant, AFP: Alpha feto protein

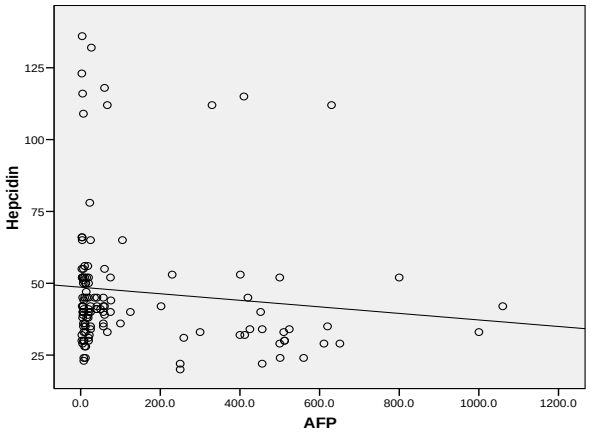

Fig. 1: Correlation between Hepcidin and AFP.

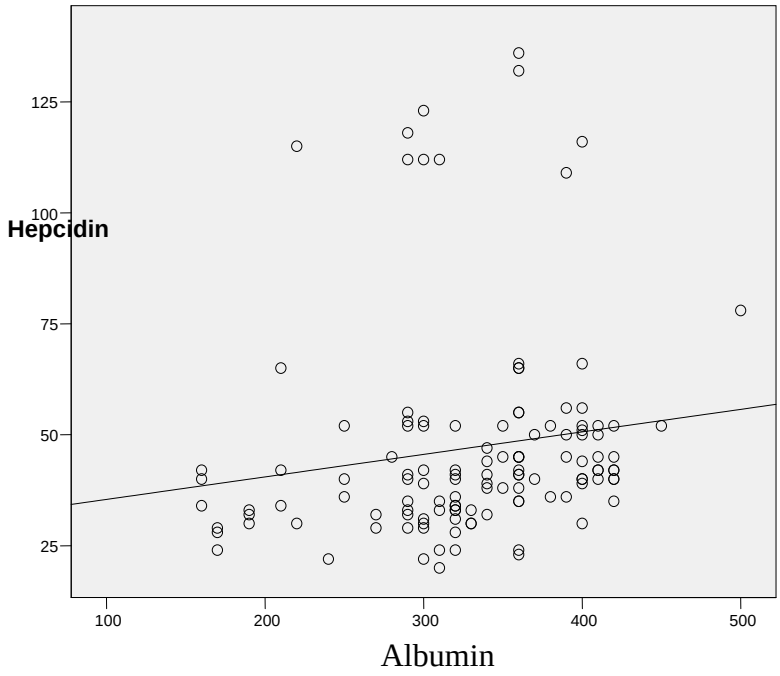

Fig. 2: Correlation between Hepcidin and Albumin.

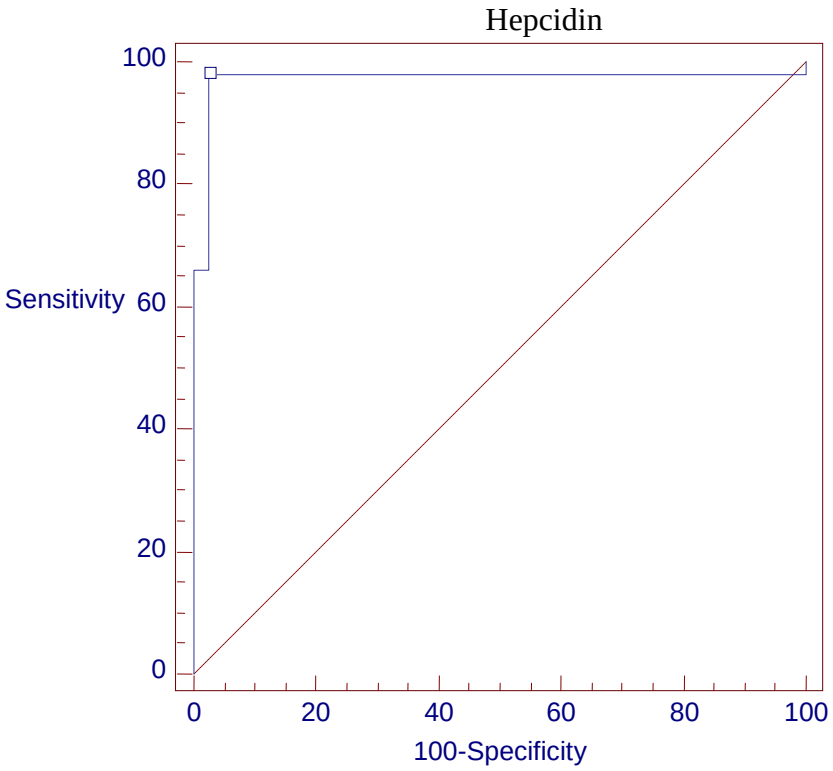

Fig. 3: Receiver operating characteristic curve of serum Hepcidin.

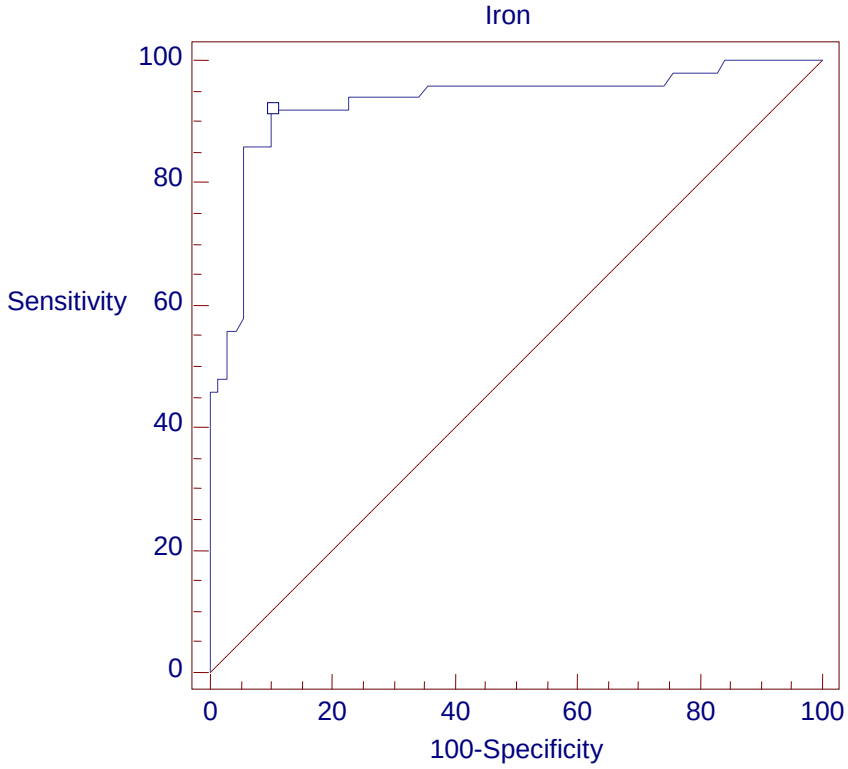

Fig. 4: Receiver operating characteristic curve of serum Iron.

Discussion:

Chronic hepatitis C (CHC) still is a major medical problem. Worldwide, chronic infection with the hepatitis C virus is the second most common cause of HCC, accounting for 20% of all HCC (Sherman, 2012). About 50-80% of patients with primary HCV infection develop chronic infection; about 25% of patients with chronic infection develop cirrhosis within 10 to 30 years; and 5-10% of patients with cirrhosis develop hepatocellular carcinoma (HCC) (Dang *et al.,* 2012). Hepcidin is a pivotal regulator of iron metabolism because it controls the efflux of iron from enterocytes, hepatocytes, and macrophages by internalization and degradation of the iron exporter (ferroportin), and also regulates the plasma iron level (Pietrangelo and Trautwein,2004; Falzacappa *et al.,* 2007). The role of hepcidin in human cancer deserves to be studied, since there have been only few reports in this context (Kijima *et al.,*2008; Ward *et al.,* 2008). It is well known that HCC develops in more than 40% of patients with hemochromatosis (Ishak *et al.,* 1995). On the other hand, iron is an essential nutrient for cell growth, and cancer cells in particular require iron in order to proliferate (Le *et al.,* 2002). Our study groups (HCC, LC, CHC patients and healthy controls) were matched for age, sex, smoking and alcohol consumption while regarding clinical symptoms and signs, there was a highly significant difference was found regard encephalopathy, bleeding and elevated PT with HCC group showing the highest prevalence of symptoms and signs compared to other groups. The present study revealed that, the concentration of hepcidin was significantly decreased in CHC patients compared with control group ($p<0.01$). Our result indicating that, the suppression of hepcidin by hepatitis C virus is likely to be an important factor of liver iron accumulation in this condition (Girelli *et al.,* 2009) and these results were in agreement with Girelli *et al.,* (2009) who indicate that, serum hepcidin was significantly lower in CHC patients than in controls (means with 95% confidence intervals: 33.7, 21.5–52.9 versus 90.9, 76.1–108.4 ng/mL respectively; $p < 0.001$).

The results of our study revealed significantly decreased serum hepcidin values in CHC, HCC and LC patients compared to the controls ($p <0.05$) and this in agreement with Fujita *et al.,* (2008) and Olmez *et al.,* (2010) but in contrast to the finding of Lin *et al.,* (2009) who reported significantly increased serum prohepcidin values in CHC patients compared to the control subjects. To explain the differences between our results and those of other investigators regarding serum hepcidin and its relation to hepatic pathological changes we can, theoretically, say that in the early phase of CHC, hepcidin may be prominently suppressed by HCV but as iron accumulates, the negative influence of viral factors may be masked by the positive stimulation of iron while, in advanced stages such as cirrhosis, hepcidin may be further decreased by impaired protein synthesis due to markedly reduced functional hepatic mass.

Hepcidin levels been reported to correlate with the liver iron concentration and the parameters of hepatic function (e.g. serum albumin) (Detivaud *et al.,* 2005). In the current study, our findings indicate a positive correlation between Hepcidin and Albumin this in agreement with a study done by Détivaud *et al.,* (2005) who found that parameters reflecting hepatic function were correlated with hepcidin levels thus, serum albumin was positively correlated with hepcidin levels.

Our findings showed highly significant difference of AST, ALT, T.bili., D.bili., INR, and GGT levels in HCC, LC and CHC patients groups compared to healthy control ($p < 0.001$). AST, ALT, T.bili., D.bili., INR and GGT were highly significant increase in HCC group while regarding to Albumin, WBCs and platelets there was significant decrease in their levels in HCC group when compared to other groups and this in agreement with a study done by AbdEl- Monem, *et al.,* (2009) who found that the comparison between liver function tests in the studied groups revealed a significant increase in ALT, AST, ALP, GGT, T. Bilirubin and AFP in patients with CHC and HCC compared to controls, with the highest value in HCC group. While the serum albumin and prothrombin concentration was significantly decreased (AbdEl- Monem, *et al.,* (2009).

In the current study, there was a highly significant negative correlation between Hepcidin level and each of AFP, Iron, AST and ALT while study by Aoki et al., indicate that, Hepcidin mRNA expression in the liver did not correlate with aspartate aminotransferase, alanine aminotransferase (Aoki *et al.,* 2005). In fact, hepcidin synthesis is suppressed at high iron levels (Nemeth *et al.,* 2003; Pigeon *et al.,* 2003). Thus, the mechanisms regulating hepcidin do not appear to be directly responsive to iron expression of hepcidin in the body is complicated and indirect.

Suppression of hepcidin transcription contradicts the previously proposed scheme for iron homeostasis in cancer cells, because cancer cells must retain iron in order to proliferate. However, suppression of hepcidin is rational because duodenal enterocytes transfer iron to plasma, resulting in an increase of total body iron content. One explanation was provided by Weizer-Stern, *et al*. who reported that activation of the tumor suppressor gene p53 stimulates the expression of hepcidin (Weizer *et al.,* 2007). In the current study regarding to the data of ROC analysis for AFP in (Table-9) we found that, AFP at a cut-off value of 21 ng/ml had sensitivity of 66.7% and specificity of 72%, ($p<0.001$) for detection of HCC cases. Similar results were obtained by Toyoda *et al.,* (2007)., who reported that AFP plays a limited role in detection and diagnosis of HCC, therefore, use of AFP alone for hepatocellular carcinoma surveillance is not recommended (Adams *et al.,* 1992).

ROC curve of Hepcidin showed, that the diagnostic ability of Hepcidin as amarker in discrimination diagnosis of HCC cases from other liver diseases at cutoff level of ≤ 42.7 ng/ml with 92 % sensitivity and 90% specificity, ($p<0.0002$) for detection of HCC cases while ROC curve of Iron showed the best cut-off value (325 ng/ml) had sensitivity of 90%, specificity of 89%, ($p<0.0001$). Taken together, these results suggest, that hepcidin expression appears to be appropriately responsive to iron status and disease progression in cirrhosis and HCC patients.

In conclusion, serum hepcidin level is decreased in HCC cancerous more than in non cancerous as CHC, LC and normal liver tissues. Moreover, there is a significant relationship between hepcidin and liver function. Suggesting that, suppression of serum hepcidin may be linked to disease progression and development of HCC. In addition, we recommend further work to evaluate the efficacy of hepcidin as marker for early detection of HCC and in reducing iron overload.

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References

Adams, D.H., E. Mainolfi, P. Burra, J.M. Neuberger, R. Ayres, E. Elias, R. Rothlein, 1992. Detecting of circulating intercellular adhesion molecule-1 in chronic liver diseases. Hepatology, 16: 810-814.

Aoki, C.A., L. Rossaro, R. Ramsamooj, D. Brandhagen, *et al*., 2005. Liver hepcidin mRNA correlates with iron stores, but not inflammation, in patients with chronic hepatitis C. J Clin Gastroenterol., 39(1): 71.

Ashby, D.R., D.P. Gale, M. Busbridge, K.G. Murphy, N.D. Duncan, T.D. Cairns, D.H. Taube, S.R. Bloom, F.W. Tam, R.S. Chapman, P.H. Maxwell, P. Choi, 2009. "Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease". Kidney Int., 75(9): 976-81.

Charlton, M., 2001. Hepatitis C infection in liver transplantation. Am J Transplant., 1: 197-203.

Dang, S.S., W.J. Wang, X.F. Wang, *et al*., 2012. Telaprevir for Chronic Hepatitis C with Genotype 1: A Meta-Analysis. Hepatogastroenterology, 59(114): 1-23.

Detivaud, L., Elizabeta nemeth, Karim Boudjema, Olvier Lore al, *et al*., 2005. Hepcidin levels in humans are correlated with hepatic iron stores, haemoglobin levels, and hepatic function. Blood, 106: 746-748.

Ehab Abd El Atti *et al*, 2013. Serum and ascitic fluid hepcidin in HCV positive liver cirrhosis with and without HCC, European Journal of Preventive Medicine, 1(3): 63-69, doi: 10.11648/j.ejpm.20130103.13.

Elhamy Abd El-Monem, EL-Sayed Tharwa, Mohamed A. Farag, Amal Fawzy *et al*., 2009. Hepcidin mRNA Level as A Parameter of Disease Progression in Chronic Hepatitis C and Hepatocellular Carcinoma, Journal of the Egyptian Nat. Cancer Inst., 21(4): 333-342.

Falzacappa, V.M.V., M.V. Spasic, R. Kessler, J. Stolte, M.W. Hentze, M.U. Muckenthaler, 2007. STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. Blood, 109: 353-358.

Fujita, N., R. Sugimoto, S. Motonishi, *et al*., 2008. Patients with chronic hepatitis C achieving a sustained virological response tointerferon and ribaviron therapy recover from impaired hepcidin secretion. J. heoatol, 49: 702-710.

Girelli, D., M. Pasino, J.B. Goodnough, G. Fattovich, *et al*., 2009. Reduced serum hepcidin levels in patients with chronic hepatitis C. J Hepatol., 51(5): 845-52.

Hsi-Huang, T., C. Jan-Gowth, H. Yaw-Huei, *et al*., 2009. Expression of hepcidin and other iron-regulatory genes in human hepatocellular carcinoma and its clinical implications JCR and Clin Oncol., 135: 1413-1420.

Ishak, K., A. Baptista, L. Bianchi, *et al*., 1995. Histological grading and staging of chronic hepatitis. J Hepatol., 22: 696-699.

Kijima, H., H. Sawada, N. Tomosugi, K. Kubota, 2008. Expression of hepcidin mRNA is uniformly suppressed in hepatocellular carcinoma BMC Cancer, 8.

Kumar, V., N. Fausto, A. Abbas,(editors), 2003. Robbins & Cotran Pathologic Basis of Disease (7th ed.). Saunders, pp: 914-7.

Lauer, G.M., B.D. Walker, 2001. Hepatitis C virus infection. N Engl J Med., 345: 41-52.

Le, N.T., D.R. Richardson, 2002. The role of iron in cell cycle-progression and the proliferation of neoplastic cells.Biochim Biophys Acta., 1603: 31-46.

Lin, T., L.Y. liao, J.M. Chou, *et al*., 2009. Serum prehepcidin levels correlates with hepatic iron stores in chronic hepatatites C patients. Hepatogastroenterology, 56(93): 1146-51.

Metwelly, M.A., C.O. Zein, N.N. Zein, 2004. Clinical significance of hepatic iron deposition and serum iron values in patients with chronic hepatitis C infection. Am J Gastroenterol., 99: 498-503.

Nemeth, E., E.V. Valore, M. Territo, G. Schiller, A. Lichtenstein, T., Ganz, 2003. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute phase protein. Blood, 101: 2461-2463.

Nemeth, E., T. Ganz, 2006. Regulation of iron metabolism by hepcidin. Annu. Rev. Nutr., 26(1): 323-42.

Olmez, F., O. Fatih, S. Gurel, *et al*., 2010. Plasma prohepcidin levels in patients with chronic viral hepatitis: relation with liver fibrosis.European Journal of gastroenterology, 108: 1104-1109.

Pietrangelo, A., C. Trautwein, 2004. Mechanism of disease: The role of hepcidin in iron homeostasis-implications for hemochromatosis and other disorders. Nat Clin Prac Gastroenerol Hepatol., 1: 39-45.

Pigeon, C., G. Ilyin, B. Courselaud, *et al*., 2003. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem., 276: 7811-7819.

Rossi, E., 2005. "Hepcidin-the iron regulatory hormone". Clin. Biochem. Rev., 26(3): 47-9.

Shepard, C.W., L. Finelli, M.J. Alter, 2005. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis., 5: 558-567.

Sherman, M., 2012. Chronic Hepatitis C Virus: Advances in Treatment, Promise for the Future, 5(14): 47-60.

Toyoda, H., T. Kumada, Y. Osaki, H. Oka and M. Kudo, 2007. Role of tumor markers in assessment of tumor progression and prediction of outcomes in patients with hepatocellular carcinoma. Hepatol Res., 37(Suppl 2): 166-171.

Ward, D.G., K. Roberts, M.J. Brookes, H. Joy, C. Tselepis, *et al*., 2008. Increased hepcidin expression in colorectalcarcinogenesis. World J Gastroenterol., 14: 1339-1345.

Weizer-Stern, O., K. Adamsky, O. Margalit, G. Rechavi, *et al*. Hepcidin, a key regulator of iron metabolism,is transcriptionally activated by p53. Br J Haematol., 138: 253-262.