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Circulating Leptin in Patients with Liver Cirrhosis and Hepatocellular Carcinoma.

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ABSTRACT

Hepatocellular carcinoma is the fifth most common cancer worldwide. As inevitable consequence of insulin resistance, hyperinsulinemia plays an important role in occurrence and prognosis of cancer. The aim of this study was to determine the value of serum leptin assessment in patients with HCV- related decompensated liver cirrhosis and hepatocellular carcinoma. 90 adult patients with HCV- related end stage liver disease were enrolled in this study; 40 patients had HCV- related decompensated cirrhosis and 50 patients had hepatocellular carcinoma. Serum leptin level was measured for all participants and was correlated with all other studied parameters. Serum leptin and HOMA-IR were found to be significantly higher among patients with hepatocellular carcinoma. Patients with hepatocellular carcinoma had also significant negative correlations between serum leptin and both fasting triglyceride and fasting glucose levels. In conclusion Patients with hepatocellular carcinoma have significant higher serum leptin levels and HOMA-IR than patients with HCV-related decompensated cirrhosis.

Keywords: Leptin, HCC, cirrhosis, Insulin Resistance.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of liver cancer, and the second common type of cancers affecting the gastrointestinal tract [1]. Inflammatory and angiogenic changes due to insulin resistance and fatty liver disease are associated with an increased risk of liver cancer. Cirrhosis remains the most important risk factor for HCC although cases of HCC arising without cirrhosis raise the possibility of a direct carcinogenesis secondary to nonalcoholic fatty liver disease (NAFLD) [2].

Leptin is best known as a regulator of food intake and energy expenditure via hypothalamic mediated effects. It is currently well known that this adipokine has many other effects, often as a consequence of peripheral actions. These include angiogenesis, hematopoiesis, and many other effects on the reproductive, cardiovascular and immune systems. Moreover, a study considered leptin as a fibrogenic factor in all types of chronic liver disease [3].

Based on analysis of hepatic tissue, somatic mutations accumulate in leptin receptor gene (LEPR) in cirrhotic liver with chronic HCV infection. These mutations could disrupt LEPR signaling pathways and increase susceptibility to carcinogenesis [4]. High levels of serum leptin could also over-regulate the signaling and expression of active Ob-R. These phenomena lead to the deregulation of leptin signaling and contribute to HCC progression through its crosstalk with multiple signaling pathways, as discussed in breast cancer [5] or colorectal cancer [6]. The aim of this study was to determine the value of serum leptin assessment in patients with HCV- related decompensated liver cirrhosis and hepatocellular carcinoma and to correlate this level with the metabolic profiles of these patients.

MATERIAL AND METHODS

This study was a randomized, cross-sectional comparative study and it was conducted at the Gastroenterology and Hepatology Unit, Department of Internal Medicine, Ain Shams University Hospitals, Cairo, Egypt.

The study was performed in the period between March to November 2016 according to the ethical standards for human experimentation approved by the human research committee of Ain Shams University Hospitals and informed consents were obtained from all participants.

90 adult patients with HCV-related liver cirrhosis were randomly recruited from the Hepatology outpatients' clinic. Patients were divided into two groups as follows Group A: 40 patients with end stage liver disease according to Child-Pugh Score, HCC excluded in these patients at time of recruitment in the study; exclusion of HCC based on the absence of any hepatic focal lesion in repeated abdominal ultrasonography scanning. Group B: 50 patients with end stage liver disease and well established diagnosis of HCC; diagnosis of HCC were based on the appearance of typical vascular pattern of enhancement in triphasic spiral CT scan of the abdomen.

Patients were excluded from the study if they had any of the following conditions: diabetes mellitus, current or past history of alcohol consumption, current or past history of any malignant diseases (solid or humoral) other than HCC, organ transplant recipients, patients receiving lipid-lowering agents (i.e., statins or fibrates), patients who had undergone any form of bariatric surgery, patients on long-term steatosis-inducing drugs (i.e., corticosteroids, tamoxifen, amiodarone, and valproic acid), I.V. drug users, patients with HIV infection, patients who received any form of treatment for HCC, obese patients (BMI >30), patients with other liver diseases such as alcoholic liver disease, non-alcoholic fatty liver disease, drug-induced hepatitis, other viral hepatitis, hereditary hemochromatosis, Wilson's disease, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and alpha-1 antitrypsin deficiency.

All patients were subjected to the following:

• History taking, clinical examination, calculation of body mass index (Body mass index = weight in kilogram/height in meters^2) , abdominal ultrasonography scan, laboratory investigations including complete blood picture, liver function tests, fasting and 2 h postprandial blood glucose level, fasting blood insulin, kidney

function tests, fasting triglycerides, and cholesterol levels, HBsAg, HCV, and HIV antibodies using ELISA technique, alpha-fetoprotein, prothrombin time, and INR.

• Oral glucose tolerance test (OGTT) was done for all patients. Patients meeting the American Diabetes Association (ADA) criteria for diagnosis of diabetes mellitus, Impaired Fasting Glucose (IFG), and/or Impaired Glucose Tolerance (IGT) were excluded from this group. The test was performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. Criteria for the diagnosis of diabetes were as follows:

Fasting plasma glucose (FPG) >126 mg/dl or 2 h plasma glucose >200 mg/dl during OGTT, IFG: FPG 100–125 mg/dl, IGT: 2 h plasma glucose 140–199 mg/dl [7].

IR was calculated using the Homeostasis Model of Assessment-Insulin Resistance index (HOMA-IR index) as follows: HOMA-IR = fasting glucose (mmol/dl) × fasting insulin (μU/ml)/22.5. Patients were considered as having IR when HOMA-IR >2.5[8].

• Model for End-stage Liver Disease (MELD) and Child- Pugh scores were measured to assess the severity of liver cirrhosis. Child–Pugh score was calculated based on patients' clinical and laboratory data (ascites, hepatic encephalopathy, serum albumin, serum bilirubin, and prothrombin time). MELD score was calculated according to original formula proposed by the Mayo clinic group where MELD score = 3.8 × log (serum bilirubin) + 11.2 × log (INR) + 9.6 × log (serum creatinine) [9].

• Serum leptin level was measured via DRG leptin ELISA technique. Normal values: Males: 3.84 ± 1.79 ng/mL, Female: 7.36 ± 3.73 ng/mL

Statistical analysis

Data were revised, coded, tabulated, and introduced to a PC using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Quantitative variables were expressed as mean ± standard deviation. Qualitative variables were expressed as frequencies and percents. Student-t (t) test was used to compare a continuous variable between two study groups. Chi-square test ($\chi 2$) was used to examine the relationship between categorical variables. Correlation analysis using spearman's (r) method was used to assess the strength of association between two quantitative variables. The probability of error (P) was expressed as follows: P value >0.05: non-significant, P value ≤0.05: significant, and P value <0.01: highly significant. Sensitivity, specificity, positive and negative predictive values, and accuracy were calculated as follows: sensitivity = true positive/true positive + false negative. Specificity = true negative/true negative + false positive. Positive predictive value = true positive/true positive + false positive. Negative predictive value = true negative/true negative + false negative. Accuracy = true positive + true negative/all cases examined. The overall diagnostic performance of a test was assessed by receiver-operating characteristics (ROC) curve analysis.

RESULTS

90 patients with HCV- related end stage liver disease were included in this study and were divided into 2 groups; group A included 25(62.5%) males and 15(37.5%) females with a mean age of 50.7± 7.1 year, all patients of this group had HCV-related decompensated liver cirrhosis. Group B included 29 (58%) males and 21 (42%) females with a mean age of 53.2 ± 8.5 year, all patients of this group had HCC. The statistical differences between both groups regarding age and gender were insignificant (P value >0.05).

The statistical differences between both studied groups regarding liver function tests and BMI are shown in table 1.

Significant differences were found between both groups as regards HOMA-IR, fasting blood glucose and fasting insulin levels. Also serum leptin level was significantly higher among group B as compared to group A (table 2).

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Table 1: Comparison between the two groups regarding liver function test and MBI

		Group A	Group B	t	P-value
ALT	**Range**	18 – 87	18 – 92	0.221	0.826
	Mean ±SD	53.150 ± 16.342	52.340 ± 18.038		
AST	**Range**	23 – 98	32 – 98	-0.737	0.463
	Mean ±SD	66.350 ± 16.440	69.100 ± 18.470		
Total billirubin	**Range**	1 - 12.5	1.1 - 12.5	-0.709	0.480
	Mean ±SD	3.978 ± 2.136	4.372 ± 2.955		
Direct billirubin	**Range**	0.6 - 7.5	0.4 - 7.5	0.077	0.938
	Mean ±SD	2.573 ± 1.526	2.544 ± 1.886		
Albumin	**Range**	1.5 - 3.1	1.5 - 3.2	-0.531	0.597
	Mean ±SD	2.418 ± 0.375	2.462 ± 0.411		
INR	**Range**	1.14 - 3.1	1.01 - 3.2	-2.442	0.017
	Mean ±SD	1.631 ± 0.440	1.894 ± 0.557		
ALP	**Range**	34 - 189	97 - 476	-5.065	<0.001
	Mean ±SD	113.950 ± 38.642	191.100 ± 89.835		
GGT	**Range**	5 – 76	29 - 169	-7.537	<0.001
	Mean ±SD	31.825 ± 18.449	71.900 ± 29.283		
AFP	**Range**	1.15 - 120	120 – 7364	-6.239	<0.001
	Mean ±SD	12.613 ± 21.047	1221.40± 1223.405		
BMI	**Range**	19 – 32	17 - 32	2.167	0.033
	Mean ±SD	26.100 ± 3.342	24.520 ± 3.512		

Table 2: Comparison between the two groups regarding HOMA-IR and serum leptin level

	Group A		Group B	t	P-value
HOMA.IR	**Range**	0.7 - 11.3	0.8 - 12.9	-2.412	0.018
	Mean ±SD	4.485 ± 3.189	6.146 ± 3.292		
Fasting glucose	**Range**	3.8 – 6.3	3.8 - 5.8	3.280	0.001
	Mean ±SD	4.912 ± 0.605	4.562 ± 0.403		
Fasting Insulin	**Range**	4-45	4- 55	-3.015	0.003
	Mean ±SD	20.613±14.370	30.190±15.441		
Fasting Leptin	**Range**	2 – 20	7.5 – 36	-4.285	<0.001
	Mean ±SD	10.075 ± 6.003	15.170 ± 5.267		

Group A had insignificant correlations between serum leptin level and all other studied parameters. On the other side, group B had significant negative correlations between serum leptin level and both fasting triglyceride and fasting blood glucose levels (table 3).

Both studied groups showed highly significant positive correlation between HOMA-IR and fasting insulin level. Also, a highly significant positive correlation was found between HOMA-IR and BMI among group B (table 4).

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Table 3: Correlation between serum leptin level and other studied parameters

	Group A		Group B	
	R	P-value	R	P-value
HOMA.IR	0.148	0.361	-0.134	0.353
ALT	0.088	0.590	-0.173	0.230
AST	0.003	0.985	-0.146	0.313
Albumin	-0.266	0.097	0.165	0.252
INR	0.106	0.515	-0.266	0.062
Total billirubin	-0.248	0.122	-0.146	0.313
Direct billirubin	-0.218	0.177	-0.119	0.410
Fasting Cholestrol	-0.052	0.752	0.020	0.892
Fasting Triglyceride	-0.241	0.134	-0.419	0.002
AFP	0.118	0.469	-0.004	0.977
Fasting glucose	-0.267	0.095	-0.293	0.039
BMI	-0.198	0.221	-0.019	0.895
ALP	0.092	0.571	-0.034	0.817
GGT	0.259	0.106	-0.019	0.896

Table 4: Correlation between HOMA-IR and other studied parameters

	Group A		Group B	
	R	P-value	R	P-value
Fasting Insulin	0.970	<0.001	0.983	<0.001
ALT	0.278	0.082	-0.037	0.800
AST	0.202	0.211	0.053	0.717
Albumin	0.274	0.088	0.065	0.654
INR	-0.134	0.410	0.121	0.402
Total billirubin	0.196	0.225	-0.009	0.952
Direct billirubin	0.252	0.116	-0.058	0.687
Fasting Cholestrol	-0.334	0.035	0.059	0.682
Fasting Triglyceride	0.230	0.154	-0.101	0.487
AFP	-0.137	0.398	-0.004	0.979
Fasting glucose	0.172	0.288	0.236	0.099
BMI	0.186	0.251	0.511	<0.001
ALP	0.249	0.122	-0.047	0.743
GGT	0.114	0.485	-0.176	0.222
LEPTIN	0.148	0.361	-0.134	0.353

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Figure 1: ROC curve analysis for leptin level in detecting IR among patients group A.

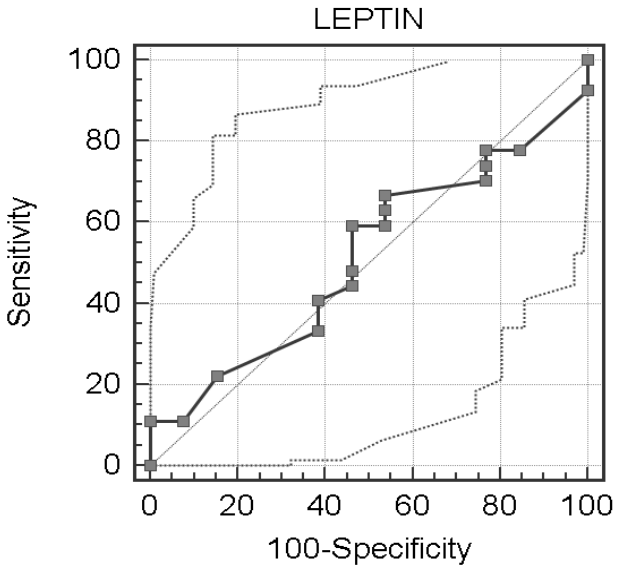

Figure 2: ROC curve analysis for leptin level in detecting IR among patients group B.

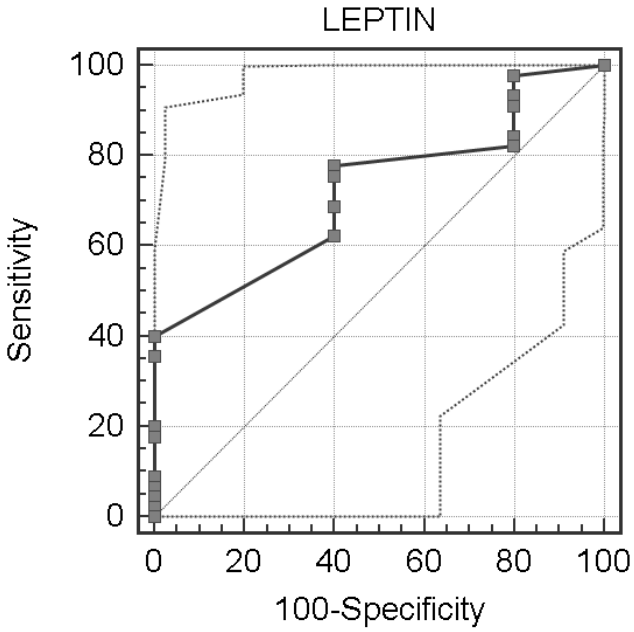

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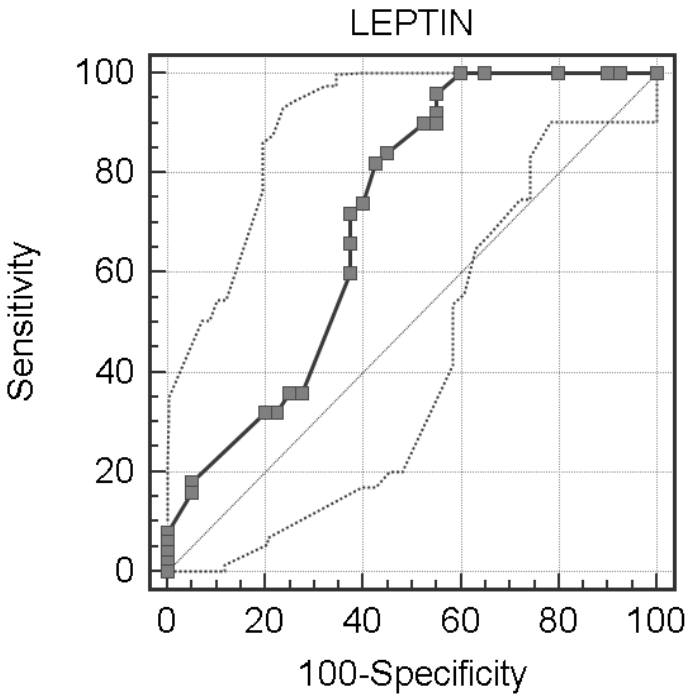
Figure 3: Validity of serum leptin level in prediction of HCC



At a cut off value ≤ 10 µg/ml, serum leptin had 59.26 % sensitivity, 53.85 % specificity, 72.7 % PPV and 38.9 % NPV for prediction of IR in patients with HCV-related decompensated liver cirrhosis (with an overall accuracy of 51.3%) (figure 1). On the other hand, serum leptin level at a cutoff value >15 µg/ml had 40 % sensitivity, 100 % specificity, 100% PPV and 15.6 % NPV in detecting insulin resistance among patients with HCC (with an overall accuracy of 72.2%) (figure 2).

The best cut off value of serum leptin for prediction of HCC in patients with HCV- related decompensated cirrhotic was >7.5 µg/ml with 96 % sensitivity , 45 % specificity , 90% NPV , 68.6 % PPV and an overall accuracy of 71.2 % (figure 3).

DISCUSSION

HCV is a major cause of chronic hepatitis and HCC. IR may synergize with viral hepatitis in HCC development [10] and considered to be a critical factor in the progression of fibrosis and the enhancement of the risk of HCC [11].On the other side, leptin was found to play a crucial role in the development of cancer; its role in carcinogenesis is based on its oncogenic, mitogenic, proinflammatory, and pro-angiogenic actions[12]. In vitro studies have demonstrated the role of leptin in HCC proliferation, migration, and angiogenesis. In addition, leptin signaling and its cross-talks with many signaling pathways, play critical roles in HCC cell growth, invasion, angiogenesis, and metastasis. There are still a number of gaps to fill in the field of leptin signaling in HCC [13]. The present study was designed to determine the value of serum leptin assessment in patients with HCV -related liver cirrhosis and HCC.

The present study revealed significant higher mean values of serum leptin among patients with HCC as compared to cirrhotic patients without HCC. This finding goes in agreement with Sadik NA et al. and Wang SN et al. [14, 15] who reported significant increase in serum leptin levels in cirrhotic patients with HCC. Wang SN et al., 2006 found also a significant correlation between leptin levels and intra-tumor micro vessel density. Furthermore, leptin expression was determined as a predictor for improved overall survival of patients with HCC. As a result, it was suggested that high leptin expression in HCC tissues could predict better overall survival [15]. On the same line, Chen et al [16] reported an over-expression rate of leptin and Ob-R in HCC patients. Ob-R over-expression was significantly correlated to the tumor size and TNM stage , but not to age,

body mass index, α-fetoprotein, hepatitis B surface antigen status, tumor grade, vascular invasion, or liver cirrhosis.

On the opposite side, Wang YY and Lin SY found that increased serum leptin level was significantly correlated with cirrhotic changes but not with HCC [17]. This contradiction may be largely attributed to the differences between the studies as regard patients' selection criteria, number of patients included, race, ethnicity and HCV genotypes.

Hyperinsulinemia plays a critical role as a risk factor in the onset and/or progression of HCC through up-regulation of insulin signal cascades. This could enhance fibrogenesis by stimulating the release of connective tissue growth factor and fibrogenic growth factor from hepatic stellate cells [18] Also, IR enhances fibrosis progression in patients with HCV through stimulation of hepatic steatosis, hyperleptinemia, TNF production and reduced expression of PPAR ɣ receptors [19].

The current study showed significant higher fasting serum insulin level and HOMA-IR among cirrhotic patients with HCC as compared to cirrhotic patients without HCC. This finding is consistent with Hayashi T and co-workers who reported significant higher fasting serum insulin level (≥15.0 µU/mL) and significant higher HOMA-IR (≥2.5) among patients with HCC [11]. Also, Gupta SP and co-workers reported that high insulin levels (>6.10 µU/ml) were seen to be associated with a 2.36 fold risk of HCC when compared with fasting insulin levels of <2.75 µU/ml. Furthermore, the insulin levels 2.75-4.10 µU/ml also conferred a 1.57 fold risk for HCC when compared with lowest fasting insulin levels of (<2.75 µU/ml) [20].

The present study revealed the best cutoff value of serum leptin in predicting IR among patients with CHC to be ≤ 10 ng/mL. This value had 59.26 % sensitivity, 53.85 % specificity and an overall accuracy of51.3% for detection of insulin resistance among patients with chronic HCV infection. On the other side, the best cutoff value of serum leptin in predicting IR among patients with HCC was > 15 ng/mL. This value had 40 % sensitivity, 100 % specificity and an overall accuracy of 72.2% for detection of insulin resistance among patients with HCC. Furthermore , The best cut off value of serum leptin for prediction of HCC in patients with HCV- related decompensated cirrhotic was >7.5 µg/ml with 96 % sensitivity , 45 % specificity and an overall accuracy of 71.2 % . These results strongly points to the potential role of leptin and leptin resistance in the pathogenesis of HCC in patients with HCV-related cirrhosis.

The proposed mechanisms of leptin resistance include perturbations in developmental programming, alterations in cellular Ob-Rb signaling, alterations in the transport of leptin across the blood–brain barrier, and others [21]. In peripheral tissues, high levels of serum leptin could over-regulate the signaling and expression of active Ob-R. These phenomena lead to the deregulation of leptin signaling, thereby significantly contribute to HCC progression through its crosstalk with multiple signaling pathways, as discussed in other types of tumors [5, 6].

The present study had few limitations. First, degrees of hepatic necro-inflammation and steatosis were not evaluated and were not correlated with serum leptin level. Second, the small sample size which consisted of adult Egyptian patients with HCV-related liver cirrhosis and thus applicability to other populations requires further work. Finally, the lack of data related to serum HCV load, which may not significantly impact the results. Indeed, HCV quantity is not an independent predictor of pathology [22].

CONCLUSION

Patients with hepatocellular carcinoma have significant higher serum leptin level and HOMA-IR than patients with HCV-related decompensated cirrhosis.

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COMPLIANCE WITH ETHICAL STANDARDS

Funding: This research received no specific grant from any funding agency in the public, commercial, or not for profit sectors.

All authors declare that they have no conflict of interest regarding this work.

Ethical approval: All procedures performed in this study were in accordance with the ethical standards of Ain Shams University Hospitals and with the 1964 Helsinki declaration and its later amendments.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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