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Assessment of serum leptin in patients with chronic hepatitis B virus infection.

Ahmed Moanis, Reham Al Swaff*, Zainab Alii-El Din, Hany Aly, Hany Samir, and Khaled Raafat.

Internal medicine department, Faculty of medicine, Ain shams university, Cairo, Egypt

ABSTRACT

The relationship between serum leptin and insulin resistance in patients with chronic viral hepatitis seems obscure. This study aimed to determine the value of assessment of circulating leptin in patients with chronic hepatitis B virus infection and to correlate leptin levels with the metabolic profile of these patients. A hospital based case- control analytical study was conducted on ninety subjects who were divided into 2 groups as follow: group I included sixty patients with documented chronic HBV infection. Group II included thirty healthy subjects who served as a control group. All participants were subjected to the following: calculation of body mass index, visceral adiposity index and fat mass index. Laboratory investigations were done including fasting serum leptin, PCR for hepatitis B virus DNA and calculation of HOMA-IR index. Statistically significant higher levels of fasting serum leptin and HOMA-IR index were found among cases as compared to controls. HBV DNA was the single most important variant that affected serum leptin level in HBe Ag negative cases. Fasting serum leptin at cut off value >43 ng/ml had 66.67% sensitivity, 53.85% specificity in predicting insulin resistance among HBe Ag positive patients. On the other hand, serum leptin at cut off value >77.4 ng/ml had 57.14% sensitivity, 100% specificity in predicting insulin resistance among HBe Ag negative patients. In conclusions, patients with chronic HBV infection had increased levels of serum leptin and higher HOMA-IR index. HBV DNA was the most important variant that affected serum leptin in HBe Ag negative cases.

Keywords: leptin, HBV, HBe Ag, HOMA-IR

Corresponding author

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INTRODUCTION

Leptin is a 16 KDa hormone secreted from adipocytes of white fat [1]. Circulating leptin levels are related to the adipose tissue mass, and signal the central nervous system of the presence of sufficient energy [2]. Leptin major action is to regulate food intake and energy expenditure. It has also other roles as it regulates the circadian rhythms of the gonadotropic, thyrotrophic and adrenal axes. In addition, leptin plays a crucial role in glucose homeostasis and insulin sensitivity [3]. Circulating leptin levels correlate with fasting insulin, body mass index, triglycerides, HDL cholesterol, and hypertension [4].

Serum levels of adipokines (i.e:- leptin, adiponectin, resistin) increases with various hepatic disorders including chronic viral hepatitis B, they represent a bridge between inflammation, insulin resistance and metabolic state [5].

The relationship between leptin and insulin resistance in patients with viral hepatitis remains obscure. Increased levels of circulating leptin have been found in patients with chronic hepatitis C as compared with healthy controls in some studies [6], whereas in other studies, comparable or even lower leptin levels have been reported, which points out that the role of leptin in hepatic steatosis, inflammation, fibrosis and insulin resistance in patients with chronic hepatitis is still obscure [7].

Hepatitis B virus (HBV) infection is associated with type 2 diabetes; however, the mechanism by which HBV affects glucose homeostasis is not clearly understood [8]. Some studies reported insignificant relation between insulin resistance and HBV among HBsAg positive patients. However, others reported higher levels of fasting insulin and HOMA-IR index among patients infected with HBV [9]. The main aim of this study was to determine the value of assessment of serum leptin levels in patients with chronic hepatitis B virus infection and to correlate these levels with insulin resistance.

MATERIAL AND METHODS

This study was a hospital based case- control analytical study that was conducted on ninety subjects at the Gastroenterology and Hepatology unit, department of internal medicine, Ain Shams University hospitals, Cairo, Egypt. Ninety adult Egyptian subjects were recruited and divided into 2 groups as follow: group I: sixty patients with documented chronic HBV infection of at least 6 month duration (diagnosis of chronic HBV infection relied on documented persistence of HBV surface antigen for > 6 months). Group II: thirty healthy subjects who served as a control group. The study was performed 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.

Subjects with any of the following conditions were excluded: diabetes mellitus, current or past history of alcohol consumption, current or past history of any malignant diseases, organ transplant recipients, patients receiving lipid lowering agents, patients who received any form of antiviral therapy for chronic HCV or HBV infection, patients who had any form of bariatric surgery, obese subjects , patients with any hepatic focal lesion detected by abdominal ultrasonography or any other imaging modality, patients on long term steatosis inducing drugs, I V drug users, patients with HIV infection, patients who received any form of treatment for HCC, recent alteration in body weight ($\geq$ 10 % change in body weight within the last 6 months preceding the study), pregnant and/or nursing females.

All participants were subjected to the following: history taking, clinical examination including waist circumference measurement, calculation of body mass index (BMI) [10], calculation of Fat Mass Index (FMI) [11], laboratory investigations including: complete blood picture, liver function tests, kidney function tests, fasting and 2 hours post prandial blood glucose level, fasting total cholesterol, fasting triglycerides, fasting high-density lipoprotein (HDL), fasting low- density lipoprotein (LDL), fasting very low- density lipoprotein (VLDL) HBsAg, HBsAb, HBcAb, HBeAg and HBeAb, serum HBV DNA concentrations (for group I only), Alpha-fetoprotein, HCV and HIV antibodies using ELISA technique, and abdominal ultrasound.

Serum leptin level was measured using DRG leptin ELISA kit according to the manufacturer's protocol.

Calculation of insulin resistance was done using HOMA-IR [HOMA-IR = fasting glucose (mmol/dL) × fasting insulin (µU/ml)/22.5] [12]. Patients were considered to have insulin resistance when HOMA-IR > 2.5[13].

Visceral adiposity index (VAI) was calculated using waist circumference in cm, triglycerides in mmo/L, BMI, HDL in mmol/L [14].

Statistical analysis

Data was collected, coded and entered to a personal computer (P.C.) IBM compatible 2.6 GHZ. Data was analyzed with the program (SPSS) statistical package 17 for social science. Statistical presentation and analysis of the present study was conducted using the mean, standard deviation, student t- test (t), Chi-square (x^2), Linear Correlation Coefficient (r), Analysis of variance [ANOVA] test and ROC curve analysis. Significance level (P) value was considered significant if P < 0.05.

RESULTS

Group I included twenty eight males and thirty two females while group II included fourteen males and sixteen females with statistical insignificance between the two groups as regards gender (P=1.00**).** The statistical differences between the two groups regarding body composition measurements are shown in table 1.

Fasting serum leptin, serum insulin and HOMA –IR index were all significantly higher in patients with chronic HBV infection than in healthy subjects (table 2).

Twenty three cases of the first group were positive and thirty seven cases were negative for HBeAg. Fasting serum leptin, serum insulin and HOMA-IR HOMA –IR index were not affected by HBeAg status (table 3).

Fasting serum leptin correlated positively with HBV DNA, markers of insulin resistance, LDL cholesterol and body composition measurements. A negative correlation was found between fasting leptin and HDL cholesterol. These correlations were true for both HBeAg positive and negative cases (table 4).

On applying multivariate analysis, the single most important variant that affected serum leptin level in both studied groups was visceral adiposity index. However, it is worth mentioning that HBV DNA was the single most important variant that affected serum leptin level in HBe Ag negative cases (table 5).

Table 1: Comparison between the two groups regarding age and body composition measurements

	Group I Mean ±SD	group II Mean ±SD	t	P-value
BMI	24.308 ± 3.022	24.800±2.640	-0.758	0.451
Fat mass index	8.496±2.338	9.263±2.906	-1.351	0.180
Waist circumference (cm)	87.500±13.716	91.200±14.554	-1.182	0.240
Visceral adiposity index	5.193±2.060	6.430±2.576	-2.466	0.016

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Table 2: Comparison between the two groups regarding fasting leptin and markers of insulin resistance

	Group I Mean ±SD	Group II Mean ±SD	t	P-value
Leptin (ng/ml)	57.414±29.603	37.730±19.215	3.306	0.001
Fasting blood glucose (mmol/l)	5.003±0.715	5.013±0.780	-0.061	0.952
Fasting Serum insulin (µIU/ml)	12.880±9.220	9.377±1.995	2.052	0.043
HOMA-IR	3.451±2.199	2.581±0.838	2.086	0.040

Table 3: Comparison between HBeAg positive and negative cases regarding fasting serum leptin, serum insulin and HOMA-IR

	HBeAg						T-Test	
	Negative			Positive				
	Mean	±	SD	Mean	±	SD	t	P-value
Leptin	52.400	±	29.386	60.531	±	29.706	-1.035	0.305
S.insulin	12.152	±	8.640	13.332	±	9.651	-0.479	0.634
HOMA-IR	3.242	±	2.087	3.580	±	2.285	-0.576	0.567

Table 4: Correlation between fasting serum leptin level and other studied parameters

Leptin								
	Group I		HBe Ag positive cases		HBe Ag negative cases		Group II	
	r	P-value	r	P-value	r	P-value	r	P-value
S. insulin	0.521	<0.001	0.559	<0.001	0.446	0.033	0.284	0.128
HOMA-IR	0.471	<0.001	0.483	0.002	0.438	0.036	0.745	<0.001
PCR HBV	0.486	<0.001	0.366	0.026	0.762	<0.001	-	-
HbA1c	0.631	<0.001	0.490	0.002	0.846	<0.001	0.786	<0.001
FBS (mmol/l)	0.384	0.002	0.391	0.017	0.343	0.109	0.745	<0.001
2hr PP	0.313	0.015	0.288	0.084	0.354	0.098	0.054	0.778
T.chol	0.511	<0.001	0.552	<0.001	0.418	0.047	0.735	<0.001
HDL	-0.453	<0.001	-0.433	0.007	-0.447	0.032	-0.728	<0.001
LDL	0.501	<0.001	0.498	0.002	0.503	0.014	0.672	<0.001
TG	0.295	0.022	0.258	0.123	0.442	0.035	0.562	0.001
BMI	0.598	<0.001	0.627	<0.001	0.539	0.008	0.796	<0.001
Fat mass index	0.612	<0.001	0.637	<0.001	0.560	0.005	0.668	<0.001
Waist circumference	0.672	<0.001	0.676	<0.001	0.668	<0.001	0.753	<0.001
Visceral adiposity index	0.700	<0.001	0.758	<0.001	0.578	0.004	0.837	<0.001

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Table (5): Multivariate analysis of serum leptin with other studied parameters.

Cases	Unstandardized Coefficients		Standardized Coefficients	t	P-value
	B	Std. Error	Beta		
S.insulin	-0.312	0.913	-0.097	-0.341	0.734
HOMA-IR	1.047	3.513	0.078	0.298	0.767
PCR HBV	0.000	0.000	0.180	1.834	0.072
Fat mass index	1.955	1.743	0.154	1.122	0.267
Waist circumference	0.486	0.322	0.225	1.509	0.137
Visceral adiposity index	6.209	1.779	0.432	3.491	0.001
HBeAg positive	**B**	**Std. Error**	**Beta**	**t**	**P-value**
S.insulin	-0.140	1.220	-0.045	-0.115	0.910
HOMA-IR	0.449	4.648	0.034	0.097	0.924
PCR HBV	0.000	0.000	0.016	0.128	0.899
Fat mass index	2.191	1.973	0.175	1.110	0.276
Waist circumference	0.518	0.353	0.243	1.465	0.153
Visceral adiposity index	6.652	1.989	0.523	3.345	0.002
HBeAg negative	**B**	**Std. Error**	**Beta**	**t**	**P- value**
S.insulin	-0.661	1.346	-0.194	-0.491	0.630
HOMA-IR	3.438	5.259	0.244	0.654	0.523
PCR HBV	0.000	0.000	0.571	3.528	0.003
Fat mass index	1.642	3.813	0.129	0.431	0.673
Waist circumference	0.286	0.787	0.133	0.363	0.721
Visceral adiposity index	3.192	4.219	0.161	0.757	0.460
Controls	**B**	**Std. Error**	**Beta**	**t**	**P-value**
S.insulin	-1.148	1.258	-0.119	-0.912	0.371
HOMA-IR	6.355	5.643	0.277	1.126	0.271
Fat mass index	1.067	1.032	0.161	1.034	0.311
Waist circumference	-0.101	0.347	-0.077	-0.292	0.773
Visceral adiposity index	4.691	1.433	0.629	3.272	0.003

Figure 1: ROC curve analysis of serum leptin in prediction of insulin resistance among cases.

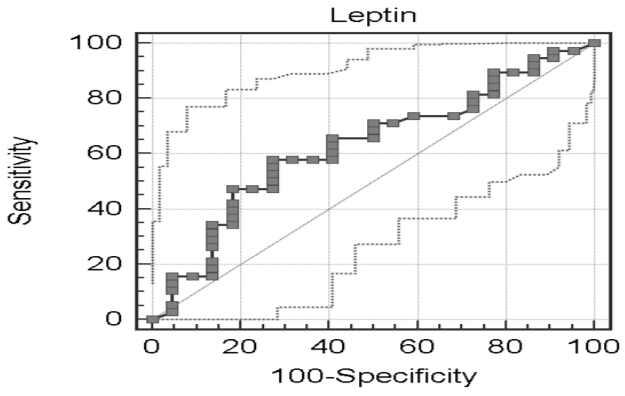

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Figure 2: ROC curve analysis of serum leptin level in prediction of insulin resistance among control.

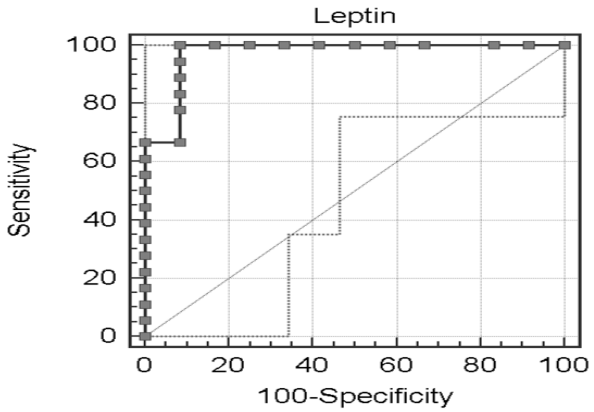


Figure 3: ROC curve analysis of serum leptin level in prediction insulin resistance among HBe Ag positive cases

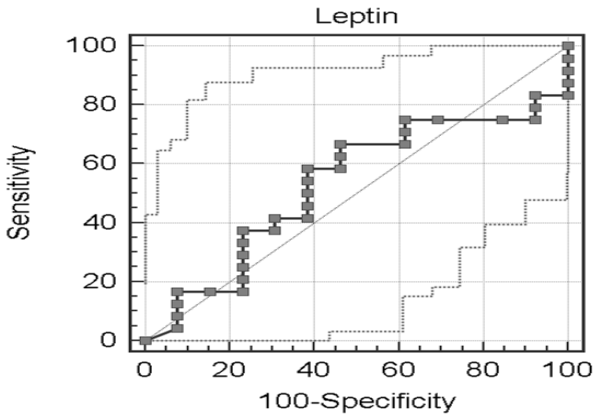
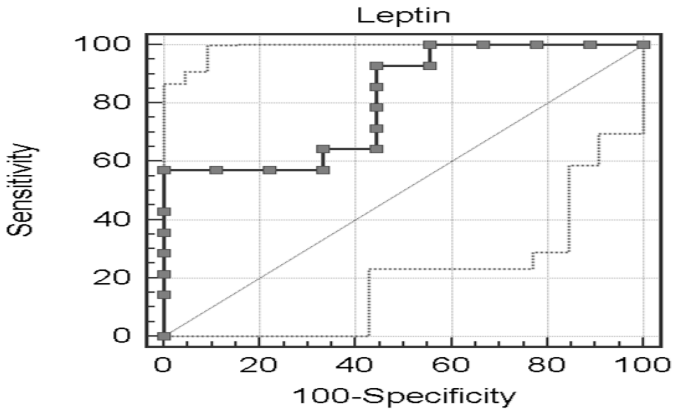


Figure 4: ROC curve analysis of serum leptin level in prediction of insulin resistance among HBe Ag negative cases.



ROC curve analysis of serum leptin for prediction of insulin resistance showed the following: fasting serum leptin level at a cut off value >57 ng/ml had 57.89% sensitivity, 72.73% specificity, 78.6% PPV, 50% NPV in predicting insulin resistance among cases (with an overall accuracy of 62.9%) (figure 1). As for controls: serum leptin level at cut off value >27.5 ng/ml had100% sensitivity, 91.67% specificity, 94.7% PPV, 100% NPV in predicting of insulin resistance among controls (with an overall accuracy of 97.2%) (figure 2).

With further subdivision of the first group according to HBe Ag status the following was revealed : serum leptin at a cut off value >43 ng/ml had 66.67% sensitivity, 53.85% specificity, 72.7% PPV, 46.7% NPV in

predicting insulin resistance among HBe Ag positive cases (with an overall accuracy of 53%) (figure 3). On the other hand and at a cut off value >77.4 ng/ml, serum leptin had 57.14% sensitivity, 100% specificity, 100% PPV, 69% NPV in predicting insulin resistance among HBe Ag negative cases (with an overall accuracy of 81%) (figure 4).

DISCUSSION

Chronic hepatitis B is one of the main causes of liver cirrhosis and hepatocellular carcinoma [15]. Disease progression has been shown to be associated with host factors (i.e.: age, gender), viral factors (i.e.: viral load, genotype, core promoter), lifestyle (alcohol and smoking), and viral super-infection (hepatitis C, hepatitis D, and human immunodeficiency virus) [16]. Metabolic factors also affect the natural history of chronic hepatitis B (CHB) infection [17] .The mechanism of how metabolic factors influence disease progression is not clearly understood. Adipose tissue secretes bioactive proteins known as adipokines, including leptin, adiponectin and resistin. These proteins exert different effects on insulin resistance and inflammation. As hepatic steatosis is common in patients with chronic hepatitis B infection [18, 19], the role of adipokines in chronic hepatitis B mandates further investigation.

The behavior of circulating leptin in the course of liver disease due to viral hepatitis is still under investigation; the current study aimed at determining the value of assessment of serum leptin in patients with CHB infection. Patients with diabetes mellitus, a BMI ≥30 kg/m2, and active alcohol drinking were excluded to eliminate confounding factors in the evaluation of serum leptin. In this study leptin levels were significantly higher in Egyptian patients with CHB infection as compared to control subjects, which is consistent with many previous reports from other countries [11, 20-23]. The mechanisms involved in the elevation of serum leptin in the course of liver disease due to HBV are obscure. Modified stellate cells may express leptin and contribute to the rise in circulating leptin [24]. A cytokine-leptin link hypothesis has also been suggested; activated tumor necrosis factor-α (TNF-α) can cause excess release of leptin from adipose tissue [25]. Although we could not investigate the cytokine levels of our patients, these published data may explain the higher levels of leptin observed in our patients with chronic HBV infection. Furthermore, Henriksen et al. suggested that the elevated circulating leptin in patients with alcoholic liver disease was most likely caused by a combination of decreased renal excretion and increased release from subcutaneous abdominal, femoral, gluteal, retroperitoneal, pelvic and upper limb fat tissue [26].

Insulin resistance is the principal indicator for development of metabolic syndrome [27]. Over-expression of the hepatitis B virus X protein induces peroxisome proliferator activated receptor gamma (PPARγ) gene expression and transcriptional activation, leading to up-regulation of the fatty acid uptake-associated gene CD36 and of several adipogenic genes [28]. However, it has been shown in large Chinese cohorts that chronic hepatitis B patients have a lower prevalence of metabolic syndrome than does the general population [29, 30]. This effect was largely driven by lower serum triglycerides in chronic hepatitis B patients. In total contradiction with the Chinese study, the present study revealed significant positive correlation between fasting triglycerides and serum leptin levels in HBeAg negative patients and in healthy subjects but not in HBeAg positive patients. Furthermore, fasting leptin had significant positive correlation with LDL cholesterol and significant negative correlation with HDL cholesterol. Also, patients with CHB infection had significantly higher markers of insulin resistance than healthy subjects. The reason for this discrepancy is not clear. Perhaps the different selection criteria and the different form of chronic HBV genotype infection may offer reasonable explanation. These findings strongly points to the importance of studying each component of the metabolic syndrome in patients with CHB infection.

The present study revealed insignificant differences in serum leptin levels and HOMA-IR between patients with positive and negative HBeAg, the same finding was reported by Wong V. and co-workers [31]. The current study revealed also a significant positive correlation between serum leptin and HBV DNA level; this correlation was highly significant for HBe Ag negative patients. This result totally disagreed with Wong V. and co-workers 31. In that study, HBV DNA had positive correlation with serum adiponectin, but not with leptin, resistin or HOMA-IR. The same contradictory result was also reported by Hsu C. et al, 2015 [32]; serum HBV DNA and HBsAg levels were significantly associated with higher serum adiponectin and visfatin levels, but lower serum leptin levels; the association remained unchanged after adjustment for age, gender and BMI . Further studies with follow-up data of adipocytokines in different phases of chronic HBV infection are required to elucidate the possible causal relationship between different adipocytokines and HBV infection.

HBV is an important etiology of HCC [33]. Metabolic syndrome and related factors such as type II diabetes and obesity have also received much attention as important risk factors for HCC [34]. Hyperinsulinemia plays a critical role in the induction 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 [35].the current study found significant higher levels of fasting serum insulin and HOMA –IR index in patients with chronic HBV infection. Moreover, significant positive correlations were found between serum leptin and markers of insulin resistance. These results highlight the potential role of chronic HBV infection in the induction and/or enhancement of leptin resistance in patients with HBV-related liver diseases. The suggested mechanisms of leptin resistance include perturbations in developmental programming, alterations in cellular Ob-Rb signaling and alterations in the transport of leptin across the blood–brain barrier [36]. 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 [37,38].

In addition to the classical knowledge of the positive correlation of BMI and serum leptin level, it was also shown that this is accurate for adult patients with chronic hepatitis B or C [39, 40]. This correlation was also preserved in Egyptian patients with chronic HBV infection (irrespective of HBe Ag status).

The present study has few limitations. First, diabetic patients were excluded. The possibility that serum leptin, insulin resistance, and metabolic syndrome may have a stronger role in liver injury in patients with diabetes cannot be excluded. However, the current study avoided the potential interference of anti-diabetic drugs on the assessment of leptin and insulin resistance. Second, all subjects were Egyptians. In NAFLD studies, an association between disease severity and different gene polymorphisms has been reported in different ethnic groups [41, 42].

CONCLUSION

Patients with chronic HBV infection had increased levels of serum leptin and higher HOMA-IR index. Hepatitis B virus DNA level was the most important variant that affected leptin levels in HBe Ag negative cases**.**

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