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Serum leptin and homeostasis model assessment-IR as novel predictors of early liver fibrosis in chronic hepatitis B virus infection

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ABSTRACT

Background: The relationship between hepatitis B virus (HBV) infection, leptin and insulin resistance remains unclear. We hypothesised links between serum leptin and insulin resistance in non-diabetic patients with chronic viral hepatitis B infection and their relation to liver fibrosis.

Methods: We recruited 190 untreated patients with chronic HBV infection and 72 healthy controls. Serum leptin, fasting glucose, insulin, liver function tests (LFTs), C-peptide and Homeostasis model assessment-IR (HOMA-IR) were measured/calculated by ELISA and standard techniques.

Results: Serum leptin, C-peptide (both $P < 0.001$), HOMA-IR ($P = 0.021$) and several LFTs were increased in patients with chronic HBV-infection. In multivariate regression analysis, both HOMA-IR ($P = 0.003$) and leptin ($P = 0.002$) were significant independent predictors of HBV infection. There were significant positive correlations ($P < 0.01$) between leptin and HOMA-IR ($r = 0.81$), between serum leptin and METAVIR activity ($r = 0.95$), and between HOMA-IR and BMI ($r = 0.75$), fasting glucose ($r = 0.005$), and fasting insulin ($r = 0.81$). Several LFTs, glucose and insulin correlated modestly ($r = 0.61–0.69$, $P < 0.05$) with leptin.

Conclusion: Serum leptin may be related to the rate of fibrosis progression in nondiabetic patients with chronic HBV infection. Follow-up by serial measurement of serum leptin and HOMA-IR in non diabetic HBV-infected patients may be used as a non-invasive marker of early liver fibrosis.

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KEYWORDS
Leptin; homeostasis model assessment–IR; hepatitis B virus; liver fibrosis

Introduction

Hepatitis B virus (HBV) infection affects 350 million people around the world [1]. Chronic HBV infection may lead to chronic hepatitis, cirrhosis, liver decompensation and hepatocellular carcinoma [2]. Leptin is a cytokine hormone secreted from adipocytes and regulates insulin secretion and tissue responsiveness to insulin [3,4]. Serum leptin levels significantly correlate with insulin resistance [5]. The association of leptin and insulin resistance in patients with chronic viral hepatitis is not clear, both presence and absence of relationship between serum leptin and insulin levels have been suggested in patients with chronic viral hepatitis [6]. Leptin may play a role in the pathogenesis of hepatic steatosis and steatohepatitis in the absence of viral infection, and has been implicated in liver fibrogenesis [7,8]. Patients with mild chronic hepatitis have a higher homeostasis model of the assessment insulin resistance (HOMA) index than healthy controls matched for age and BMI [9]. Chronic HCV infection is known to link with insulin resistance and several studies show that the presence of insulin resistance is associated with increased rates of fibrosis in patients with chronic HCV infection [10]. In contrast, the role of serum leptin and insulin resistance in HBV infection is unclear. As the relationship between HBV and HOMA is unclear, we hypothesised links between serum leptin and insulin resistance in non-diabetic patients with untreated chronic viral hepatitis B infection, and potential utility as predictor of liver fibrosis and steatosis.

Patients and methods

We recruited 190 patients who were not candidates for treatment with chronic hepatitis HBV infection who had undergone liver biopsy with no significant fibrosis (METAVIR status F1-F2). The patients attended the Tropical Medicine and Internal Medicine, Mansoura University Hospital; Tropical Medicine Menufia University from January, 2013 to October 2015. The control group was 72 healthy volunteers, non-diabetic, age, sex and BMI matched, recruited among the inpatients and the out-patients of the department. Eligible patients were ≥ 18 years of age

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diagnosed with chronic HBV infection (positive HBsAg and serum HBV-DNA by PCR for more than 6 months). None of the patients were previously treated for HBV infection. A complete medical history was taken and physical examination carried out in all patients and controls. Exclusion criteria were significant fibrosis (F3-F4), diabetes mellitus [11], BMI ≥ 30 kg/m^2, decompensated liver cirrhosis (ascites, encephalopathy, bleeding varices), chronic HCV infection (diagnosed by anti-HCV and HCV-PCR), combined chronic hepatitis B and C, HDV infection, HIV infection, hepatitis A virus infection, auto-immune hepatitis, non-alcoholic fatty liver diseases (excluded based on histopathological findings), patients with uncontrolled psychiatric disorders, cardiac diseases (cardiomyopathy, arrhythmias, ischemia, myocarditis and significant valvular disease), comorbid severe diseases (renal failure, hypertension), hepatocellular carcinoma, congenital liver disease, a history of alcohol intake and pregnancy. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from all patients.

After 12-h overnight fasting, a blood sample was taken for routine analyses, including LFTs (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin), albumin, prothrombin time and INR. Blood tests included serum creatinine; blood glucose (fasting and postprandial blood sugar), and complete blood count. Fasting samples of serum obtained after centrifugation were stored in aliquots at −70° C until assayed. Serum insulin was measured using an immunoradiometric assay kit (Insulin Riabead II kit; Dainabot, Tokyo, Japan). The intra and inter-assay coefficients of variation of the assay were 2.0% and 2.1%, respectively. Serum leptin levels were determined by a solid phase ELISA (BioSource Europe S.A. 8 B-1400 Nivelles Belgium). The intra-assay coefficient of variation (CV) was 6.9%, while inter-assay CV was 8.7%. Viral markers for hepatitis A virus, HBV (HBsAg and HBeAg), HCV and HIV, were performed using an ELISA technique, (ELISA Kit, Abbott Diagnostics). ANA was measured by an enzyme-linked immunosorbent assay supplied by Orgentec Diagnostic (Mainz, Germany). Serum HBV DNA concentrations viral load were measured by a commercially available quantitative assay (Amplicor HBV MONITOR assay, Roche Diagnostics GmbH Mannheim, Germany).

Baseline anthropometric measurements, including the height and weight for calculating the body mass index (BMI), were recorded. Overweight and obesity was defined as a BMI ≥ 25 and ≥ 30 kg/m^2, respectively, for both men and women [12]. The HOMA-IR was calculated on the basis of fasting values of plasma glucose and insulin according to the HOMA model formula: HOMA score = fasting insulin (mIU/L) × fasting glucose (mmol/L)/22.5 [13,14].

A percutaneous ultrasound guided liver biopsy (≥ 15 mm in length) was obtained from all patients. Liver biopsies were paraffin-embedded and stained with hematoxylin, eosin and Masson's trichrome stains; additional stains were used when required. The biopsies were reviewed by one blinded pathologist. The degree of histologic hepatic inflammation and fibrosis was scored using the METAVIR scoring system. According to the degree of hepatocyte necrosis and lymphocyte infiltration, the level of inflammation was classified from A0 to A3, with a higher score indicating more severe inflammation. Fibrosis was graded from F0 to F4 as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, cirrhosis [15]. The extent of hepatic steatosis was assessed and graded according to Kleiner et al. [16].

Statistical analysis was performed using SPSS version 10, 1999 (SPSS Inc., Chicago, IL, USA). Parametric data were analyzed using Kolmogorov – Smirnov test. Continuously variable data are presented in the form of mean with standard deviation. Student's t-test was used for comparison of two groups. Categorical data were presented in the form of number and percentage and analysed by the chi–square test. The variables (i.e. liver enzymes, degree of inflammation and stage of fibrosis) were analysed using simple and multiple linear regression methods, with odds ratio (OR) and 95% confidence interval (CI), where appropriate. All P values were two-tailed and P value less than 0.05 was considered statistically significant.

Results

Table 1 shows the clinical and laboratory data of the studied groups. There was no significant difference in age, sex and BMI, prothrombin time, fasting blood sugar and fasting insulin but there were significant increases in serum ALT, AST, total bilirubin, HOMA-IR, leptin, and C-Peptide, and a significant decrease in albumin. In multivariate regression analysis, both HOMA-IR (P = 0.003) and leptin (P = 0.002) were significant predictors of HBV infection. Mild steatosis (steatosis 5–33% of hepatocytes) was found in 15 patients (7.9%). Table 2 shows the correlations between leptin, HOMA-IR, and other parameters in the patient group. There was a significant negative correlation between leptin and albumin and non significant negative correlation with C peptide. However, there was a significant positive correlation with ALT, AST, fasting glucose and fasting insulin, HOMA-IR and a very strong correlation with disease stage as defined by METAVIR activity, although the correlation between METAVIR staging 1 and 2 was weak. HOMA-IR correlated with BMI, with glucose and insulin (as expected, as these indices generate the HOMA-IR index) and with METAVIR activity. Figure 1 shows ROC/AUC analysis for leptin levels with a cut off

Table 1. Clinical and laboratory data of patients studied and controls.

Parameter	Chronic hepatits B infection (N = 190)	Control group (N = 72)	p value
Age (yr)	31.5 ± 9.7	34.1 ± 8.5	0.071
Male/female	130/60	42/30	0.068
BMI (kg/m^2)	27.6 ± 2.8	26.6 ± 1.9	0.071
ALT (U/ml)	47 ± 29	28 ± 7	0.003
AST (U/ml)	38 ± 8	26 ± 6	< 0.001
PT (seconds)	12.4 ± 0.7	11.5 ± 0. 6	0.21
Albumin (g/dL)	40.0 ± 4.1	44.0 ± 3.0	< 0.001
Total bilirubin (µmol/L)	60 ± 3	40 ± 6	0.021
FBG (mmol/l)	4.1 ± 0.4	3.8 ± 0.4	0.31
F. insulin (µU/l)	14.0 ± 1.5	9.0 ± 3.6	0.091
HOMA-IR	3.1 ± 0.7	1.9 ± 0.7	0. 021
Leptin (ng/mL)	15 ± 2.3	10.9 ± 3.2	< 0.001
C-Peptide (pmol/l)	961 ± 195	707 ± 233	< 0.001

BMI: Body mass index; ALT: Alanine transaminase; PT: Prothrombin activity; FBG: fasting blood glucose; FI: Fasting insulin; HOMA-IR, Homeostasis model assessment insulin resistance index.

Table 2. Correlations between serum leptin and clinical & laboratory data in chronic hepatitis B infection.

Parameter		Serum leptin *r: p*	HOMA-IR *r: p*
Age		0.42: 0.149	0.39: 0.201
BMI		0.35: 0.31	0.75: 0.002
Albumin		−0.66: 0.035	0.45: 0.322
ALT		0.61: 0.045	0.44: 0.38
AST		0.69: 0.015	0.31: 0.39
C-peptide		−0.39: 0.26	0.54: 0.043
Fasting glucose		0.61: 0.045	0.72: 0.005
Fasting insulin		0.66: 0.037	0.81: 0.001>
HOMA-IR		0.81: 0.004	-
Prothrombin time		0.31: 0.38	0.41: 0.253
Liver biopsy results	*METAVIR (activity)*	0.95: < 0.001	0.76: 0.011
	METAVIR (staging 1–2)	0.72: 0.023	0.33: 0.41
	Steatosis (Mild)	0.06: 0.85	0.34: 0.33

r: correlation coefficient. See Table 1 for other abbreviations

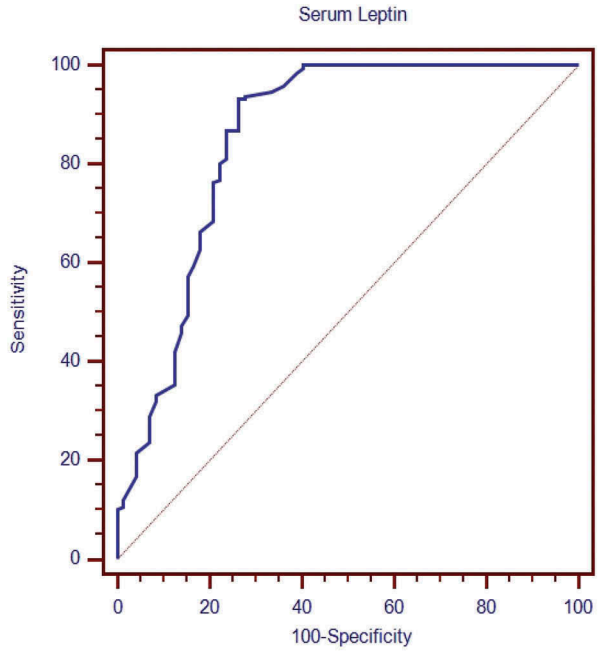

Figure 1. ROC/AUC analysis for serum leptin levels.

of > 11.5 ng/mL. The AUC was 0.85 (95% CI 0.81–0.89), P < 0.001, with 93.2 sensitivity and 73.6 specificity, a positive predictive value of 90.3 and negative predictive value of 80.3.

Discussion

As the leading pathophysiological consequence of HBV infection is liver damage, followed by cirrhosis and hepatocellular carcinoma, markers of this process are desirable [17]. The role of serum leptin during chronic HBV infection is unclear. We add to the literature showing that leptin and HOMA-IR are increased in patients with chronic HBV infection compared to a healthy control group, and that leptin is strongly linked to disease activity. Chronic HCV infection is well known to link with insulin resistance and increasing the risk of developing diabetes mellitus. In contrast, chronic HBV infection follows a different course that of chronic HCV infection, and contrasting studies report similar or lower insulin resistance rate in HBV patients versus with HCV patients [18], and similar or higher insulin resistance rates in HBV patients compared with healthy controls [19,20]. Moreover, some studies show that insulin resistance is not different between patients with chronic HBV infection and healthy controls [21] and other studies demonstrate that chronic HBV infection is associated with insulin resistance [22]. Although some studies found no association between leptin and fibrosis in hepatitis B or C infection [23,24] we found a significant positive correlation between leptin and METAVIR activity and staging in patients with chronic HBV infection, and so confirm a previous small study [4].

The relationship between leptin and insulin resistance in chronic HCV infection is unclear; both the presence [23] and absence [25] of an association between leptin and insulin levels have been observed. However, data on the relationship of leptin and insulin resistance in patients with chronic HBV infection are scarce. We found a significant positive correlation between serum leptin and both HOMA-IR and fasting glucose. In a study of 25 subjects, Segal et al. demonstrated that insulin resistance is associated with elevated plasma leptin levels [26], whilst Zuo et al. found that leptin levels among insulin resistant subjects were almost double compared to those who were not insulin resistant at the same level of adiposity in both men and women [27]. This is consistent with Wong et al. who found a moderate positive correlation between leptin levels and hepatic necroinflammation in patients with chronic HBV [18]. Leptin has been found to be higher in patients with cirrhosis during the course of chronic viral hepatitis B [28]. Moreover, another study showed a significant association between serum leptin and fibrosis stage in HCV- and HBV–infected patients

[4]. We found a significant positive correlation between HOMA-IR and METAVIR activity: previous studies also demonstrated that insulin resistance is associated with a more rapid progression of liver fibrosis [29,30]. We extend these data, showing a significant positive correlation between serum leptin and METAVIR activity and staging.

The mechanisms involved in the increase of leptin according to the stage of hepatic fibrosis are obscure. In the rat, the modified stellate cells may express leptin, the cirrhotic liver may be expected to produce leptin and contribute to the rise in circulating levels with a significant hepatic venous spillover [31]. Notably, we failed to correlate leptin with BMI, although this does not exclude the possibility that raised levels arise from adipocytes (3). Mild hepatic steatosis was present in 14.2% of our patients, and was unrelated to leptin or HOMA-IR. Taken together, the relationship between HBV and insulin resistance remains inconclusive and awaits further studies to clarify.

We recognize a number of limitations in our study, such as the relatively small sample size, and our wish to exclude patients with overt hepatic damage and the obese. Nevertheless, our work represents an advance in biomedical science as it supports the hypothesis that leptin may be related to the rate of hepatic fibrosis progression in non-diabetic patients with chronic HBV infection, and so promotes the view that routine leptin measurement be considered an important part of the investigation of liver disease.

Summary table

What is known about this subject:
- Chronic HBV infection causes liver damage and, ultimately, hepatocellular carcinoma
- Leptin levels are raised in chronic hepatitis virus infection
- The relationship of HBV infection with leptin and insulin resistance remains unclear

What this paper adds:
- Serum Leptin and HOMA-IR are both independent predictors of chronic hepatitis B infection
- Leptin is a stronger predictor of the degree of liver fibrosis than HOMA-IR

Disclosure statement

No potential conflict of interest was reported by the authors.

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