Original Article

Central Obesity and Advanced Liver Stiffness in Hepatitis B: Result from Golestan Hepatitis B Cohort Study

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Abstract

Background: Chronic infection with the hepatitis B virus and obesity may both contribute synergistically to liver disease, although relatively few studies have investigated this hypothesis. Therefore, in this study, we evaluated the relationship between central obesity and the liver stiffness in the Golestan Hepatitis B cohort study (GHBCS).

Methods: Our study included 304 chronic hepatitis B (CHB) patients enrolled from GHBCS. Liver stiffness measurement (LSM) and laboratory tests were performed after a follow-up of 4 years (2012). The hepatitis B viral load was measured at the baseline and follow-up using the real-time PCR method. Waist circumference ≥ 102 cm in men and ≥ 89 cm in women (central obesity) was considered to be abnormal. Advanced liver stiffness (ALS) was defined as LSM ≥ 8 KPa. Statistical analysis was performed using SPSS-V17. Logistic regression was used to test predictors of advanced liver stiffness (LSM ≥ 8 KPa). Linear regression was used to test the predictive value of variables in ALT (as a continuous variable). *P*-value of less than 0.05 was considered statistically significant.

Results: Among these CHB patients, 19 (7.4%) cases with a mean (±SD) age of 49.5 (±6.3) developed ALS after 4 years of follow-up. Multivariate analysis showed a significant predictive role of central obesity and viral load in ALS.

Conclusions: Central obesity is related to the liver stiffness in chronic hepatitis B patients.

Keywords: Central obesity, chronic Hepatitis B, liver fibrosis, liver stiffness, waist circumference

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Introduction

More than 350 million individuals are chronically infected with the Hepatitis B virus (HBV) worldwide. A proportion of these individuals develop progressive liver diseases,[1] so it is critical to identify the determinants of disease progression in order to develop preventive approaches.[2]

Published data have shown a prominent role of metabolic factor in the natural course of HBV along with the viral factors.[3–5] Furthermore, the key metabolic genes are regulated similar in HBV infected hepatocytes and in patients with metabolic disorders. It has been shown that hypertriglyceridemia, central obesity and diabetes mellitus could lead to hepatocyte damage through oxidative stress and fat accumulation (steatosis).[3,6] Although, there would be a relationship between metabolic factors and HBV. As HBV viral load has been reported to be lower in obese patients (HBeAg seropositive carriers) and those with hypertriglyceridemia (HBeAg seronegative carriers).[6]

The incidence of obesity and type 2 diabetes are increasing in developing countries due to the industrial lifestyle. In addition, obesity plays role in hepatocelular carcinoma (HCC)[3,4,6] so it seems certain that the number of patients having both chronic hepatitis B (CHB) and obesity is increasing too.[3,4] There are far less data available about the interrelationship of metabolic factors and HBV comparing to hepatitis C.[3,7]

Metabolic risk factors are concomitant with increased visceral fat.[8] Adipose tissue distribution influences metabolic complications. Visceral adiposity is a better predictor of fatty liver than body mass.[8–10] Those with visceral fat adiposity appear to be at greater risk for fatty liver, due to their ability for transporting free fatty acids directly into the portal vein for conversion to triglycerides. Abdominal fat is thought to be associated with both liver steatosis and further consequences such as insulin resistance,[9] as it has been proposed a key indicator of fat deposition in hepatocytes.[11]

The exact role of fat accumulation and distribution through the body in the natural history of CHB remains to be clarified in longitudinal studies. However, such studies are scarce. Here, we followed a cohort of naïve patients with chronic hepatitis B to evaluate the relationship between central obesity and the liver stiffness.

Materials and Methods

Study population

Out of 3505 participants of Golestan Hepatitis B cohort study (Golestan HBCS), 304 treatment-naïve CHB patients, defined as being HBsAg positive and HBeAg negative, were randomly selected according to current guidelines.[12,13] The treatment-naïve CHB patients did not have co-infection with human immune-deficiency virus (HIV), hepatitis C virus, hepatitis D virus, hepatitis G

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virus; autoimmune hepatitis; alcohol consumption; recent blood transfusion; pregnancy; and hemochromatosis.

Anthropometric data, including body weight, height, and waist circumference, were measured according to standardized procedures. Body mass index (BMI) was calculated by dividing weight (kg) to height (m^2). Waist circumference (WC) was measured at the midpoint between the superior aspect of the iliac crests and the lower lateral margins of the ribs using an inelastic tape (TBW Import Ltd) that was 0.5 cm in width and 200 cm in length, according to World Health Organization (WHO) recommendations.[14]

Central obesity was defined as having an abnormal waist circumference based on the updated definition of Metabolic Syndrome in the National Cholesterol Education Program Adult Treatment Panel III (NCEPAT III) criteria: waist circumference > 102 cm in men or > 89 cm in women and blood pressure ≥ 130/85 mmHg or current use of antihypertensive medications have been considered abnormal.[15]

Blood samples were collected and assessed for complete blood count including: platelet, liver and kidney function tests, as well as blood chemistry including lipid profiles. Written informed consent was obtained from all individuals. Study was conducted according to the Declaration of Helsinki. This study was approved by the Institutional Review Board and the Ethics Committee of Digestive Disease Research Institute (DDRI), Tehran University of Medical Sciences.

Study design

At the first visit, demographic data was recorded and HBsAg was assessed. After 4 years, liver stiffness was assessed by transient elastography; and 10 mL of peripheral venous blood was collected. Serum and plasma were stored at -70°C until further processing.

Liver Stiffness Measurement (LSM)

Liver stiffness was measured by a trained medical doctor using the FibroScan® 502 machine (EchoSense, Paris, France, 5 MHz) after at least 3 hours of fasting. According to the manufacturer's guidelines, the M and XL probes were used for individuals with a thoracic perimeter of less than or above 110 cm, respectively. With the patient lying in the dorsal decubitus position with maximal abduction of the right arm, the probe was placed on the skin overlying the right lobe of the liver, through the intercostal spaces. At least 10 measurements were done for each patient and the median value was recorded. Values were considered valid if the inter-quartile range (IQR) was less than 30% of the median reading and the success rate was at least 60%. The median value was calculated automatically and expressed in kilopascals (kPa). Transient elastography was not performed in cases of any degree of ascites; ferromagnetic tools in the body; pregnancy; or morbid obesity (Body Mass Index ≥ 35 kg/m^2) as suggested by the manufacturer. The cut-off for the advanced liver fibrosis was set as equal or greater than 8 KPa.

HBV-DNA Quantification

HBV DNA was extracted from 200 mL of serum using the QIAampDNA Blood Mini Kit (QIAGEN, Valencia, CA, USA), and quantified in the Light-Cycler (Roche Diagnostics, Mannheim, Germany) by the artus RealArt™HBV LC PCR (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The linear range of this assay was $10^2 – 10^9$ copies/mL. The HBV DNA level was assessed both qualitatively and quantitatively, at baseline and follow up steps.

Statistical analysis

Statistical analysis was performed using SPSS-V17. Data were expressed as mean ± SD and number (percent) for continues and categorical data, respectively. Chi-square test and t-tests were used. Those variables with a *P*-value < 0.2 or biological plausibility in the univariate analysis were included in the multivariate analysis. Logistic regression was used to test predictors of advanced liver stiffness (LSM ≥ 8 KPa) and ALT (as a continuous variable). *P*-value of less than 0.05 was considered statistically significant.

Results

Patients' baseline characteristics and prevalence of abdominal obesity

Overall, 304 treatment-naïve CHB patients were studied. Mean age (±SD) was 52 (±8.13) years and 149 (49%) were male. All patients were urban residents. At baseline, 142 (46.7%) had high blood pressure. The mean (±SD) BMI was 28.5 ± 5.3 kg/m^2. Abdominal obesity was seen in 101 (68%) of men and 103 (66.5%) of women. Patients' baseline characteristics are summarized in Table 1.

Baseline abdominal obesity and advanced liver stiffness

Out of 250 successful measurements (7.6%), 19 patients were diagnosed with an advanced liver stiffness (LSM ≥ 8KPa). Central obesity and advanced liver stiffness showed a significant relationship in Chi-square test (*P*-value = 0.048) (Table 2).

Predictive role of viral and host factors in advanced liver stiffness (logistic regression)

In univariate analysis, we examined the predictive values of each variable that assumed to play a role in further liver stiffness including: age, gender, ethnicity, BMI, abdominal obesity, hypertension, viral load, alcohol drinking, opiate consumption and tobacco smoking.

We found that waist circumference, BMI, viral load, alcohol consumption, opiate consumption and hypertension had a *P*-value less than 0.2 or biological plausibility. These variables were then entered into a multivariate model (logistic regression test), except for BMI (due to the interaction with waist circumference). In multivariate analysis, associations of central obesity (Adjusted OR = 4.3, *P*-value = 0.034) and viral load (Adjusted OR = 5.02, *P*-value = 0.016) with advanced liver stiffness remained statistically significant (Table 3).

Predictive role of viral and host factors in ALT measures (logistic regression)

In the univariate analysis, BMI, gender, age, viral load and opiate consumption showed a *P*-value < 0.5. In multivariate analysis, viral load (*P*-value = 0.008), gender (*P*-value = 0.005) and age (*P*-value = 0.042) remained significant (Table 4).

Discussion

Results of the present study showed a significant association between the waist circumference and advanced liver stiffness.

Table 1. Baseline characteristics of chronic hepatitis B patients in Golestan Hepatitis B Cohort Study

Variables	N (%)
Age (years)	
< 45	64 (21.1)
≥ 45	240 (78.9)
Ethnicity	
Turkmen	190 (62.5)
Non-Turkmen	114 (37.5)
BMI (kg/m²)	
Normal	80 (26.3)
Obese/overweight	224 (73.7)
HTN	
No	142 (46.7)
Yes	162 (53.3)
Waist circumference	
Normal	109 (35.9)
Abnormal (central obesity)	195 (64.1)
Log10 viral load (IU/mL)	
< 2000	283 (93.1)
≥ 2000	21 (6.9)

Table 2. Advanced liver fibrosis (at follow-up) in CHB patients regards to the central obesity at baseline

		Liver Stiffness Measurement (KPa)		Total	*P*-value
		< 8, N (%)	≥ 8, N (%)		
Waist circumference	**Normal**	89 (96.7)	3 (3.3)	92 (100)	0.048
	Central obesity	142 (90)	16 (10)	158 (100)	
	Total	231 (92.4)	19 (7.6)	250 (100)	

Table 3. Logistic regression model of factors related to the advanced liver stiffness in cases of chronic hepatitis B

Variables	Univariate			Multivariate		
	Crude OR	95% CI	*P*	Adjusted OR	95% CI	*P*
Age (year)						
< 45	0.8	0.27–2.3	0.67	- - -	- - -	- - -
≥ 45						
Gender						
Male (reference)	1.32	0.51–3.4	0.57	- - -	- - -	- - -
Female						
Ethnicity						
Turkmen (reference)	1.25	0.45–3.23	0.65	- - -	- - -	- - -
Fars						
BMI (kg/m²)						
Normal (< 25)	3.33	0.75–14.81	0.11	- - -	- - -	- - -
Overweight/Obese (≥ 25)						
Waist circumference (cm)						
Normal (reference)	3.34	0.95–11.8	0.06	4.3	1.08–16.6	0.03
Central obesity						
Hypertension						
No (reference)	1.74	0.67–4.5	0.25	2.03	0.75–5.5	0.16
Yes						
Viral load						
< 2000 IU/L	3.6	1.06–12.06	0.04	5.02	1.36–18.62	0.016
≥ 2000 IU/L						
Alcohol						
No (reference)	2.6	0.53–12.83	0.24	5.08	0.82–31.22	0.8
Yes						
Opiate						
No (reference)	0.44	0.6–3.42	0.44	0.41	0.04–3.97	0.45
Yes						
Cigar						
No (reference)	1.31	0.41–4.17	0.64	- - -	- - -	- - -
Yes						

Table 4. Logistic regression model of factors related to the ALT serum level in cases of chronic hepatitis B

Variables	Univariate			Multivariate		
	Crude Standardized β	95% CI	*P*	Adjusted Standardized β	95% CI	*P*
Age(year) < 45(reference) ≥ 45	-0.104	23.96–34.58	0.070	-0.114	-11.9– -0.35	0.042
Gender Male (reference) Female	-0.131	25.72–41.17	0.023	-0.164	-12.03– -2.18	0.005
Ethnicity Turkmen (reference) Fars	-0.010	-5.5–4.6	0.86	- - -	- - -	- - -
BMI (kg/m^2) Normal (< 25) Overweight/Obese (≥ 25)	0.16	2.34–13.32	0.005	0.142	1.52–12.61	0.015
Waist circumference (cm) Normal (reference) Central obesity	0.033	-3.62–6.6	0.57	- - -	- - -	- - -
Hypertension No (reference) Yes	-0.035	-6.44–3.37	0.54	- - -	- - -	- - -
Viral load < 2000 IU/L ≥ 2000 IU/L	0.147	2.99–22.09	0.01	0.15	- - -	0.008
Alcohol No (reference) Yes	0.001	-10.05–10.17	0.1	- - -	- - -	- - -
Opiate No (reference) Yes	-0.11	-16.12– -0.5	0.05	-0.082	-17.48– -0.7	0.16
Cigar No (reference) Yes	-0.009	-6.94–5.87	0.87	- - -	- - -	- - -

In their cross-sectional cohort study on 94 chronic inactive HBV carriers in Spain (2014), Mena, et al. reported a significant association between central obesity and advanced liver fibrosis. However, no relation was found between factors like age, sex, transaminases, HBV DNA level or genotype and the liver fibrosis in their study.[16] In China (2014), Wong, et al. followed 663 naïve CHB patients for about four years and reported a significant association between coincidental metabolic syndrome, central obesity and low level of high-density lipoprotein cholesterol with liver fibrosis progression independent of viral load change and ALT level.[17]

There are also other reports about the adverse effect of general obesity (high BMI) on the liver-related morbidity and mortality contributing to the non-alcoholic fatty liver, non-alcoholic steatohepatitis, cirrhosis and HCC.[18–20]

In a retrospective study on 136 eAg negative CHB in Taiwan (2011), the authors reported more advanced liver fibrosis stages and grades in those with higher BMI and viral load and observed a synergistic association of BMI and viral load on the progression of liver disease.[20] In another recent published paper from Turkey (2014), Demir, et al. reported a positive correlation between advanced fibrosis (which has been seen in 38.4%) and higher BMI, AST and ALT level, as well as HBV DNA level in 456 CHB patients.[21] Furthermore, Chinese patients with chronic hepatitis B and BMI > 28 kg/m^2 and abnormal levels of ALT had higher liver stiffness values in a study from Ding, et al.[22]

Nau, et al. (2014) also observed an association between the presence of liver steatosis and higher BMI in a retrospective cross-sectional study on 83 CHB patients in Brasil, suggesting a role for alteration in the metabolic factors in liver disease progression of HBV cases.[23]

However, a cross-sectional study in Indonesia of 174 CHB patients (56% HBeAg negative) reported an association between higher BMI and liver steatosis, but no association with fibrosis.[24] Considering all these data together, obesity and specifically central obesity may play an important role in the progression of liver diseases.

A significant association was found between the baseline viral load (log10) in CHB patients and advanced liver fibrosis (LSM ≥ 8 KPa) in a 4 years follow-up. To the best of our knowledge, due to the lack of longitudinal and cohort studies, this association has not been studied extensively. There is just one other cross-sectional study from China that found a correlation between steatosis and the hepatitis B virus (HBV)–DNA levels.[18] Future studies on the current topic are therefore recommended. Our major limitation was a small amount of CHB cases with possible or probable cirrhosis. Although it has been previously showed that liver fibrosis progression would be uncommon within 3 – 4 years of follow-up in inactive chronic HBeAg-negative hepatitis B patients with baseline HBV-DNA level < 20,000 IU/mL.[25] Other limitations include our lack of data on liver stiffness and other laboratory markers of liver disease at baseline. In addition, our results may not apply to other populations, particularly for patients who are HBeAg positive or infected with other genotypes of HBV.

Key strengths of our study include the comprehensive character-

ization of HBV infection and liver disease. Our study also benefited from a population-based setting and enrollment of participants from a homogenous ethnic group who were all HBeAg negative and infected by HBV genotype D.

In conclusions, in the present study, we observed an association between central obesity and advanced liver stiffness in the context of hepatitis B virus infection. Patients with HBV and coexisting central obesity are recommended to reduce their weight, for prevention of liver disease and other chronic disease.

Conflict of interests: *There was no conflict of interests.*

Authors' contributions

S. B. and A. M. have made substantial contributions to conception and design and acquisition of data and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. H. P. and N. F. contributed to the drafting and revisions of the manuscript and provided important intellectual content. G. R. contributed to analysis and interpretation of data. R. M. has given final approval of the version to be published.

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