

Original paper

Serum concentrations of selected adipokines in virus-related liver cirrhosis and hepatocellular carcinoma

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Abstract

Aim of the study: Hepatotropic viruses cause metabolic disturbances such as insulin resistance and hepatosteatosis. Moreover, metabolic factors, such as insulin resistance, obesity, and type 2 diabetes mellitus, increase the risk for hepatocellular carcinoma (HCC) in patients with virus-related liver cirrhosis. Cytokines secreted by the adipose tissue (adipokines) may be implicated in these metabolic disturbances, but there is little evidence regarding the role of adipokines in virus-related cirrhosis and HCC. Thus, we studied whether serum concentrations of selected adipokines were altered in patients with virus-related liver cirrhosis, including patients with HCC.

Material and methods: We included 43 patients with liver cirrhosis due to chronic hepatitis B or chronic hepatitis C. Of these patients, 36 had HCC and 7 did not have any malignant lesions. In addition to routine clinical and laboratory variables, we analyzed serum concentrations of betatrophin, insulin, vaspin, visfatin, and irisin.

Results: Compared with healthy controls, patients with HCC had significantly increased vaspin concentrations and significantly reduced irisin concentrations. Compared with controls, patients with virus-related cirrhosis, with or without HCC, had significantly increased concentrations of insulin and betatrophin. The serum visfatin concentration was non-significantly higher in patients with virus-related cirrhosis than in controls. None of the studied adipokines was a significant predictor of HCC. Serum concentrations of the studied adipokines were not related to cirrhosis severity or HCC stage.

Conclusions: Metabolic parameters, including serum adipokine concentrations, are altered in patients with virus-related liver cirrhosis. Adipokines might be related to the HCC risk in these patients.

Key words: adipokines, hepatocellular carcinoma, hepatitis B virus, hepatitis C virus, cirrhosis, metabolic factors.

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Introduction

Primary liver cancer is the third most common cause of cancer-related death, and it is the sixth most common cancer worldwide, with a growing incidence [1]. Hepatocellular carcinoma (HCC) is the most frequent histological type of primary liver cancer (75%), and its risk is increased substantially in patients with chronic hepatitis B (CHB) and chronic hepatitis C (CHC) [2]. Hepatic viruses cause HCC by integrating with the host genome (CHB) and by inducing chronic inflammation (CHB, CHC), which is associated with disturbances in hepatocyte growth and regeneration [3].

In patients with viral hepatitis, there is evidence that metabolic factors may further increase the HCC risk. For instance, the risk for HCC is increased by overweight, obesity, and type 2 diabetes mellitus [4-6]. It is hypothesized that viral hepatitis might promote carcinogenesis through metabolic effects in the liver. For example, the hepatitis C virus (HCV) induces insulin resistance directly, by interfering with insulin signaling pathways, and indirectly, by increasing the production of pro-inflammatory cytokines [7]. The hepatitis B virus (HBV) is also associated with increased insulin resistance [8]. Notably, insulin resistance increases the HCC risk in patients with CHB and

CHC [9, 10]. Moreover, HCV and, to a lesser extent, HBV promote steatohepatitis, which may increase oxidative stress, inflammation, and fibrosis and lead to the development of liver cancer [7]. The mechanisms inducing insulin resistance and steatohepatitis might be responsible for the increased prevalence of obesity in patients with viral hepatitis. Adipose tissue is metabolically active, and it secretes cytokines (adipokines) that may promote or inhibit hepatic carcinogenesis. For example, substantial evidence shows that leptin and adiponectin might promote steatohepatitis and thus HCC development in patients with viral hepatitis [11]. Although other adipokines might be involved in the pathogenesis of the metabolic disturbances that increase the risk of HCC, their effects on HCC risk in viral hepatitis have been less studied. For example, betatrophin has been shown to correlate positively with insulin resistance in CHC [12]. Vaspin and irisin, in contrast, might improve insulin resistance, which could reduce the risk of HCC in patients with viral hepatitis [13-15]. Data regarding the role of some adipokines in the pathogenesis of HCC among patients with viral hepatitis are conflicting. For instance, visfatin is increased in patients with CHC and HCC, but higher visfatin concentrations are also associated with lower necro-inflammatory activity in the liver [16, 17]. Thus, we investigated whether concentrations of betatrophin, vaspin, irisin, and visfatin were altered and associated with the HCC risk in patients with cirrhosis due to CHB or CHC who had focal liver lesions suggestive of liver cancer.

Material and methods

Patients

We reviewed the data of all patients with cirrhosis and focal liver lesions who were admitted to our department between January 2012 and December 2017. The lesions were found on abdominal ultrasound or abdominal computed tomography. We included only the patients with CHC and CHB. CHB was defined as the presence of both the hepatitis B surface antigen and antibodies against the hepatitis B core antigen in the blood. CHC was defined as the presence of both HCV RNA in the blood, detected by reverse transcription polymerase chain reaction, and antibodies against the hepatitis C virus. All patients with CHC were infected with genotype 1b HCV. Ten healthy volunteers served as a control group. We assessed the severity of cirrhosis with the Child-Pugh score, which takes into account serum concentrations of bilirubin and albumins, prothrombin time, and the presence of ascites

and hepatic encephalopathy. The cancer stage was assessed according to the Barcelona Clinic Liver Cancer (BCLC) score, which takes into account the performance status and Child-Pugh score of the patient, and the radiologic tumor extent. We calculated the body mass index (BMI), and recorded the presence of type 2 diabetes and hypertension. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

Laboratory measurements

Serum samples were obtained from peripheral blood by centrifugation and stored at -80°C until used. Using commercially available enzyme-linked immunosorbent assays (ELISA), we measured serum concentrations of vaspin (Cat. No. RD101097200R, detection limit: 0.01 ng/ml, BioVendor, Czech Republic), visfatin (Cat. No. RAG004R, detection limit: 30 pg/ml, BioVendor, Czech Republic), irisin (Cat. No. RAG018R, detection limit: 1 ng/ml, BioVendor, Czech Republic), betatrophin (Cat. No. SK00528-06, detection limit: 160 pg/ml, Aviscera Bioscience Inc., USA), and insulin (Cat. No. ME E-0900, detection limit: 1.76 $\mu\text{IU}/\text{ml}$, LDN Labor Diagnostika Nord, Germany) in all participants. Moreover, in the patients, we measured routine blood variables such as white blood cell count (WBC), platelet count (PLT), hemoglobin (HGB) concentration; activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyl transpeptidase (GGTP), and alkaline phosphatase (ALP); concentrations of fasting glucose, urea, creatinine, bilirubin, total cholesterol (TCh), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-Ch); prothrombin activity index (PT%); albumin and total protein concentration; and cancer markers (α -fetoprotein [AFP], carcinoembryonic antigen [CEA], cancer antigen 19-9 [CA 19-9]).

Statistical analysis

The Kolmogorov-Smirnov test was used to test for normality. Student's *t*-test or the Mann-Whitney *U*-test was used for comparing patients with or without HCC, as appropriate. Analysis of variance (ANOVA) or the Kruskal-Wallis test was used to compare patients with liver cirrhosis and HCC, patients with liver cirrhosis without HCC, and healthy controls. These tests were also used to compare patients with different Child-

Pugh and BCLC scores. Dunn's post hoc test was used with Holm's adjustment for multiple comparisons when ANOVA or the Kruskal-Wallis test showed significant differences. Spearman's rho correlation coefficient was used to analyze the relationship between adipokine concentrations and clinical and laboratory variables. A logistic regression model was built to predict the occurrence of HCC among patients with liver cirrhosis and focal liver lesions. $P < 0.05$ was considered statistically significant. All calculations were performed using R version 3.5.0.

Results

We included 43 patients with HBV-related ($n = 8$) or HCV-related ($n = 35$) cirrhosis and focal liver lesions. Of those patients, 36 had HCC on histopathology, and 7 did not have malignant lesions. There were no significant differences between patients with HCC, patients without HCC, and controls in terms of sex, age, and BMI (Table 1).

In the group without HCC, consisting of 7 patients (2 HBV-infected and 5 HCV-infected people), at first we also suspected neoplasms, but as this was excluded by focal lesion biopsy, the group became our control group, next to healthy people.

All patients in the HBV/HCC group (8 people) were treated with nucleotide and nucleoside analogues and had viremia undetectable in serum.

Patients with virus-related liver cirrhosis, both with and without HCC, had significantly higher concentrations of betatrophin and insulin than did healthy controls (Table 1). Compared with healthy controls, patients with HCC, but not patients without HCC, had significantly lower concentrations of irisin. Patients

with HCC had significantly higher concentrations of vaspin than did healthy controls. The concentration of visfatin was higher in patients with virus-related cirrhosis than in controls (not significant).

Among patients with virus-related liver cirrhosis, both with and without HCC, the visfatin concentration correlated with the WBC ($\rho = 0.32$, $p = 0.039$) and GGTP ($\rho = 0.34$, $p = 0.03$). The betatrophin concentration correlated with the insulin concentration ($\rho = 0.44$, $p = 0.001$), glucose concentration ($\rho = 0.35$, $p = 0.03$), WBC ($\rho = -0.38$, $p = 0.02$), PLT ($\rho = -0.44$, $p = 0.004$), PT% ($\rho = -0.39$, $p = 0.014$), and albumin concentration ($\rho = -0.42$, $p = 0.007$). The concentrations of vaspin and irisin did not correlate with any of the studied variables (Table 2).

Among patients with virus-related liver cirrhosis, both with and without HCC, there were no differences in the studied variables according to the Child-Pugh score (Table 3). Similarly, there were no significant differences in the studied variables depending on the BCLC stage (Table 4).

Compared with patients without HCC, those with HCC had significantly higher concentrations of total cholesterol and AFP (Table 5). We did not find significant differences between patients with or without HCC in the remaining variables. In a logistic regression model with age, sex, and total cholesterol and AFP concentrations, only AFP remained a significant predictor of HCC (Table 6).

Discussion

In our study we analyzed patients with liver tumor regardless of their viral etiology – either HBV- or HCV-related – because, although the pathways of

Table 1. Anthropometric variables and serum concentrations of adipokines in patients with liver cirrhosis, patients with hepatocellular carcinoma, and healthy controls

Variable	Patients with HCC ($n = 36$)	Patients without HCC ($n = 7$)	Healthy controls ($n = 10$)	p
Age (years), median (Q1-Q3)	60.0 (57.0-67.0)	59.0 (51.5-70.0)	42.0 (32.2-59.8)	0.062
Sex, n (%) of women	10 (27.8%)	2 (28.6%)	5 (50.0%)	0.403
BMI (kg/m^2), median (Q1-Q3)	29.0 (27.8-32.2)	29.0 (28.0-33.5)	26.0 (24.0-28.8)	0.115
Vaspin (ng/ml), median (Q1-Q3)	0.4 (0.2-0.6)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.005*
Visfatin (ng/ml), mean \pm SD	4.3 \pm 1.9	4.4 \pm 1.5	3.1 \pm 1.5	0.097
Irisin ($\mu\text{g}/\text{ml}$), median (Q1-Q3)	2.3 (1.7-3.5)	2.1 (2.0-2.9)	4.5 (2.5-4.7)	0.045*
Betatrophin (ng/ml), median (Q1-Q3)	36.5 (24.1-48.6)	25.0 (22.7-43.6)	12.3 (11.0-12.7)	< 0.001**
Insulin (ng/ml), median (Q1-Q3)	1.0 (0.5-1.3)	0.7 (0.4-1.6)	0.4 (0.4-0.6)	0.008**

Q1-Q3 – interquartile range, BMI – body mass index, HCC – hepatocellular carcinoma

*significant difference (Dunn's test, $p < 0.05$) between healthy controls and patients with HCC

**significant difference (Dunn's test, $p < 0.05$) between healthy controls and both patients with and without HCC

Table 2. Spearman rho correlation coefficients between adipokine concentrations and clinical and laboratory variables in patients with liver cirrhosis, including patients with hepatocellular carcinoma

Variable	Vaspin (ng/ml)		Visfatin (ng/ml)		Irisin (µg/ml)		Betatrophin (ng/ml)	
	rho	p	rho	p	rho	p	rho	p
Insulin (ng/ml)	-0.08	0.56	0.08	0.54	-0.18	0.20	0.44	0.001
WBC (10 ⁶ /µl)	0.18	0.25	0.32	0.03	0.22	0.16	-0.38	0.02
HGB (mg/dl)	0.21	0.18	-0.02	0.85	0.06	0.66	-0.11	0.48
PLT (106/µl)	0.03	0.82	0.26	0.08	0.09	0.55	-0.44	0.004
ALT (IU/l)	0.13	0.40	0.09	0.52	0.19	0.22	0.16	0.29
AST (IU/l)	-0.01	0.90	0.08	0.59	0.06	0.67	0.09	0.54
ALP (IU/l)	< -0.01	0.99	-0.10	0.49	-0.24	0.11	< -0.01	0.98
GGTP (IU/l)	0.14	0.37	0.34	0.03	-0.15	0.31	-0.25	0.12
Glucose (mg/dl)	0.04	0.80	< -0.01	0.98	-0.08	0.58	0.35	0.03
Urea (mg/dl)	0.05	0.75	0.03	0.82	< -0.01	0.99	0.17	0.28
Creatinine (mg/dl)	-0.08	0.61	0.07	0.61	-0.06	0.68	0.17	0.29
Bilirubin (mg/dl)	-0.08	0.58	-0.13	0.40	-0.09	0.53	-0.16	0.31
TChol (mg/dl)	0.18	0.24	0.03	0.83	0.03	0.80	-0.19	0.22
TG (mg/dl)	-0.01	0.92	0.15	0.31	0.12	0.43	-0.05	0.73
HDL (mg/dl)	< 0.01	0.99	0.09	0.55	< -0.01	0.99	0.22	0.15
PT (%)	0.242	0.12	0.18	0.24	-0.12	0.43	-0.39	0.01
Total protein (g/dl)	-0.209	0.19	-0.28	0.05	0.12	0.42	-0.29	0.06
Albumin (g/dl)	< 0.01	0.98	0.20	0.18	0.01	0.90	-0.42	0.006
AFP (ng/ml)	0.10	0.51	0.11	0.47	-0.05	0.74	-0.11	0.47
CEA (ng/ml)	0.24	0.12	-0.13	0.39	0.26	0.08	0.13	0.41
CA 19-9 (IU/ml)	0.15	0.33	0.12	0.42	0.02	0.85	-0.16	0.30
BMI (kg/m ²)	0.02	0.84	0.05	0.71	-0.14	0.31	0.09	0.50

WBC – white blood cell count, HGB – hemoglobin, ALT – alanine transferase, AST – aspartate transferase, ALP – alkaline phosphatase, GGTP – γ-glutamyl transpeptidase, TChol – total cholesterol, TG – triglycerides, HDL – high-density lipoprotein, PT – prothrombin time, AFP – α-fetoprotein, CEA – carcinoembryonic antigen, CA – cancer antigen, BMI – body mass index

oncogenesis are different in both etiological cases, the metabolic changes in patients with cirrhosis and HCC of a different cause are similar.

This study investigated whether virus-related liver cirrhosis and HCC were associated with changes in serum adipokine concentrations and whether serum adipokine concentrations were related to routine laboratory and clinical metabolic variables. We found that virus-related liver cirrhosis was associated with increased serum concentrations of betatrophin, insulin, vaspin, and visfatin. Moreover, in patients with HCC, the serum irisin concentration was reduced.

The increased concentrations of insulin in virus-related cirrhosis might be due to direct interference of the virus with insulin signaling pathways (HCV) and an indirect effect of chronic inflammation (HBV, HCV) [7]. We found that patients with virus-related liver cirrhosis, both with and without HCC, had increased insulin concentrations compared with healthy controls. This is in line with the ob-

servations on increased insulin resistance in CHC, CHB, and HCC [7, 8]. We did not find any relationship between serum insulin concentration and cirrhosis severity or HCC progression. Likewise, previous evidence shows that hyperinsulinemia develops regardless of cirrhosis severity [18]. In line with previous studies in viral hepatitis, we found that serum insulin concentrations correlated with the concentrations of betatrophin; however, this correlation was also found in other diseases [12, 19]. Similar to previous studies, we found that patients with virus-related cirrhosis, including those with HCC, had increased betatrophin concentrations compared with healthy controls [12, 20]. In the study by Arias-Loste *et al.*, higher betatrophin concentrations were related to greater cirrhosis severity [20]. Likewise, in our study, betatrophin concentrations were higher, though non-significantly, in patients with higher Child-Pugh scores. We found that betatrophin concentrations correlated negatively with PT%, a marker of liver injury, although betatrophin itself is produced mainly by the

Table 3. Differences in clinical and laboratory variables in patients with liver cirrhosis, including those with hepatocellular carcinoma, and different Child-Pugh scores

Variable	n	Child-Pugh A	n	Child-Pugh B	n	Child-Pugh C	p
BMI (kg/m ²), median (Q1-Q3)	21	28.0 (27.0-33.0)	19	29.0 (28.0-32.5)	3	29.0 (28.5-37.0)	0.573
Age (years), median (Q1-Q3)	21	59.0 (55.0-64.0)	19	65.0 (57.5-72.0)	3	57.0 (52.5-57.5)	0.065
Sex, n (%) of women	21	7 (33.3)	19	3 (15.8)	3	2 (66.7)	0.140
Vaspin (ng/ml), median (Q1-Q3)	19	0.2 (0.2-0.6)	19	0.4 (0.1-0.9)	3	0.1 (0.1-0.2)	0.332
Visfatin, mean ±SD	21	4.5 ±1.8	19	4.2 ±2.0	3	4.5 ±2.0	0.893
Irisin (µg/ml), median (Q1-Q3)	21	2.1 (1.8-3.9)	19	2.4 (1.7-2.7)	3	2.0 (1.8-3.1)	0.578
Betatrophin (ng/ml), median (Q1-Q3)	21	33.7 (23.4-47.2)	17	35.4 (25.0-48.7)	2	46.7 (45.0-48.3)	0.318
Insulin (ng/ml), median (Q1-Q3)	20	0.7 (0.5-1.1)	19	1.0 (0.5-1.5)	3	1.3 (0.9-2.2)	0.781
Type 2 diabetes, n (%)	21	7 (33.3)	19	6 (31.6)	3	1 (33.3)	0.993
Hypertension, n (%)	21	6 (28.6)	19	6 (31.6)	3	1 (33.3)	0.972
Tchol (mg/dl), median (Q1-Q3)	21	154.0 (128.1-169.0)	19	168.0 (123.5-204.0)	3	98.3 (91.7-108.2)	0.073
TG (mg/dl), median (Q1-Q3)	21	107.0 (64.7-131.0)	19	101.0 (79.6-136.9)	3	113.0 (81.0-120.3)	0.721
HDL (mg/dl), median (Q1-Q3)	21	40.2 (33.9-50.7)	19	35.7 (32.4-41.5)	3	23.9 (23.4-30.6)	0.090

BMI – body mass index, Tchol – total cholesterol, TG – triglycerides, HDL – high-density lipoprotein

Table 4. Differences in clinical and laboratory variables in patients with liver cirrhosis, including those with hepatocellular carcinoma, and different BCLC scores

Variable	n	BCLC A	n	BCLC B	n	BCLC C	p
BMI (kg/m ²), median (Q1-Q3)	8	28.5 (25.2-30.5)	14	29.5 (28.0-31.8)	14	29.0 (28.0-33.8)	0.609
Age (years), median (Q1-Q3)	8	60.0 (53.0-64.2)	14	65.0 (58.0-70.8)	14	58.5 (52.5-59.8)	0.134
Sex, n (%) of women	8	3 (37.5)	14	2 (14.3)	14	5 (35.7)	0.352
Vaspin (ng/ml), median (Q1-Q3)	8	0.5 (0.3-0.6)	13	0.4 (0.2-0.6)	14	0.2 (0.1-0.5)	0.504
Visfatin, mean ±SD	8	4.8 ±1.9	14	4.1 ±1.4	14	4.3 ±2.4	0.653
Irisin (µg/ml), median (Q1-Q3)	8	3.4 (1.8-4.4)	14	2.5 (1.8-2.9)	14	2.0 (1.6-2.8)	0.246
Betatrophin (ng/ml), median (Q1-Q3)	8	39.4 (22.7-47.0)	13	34.3 (25.0-41.7)	13	43.4 (23.8-48.7)	0.744
Insulin (ng/ml), median (Q1-Q3)	8	1.1 (0.6-1.2)	14	0.8 (0.5-1.2)	14	1.0 (0.5-1.3)	0.657
Type 2 diabetes, n (%)	8	4 (50.0%)	14	10 (71.4%)	14	10 (71.4%)	0.526
Hypertension, n (%)	8	2 (25.0%)	14	3 (21.4%)	14	7 (50.0%)	0.235
Tchol (mg/dl), median (Q1-Q3)	8	153.3 (127.3-161.1)	14	161.9 (137.0-200.9)	14	157.1 (121.5-190.0)	0.546
TG (mg/dl), median (Q1-Q3)	8	94.4 (67.8-122.3)	14	116.0 (83.2-132.2)	14	120.3 (78.2-134.2)	0.785
HDL (mg/dl), median (Q1-Q3)	8	40.5 (29.6-45.2)	14	37.0 (32.8-42.2)	14	36.0 (32.7-47.0)	0.999

BCLC – Barcelona Clinic Liver Cancer, BMI – body mass index, Tchol – total cholesterol, TG – triglycerides, HDL – high-density lipoprotein

liver. We suspect that increased betatrophin production in patients with liver diseases may be induced by hyperinsulinemia. Moreover, betatrophin concentrations correlated negatively with indicators of chronic inflammation (WBC, PLT), which may be due to a compensatory anti-inflammatory action of betatrophin [21]. Although previous studies showed a correlation between betatrophin concentration and triglyceride concentration [22], we did not find such a relationship in our study.

Irisin, which is released from muscles during exercise, may reduce insulin resistance [13]. In our study, patients with virus-related liver cirrhosis, including those

with HCC, had lower irisin concentrations than did healthy controls. However, only the difference between patients with HCC and healthy controls was significant. Moreover, we observed that irisin concentrations were non-significantly lower in patients with greater cirrhosis severity. To our knowledge, irisin has not been studied in patients with viral hepatitis, but irisin concentrations are reduced in non-alcoholic fatty liver disease [23]. Because irisin is expressed primarily in skeletal muscles, muscle loss, which progresses with the severity of liver disease, might explain the reduced irisin concentrations in patients with cirrhosis and HCC [24].

Table 5. Differences in clinical and laboratory variables between patients with liver cirrhosis alone and patients with liver cirrhosis and HCC

Variable	Patients without HCC (n = 7)	Patients with HCC (n = 36)	p
BMI (kg/m ²), median (Q1-Q3)	29.0 (28.0-33.5)	29.0 (27.8-32.2)	0.692
Age (years), median (Q1-Q3)	59.0 (51.5-70.0)	59.0 (57.0-65.2)	0.947
Sex, n (%) of women	2 (28.6)	10 (27.8)	1.000
Vaspin (ng/ml), median (Q1-Q3)	0.1 (0.1-0.2)	0.4 (0.2-0.6)	0.090
Visfatin (ng/ml), mean ±SD	4.4 ±1.5	4.3 ±1.9	0.870
Irisin (µg/ml), median (Q1-Q3)	2.1 (2.0-2.9)	2.3 (1.7-3.5)	0.669
Betatrophin (ng/ml), median (Q1-Q3)	25.0 (22.7-43.6)	36.5 (24.1-48.6)	0.733
Insulin (ng/ml), median (Q1-Q3)	0.7 (0.4-1.6)	1.0 (0.5-1.3)	0.472
Type 2 diabetes, n (%)	2 (28.6%)	12 (33.3%)	1.000
Hypertension, n (%)	1 (14.3%)	12 (33.3%)	0.579
ALT (IU/l), median (Q1-Q3)	58.0 (36.0-86.5)	60.0 (40.0-91.2)	0.818
AST (IU/l), median (Q1-Q3)	68.0 (56.5- 99.5)	96.5 (51.8-139.5)	0.831
ALP (IU/l), mean ±SD	119.7 ±97.0	145.8 ±80.8	0.524
GGTP (IU/l), median (Q1-Q3)	50.0 (37.5-122.0)	95.5 (64.2-170.8)	0.183
TChol (mg/ml), median (Q1-Q3)	85.1 (77.2-140.7)	155.7 (128.2-179.5)	0.023
TG (mg/ml), median (Q1-Q3)	77.0 (49.0-120.9)	114.0 (74.9-133.0)	0.300
HDL (mg/ml), median (Q1-Q3)	39.7 (32.6-47.6)	37.6 (32.2-45.2)	0.543
PT (%), mean ±SD	69.4 ±15.1	74.9 ±13.8	0.402
Total protein (g/dl), mean ±SD	7.1 ±0.8	7.1 ±1.1	0.996
Albumin (g/dl), mean ±SD	3.3 ±0.5	3.0 ±0.5	0.153
AFP (ng/ml), median (Q1-Q3)	5.7 (4.4-14.9)	42.1 (15.0-188.4)	0.005
CEA (ng/ml), median (Q1-Q3)	4.6 (2.2-7.3)	3.1 (2.2-4.6)	0.479
CA 19-9 (U/l), median (Q1-Q3)	10.6 (6.2-32.7)	16.0 (6.9-25.3)	0.717

BMI – body mass index, ALT – alanine aminotransferase, AST – aspartate aminotransferase, WBC – white blood cell count, HGB – hemoglobin, ALT – alanine transferase, AST – aspartate transferase, ALP – alkaline phosphatase, GGTP – gamma glutamyl transpeptidase, TChol – total cholesterol, TG – triglycerides, HDL – high-density lipoprotein, PT – prothrombin time, AFP – α -fetoprotein, CEA – carcinoembryonic antigen, CA – cancer antigen

Table 6. Logistic regression model for prediction of HCC in patients with liver cirrhosis and focal liver lesions

Variable	Unadjusted odds ratio (95%CI)	adjusted odds ratio (95%CI)	p*
Sex	1.04 (0.17, 6.26)	1.19 (0.15, 9.68)	0.87
Age (years)	1.00 (0.94, 1.07)	0.98 (0.91, 1.06)	0.66
TChol (mg/dl)	1.02 (1.00, 1.05)	1.02 (0.99, 1.04)	0.20
AFP (ng/ml)	1.07 (0.99, 1.15)	1.05 (0.97, 1.14)	0.02

CI – confidence interval, Tchol – total cholesterol, AFP – α -fetoprotein, *likelihood-ratio test

Vaspin, which is expressed primarily in the visceral adipose tissue, improves glucose tolerance and insulin sensitivity, and it decreases the synthesis of pro-inflammatory cytokines [14, 25]. In viral hepatitis, vaspin might be synthesized in response to increased insulin resistance [25]. In CHC, vaspin was shown to be reduced and positively related to greater liver fibrosis [25]. In our study, vaspin concentrations were not reduced in patients with viral cirrhosis, but our study

also included patients with CHB, which might explain our findings. The role of vaspin in carcinogenesis is unclear, but serum vaspin concentrations are increased in patients with colorectal cancer [26]. In our study, patients with HCC had significantly higher vaspin concentrations than did healthy controls, but the vaspin concentration was not a significant predictor of HCC.

Visfatin has insulin-sensitizing and pro-inflammatory effects, and it is increased in patients with liver

cirrhosis due to CHB or CHC [17, 27, 28]. Moreover, in patients with CHB or CHC, increased visfatin concentrations might be related to more advanced inflammation and greater HCC risk [16]. Similarly, in our study, patients with virus-related cirrhosis, including those with HCC, had higher visfatin concentrations than did controls (not significant). Moreover, in our study, visfatin concentration correlated positively with a marker of chronic inflammation (WBC) [29]. Visfatin concentrations might be increased selectively in viral cirrhosis, because visfatin concentrations are decreased in non-viral cirrhosis [30].

Our study was observational, and we were not able to investigate the direct effects of adipokines on metabolic variables. The study was conducted in one center and included a relatively small number of participants. We were therefore not able to investigate the associations of the clinical and laboratory variables with cirrhosis severity and HCC risk in multivariate analyses. Moreover, we included only patients with viral cirrhosis. HCV, in addition to causing insulin resistance and steatosis, might influence adipokine production in the adipose tissue, and HBV might directly promote HCC by integrating with the host genome. We were not able to compare the individual effects of HCV and HBV in our study because of the low number of patients infected with each virus type ($n = 35$ and $n = 8$, respectively). Thus, further studies are needed to investigate the role of adipokines in alcohol-induced liver cirrhosis and non-alcoholic fatty liver disease. Regarding irisin, which is mainly synthesized by skeletal muscles, we did not account for the differences in muscle mass between patients and controls.

In conclusion, our findings support the view that metabolic parameters, such as insulin resistance and concentrations of betatrophin, vaspin, visfatin, and irisin, are altered in patients with virus-related liver cirrhosis. Moreover, these adipokines might be related to the risk of HCC in these patients.

Our research was preliminary in nature, and its aim was to obtain information on the usefulness of these markers in patients with hepatocellular carcinoma. Further work requires the enlargement of the groups, especially the HBV-infected ones (currently the number of patients in this group is decreasing), and the comparison of the groups with regard to etiology and hepatic cirrhosis, which is a separate project.

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Disclosure

The authors declare no conflict of interest.

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