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ORIGINAL ARTICLE

Prospective Study

Adipokines levels are associated with the severity of liver disease in patients with alcoholic cirrhosis

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Abstract

AIM: To investigate the adipokine levels of leptin, adiponectin, resistin, visfatin, retinol-binding protein 4 (RBP4), apelin in alcoholic liver cirrhosis (ALC).

METHODS: Forty non-diabetic ALC patients [median age: 59 years, males: 35 (87.5%), Child-Pugh (CP) score: median 7 (5-12), CP A/B/C: 18/10/12, Model for End-stage Liver Disease (MELD): median 10 (6-25), follow-up: median 32.5 mo (10-43)] were prospectively included. The serum adipokine levels were estimated in duplicate by ELISA. Somatometric characteristics were assessed with tetrapolar bioelectrical impedance analysis. Pearson's rank correlation coefficient was used to assess possible associations with adipokine levels. Univariate and multivariate Cox regression analysis was used to determine independent predictors for overall

survival.

RESULTS: Body mass index: median 25.9 (range: 20.1-39.3), fat: 23.4% (7.6-42.1), fat mass: 17.8 (5.49-45.4), free fat mass: 56.1 (39.6-74.4), total body water (TBW): 40.6 (29.8-58.8). Leptin and visfatin levels were positively associated with fat mass ($P < 0.001$/$P = 0.027$, respectively) and RBP4 with TBW ($P = 0.025$). Median adiponectin levels were significantly higher in CPC compared to CPA (CPA: 7.99 ± 14.07, CPB: 7.66 ± 3.48, CPC: 25.73 ± 26.8, $P = 0.04$), whereas median RBP4 and apelin levels decreased across the spectrum of disease severity ($P = 0.006$/$P = 0.034$, respectively). Following adjustment for fat mass, visfatin and adiponectin levels were significantly increased from CPA to CPC (both $P < 0.001$), whereas an inverse correlation was observed for both RBP4 and apelin (both $P < 0.001$). In the multivariate Cox regression analysis, only MELD had an independent association with overall survival (HR = 1.53, 95%CI: 1.05-2.32; $P = 0.029$).

CONCLUSION: Adipokines are associated with deteriorating liver function in a complex manner in patients with alcoholic liver cirrhosis.

Key words: Adipokines; Adiponectin; Cirrhosis; Leptin; Resistin

Core tip: Ongoing data suggest that obesity and insulin resistance are associated with a more rapid progression of the fibrogenic process in chronic liver diseases, with different adipokines contributing to the complex pathophysiology of hepatic injury and repair. In cirrhosis, accumulating data demonstrate an alteration in the levels of different adipokines; although most studies did not exclude patients with baseline diabetes which is itself associated with altered adipokines. Alcoholic cirrhosis has been the least studied. The present study evaluates the serum levels of six adipokines in non-diabetic patients with alcoholic cirrhosis and investigates their potential association with liver disease severity.

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INTRODUCTION

Adipokines are polypeptide hormones produced predominantly by adipose tissue. Apart from fat cells, adipose tissue is composed of stromal cells, including macrophages, fibroblasts and infiltrating monocytes, all of which contribute to adipokines' production[1]. In obesity, excess fat represents a dysfunctional inflammatory tissue with alterations in the pattern of adipokines' expression which contributes to obesity-related disorders[2].

Ongoing data suggest that obesity and insulin resistance are associated with a more rapid progression of the fibrogenic process in chronic liver diseases[3], with different adipokines contributing to the complex pathophysiology of hepatic injury and repair[4,5]. A multicentre study[6] of 15866 patients with chronic liver disease showed that diabetes mellitus, insulin resistance, obesity and metabolic syndrome were independent predictors of liver-related mortality in chronic hepatitis C, non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD).

Alcohol-induced liver injury is related to a variety of molecular factors including cytokines, adipokines, chemokines and neurotransmitters[7]. In cirrhosis, accumulating data demonstrate an alteration in the levels of different adipokines[8,9], although most studies did not exclude patients with baseline diabetes which in itself is associated with altered adipokines. Leptin has been shown to be a hepatic pro-fibrogenic agent in experimental studies[10-13]. Alcoholic cirrhosis is associated with increased levels of leptin compared to controls without liver disease, independently of body mass index (BMI)[14-17]. However, studies of leptin in patients with NAFLD and chronic hepatitis C have shown conflicting results, with some studies demonstrating a strong association with severity of fibrosis[18,19], while others failed to show such a correlation[20-22]. Adiponectin is an adipocyte-specific protein, which in contrast to leptin, is reduced in obesity[23]. Several animal studies have assessed the hepatoprotective and antifibrogenic effects of this adipokine[24,25]. The role of adiponectin in alcoholic liver disease as yet has not been elucidated. Buechler *et al*[26] found that adiponectin levels were significantly higher in heavy drinkers without advanced liver damage and in patients with alcoholic cirrhosis, and that adiponectin levels in alcoholics were significantly associated with the daily amount of alcohol consumption and declined with alcohol abstinence. In animal models, resistin is increased in obesity[27], antagonizes the action of insulin by decreasing tissue insulin sensitivity[28] and modulates hepatic stellate cells behavior towards a more fibrogenic phenotype[29]. However, in human studies resistin does not seem to have a role in insulin homeostasis, but it is associated with inflammatory responses[30]. Tsochatzis *et al*[4] showed that moderate/severe fibrosis (Ishak stages 4-6) in patients with viral hepatitis was significantly associated with lower resistin levels irrespective of gender or other factors. In another study[31], plasma resistin levels were increased in patients with cirrhosis compared to controls in a stepwise fashion in line with worsening Child-Pugh

stage. Similar findings were published by Yagmur *et al*[32] who also showed that resistin correlated inversely with markers of hepatic biosynthetic capacity, and positively with portal hypertension and 6-year mortality.

Visfatin, apelin and RBP4 are newly described adipokines and the data on their impact in liver fibrogenesis is scarce. In experimental and human studies, a link between RBP4 and insulin resistance has been demonstrated[33,34], whereas in other studies RBP4 levels were associated with hepatic steatosis[35]. Apelin is also up-regulated in obesity and insulin resistance[36]. Principe *et al*[37] found that apelin levels and apelin receptor mRNA levels were higher in rats with cirrhosis than in controls. Also, rats with cirrhosis treated with apelin receptor antagonist showed diminished hepatic fibrosis, improved cardiovascular performance and renal function, and lost ascites.

The aim of this study was to assess the serum levels of six adipokines [leptin, adiponectin, resistin, visfatin, retinol binding protein 4 (RBP4), apelin] in patients with alcoholic cirrhosis and to investigate a potential association of adipokines with the severity of liver disease and survival during follow-up.

MATERIALS AND METHODS

The current prospective, observational cohort study was performed at the University Hospital of Patras (Patras, Greece) and planned at the Royal Free Sheila Sherlock Liver Centre (London, United Kingdom). The study was reviewed and approved by the Institutional Review Board of the University Hospital of Patras. Forty consecutive patients with alcoholic cirrhosis attending the hepatology outpatient clinics from May 2010 until September 2011 were evaluated and followed-up until December 2013. The diagnosis of cirrhosis was based upon compatible histological findings, clinical evaluation, laboratory data or imaging findings. All included patients had a stable clinical condition (no active infection, alcoholic hepatitis, recent variceal bleeding or other acute decompensating event) and 6 mo of abstinence from alcohol drinking. Informed consent was obtained from each participant. Patients with overt diabetes mellitus (fasting glucose $\geqslant$ 126 mg/dL) and/or use of insulin or oral antidiabetic/insulin-sensitizing medication at study entry, or in the past, were excluded from the protocol. No patient had heart failure, renal failure, proteinuria, active bacterial infections, evidence of other endocrine disorder or received hormone replacement therapy. Patients with other causes of chronic liver disease apart from alcohol abuse were excluded from the study. Patients with hepatocellular carcinoma (HCC) within Milan criteria, no major vessel involvement and no metastasis were included.

Blood samples for the measurement of adipokines and other laboratory assessments (including biochemical parameters) were taken at enrollment, as part of the study protocol. Follow-up of these patients was according to the standard clinical practice. Patients were followed-up until death or until December 2013. Demographic, endoscopic and histological data were gathered based on patient charts. The severity of liver disease was assessed by the Child-Pugh class and the Model for End-stage Liver Disease (MELD).

Anthropometry assessment

BMI was calculated as weight (in kilograms)/height (in m^2). According to the World Health Organization definition[38], patients were stratified using the following scale: (1) underweight (BMI < 18.5 kg/m^2); (2) normal weight (BMI = 18.5-24.9 kg/m^2); (3) overweight (BMI = 25-29.9 kg/m^2); and (4) obese (BMI $\geqslant$ 30 kg/m^2). Tetrapolar bioelectrical impedance analysis (BIA) was used to analyze body composition [assessment of fat mass, fat%, free fat mass (FFM) and total body water (TBW)] with a radiofrequency current of 800 mA and a frequency of 50 kHz, between a set of electrodes attached to the dorsum of the hand and foot (Tanita, BC-418 MA). Calculations were performed using previously described formulas[39]. Body composition analysis was performed after an overnight fast at the same time point of blood sampling. BIA was used as a more reliable method to assess body composition in patients with liver cirrhosis. Recent studies[40] have shown that BIA is a reliable method for calculation of body cell mass in cirrhosis, at least in patients without clinically significant fluid overload.

Measurement of serum concentration of adipokines

All blood samples were measured in the morning following an overnight fast. Plasma and serum were immediately separated by centrifugation for 10 min at 3000 rpm (4 ℃) and then stored at -30 ℃ until the time of assessment. Serum leptin, adiponectin, resistin and PBR4 concentrations were measured using a quantitative sandwich enzyme immunoassay (ELISA), according to the instructions of the manufacturer (Millipore Corporation, Billerica, MA, United States). Serum visfatin and apelin were determined using ELISA (Phoenix Europe GmbH, Karlsruhe, Germany).

Statistical analysis

Numerical data were expressed as median and range (minimum to maximum) and categorical data as counts and percentages. All variables were tested for normal distribution using the Kolmogorov-Smirnov test. Categorical variables were tested using the χ^2 and Fisher's exact test. Continuous variables with and without normal distribution were compared using Student's *t*-test or the Mann-Whitney *U* test, respectively. Pearson's rank correlation coefficient was used to assess possible associations between different quantitative parameters. All parameters found to have significant associations with adipokine levels as the dependent variable in the univariate analysis, were included in the multivariate linear regression analysis. A subgroup analysis of

Table 1 Baseline clinical and laboratory characteristics of study population

	All patients (n = 40)	CP A (n = 18)	CP B (n = 10)	CP C (n = 12)	P value[1]
Sex (M/F), n (%)	35/5 (87.5/12.5)	16/2 (88.9/11.1)	9/1 (90/10)	10/2 (83.3/16.7)	0.870
Age, median years (range)	59 (37-75)	59.5 (43-75)	60.5 (37-71)	59 (45-70)	0.838
Smoking, n (%)	15 (37.5)	7 (38.9)	3 (30)	5 (41.7)	0.954
HCC, n (%)	5 (12.5)	1 (5.6)	1 (10)	3 (25)	0.277
Hemoglobin (11.8-17 g/dL)	12.35 (9.3-16.6)	14.3 (9.8-16.6)	12.75 (10.2-15.1)	10.1 (9.3-13.2)	0.002
WBC count (4 × 10^9/L-11 × 10^9/L)	5.888 (3.2-15.18)	6.66 (3.2-12.97)	5.31 (4.1-8.97)	5.66 (4.15-15.18)	0.093
Platelet count (150 × 10^9/L-400 × 10^9/L)	113 (81-246)	148 (87-246)	117.5 (70-198)	127 (75-218)	0.306
INR (1-1.3)	1.28 (0.87-2.32)	1.09 (0.87-1.59)	1.2 (1.07-1.92)	1.58 (1.24-2.32)	0.001
PT (s)	15.25 (9.8-24.8)	11.9 (9.8-18.3)	15.15 (12.2-17)	18.6 (15.2-24.8)	< 0.001
Total bilirubin (0.1-1.3 mg/dL)	1.1 (0.28-8.6)	0.72 (0.28-1.19)	1.23 (0.85-3.5)	2.4 (1.6-8.6)	< 0.001
Albumin (3.5-5.5 g/dL)	3.65 (0.9-4.6)	4 (3.4-4.6)	3.6 (2.6-4.3)	2.8 (0.9-3.2)	< 0.001
Creatinine (0.9-1.6 mg/dL)	0.82 (0.68-1.8)	0.88 (0.7-1.7)	0.82 (0.7-1.4)	0.8 (0.68-1.8)	0.967
Urea (15-54 mg/dL)	28 (16-110)	28 (18-64)	26.5 (21-47)	29 (16-110)	0.981
SGOT (5-40 U/L)	36 (15-149)	32.3 (15-48)	59 (24-149)	58 (18-128)	0.017
SGPT (5-40 U/L)	33 (8-156)	32 (8-60)	46 (23-156)	32 (11-64)	0.298
ALP (U/L)	116 (37-303)	104 (41-236)	113.5 (75-244)	123 (37-303)	0.324
GGT (U/L)	94 (12-328)	82.5 (12-231)	135 (61-328)	108 (28-199)	0.057
Glucose (75-115 mg/dL)	99 (60-126)	99 (71-126)	105 (94-126)	92 (60-118)	0.170
Serum sodium (134-152 mmol/L)	138 (129-146)	140.8 (134.5-146)	137.4 (135-141)	135 (129-142)	0.012
Cholesterol (mg/dL)	146 (66-341)	188.5 (130-341)	133 (91-173)	146 (66-168)	0.041
Triglycerides (mg/dL)	88.5 (43-142)	106 (43-142)	74.5 (47-139)	76 (46-116)	0.525
Child-Pugh score (range)	7 (5-12)	NA	NA	NA	NA
MELD score (range)	10 (6-25)	7 (6-16)	11 (8-18)	18 (13-25)	< 0.001
Ascites, n (%)	14 (35)	0	4 (40)	10 (83.3)	< 0.001
Encephalopathy, n (%)	2 (5)	1 (5.6)	0	1 (8.3)	0.664

[1]P value: correlation of variables regarding different stages of liver disease. CP: Child-Pugh; HCC: Hepatocellular carcinoma; WBC: White blood cells; INR: International normalized ratio; PT: Prothrombin time; SGOT: Aspartate aminotransferase; SGPT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl-transferase; MELD: Model for End-stage Liver Disease; BMI: Body mass index; NA: Not applicable.

possible associations between baseline characteristics and adipokine levels in patients with ascites was performed. Univariate Cox regression analysis was used to determine possible predictors of overall survival. Variables significant at the 0.1 level were included in the multivariate analysis using Cox regression analysis. The results are presented as hazard ratios (HR) with 95%CI. All statistical tests were two-sided and significance level was set at < 0.05 or less. The SPSS statistical package (version 19.0 for Windows; SPSS Inc, Chicago, Illinois) was used. The statistical methods of this study were reviewed by E.J. from Medical School of University of Patras.

RESULTS

Baseline clinical and laboratory characteristics of study population are shown in Table 1. Five patients (12.5%) had HCC at baseline. All patients had early HCC (stage A) according to BCLC (Barcelona Clinic Liver Cancer) staging system for HCC[41]. Table 2 presents the measurements provided by BIA. Patients with different stages of liver disease (assessed by Child-Pugh classification) were matched regarding somatometric characteristics.

Patients with (n = 14) and without ascites (n = 26) had no significant differences regarding height (P = 0.085), BMI (P = 0.105), fat% (P = 0.218), fat mass (P = 0.124) or FFM (P = 0.271). However, there was a significant association of TBW and weight with ascites (P = 0.012 and P = 0.045, respectively). Sex was not significantly associated with the levels of adipokines apart from leptin levels, which were significantly higher in female subjects (females: 11.85 ± 16.7 ng/mL *vs* males: 4.98 ± 11.3 ng/mL, P = 0.04).

Adipokines and somatometric characteristics

Normal-weight subjects had significantly lower leptin levels compared to overweight (3.56 ± 3.13 ng/mL *vs* 8.33 ± 10.9 ng/mL, P = 0.022) and obese patients (3.56 ± 3.13 ng/mL *vs* 33.18 ± 16.79 ng/mL, P = 0.003), whereas a positive correlation was observed between leptin levels and fat mass (P < 0.001). Normal-weight subjects also had significantly lower visfatin concentrations compared to obese (13.31 ± 27.37 ng/mL *vs* 22.18 ± 53.66 ng/mL, P = 0.04), and visfatin levels were positively associated with fat mass (P = 0.027). RBP4 had a statistically significant association with total body water (TBW) (Table 3).

Correlations of adipokines with the severity of cirrhosis

Leptin levels were positively correlated with prothrombin time (P = 0.038) and inversely with albumin levels (P = 0.014). Adiponectin was significantly correlated with prothrombin time (P = 0.043), international normalized ratio (INR) (P = 0.028), aspartate aminotransferase (SGOT) (P < 0.001), alanine aminotransferase (SGPT) (P = 0.006), alkaline phosphatase (ALP) (P = 0.04) and total bilirubin levels (P = 0.016). A significant correlation was observed between RBP4 levels and hemoglobin

Table 2 Baseline somatometric characteristics of study population

	All patients (n = 40)	CP A (n = 18)	CP B (n = 10)	CP C (n = 12)	P value[1]
BMI (kg/m^2) (range)	25.89 (20.08-39.31)	26.29 (21.34-30.52)	27.39 (20.76-39.3)	25.29 (20.08-35.64)	0.449
BMI (kg/m^2) (%)					
18.5-24.9	33.3	21.4	40	44.4	0.261
25-29.9	51.5	71.4	30	44.4	
> 30	15.2	7.1	30	11.1	
Fat, % (range)	23. 4 (7.6-42.1)	24.3 (18.7-32.9)	23 (16.3-38.5)	19.9 (7.6-42.1)	0.522
Fat mass, kg (range)	17.81 (5.49-45.4)	17.97 (10.73-29.02)	14.62 (11.41-45.4)	13.87 (5.5-35.74)	0.454
FFM, kg (range)	56.145 (39.56-74.4)	54.475 (46.67-67.31)	58.59 (48.89-74.4)	55.83 (39.56-66.7)	0.234
TBW, kg, (range)	40.63 (29.83-58.82)	40.875 (34.36-45.36)	41.025 (30.52-58.82)	39.01 (29.83-43.45)	0.300

[1]P value: correlation of variables regarding different stages of liver disease. CP: Child-Pugh stage; BMI: Body mass index; FFM: Fat-free mass; TBW: Total body water.

Table 3 Association of adipokines with demographic, biochemical and anthropometric characteristics

	Leptin	Adiponectin	Resistin	Visfatin	RBP4	Apelin
	r (P value)	r (P value)	r (P value)	r (P value)	r (P value)	r (P value)
Age (yr)	-0.012 (0.94)	0.206 (0.203)	0.127 (0.434)	-0.014 (0.933)	0.178 (0.273)	-0.059 (0.716)
Hemoglobin (g/dL)	-0.299 (0.081)	-0.055 (0.75)	-0.138 (0.422)	0.314 (0.066)	0.582 (< 0.001)	0.197 (0.249)
WBC count	-0.268 (0.115)	-0.077 (0.653)	0.262 (0.117)	-0.027 (0.875)	0.195 (0.247)	-0.021 (0.902)
Platelet count	-0.298 (0.082)	-0.097 (0.575)	0.165 (0.337)	-0.007 (0.966)	0.238 (0.163)	-0.006 (0.973)
INR	0.349 (0.059)	0.395 (0.028)	-0.056 (0.767)	0.185 (0.327)	-0.493 (0.008)	0.136 (0.466)
PT (s)	0.387 (0.038)	0.373 (0.043)	0.055 (0.772)	0.172 (0.373)	-0.473 (0.005)	0.024 (0.899)
Total bilirubin (mg/dL)	-0.081 (0.648)	0.403 (0.016)	0.204 (0.24)	-0.07 (0.694)	-0.37 (0.029)	-0.122 (0.484)
Albumin (g/dL)	-0.424 (0.014)	-0.179 (0.311)	-0.115 (0.516)	-0.215 (0.229)	0.32 (0.065)	0.132 (0.457)
Creatinine (mg/dL)	0.271 (0.105)	0.055 (0.741)	0.241 (0.145)	0.027 (0.872)	0.251 (0.128)	-0.177 (0.288)
Urea (mg/dL)	0.263 (0.116)	0.268 (0.103)	0.147 (0.378)	0.011 (0.948)	0.149 (0.371)	-0.158 (0.342)
SGOT (U/L)	0.031 (0.859)	0.589 (< 0.001)	0.145 (0.392)	0.177 (0.302)	-0.169 (0.319)	0.186 (0.269)
SGPT (U/L)	0.186 (0.279)	0.443 (0.006)	-0.064 (0.707)	0.18 (0.292)	0.039 (0.817)	0.248 (0.139)
ALP (U/L)	0.072 (0.716)	0.381 (0.04)	0.069 (0.722)	0.252 (0.196)	-0.259 (0.175)	-0.013 (0.946)
GGT (U/L)	0.174 (0.358)	-0.222 (0.231)	0.083 (0.658)	0.151 (0.424)	0.292 (0.111)	-0.028 (0.882)
Glucose (mg/dL)	0.064 (0.723)	-0.184 (0.298)	-0.212 (0.228)	0.131 (0.466)	0.115 (0.516)	-0.108 (0.543)
Serum sodium (mmol/L)	-0.183 (0.299)	-0.001 (0.997)	-0.154 (0.376)	0.078 (0.662)	0.174 (0.316)	0.211 (0.224)
Cholesterol (mg/dL)	-0.159 (0.529)	0.208 (0.392)	-0.163 (0.505)	-0.22 (0.38)	0.036 (0.882)	-0.131 (0.594)
Triglycerides (mg/dL)	-0.421 (0.092)	-0.365 (0.137)	0.117 (0.645)	-0.318 (0.213)	0.401 (0.099)	0.096 (0.706)
Height (cm)	-0.76 (0.679)	-0.357 (0.041)	-0.083 (0.647)	-0.269 (0.136)	0.206 (0.25)	0.02 (0.912)
Weight (kg)	0.607 (P < 0.001)	-0.03 (0.867)	-0.019 (0.917)	0.152 (0.405)	0.306 (0.083)	0.136 (0.45)
BMI (kg/m^2)	0.705 (< 0.001)	0.163 (0.365)	0.01 (0.956)	0.304 (0.09)	0.202 (0.26)	0.149 (0.408)
Fat, %	0.783 (< 0.001)	0.186 (0.325)	-0.074 (0.696)	0.391 (0.036)	0.261 (0.164)	0.211 (0.263)
Fat mass, kg	0.794 (P < 0.001)	0.112 (0.556)	-0.071 (0.708)	0.41 (0.027)	0.333 (0.072)	0.22 (0.243)
FFM, kg	0.136 (0.472)	-0.1 (0.6)	-0.063 (0.743)	0.001 (0.998)	0.153 (0.42)	0.102 (0.592)
TBW, kg	0.312 (0.082)	-0.198 (0.27)	0.083 (0.648)	-0.004 (0.981)	0.389 (0.025)	0.1 (0.58)

RBP4: Retinol-binding protein 4; WBC: White blood cells; INR: International normalized ratio; PT: Prothrombin time; SGOT: Aspartate aminotransferase; SGPT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl-transferase; FFM: Fat-free mass; BMI: Body mass index; TBW: Total body water.

(P < 0.001), prothrombin time (P = 0.005), INR (P = 0.008) and total bilirubin levels (P = 0.029) (Table 3). The serum levels of adipokines and their distribution according to the severity of liver disease (assessed by Child-Pugh classification) are shown in Table 4. Median adiponectin levels were significantly higher in Child-Pugh C compared to Child-Pugh A (P = 0.04), whereas median RBP4 and apelin levels decreased across the spectrum of severity of liver disease (RBP4, P = 0.006; apelin, P = 0.034) (Figure 1). Furthermore, median adiponectin levels were positively associated with MELD score (r = 0.539; P < 0.001) (Figure 2A), whereas an inverse correlation was observed between RBP4 levels and MELD score (r = -0.439; P = 0.006) (Figure 2B). Results did not substantially change when patients with HCC (n = 5) were excluded from the analysis (data not shown). Patients with ascites had significantly higher adiponectin levels (P = 0.008) and decreased RBP4 levels (P = 0.001) compared to non-ascitic patients; leptin, resistin, visfatin and apelin levels were not significantly different between ascitic and non-ascitic patients (Table 5).

Following adjustment for fat mass, the median visfatin and adiponectin levels were significantly increased from Child-Pugh A to Child-Pugh C (both P < 0.001), whereas an inverse correlation with Child-Pugh classification was observed for both RBP4 and apelin levels (both P < 0.001). Leptin and resistin levels were not associated with liver disease severity despite adjustment for BMI.

Table 4 Serum levels of adipokines in study population

	All patients (n = 40)	CP A (n = 18)	CP B (n = 10)	CP C (n = 12)	P value[1]
Leptin, ng/mL (range)	6.22 (1.04-53.87)	6.64 (1.43-30.75)	8.68 (3.07-53.87)	3.11 (1.04-42.53)	0.396
Adiponectin, μg/mL (range)	10.23 (2.13-77.75)	7.99 (2.13-47.67)	7.66 (4.9-76.85)	25.73 (3.48-77.75)	0.040
Resistin, ng/mL (range)	0.78 (0.43-1.07)	0.82 (0.43-1.03)	0.74 (0.47-1.004)	0.88 (0.52-1.07)	0.460
Visfatin, ng/mL (range)	4.5 (0.1-109)	3.74 (0.1-100)	12.32 (0.1-100)	4.5 (0.1-109)	0.536
Apelin, ng/mL (range)	5.595 (1.53-9.74)	5.83 (2.23-9.27)	6.94 (2.43-9.74)	3.97 (1.53-6.74)	0.034
RBP4, μg/mL (range)	5.12 (1.88-13.04)	6.48 (2.6-13.04)	6.56 (3.9-12.12)	2.89 (1.94-9.42)	0.006

[1]P value: Correlation of variables regarding different stages of liver disease. CP: Child-Pugh stage; RBP4: Retinol-binding protein 4.

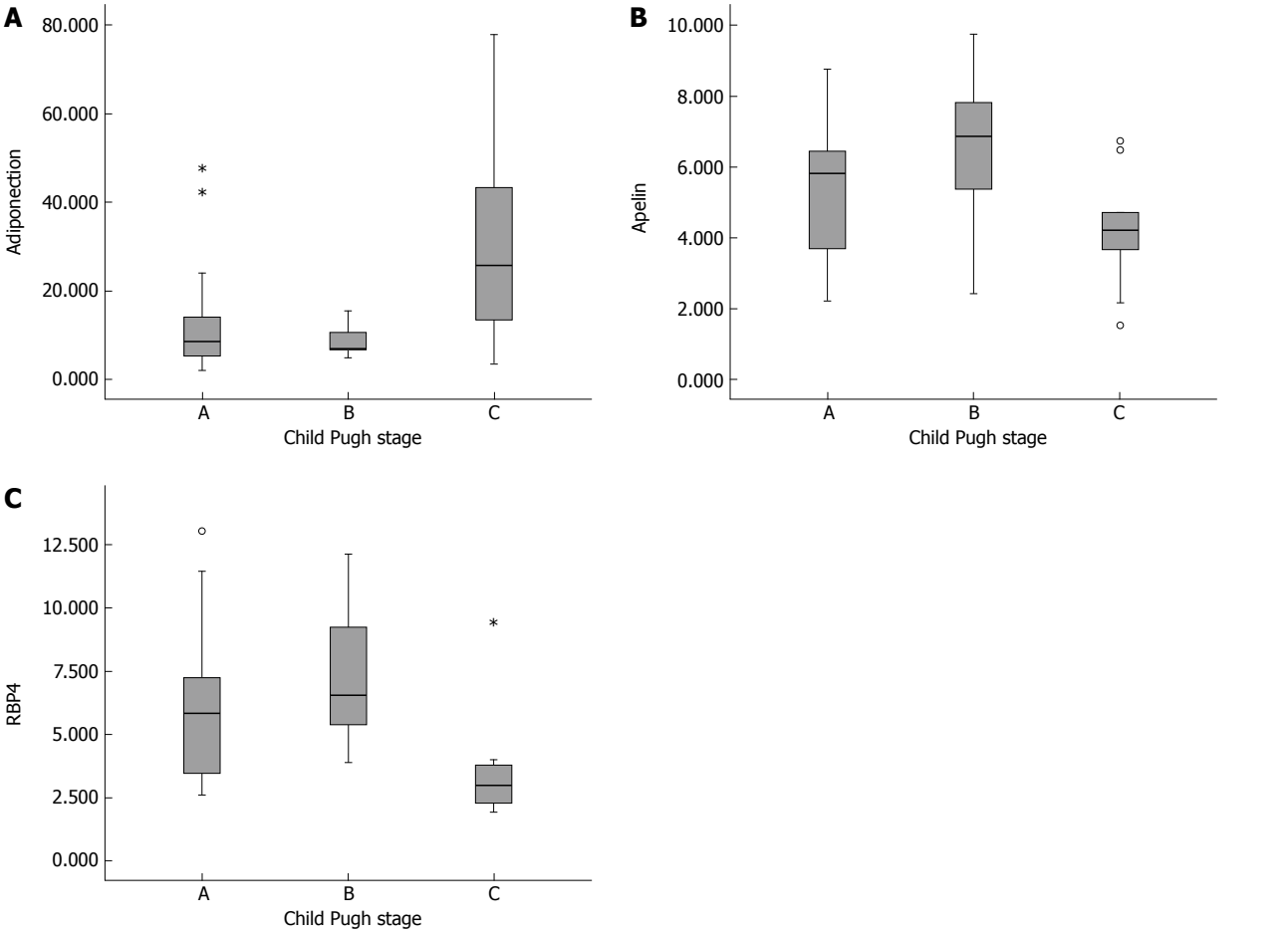

Figure 1 Box plots of adiponectin, apelin and retinol-binding protein 4 levels in relation to Child-Pugh stage in patients with alcoholic liver cirrhosis. A: Adiponectin [Child-Pugh (CP) A: 7.99 ± 12.99, CP B: 7.66 ± 21.85, CP C: 25.73 ± 24.6, P = 0.04]; B: Apelin (CP A: 5.83 ± 2.23, CP B: 6.94 ± 2.36, CP C: 3.97 ± 1.78, P = 0.034); C: Retinol-binding protein 4 (CP A: 6.48 ± 3.2, CP B: 6.56 ± 3.37, CP C: 2.89 ± 2.07, P = 0.006).

Multivariate analysis

Multivariate analyses were as follows: (1) Leptin: When sex, fat mass, PT and albumin levels were included in multiple linear regression analysis, leptin was significantly associated with fat mass (b = 0.603; P < 0.001) and albumin levels (b = -0.449; P = 0.002); (2) Adiponectin: Only advanced Child-Pugh stage was significantly correlated with adiponectin levels (b = 0.398; P = 0.032), when HCC, SGPT, SGOT and ALP were included in a multivariate model; (3) Visfatin: When fat mass and Child-Pugh stage were included in the analysis, only fat mass remained statistically significant (b = 0.403; P = 0.027); and (4) RBP4: In the multiple linear regression model including TBW, Child-Pugh stage, and HCC, only Child-Pugh stage was significantly associated with RBP4 levels (b = -0.328; P = 0.04).

Correlation of adipokines with overall survival

During a median follow-up of 32.5 mo (range: 10-43 mo), 7 patients (17.5%) died. In the univariate analysis, the factors significantly associated with overall survival were: adiponectin (HR = 1.046, 95%CI: 1.009-1.085; P = 0.013), INR (HR = 1.8, 95%CI: 1.5-3.2; P = 0.037), total bilirubin (HR = 2.1, 95%CI: 1.34-3.3; P = 0.001), Child-Pugh score (HR = 1.376, 95%CI: 1.003-1.089; P = 0.048) and MELD score (HR = 1.37, 95%CI: 1.118-1.68;

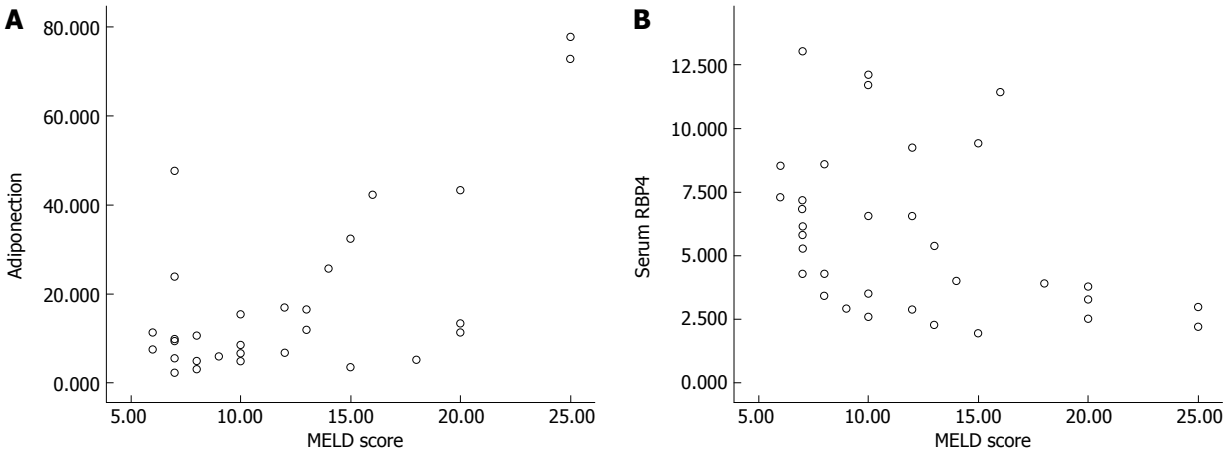

Figure 2 Correlation of serum adiponectin and retinol-binding protein 4 levels with Model for End-stage Liver Disease score in patients with alcoholic liver cirrhosis. A: Adiponectin (r = 0.539; P < 0.001); B: Retinol-binding protein 4 (r = - 0.439; P = 0.006).

Table 5 Serum levels of adipokines in patients with and without ascites

	Patients with ascites (n = 14)	Patients without ascites (n = 26)	P value
Leptin (range)	3.54 (1.04-42.53)	7.41 (1.43-53.87)	0.356
Adiponectin (range)	21.08 (4.06-77.75)	7.14 (2.125-72.8)	0.008
Resistin (range)	0.79 (0.47-1.06)	0.78 (0.43-1.07)	0.799
Visfatin (range)	10.4 (0.1-109)	4.01 (0.1-100)	0.219
Apelin (range)	4.01 (1.53-7.5)	6.09 (2.23-9.74)	0.089
RBP4 (range)	3.04 (1.88-8.6)	6.69 (2.6-13.04)	0.001

RBP4: Retinol-binding protein 4.

P = 0.002). In the Cox regression multivariate analysis including MELD, Child-Pugh score and adiponectin, only MELD was found predictive for survival (HR = 1.53, 95%CI: 1.05-2.32; P = 0.029).

DISCUSSION

In this study, we clearly show that there is a significant correlation of adiponectin, RBP4 and apelin levels with the severity of liver disease, independently of body mass index in the setting of alcoholic cirrhosis. This is the first study to evaluate together all the adipokines which have been associated with liver fibrogenesis, excluding patients with diabetes mellitus as a confounder, thus exploring the impact of adipose tissue on the pathophysiology of liver injury and repair.

Leptin resistance is a well-known feature of obesity[42], confirmed in our study by the significantly higher levels of leptin in overweight and obese patients and the strong association of leptin levels with fat mass. Another major determinant of leptin levels is gender, with female subjects having higher serum concentrations[43], a similar finding in our study. The relationship of leptin with the severity of liver disease is debated. McCullough *et al*[14] found no relationship with the degree of liver failure, whereas Campillo *et al*[43] observed a significant correlation in female patients with alcoholic cirrhosis. In our study, leptin levels were significantly associated with low albumin and high prothrombin time levels. In the multivariate analysis, low albumin levels and high fat mass were independently associated with leptin levels. This indirectly reflects that the decreased hepatic metabolism and the increased release from fat tissues are the most likely mechanisms of increased leptin levels in cirrhosis[16].

Adiponectin has been shown to be elevated in cirrhosis independently of the etiology of liver disease[44] and it was recently found to be independently associated with hepatic fat loss in advanced stages of NAFLD[45]. We showed a significant association with the severity of liver disease-assessed by Child-Pugh and MELD, as well as with individual components of Child's classification, independently of BMI. Adiponectin levels increased proportionally with the Child's classification stage, which was the only independently associated factor with adiponectin levels in the multivariate model. This finding confirms the results of others[26,44,46,47]. A positive association was also found with higher levels of serum transaminases, probably reflecting a link of adiponectin with hepatic inflammation. Tacke *et al*[47] reported that patients with biliary cirrhosis had the highest levels of adiponectin, suggesting that biliary secretion is involved in adiponectin clearance. We found a significant association of adiponectin with bilirubin levels and ALP, suggesting that adiponectin might be related to cholestasis. In our study, no association of adiponectin with BMI or fat mass was found. It seems that in liver cirrhosis, there is no increased production of adiponectin related to fat mass, but the elevated levels are linked to reduced hepatic metabolism. In addition, considering the hepatoprotective effect of adiponectin[24,25], it is likely that the increased levels of this adipokine in cirrhosis might reflect an anti-inflammatory response to liver injury, which is dependent to the degree of disease severity.

In our study resistin levels were not associated with somatometric characteristics. This reflects the findings

from other studies[48,49], in which adipocytes were not the main sites of resistin synthesis, while inflammatory cells seem to be the major source of human resistin[50]. Although there is data showing that increased levels of resistin in cirrhosis are associated with the severity of liver disease[4,31,32], this was not confirmed by our study, but this may be due to a type 2 error.

Visfatin is increased in obesity-related disorders[51]. This was confirmed by this study, which showed a significant correlation of visfatin levels with BMI and fat mass. This is the second study to evaluate this adipokine in cirrhosis. In the other study[52], plasma levels of visfatin were assessed in 19 patients with cirrhosis and in 19 BMI, sex and age-matched controls. Circulating visfatin was 78% lower in cirrhotics ($P < 0.001$) and decreased with worsening stage of liver disease. Hepatic visfatin secretion also decreased with clinical stage; furthermore, hepatic visfatin mRNA expression was lower in cirrhosis than controls ($P < 0.05$). Serum visfatin levels were correlated with fat mass and body cell mass but not with insulin resistance[52]. We found that visfatin levels increased across the spectrum of severity of liver disease, after correction for BMI, thus implying reduced hepatic metabolism.

We showed that RBP4 levels decreased with worsening liver disease independently of BMI. Our results are similar to those of Yagmur *et al*[53] who evaluated RBP4 levels in 111 patients with chronic liver diseases and 99 age- and sex-matched control subjects. RBP4 was significantly reduced compared with controls and correlated with the stage of cirrhosis. Patients without cirrhosis showed normal RBP4 levels, which correlated with serum glucose and insulin secretion, and inversely correlated with insulin sensitivity. However, in patients with cirrhosis, RBP4 was not associated with glucose metabolism but was linked to the hepatic biosynthetic capacity or portal hypertension. We also found an association of RBP4 with TBW, which probably reflects the correlation of RBP4 with ascites. In addition the association of RBP4 with INR and total bilirubin imply that the liver is the primary source of RBP4 synthesis and its concentration declines along with disease progression and decrease of hepatic biosynthetic capacity.

This is the first study to evaluate the association of apelin levels with the severity of liver disease in patients with alcoholic cirrhosis. We found that serum apelin levels decreased along with worsening liver disease. Principe *et al*[37] suggested that the hepatic apelin system is markedly and selectively activated in cirrhosis and the increased levels mainly derive from enhanced hepatic production, implying that the hepatic apelin system is an important mediator in the initiation and maintenance of the inflammatory and fibrogenic processes. In our study apelin levels progressively decline as the biosynthetic capacity of liver decreases.

A limitation of our study is the relative small sample size. However, we prospectively studied patients with cirrhosis attributed only to alcohol abuse and our findings are similar to those of larger prospective studies. A significant difference of our study compared to others is that we excluded patients with diabetes as there is a significant association of adipokines with insulin resistance, which introduces a confounding factor in the results of most previous publications.

In conclusion, we demonstrate that adiponectin, RBP4 and apelin levels are deregulated in liver cirrhosis in accordance to the degree of liver dysfunction. The alterations in the pattern of these adipokines might be implicated in the complex process of hepatic injury and repair and this association should be studied in larger, well-designed prospective studies. The significance of novel drugs interfering with adipokine production and expression (especially the adiponectin system) in liver fibrogenesis deserves to be investigated further.

COMMENTS

Background

Adipokines are polypeptide hormones produced predominantly by adipose tissue. Ongoing data suggest that obesity and insulin resistance are associated with a more rapid progression of the fibrogenic process in chronic liver diseases, with different adipokines contributing to the complex pathophysiology of hepatic injury and repair. Increased levels of leptin, adiponectin and resistin have been reported in cirrhosis but diabetic patients have not been excluded, although diabetes alters adipokine concentrations. It is also unclear whether the severity of cirrhosis is associated with the adipokine concentrations. Alcoholic cirrhosis has been the least studied.

Research frontiers

In the area of liver cirrhosis, the research hotspot is the understanding of the molecular mechanisms that take place during the fibrogenic process as well as the complex interactions among these mechanisms. Adipokines seem to play an important role in this process and there is an ongoing *in vivo* and *in vitro* research on the way they interact with each other or with other cytokines to enhance or prevent liver fibrogenesis.

Innovations and breakthroughs

The main difference between this study and other relevant studies is the measurement of adipokine levels only in patients with alcoholic cirrhosis and not in cirrhotic patients of various etiologies. Considering that adipokine levels are associated with the etiology of chronic liver disease, the inclusion only of patients with alcoholic cirrhosis aimed to prevent bias from disease etiology as a confounding factor. Another innovation of this study relevant to others is the exclusion of patients with overt diabetes mellitus and/or use of insulin or oral antidiabetic/insulin-sensitizing medication considering that diabetes by itself alters the serum adipokine concentrations.

Applications

This study shows that adiponectin, RBP4 and apelin levels are deregulated in liver cirrhosis in accordance to the degree of liver dysfunction and, thus, implies that certain adipokines (especially the adiponectin system) are potentially therapeutic targets in the prevention or delay of progress of liver fibrogenesis in alcoholic cirrhosis.

Terminology

Adipokines are polypeptide hormones produced predominantly by adipose tissue. In obesity, excess fat represents a dysfunctional inflammatory tissue with alterations in the pattern of adipokines' expression which contributes to obesity-related disorders. Cirrhosis is the end-stage of chronic liver diseases characterized by the replacement of normal liver tissue by scar tissue resulting in the formation of liver nodules and alterations in liver architecture. However, not all cirrhotic patients have the same disease severity and thus, short-term prognosis; Different scoring systems have been developed for the prognostication of patients with end-stage liver disease including mainly Child-

Pugh class and Model for End-stage Liver Disease scoring system.

Peer-review

This is the hard work effort done throughout the study and the interesting methods. The authors evaluated the serum levels of six adipokines in non-diabetic patients with alcoholic cirrhosis and investigated their potential association with liver disease severity.

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