JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2020, 71, 1, 113-123
www.jpp.krakow.pl | DOI: 10.26402/jpp.2020.1.11

M. PAZGAN-SIMON[1,2], J. ZUWALA-JAGIELLO[3], T. MENZYK[4], M. BATOR[4], A. DERRA[4], A. LEKSTAN[5], E. GRZEBYK[3], K. SIMON[1,2], M. KUKLA[2,6,7]

SERUM BETATROPHIN AND IRISIN LEVELS IN HEPATOCELLULAR CARCINOMA

[1]Department of Infectious Diseases, Liver Diseases and Acquired Immune Deficiency, Wroclaw Medical University, Wroclaw, Poland; [2]Department of Infectious Diseases, Regional Specialistic Hospital in Wroclaw, Wroclaw, Poland; [3]Department of Pharmaceutical Biochemistry, Wroclaw Medical University, Wroclaw, Poland; [4]Medical University of Silesia in Katowice, Katowice, Poland; [5]Department of Digestive Tract Surgery, Medical University of Silesia in Katowice, Katowice, Poland; [6]Department of Internal Medicine and Geriatrics, Jagiellonian University Medical College, Cracow, Poland; [7]Department of Endoscopy, University Hospital in Cracow, Cracow, Poland

Hepatocellular carcinoma (HCC) development is a complex process with well-known risk factors, however the role of betatrophin/angiopoietin-like protein 8 and irisin has been poorly investigated thus far. The aim of this study is to measure betatrophin and irisin serum levels in HCC, cirrhotic patients and controls, assess their relationship with cancer etiology and grade, metabolic abnormalities and liver dysfunction severity. Serum betatrophin and irisin concentrations were measured with commercially available ELISA kits in 69 cirrhotic patients with HCC, 24 patients with non-viral cirrhosis and 20 healthy volunteers. The severity of liver disfunction was assessed according to Child-Pugh (C-P) score, while HCC grade according to the Barcelona clinic liver cancer (BCLC) staging system. Serum betatrophin concentration was significantly higher (33.7 ± 13.4 versus 12.3 ± 2.0 ng/ml; $P < 0.001$), while serum irisin level significantly lower in HCC patients compared to controls (2.52 ± 1.14 versus 4.46 ± 1.34 μg/ml; $P = 0.02$). Betatrophin level was also significantly elevated among cirrhotic patients compared to healthy volunteers. More evident serum betatrophin increase was found in patients with viral disease (34.8 ± 12.9 versus 26.1 ± 13.8 ng/ml; $P < 0.001$). Serum irisin concentration was significantly decreased in more advanced HCC cases (stage A versus C according to BCLC: 3.4 ± 1.3 versus 1.89 ± 1.1 μg/ml; $P = 0.02$). Decline of serum irisin (A: 3.4 ± 1.2; B: 2.42 ± 0.8; C: 1.91 ± 1.19 μg/ml; $P = 0.03$) and up-regulation of serum betatrophin levels (A: 24.1 ± 13.8; B: 39.3 ± 11.4; C: 46.2 ± 9.4 ng/ml; $P = 0.03$) were observed in patients with more advanced cirrhosis according to C-P score. We concluded that betatrophin serum level increased in cirrhotic patients, compared to controls. Since there was no difference between cirrhotic patients with and without intercurrent HCC, we suppose it may have an influence on fibrosis development, however not hepatocarcinogensis. Irisin serum level decreased in HCC patients, especially with more advanced disease grade, and was inversely related to the severity of liver disfunction.

Key words: *hepatocellular carcinoma, irisin, betatrophin/angiopoietin-like protein 8, liver cirrhosis, obesity adipokines, hepatokines, diabetes mellitus type 2*

INTRODUCTION

Hepatocellular carcinoma (HCC), the most prevalent histological subtype of primary liver malignancies, is the fifth most common form of cancer worldwide and the third most common cause of cancer-related deaths (1). The most important risk factor for HCC remains liver cirrhosis. To the other important risk factors for HCC belong infections with the hepatitis B virus (HBV) and hepatitis C virus (HCV), excessive consumption of alcohol and exposition to aflatoxin B1, but the role of another factors is increasingly emphasized - obesity, type 2 diabetes mellitus (T2DM) and hepatic manifestation of the metabolic syndrome, non-alcoholic fatty liver disease (NAFLD) (2, 3). Hepatic cancer may simply be a complication of any stage of NAFLD, unlikely to that seen in advanced stages of other chronic liver diseases. Obesity and excessive adipose tissue contribute to a chronic systemic low-grade inflammatory response that is believed to play an important role in HCC development (4, 5). This, and other pathophysiological mechanisms underlying the development of NAFLD, such as insulin resistance (IR) and hyperinsulinaemia, adipokine and hepatokine dysregulation or genetic and environmental factors, seem to play a significant role in the development of HCC (6). In HCC there is a number of staging systems available, but the only one assesses the severity of liver disease as well as the patient's performance status (PS) and cancer-related symptoms - the Barcelona Clinic Liver Cancer (BCLC) classification (7).

Betatrophin/angiopoietin-like protein 8 (ANGPTL8), produced by liver and adipose tissue, is a novel but atypical member of the angiopoietin protein family due to its lack of the fibrinogen-like domain, the aminoacids for forming disulfide bonds and the glycosylation sites (8, 9). Increased levels of

betatrophin have been observed in various clinical conditions such as obesity and T2DM, suggesting a role for this protein in pathogenesis of these diseases (10). Abnormalities in serum levels of ANGPTL8 have also been reported in significant liver fibrosis and NAFLD. According to previous publication serum betatrophin appeared to increase in NAFLD patients and may be a potential non-invasive marker for identification of NAFLD and non-alcoholic steatohepatitis (NASH) (11). The plasma levels of betatrophin are also increased in patients with liver cirrhosis and these increases were more pronounced in patients with advanced liver disease, particularly Child-Pugh (C-P) class C or those with a higher Model for End-Stage Liver Disease (MELD) score (12). This elevation seems to be connected to IR, which is a known potent stimulator of betatrophin expression in liver and fat tissue. So far, only one study has been performed to prove the direct role of betatrophin in carcinogenesis of HCC (13). ANGPTL8 may act as a mild suppressor against hepatocellular carcinoma cells *via* affecting mRNA expression of two key regulators of Wnt signaling pathway, β-catenin and WIF-1, and subsequently inhibiting proliferation of the hepatocellular carcinoma cell line HepG2.

Irisin is a recently identified novel myokine, predominantly produced and secreted from skeletal muscle as a cleavage product of fibronectin type III domain containing 5 (FNDC5) (14). Irisin appears to exert a variety of functions, however most of them has not been confirmed yet. It seems to be capable to induce browning of primarily subcutaneous and visceral white adipose tissue and thereby induces thermogenesis. Therefore, it has been proposed to mediate the beneficial effects of exercise on metabolism and may reveal a new target for obesity, and its related diseases, like T2DM or NAFLD (15). The results of previous publications investigating the association between irisin and NAFLD are inconsistent, since both increase and decrease of its level have been observed (16). Irisin is also considered in the development of other liver pathologies such as primary biliary cholangitis (PBC) or alcoholic cirrhosis (AC). Among AC patients this myokine concentration was decreased, on the other hand positive correlation of it with grade of inflammation in PBC was shown (17). As we can easily notice the role of irisin in chronic liver diseases is still unclear and requires further investigation. Interestingly, it was recently proved that skeletal muscles are not the sole source of irisin. It was reported that adipose tissue also expresses and secretes irisin, suggesting that it may function not only as a myokine, but also as an adipokine (18).

Pointing to essential role of betatrophin and irisin in metabolic regulation and energy expenditure we hypothesize that they play an important role in chronic liver diseases and HCC. Since ANGPTL8 is associated with grade of fibrosis, and hence with progression of liver cirrhosis, possibly it plays an indirect role in carcinogenesis process. In contrast, irisin may be the myokine inhibiting the development of cancer. The main aim of the study was to assess betatrophin and irisin serum levels in HCC patients and compare their levels in patients with different cancer grade and etiology. The additional purpose was to analyze relationship between metabolic abnormalities, severity of liver disfunction and betatrophin and irisin serum levels.

MATERIAL AND METHODS

Patients' selection

The study was approved by the Ethics Committee of the Medical University in Wroclaw and conformed to the ethical guidelines of the Declaration of Helsinki (KNW/0022/KB1/95/13). Informed consent was obtained for the whole study series.

The study included 69 cirrhotic patients with contrast Computed tomography (CT)/magnetic resonance imaging (MRI) proven HCC, demonstrating typical radiological symptoms - a luminal focal lesion undergoing contrast enhancement in the arterial phase and the phenomenon of leaching in the late venous phase (HCC group). The study included also 24 patients with non-viral liver cirrhosis without HCC (cirrhosis group). Cirrhosis in these patients was diagnosed on the basis of the interview (history of chronic liver disease), physical examination (typical signs of cirrhosis: *i.e.* spider naevi, palm erythema, ascites, spleno- and/or hepatomegaly, nails abnormalities, jaundice), imaging (ultrasound, CT, MRI) or endoscopy (presence of signs of portal hypertension *i.e.* gastropathy, esophagal/gastric varices) and abnormal laboratory findings. The diagnosis of chronic HBV infection was based on raised serum transaminases for at least 6 months, positivity for hepatitis B virus surface antigen (HBsAg) and anti-HB core IgG antibodies. Chronic hepatitis C was established in case of the presence of anti-HCV antibodies together with HCV-RNA for more than 6 months. The control group comprised 20 healthy volunteers. The volunteers had no complaints at the time of participation in the study as well as no history of gastrointestinal or chronic liver diseases, smoking and alcohol intake, and systemic diseases. They had a constant body mass and a good appetite. These healthy subjects had normal activity of aminotransferases, gamma-glutamyltransferase (GGTP) or alkaline phosphatase (ALP). Exclusion criteria for the study groups included: patients' no permission for contribution in research, other malignances, insulin dependent diabetes mellitus, chronic heart failure, thyroid disease, chronic renal failure, mental illnesses and cirrhosis based on primary sclerosing cholangitis (PSC) (19), PBC (20) and autoimmune hepatitis (AIH) (21). The clinical data that excluded patients from the control group were: elevated alanine aminotransferase (ALT) activity; alcohol consumption of more than 20 g/day, presence of anti-HCV antibodies, presence of HBsAg and HIV infection.

Serological assays

Irisin and betatrophin serum concentrations were assessed in duplicate by an immunoenzymatic method with commercially available enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) kits: betatrophin (active) human Elisa Kit (sensitivity: 450 pg/ml; intra-assay CV: 4 – 6%; inter-assay CV: 8 – 12%; catalogue No. SK00528-02; Aviscera Bioscience, Inc.; USA) and Irisin Elisa Kit (sensitivity: 1 ng/ml; intra-assay CV: 4.86 – 6.75%; inter-assay CV: 9.67 – 9.72%; catalogue No. RAG018R; BioVendor-Laboratorni Medicina a.s.). Insulin concentration was measured using a Diametria Insulin EIA Kit (catalogue No. DKO076; Diametra S.r.l).

The remaining biochemical parameters were measured using routine methods. The upper limit of normal (ULN) of ALT activity was set at 38 IU/l and the ULN of aspartate aminotransferase (AST) activity at 40 IU/l. The only inflammatory marker assessed in our study was white blood cells count (WBC). Insulin resistance was calculated according to the homeostasis model assessment for insulin resistance (HOMA-IR) by the formula: fasting insulin level (mUI/l) × fasting glucose level (mg/dl)/405. We divided patients into two subgroups with HOMA-IR value < 4 and ≥ 4. For further analysis, we defined two subgroups: body mass index (BMI) < 30 and BMI ≥ 30 kg/m^2. According to guidelines of the World Health Organization (WHO) (22), all of the patients with a BMI of ≥ 30 kg/m^2 were classified as being obese. The diagnosis of T2DM was made according to the WHO criteria (23), with a value of fasting blood glucose level of ≥ 126 mg/dl on at least two occasions, or ongoing treatment with hypoglycemic agents.

Table 1. General characteristics and basic laboratory tests of cirrhotic patients with hepatocellular carcinoma (HCC), cirrhotic patients without HCC and control group. All results presented as median ± SD (min.-max. values).

Parameter	**HCC group** n = 69	**Cirrhosis group** n = 24	**Control group** n = 20	**P***	**P****
Age (years)	59.0 ± 12.1 (20.0 – 88.0)	59.0 ± 14.0 (24.0 – 84.0)	40.6 ± 5.5	0.62	0.65
BMI (kg/m^2)	29.0 ± 4.49 (22.0 – 45.0)	29.0 ± 4.16 (22.0 – 38.0)	25.4 ± 4.1	0.56	0.25
Waist grith (cm)	102.0 ± 12.5 (70.0 – 139.0)	100.0 ± 10.2 (80.0 – 120.0)	78.2 ± 5.4	0.14	0.03
Irisin (μg/ml)	2.52 ± 1.14 (0.41 – 4.73)	2.88 ± 1.85 (1.59 – 4.75)	4.46 ± 1.34	0.02	0.02
Betatrophin (ng/ml)	33.7 ± 13.3 (10.9 – 49.9)	36.8 ± 26.9 (0.00 – 49.6)	12.3 ± 2.0	<0.001	<0.001
Fasting insulin (ng/mL)	0.89 ± 0.91 (0.31 – 3.94)	0.85 ± 1.38 (0.23 – 3.01)	0.44 ± 0.15	0.007	0.002
Fasting insulin (mIU/mL)	20.2 ± 20.9 (5.22 – 90.9)	19.7 ± 31.9 (5.34 – 69.5)	10.2 ± 3.45	0.007	0.002
WBC ($10^6/\mu L$)	4.30 ± 2.46 (1.78 – 10.4)	3.69 ± 2.13 (1.95 – 9.47)	5.20 ± 0.85	0.06	0.35
HGB (mg/dL)	11.8 ± 2.45 (5.70 – 16.1)	11.7 ± 4.05 (3.16 – 16.5)	14.5 ± 1.41	0.12	0.14
PLT ($10^6/\mu L$)	88.5 ± 85.0 (18.0 – 392.0)	80.5 ± 52.5 (11.2 – 293.0)	220.5 ± 50.7	0.12	0.02
ALT (IU/L)	45.0 ± 48.5 (14.0 – 303.0)	31.5 ± 36.5 (15.0 – 222.0)	22.6 ± 13.6	0.05	0.01
AST (IU/L)	63.0 ± 65.1 (16.0 – 409.0)	46.0 ± 49.5 (20.0 – 258.0)	24.0 ± 9.8	0.07	0.008
ALP (IU/L)	110.0 ± 89.4 (55.0 – 476.0)	102.0 ± 82.0 (38.0 – 363.0)	65.5 ± 25.6	0.25	0.01
GGTP (IU/L)	91.0 ± 194.6 (21.0 – 1243.0)	78.0 ± 92.0 (23.0 – 965.0)	24.6 ± 15.7	0.09	<0.001
Fasting glucose (mg/dL)	106.8 ± 43.9 (79.1 – 267.9)	112.1 ± 44.4 (56.7 – 281.0)	89.9 ± 9.5	0.45	0.02
Urea (mg/dL)	35.3 ± 30.5 (16.6 – 139.1)	33.1 ± 20.9 (14.7 – 229.3)	31.6 ± 9.4	0.34	0.45
Creatinine (mg/dL)	0.79 ± 0.27 (0.49 – 2.24)	0.79 ± 0.24 (0.55 – 37.4)	0.78 ± 0.13	0.67	0.09
Bilirubin (mg/dL)	1.58 ± 18.1 (0.43 – 152.1)	1.49 ± 1.72 (0.48 – 152.1)	1.10 ± 0.45	0.58	0.01
Cholesterol (mg/dL)	147.0 ± 50.1 (87.0 – 374.0)	143.2 ± 43.7 (2.02 – 230.0)	169.7 ± 50.5	0.17	0.65
Triglycerides (mg/dL)	105.0 ± 78.64 (40.0 – 180.0)	102.8 ± 69.5 (46.0 – 676.1)	128.6 ± 21.4	0.64	0.31
HDL (mg/dL)	37.4 ± 31.0 (12.2 – 55.0)	41.9 ± 19.8 (7.80 – 235.8)	44.9 ± 8.1	0.08	0.42
Prothrombin index (%)	75.0 ± 14.1 (40.0 – 102.8)	69.6 ± 18.0 (40.0 – 101.0)	95.7 ± 5.3	0.19	0.03
Total protein (g/dL)	7.10 ± 0.94 (4.30 – 9.80)	7.00 ± 1.05 (5.40 – 8.00)	7.30 ± 0.31	0.81	0.12
Albumin (g/dL)	3.13 ± 0.59 (1.90 – 4.50)	3.39 ± 0.57 (2.30 – 4.60)	4.85 ± 0.34	0.91	0.04
AFP (ng/mL)	16.0 ± 1145.0 (0.74 – 5845.0)	4.41 ± 5.41 (1.01 – 40.0)	2.70 ± 1.08	<0.001	<0.001
CEA (ng/mL)	2.86 ± 2.91 (0.17 – 18.9)	3.44 ± 3.32 (0.50 – 12.2)	2.45 ± 1.56	0.76	0.54
CA 19.9 (U/mL)	15.1 ± 89.3 (2.00 – 728.7)	14.5 ± 14.9 (2.49 – 200.0)	19.7 ± 4.6	0.69	0.28
HOMA-IR	5.47 ± 8.10	5.46 ± 7.98	2.53 ± 0.09	0.49	<0.001

AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CA 19.9, carbohydrate antigen 19.9; CEA, carcinoembryionic antigen; ALP, alkaline phosphatase; GGTP, gamma-glutamyltransferase; HDL, high-density lipoprotein; HGB, hemoglobin; HOMA-IR, homeostasis model assessment for insulin resistance; WBC, white blood cells; PLT, platelet count; P*, HCC versus cirrhotic patients without HCC, P**, HCC patients versus control group.

All of the outcomes were assessed according to Child-Pugh (C-P) score and BCLC classification. Child-Pugh score is widely used to assess the severity of liver dysfunction in clinical work. It consists ascites, hepatic encephalopathy (HE), prothrombin time, total bilirubin and albumin. Each measure is scored 1 – , with 3 indicating most severe derangement. The sum of these point characterizes patient to one of group: into C-P grades A (5 – 6 points), B (7 – 9 points), C (10 – 5 points). The Barcelona clinic liver cancer staging system combines the stage of the disease to a specific treatment algorithm. It is useful to select early stage patients who could benefit from curative therapies. The scale divides patients into five groups, from 0 to D, where patients at stage 0 with very early HCC are optimal candidates for resection and patients at stage D with end-stage disease will receive symptomatic treatment.

Statistical analysis

The statistical analysis was performed with STATISTICA 10.0 (StatSoft Polska Sp z o.o., Cracow, Poland). The statistical significance of the difference in studied variables were tested using the Mann-Whitney U-test and ANOVA rang Kruskal-Wallis tests for independent groups. The data were expressed as median ± standard deviation (SD). The Shapiro-Wilk test was used to evaluate the distribution. Correlations were analyzed with the Spearman rank correlation coefficient. Statistical significance was defined as values of $P < 0.05$.

The statistical data used to support the findings of this study are available from the corresponding author upon request.

RESULTS

Clinical and demographic data of analyzed group

Clinical and demographic data of 69 patients (54 men and 15 women, median age 59.0 ± 9.0 years) with viral and non-viral cirrhosis, and HCC as well as 24 patients (20 men and 4 women, median age 59.0 ± 12.4 years) with non-viral cirrhosis without HCC are summarized in *Table 1*. Additionally, some of HCC patients suffered from other diseases, such as hypertension (HT) - 20 patients (29.0%) and T2DM - 27 patients (39.1%). Among cirrhotic patients 4 (16.7%) of them suffered from HT and 9 (37.5%) from T2DM. As a leading risk factor of HCC, viral cirrhosis was presented among 43 patients (62.3%), 35 of them were HCV positive and 8 - HBV positive. The diagnosis of cirrhosis was based on imaging. Despite the fact that liver biopsy is considered as a gold standard of cirrhosis diagnosis, none of the patients had performed invasive diagnostic procedures. The control group comprised 20 healthy volunteers (10 females/10 males), age 40.6 ± 5.5 years.

General characteristic of HCC patients, cirrhotic patients and healthy volunteers are presented in *Table 1*.

Comparison of hepatocellular carcinoma patients, cirrhotic patients and control group with respect to serum irisin and betatrophin and glucose metabolism

When compared HCC patients with healthy volunteers, there were significantly increased betatrophin levels in HCC patients (33.7 ± 13.4 versus 12.3 ± 2.00 ng/ml; $P < 0.001$). Similar results were observed when compared cirrhotic patients with control group (36.8 ± 26.9 versus 12.3 ± 2.0 ng/ml; $P < 0.001$). However there was no significant difference in betatrophin concentration between HCC and cirrhotic patients (33.7 ± 13.4 versus 36.8 ± 26.9; $P = 1.00$) (*Fig. 1A*). Serum irisin level was significantly lower comparing HCC and control groups (2.52 ± 1.14 versus 4.46 ± 1.34 μg/ml; $P = 0.02$). There was no significant differences between cirrhotic patients and healthy volunteers as well as HCC and cirrhotic patients with respect to irisin levels (*Fig. 1B*). Comparison of HCC patients, cirrhotic patients and control group with respect to serum irisin and betatrophin is presented in *Table 2*. Fasting insulin level was significantly higher in HCC patients compared to control group (20.2 ± 20.9 versus 10.2 ± 3.4 mIU/ml, $P = 0.002$) and in cirrhotic patients compared to healthy volunteers (19.7 ± 31.9 versus 10.2 ± 3.4 mIU/ml, $P = 0.04$), while there was no difference between HCC and cirrhotic patients. Similary fasting glucose concentration (106.8 ± 43.9 versus 89.9 ± 9.5 mg/dl; 112.1 ± 44.4 versus 89.9 ± 9.5 mg/dl) and HOMA-IR (5.47 ± 8.10 versus 2.53 ± 0.09; 5.46 ± 7.98 versus 2.53 ± 0.09) were significantly higher among HCC as well as cirrhotic patients in comparison to control group.

Comparison between males and females with hepatocellular carcinoma

Serum betatrophin concentration was significantly higher in women compared to men (46.1 ± 9.1 versus 26.3 ± 13.4 ng/ml; $P = 0.008$). There was no significant difference in irisin concentration between males and females ($P = 0.78$) (*Table 3*).

Comparison between patients with viral and non-viral livers disease

Betatrophin serum concentration was significantly increased in patients with viral compared to those with non-viral disease etiology (34.8 ± 12.9 versus 26.1 ± 13.8; $P < 0.001$). Furthermore, this elevation became more evident if we considered only HCV positive patients (39.2 ± 11.9 versus 26.1 ± 13.8 ng/ml; $P = 0.02$). There was no significant difference in serum irisin levels when compared patients with viral and non-viral disease (2.12 ± 1.13 versus 3.02 ± 1.10 μg/ml; $P = 0.32$). Additionally, we observed significantly higher levels of alpha-fetoprotein (AFP) (37.7 ± 1414.2 versus 4.82 ± 13.1 ng/ml; $P < 0.001$) and carcinoembryonic antigen (CEA) (3.11 ± 3.36 versus 2.30 ± 1.61 ng/ml; $P = 0.03$) in the group of HCC patients with viral compared to those with non-viral disease etiology. Inversely, albumin and glucose serum concentrations were significantly decreased in HBV and HCV positive patients when compared to non-viral patients. There were

Table 2. Comparison of hepatocellular carcinoma patients, cirrhotic patients and control group with respect to serum irisin and betatrophin. All results presented as median ± SD.

Parameter	**HCC group** n = 69	**Cirrhosis group** n = 24	**Control group** n = 20	**HCC/ Cirrhosis** (P-value)	**HCC/ Control** (P-value)	**Cirrhosis/ Control** (P-value)
Betatrophin (ng/ml)	33.7 ± 13.3	36.8 ± 26.9	12.3 ± 2.0	1.00	< 0.001	< 0.001
Irisin (μg/ml)	2.52 ± 1.14	2.88 ± 1.85	4.46 ± 1.34	0.55	0.02	0.14

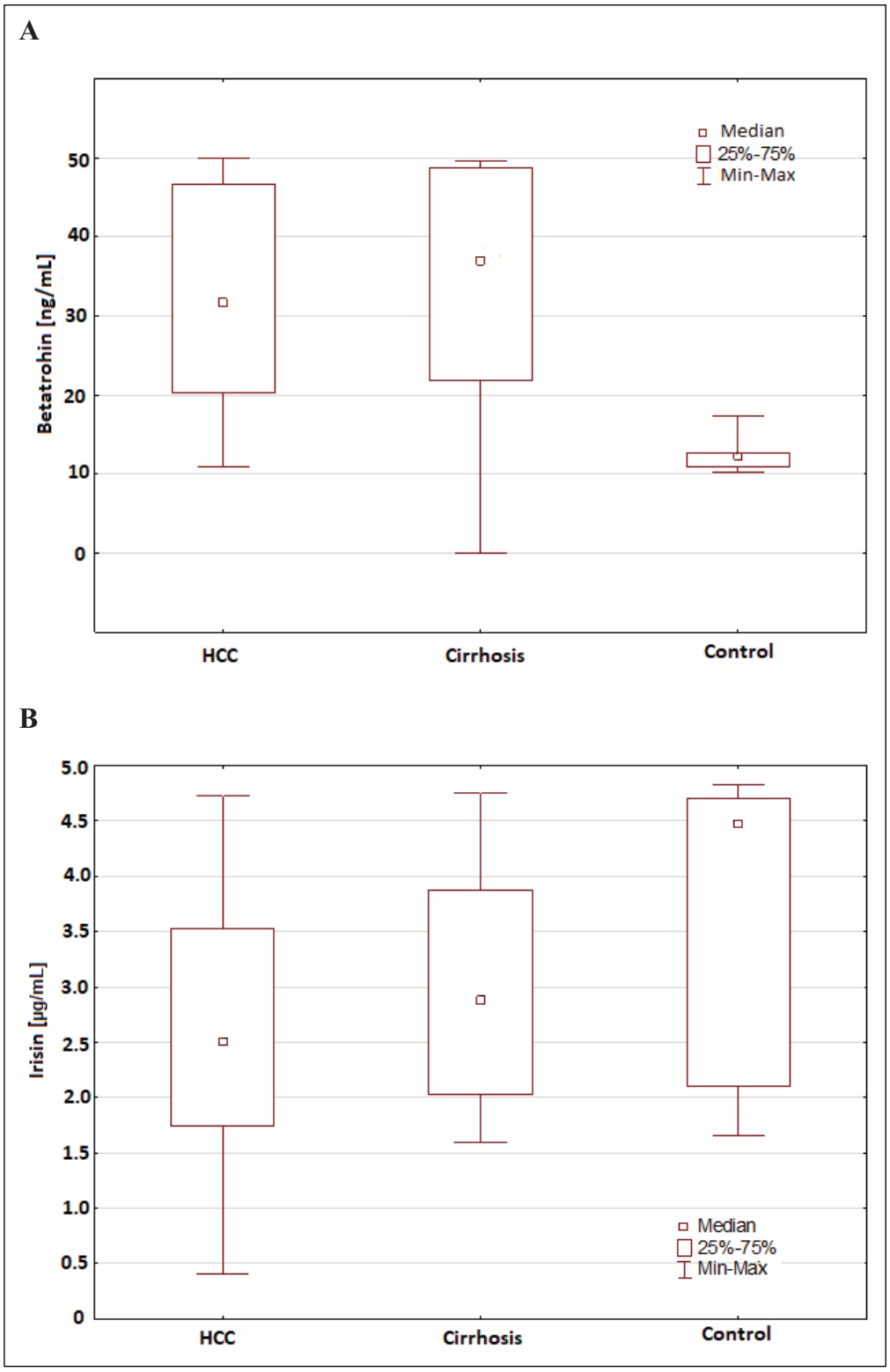

Fig. 1. (A): Comparison of betatrophin concentration between HCC patients, cirrhotic patients and healthy volunteers. (B): Comparison of irisin concentration between HCC patients, cirrhotic patients and healthy volunteers.

no significant differences with respect to insulin and HOMA-IR levels in the groups of patients with viral and non-viral disease (*Table 4*).

Comparison between patients with and without type 2 diabetes mellitus and between obese and non-obese ones

No significant difference was observed in betatrophin and irisin serum concentrations among patients with and without T2DM (30.2 ± 13.9 versus 34.1 ± 13.1 ng/ml; P = 0.74; and 2.44 ± 1.20 versus 2.68 ± 1.12 µg/ml; P = 0.81, respectively). When compared HCC patients with and without obesity there was also no significant difference in betatrophin and irisin levels (30.9 ± 12.2 versus 36.8 ± 14.3 ng/ml; P = 0.45; and 2.47 ± 1.20 versus 2.87 ± 1.08 µg/ml; P = 0.18, respectively). The patients were also divided into two groups based on fasting blood glucose level (fasting glucose < 100 and ≥ 100 mg/dl). However, neither betatrophin nor irisin serum

Table 3. Comparison of analyzed parameters between males and females with hepatocellular carcinoma. All results presented as median ± SD.

Parameter	**Males** n = 54	**Females** n = 15	**P**
Betatrophin (ng/ml)	26.3 ± 13.4	46.1 ± 9.1	0.008
Irisin (μg/ml)	2.55 ± 1.17	2.44 ± 1.04	0.78
Fasting insulin (ng/ml)	0.79 ± 0.88	1.32 ± 0.94	0.01
GGTP (IU/l)	112.5 ± 213.4	53.0 ± 53.2	0.004
Creatinine (mg/dl)	0.81 ± 4.98	0.64 ± 0.18	< 0.001
CEA (ng/ml)	2.47 ± 2.90	4.59 ± 2.70	0.008
HOMA-IR	4.88 ± 8.04	8.63 ± 7.94	0.04

CEA, carcinoembryionic antigen; GGTP, gamma-glutamyltransferase; HOMA-IR, homeostasis model assessment for insulin resistance.

Table 4. Adipokine serum levels and other laboratory test results of hepatocellular carcinoma patients with and without viral etiology of disease. All results presented as median ± SD.

Parameter	**Viral** n = 43	**Non-viral** n = 26	**P**
Betatrophin (ng/ml)	34.8 ± 12.9	26.1 ± 13.8	< 0.001
Irisin (μg/ml)	2.12 ± 1.13	3.02 ± 1.10	0.32
AFP (ng/mL)	37.7 ± 1414.2	4.82 ± 13.1	< 0.001
CEA (ng/mL)	3.11 ± 3.36	2.30 ± 1.61	0.03
Albumin (g/dl)	3.00 ± 0.5	3.55 ± 0.61	< 0.001
Fasting glucose (mg/dL)	106.8 ± 40.9	111.9 ± 48.5	0.002
Fasting insulin (ng/ml)	0.88 ± 0.95	0.92 ± 0.84	0.89
Fasting insulin (mIU/mL)	19.7 ± 22.0	21.3 ± 19.5	0.81
HOMA-IR	5.30 ± 6.72	5.49 ± 10.07	0.67

AFP, alpha-fetoprotein; CEA, carcinoembryionic antigen; HOMA-IR, homeostasis model assessment for insulin resistance.

concentrations differed significantly between these groups (27.7 ± 13.2 versus 34.8 ± 13.5 ng/ml; P = 0.28 and 2.19 ± 1.10 versus 2.81 ± 1.10 μg/ml; P = 0.07, respectively).

Comparison of hepatocellular carcinoma patients with different HOMA-IR values

Patients were divided into two groups according to HOMA-IR value: first group with HOMA-IR < 4 included 25 and second group with HOMA-IR ≥ 4 included 44 subjects. Serum betatrophin and irisin levels tended to be higher in patients with higher insulin resistance but the difference was not statistically significant (P = 0.08 and P = 0.68, respectively). The comparison of these patients is shown in *Table 5*.

Comparison of hepatocellular carcinoma patients with different platelets count and those with and without hypertension

We also tested the relationship between platelets count and serum betatrophin and irisin levels. Patients were divided into two groups: first including 28 patients with platelets count > 100000/mm^3 and second one including 41 subjects with platelets count ≤ 100000/mm^3. Serum betatrophin levels increased significantly among patients with lower platelets count (21.1 ± 12.8 versus 41.9 ± 11.2 ng/ml; P < 0.001). On the other hand, irisin levels did not differ significantly between both groups (2.98 ± 1.20 versus 2.34 ± 1.10 μg/ml; P = 0.17). Additionally, when compered patients with and without hypertension (HT), serum betatrophin level was significantly lower in those with when compared to those without HT (24.4 ± 12.0 versus 41.2 ±

Table 5. Comparison of analyzed adipokines, insulin and glucose serum concentrations in hepatocellular carcinoma patients with different homeostasis model assessment for insulin resistance (HOMA-IR) values. All results presented as median ± SD.

Parameter	**HOMA-IR < 4** n = 25	**HOMA-IR ≥ 4** n = 44	**P**
Fasting glucose (mg/dL)	96.6 ± 17.6	134.2 ± 48.5	< 0.001
Insulin (mIU/L)	11.0 ± 3.60	35.8 ± 21.4	< 0.001
Irisin (μg/ml)	2.42 ± 13.30	2.60 ± 1.11	0.08
Betatrophin (ng/ml)	24.1 ± 1.2	37.7 ± 12.9	0.68

Table 6. Irisin and betatrophin concentrations with regards to the severity of hepatocellular carcinoma, as evaluated according to the Barcelona clinic liver cancer (BCLC) staging. All results presented as median ± SD.

Parameter	**Stage A** n = 12	**Stage B** n = 20	**Stage C** n = 16	**P**
Irisin (μg/ml)	3.40 ± 1.30	2.52 ± 1.00	1.89 ± 1.10	0.03
Betatrophin (ng/ml)	27.9 ± 15.4	31.6 ± 13.1	43.4 ± 12.6	0.21

Table 7. Adipokine serum levels and high density lipoprotein (HDL) concentration with regards to the severity of cirrhosis, as assessed according to the Child-Pugh score. All results presented as median ± SD.

Parameter	**Child-Pugh A** n = 36	**Child-Pugh B** n = 27	**Child-Pugh C** n = 6	**P**
Irisin (μg/ml)	3.40 ± 1.20	2.42 ± 0.80	1.91 ± 1.19	0.03
Betatrophin (ng/ml)	24.1 ± 13.8	39.3 ± 11.4	46.2 ± 9.4	0.03
HDL (mg/dl)	42.9 ± 33.9	35.5 ± 29.1	23.4 ± 11.2	0.004

13.1 ng/ml; P = 0.02). There was no statistically significant difference in irisin serum concentration among these two groups (2.55 ± 1.20 versus 2.52 ± 1.14 μg/ml; P = 0.96). There were also no significant differences with respect to glucose, insulin and HOMA-IR levels in the groups of patients with and without HT.

Results according to Barcelona clinic liver cancer classification

The patients were also divided according to BCLC classification. Stage A was diagnosed in 12, stage B in 20 and stage C in 16 patients. None of analyzed patients were qualified for stage D. There was a significant decrease in irisin serum concentration in more advanced HCC cases, and the difference was especially evident when compared patients with stage A and stage C (3.40 ± 1.30 versus 1.89 ± 1.10 μg/ml; P = 0.02). There was no significant difference with respect to betatrophin levels in the groups of patients with different stages of BCLC classification (*Table 6*).

Results according to Child-Pugh score

According to C-P score HCC patients were divided into 3 groups. Class A was found in 36, class B in 27 and class C in 6 patients. Among patients with non-viral cirrhosis without intercurrent HCC class A was found in 13, class B in 9 and class C in 2 patients. Data pertaining to comparison of HCC patients with various C-P scores are presented in *Table 7*. When compared patients with different stage of liver disfunction according to C-P score serum irisin decreased significantly in those with more advanced liver disease. The decline of irisin level was mainly noticeable when we compared patients with class A and B (3.40 ± 1.20 versus 2.42 ± 0.80 μg/ml; P = 0.04). Comparison between class B and C of C-P score did not show any further decrease of serum irisin concentration (2.42 ± 0.80 versus 1.91 ± 1.19 μg/ml; P = 0.91). On the other hand, betatrophin level increased in patients with more severe liver cirrhosis (A: 24.1 ± 13.8 versus B: 39.3 ± 11.4 versus C: 46.2 ± 9.4 ng/ml; P = 0.03). Analyzing glucose metabolism among these three groups of patients with different C-P class, there was no significant difference in such parameters as: fasting glucose concentration (A: 106.8 ± 40.2 versus B: 111.1 ± 46.7 versus C: 110.9 ± 58.4 mg/dl; P = 0.98), insulin level (A: 16.6 ± 20.9 versus B: 23.9 ± 20.0 versus C: 25.0 ± 26.0 mIU/ml; P = 0.29) and HOMA-IR (A: 4.91 ± 6.90 versus B: 6.17 ± 9.60 versus C: 10.6 ± 7.95; P = 0.35).

DISCUSSION

To the best of our knowledge, this is the first study which provides an analysis of betatrophin in a well-characterized group of patients with HCC of different etiology. Additionally, this

study analyzed also serum irisin levels among these patients. To confine the diversity of the study group, patients with autoimmune liver diseases such as PSC, PBC and AIH, which comprise relatively small proportion of cirrhosis etiology, were excluded. As it was mentioned before the inflammatory process that occurs among these medical conditions might itself influence myokines secretion (17) and in consequence, derange final results of our study.

The intriguing finding of our study was that irisin was significantly lower in patients with HCC than in healthy volunteers and it was independent of disease etiology, since there was no significant difference in serum irisin levels when compared patients with viral and non-viral disease. The different levels of this myokine may suggest that it plays an important role in HCC pathogenesis, especially if we consider the fact that there was a significant decrease in irisin serum concentration in more advanced HCC cases according to BCLC classification. Additional hint indicating possible role of irisin in HCC development is decrease of its serum levels in patients with more advanced liver disfunction according to C-P score. However, the possibility that this decrease is secondary to liver disease cannot be completely excluded, especially if we consider the impact of skeletal muscle depletion, sarcopenia and cachexia, observed in advanced cirrhosis, on irisin levels. Decrease of irisin is directly associated with chronic loss of whole body muscle mass, due to the fact that this myokine is predominantly produced and secreted from skeletal muscle (13). An evident tendency for reduced irisin level was observed in the group of patients with non-viral cirrhosis without intercurrent HCC compared to healthy volunteers, although the difference did not reach statistical significance as we could observed between HCC and control groups. This may potentially indicate the role of this myokine in carcinogenesis. On the other hand, there was no significant difference in serum irisin levels between cirrhotic patients with and without HCC. This finding may suggest poor appropriability of irisin as a diagnostic marker of carcinogenesis in cirrhotic liver.

According to data presented by Gaggini *et al.* (24), hepatic mRNA expression of FNDC5/irisin was significantly higher in HCC patients than in controls. According to those results, only in HCC patients irisin over-expression was associated with gene expression of factors involved in lipogenesis, inflammation and cancer. Another study revealed similar over-expression of FNDC5/irisin in HCC-tissue of twenty patients with HCC of unknown etiology in comparison to non-tumor liver specimens used as the controls, without concomitant increase of serum concentration (25). Additionally, it has been shown that irisin significantly increase proliferation, invasiveness, and migration of human liver cancer cells *in vitro* through activation of the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway. In spite of the HCC- tissue over-expression of irisin in both studies, the comparison of serum level of this myokine between HCC patients and controls in our study surprisingly revealed decreased irisin concentration in HCC patients. This inconsistency between mRNA and plasma irisin levels could be explained by post-transcriptional or post-translational events (*i.e.* protein half-life, protein degradation *etc*.). Furthermore, liver is considered as target tissue for irisin and hepatobiliary mechanism is indicated as one of the main route of irisin elimination (26), which could clarify lower plasma myokine concentration. It is worth mentioning that one of the recent studies performed on the greater sample size showed significant downregulation of FNDC5/irisin expression in patients with HCC (27). On the other hand, Aydin's study (28) indicated that irisin expression was increased significantly in gastrointestinal cancer tissues, except for HCC.

Suppressive effect of irisin on cell number, migration and viability in breast cancer, osteosarcoma and lung cancer has been reported (29-32). The suppressive mechanisms included down-regulation of nuclear factor kappa B (NF-κB) activity in malignant cell lines, inhibited epithelial-to-mesenchymal transition (EMT) and reduced the invasion of cancer cells. Several studies confirmed great contribution of EMT in the progression of this cancer via allowing tumor cells to gain metastatic features (33-35). In the view of mentioned studies we may assume that explicit decrease of irisin, among HCC patients in our study, leads to disappearing of its suppressive effect on EMT and subsequently simplifying cancer progression. Furthermore, it could also explain reduction of irisin level in more advanced HCC, since our results demonstrate evident and statistically significant difference of myokine levels between each stage of HCC based on BCLC. Another intriguing findings which may explain indirect influence of lower irisin level on carcinogensis were presented by Mazur-Bialy *et al.* (36). This study indicated that irisin possesses an important antioxidazing and anti-inflammatory properties and may protect cells from the damage induced by reactive oxygen species (ROS), which elevated levels are observed in pathogenesis of some cancers.

Several human research studies focused on the potential association between circulating irisin levels and obesity or T2DM (37, 38). Last meta-analysis, involving a total of 23 observational studies (6 case control studies and 17 cross-sectional studies) with 1745 diabetic and 1339 non-diabetic individuals, showed reduction of irisin in T2DM (39). Despite, different results, the influence of metabolic disorders on serum irisin concentration is indisputable.

To exclude a potential influence of obesity and T2DM, which may interfere with irisin serum levels, we compared HCC patients with obesity and T2DM to the rest of the patients with cancer. Our results showed no significant differences in irisin serum concentration when compared those groups of HCC patients. These findings indicate that neither obesity nor T2DM interfere with decline irisin concentration in HCC patients.

According to literature irisin is suggested to be an important factor in fat and glucose metabolism and IR. Most of studies showed positive correlation between irisin and HOMA-IR (40, 41). Irisin up-regulation in response to IR might be a compensatory mechanism enhancing metabolic homeostasis. Unexpectedly in our study irisin was not up-regulated in patients with higher IR. However, we cannot exclude that relatively high IR among our patients is a direct effect of liver cirrhosis and presence of HCC that could influence final results.

Another intriguing finding, showed in our study for the first time was serum irisin to be associated with HCC grade with significant decrease in patients with more advanced cancer stage according to BCLC classification. This decline was especially emphasized comparing stage A (early stage) and stage C (advanced stage) patients. Main differences between these two stages eventuate from presence of vascular invasion and/or nodal disease and/or metastatic disease as well as worse PS according to The Eastern Cooperative Oncology Group (ECOG) PS scale in the stage C (42). As it was mentioned above, reduced inhibition of EMT due to decrease of irisin may potentiate vascular and nodal invasion or metastasis of HCC and in consequence emphatically explain the difference of irisin concentration between different stages of cancer.

Additionally, our study showed the decrease of serum irisin to be associated with the severity of cirrhosis according to the C-P scale. Meza-Junco's study, involving 116 patients with viral and non-viral HCC and cirrhosis, showed that sarcopenia is possibly associated with higher C-P scale score (43). According to Peng *et al.* (44) irisin is a factor that potentially prevents development of fibrosis in chronic kidney disease *via* inhibiting the activation of transforming growth factor beta (TGF-β) receptor. Supposable, decrease of circulating irisin may results in

escalation of fibrosis due to disappearing of its preventive role. On the other hand, it is possible that cirrhosis is the cause of decreased irisin concentrations. It is proved that irisin is also synthesized in human liver (45, 46).

Another aim of our study was to investigate the relationship between betatrophin and HCC, which has not been investigated so far. Many other authors already focused on and have confirmed the abnormal levels of betatrophin in clinical states that are widely considered to be risk factors for HCC such as obesity, T2DM, cirrhosis or NAFLD (10-12). Our study found betatrophin serum levels to be significantly elevated in HCC patients compared to controls, but not to of non-HCC cirrhotic patients. Serum betatrophin was also significantly higher comparing non-HCC cirrhotic patients to healthy volunteers.

The potential role of betatrophin in carcinogenesis may depend on the presence of hepatotropic viruses infection. We found, for the first time, serum concentration of betatrophin to be significantly higher in HCC patients with viral compared to those with non-viral disease etiology. This difference became more evident if we consider only HCV positive patients. The possible factor, which may connect changes of serum betatrophin level with HCV infection is NF-κB, which when excessively activated plays critical roles in many autoimmune and inflammatory diseases (47). Liao *et al.* (48) indicated that ANGPTL8 promotes the activation and may be related to the hyperactive NF-κB signaling during inflammatory process. According to Li *et al.* (49), HCV infection induces NF-κB activation and the subsequent liver inflammatory response. What is interesting, HCV infection results in mitogen-activated protein kinases (MAPKs) activation, which leads to subsequent activation of the IKK-IKB-NF-κB pathway, the same that was reported to be inhibited by ANGPTL8 (50, 51). Regarding the studies mentioned above, we can suspect that NF-κB is a common factor connecting pathogenesis of CHC and changes of serum betatrophin level, however the role of it is still unclear and require further investigation.

Additionally, Karlidag and Solmaz (52), in a recent study, found immunoreactivity of betatrophin in biopsy liver samples of HCV infected patients to be decreased compared to the control group. Furthermore, it is worth to notice that patients with HCC in the course of viral disease presented considerable significantly more evident increase of AFP concentration in comparison to non-viral group. This finding is not aberrant in view of publications indicating that the presence of chronic inflammatory liver diseases such as hepatitis can raise AFP levels to more than 100 ng/mL in themselves (53).

Our study demonstrated that significantly higher levels of serum betatrophin in cirrhotic patients with HCC, was associated with the severity of cirrhosis according to the C-P score. Similar results were obtained by Arias-Loste *et al.* in their study performed on 40 cirrhotic patients. They also showed that level of circulating betatrophin to be increased in patients with liver cirrhosis, correlating with the severity of cirrhosis and the emergence of IR (12).

Insulin resistance, hyperglycemia and T2DM are considered as independent risk factors for HCC. Development of HCC is thought to be related to insulin and insulin-like growth factor 1 (IGF-1), which promotes cell growth and proliferation, and inhibits apoptosis (54, 55). Many studies indicates plasma insulin and IR as factors influencing betatrophin expression. While insulin enhances its production through PI3K/Akt pathway, existing IR results in declined betatrophin level (56, 57). Our study revealed a tendency to up-regulated serum betatrophin levels in patients with higher IR. However, it is worth to mention, that most of analyzed patients had HOMA-IR > 4, so the exact interpretation of our results may be difficult. Interestingly, in our patients, when divided into two groups based on fasting blood glucose level (fasting glucose < 100 and ≥ 100 mg/dl), no difference in betatrophin level was found.

As it was mentioned above, IR and insulin influence ANGPTL8 secretion. Doubtlessly, chronic liver disease (including cirrhosis) is directly combined with glucose metabolism disorders. According to Arias-Loste *et al.* (12) 82% of cirrhotic patients showed IR alongside increased serum betatrophin levels. However, it is necessary to carry out further studies to determine any causal relationship between betatrophin and IR in HCC patients .

A marked sexual dimorphism, with betatrophin levels was showed in our study being significantly higher in women than in men. These results are in accordance with study by Gomez-Ambrosi (58). Interestingly, depending on the geographical region, HCC occurs 2 – 4 times more often in men than in women. The exact cause of this phenomenon is unknown, but it can be partly explained due to the greater affinity of men for risk factors for HCC development, such as HBV and/or HCV infections, smoking and alcohol consumption, even if the role of androgenic hormones in predisposing males to the progression to HCC is not clear (1, 2).

Our data showed that there is no significant difference in level of serum betatrophin in HCC patients with more advanced stage according to BCLC classification. Whereas the comparison of surveyed groups on C-P score demonstrated that serum betatrophin level was dependent on severity of liver disease. Such results suggest that betatrophin is not strictly associated with cancer progression, but may influence some factors leading to cancer development. Additionally, as it was mentioned above there was significant increase of betatrophin levels in the groups of HCC as well as cirrhotic patients compared to controls, whereas we did not observed any significant difference between HCC and non-HCC cirrhotic patients. In a view of presented results we can suppose that betatrophin has an impact on fibrosis progression and cirrhosis development, rather than carcinogenesis.

Unfortunately, our research has several limitations. First, the study group consists of a relatively small number of patients. Second, more than half of the patients have viral etiology of cirrhosis and HCC, while the rest developed disease due to alcohol abuse. Moreover, the lack of NAFLD patients with cirrhosis and concomitant HCC may affect the levels of irisin and betatrophin in our study. Fourth, our study does not include the assessment of muscle mass, including the possible occurrence of sarcopenia, which may have a potential effect on the level of irisin, as mentioned above. Fifth, serious limitation of our research that has to be mentioned, is application of BMI as a major parameter to assessment the metabolic profile of our patients. According to Hwang *et al.* (59) the skeletal muscle mass to visceral fat area ratio was positively correlated with the serum irisin concentration and played a key role in the association between circulating irisin and a patient's metabolic phenotype. In the view of above information and highlighting the fact that our patient, without doubt, had altered body composition, BMI seems to be invalid parameter in metabolic profile evaluation. Sixth, the presence of gender disproportion between HCC and control groups, as the percentage of male patients is distinctly higher within HCC group. This may interfere with analysis of betatrophin levels since most of publication confirmed the presence of sexual dimorphism in ANGPTL8 concentration. Seventh, there is no assessment of the expression of irisin and betatrophin in tumor and liver tissue in our study.

In conclusion, we have comprehensively studied the association between betatrophin, irisin on one side and liver cirrhosis and HCC on the other sided. To best of our knowledge, our data is the first to show up-regulated betatrophin serum levels in HCC patients when compared to healthy controls.

Serum betatrophin was positively associated with cirrhosis stage according to C-P score but not with stage of HCC according to BCLC. This observation suggest betatrophin to be important factor which influence fibrosis and cirrhosis progression but not HCC development itself, since we did not observed any difference between this hepatokine levels among cirrhotic patients with and without intercurrent cancer. On the other side serum irisin decreased in patients with HCC, whereas there was no significant difference in the group of cirrhotic patients without HCC in comparison to healthy volunteers. Moreover, serum irisin declined in more advanced cirrhosis and more advanced liver cancer. These results suggest that lower irisin levels favor faster fibrosis progression and facilitate cancer progression. Although, the impact of irisin on hepato-carcinogenesis is still not clear and requires further investigation. We also cannot exclude that presented changes in serum betatrophin and irisin levels are at least partially associated with patient's nutritional disorders.

Source of funding: The study was supported by the statutory funds of Wroclaw Medical University.

Conflict of interests: None declared.

REFERENCES

1. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; 142: 1264-1273.
2. Petruzziello A. Epidemiology of hepatitis B virus (HBV) and hepatitis C virus (HCV) related hepatocellular carcinoma. *Open Virol J* 2018; 12: 26-32.
3. Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. *J Clin Gastroenterol* 2013; 47 (Suppl.): S2-S6.
4. Stickel F, Hellerbrand C. Nonalcoholic fatty liver disease as a risk factor for hepatocellular carcinoma: mechanisms and implications. *Gut* 2010; 59: 1303-1307.
5. Ramadori G, Moriconi F, Malik I, Dudas J. Physiology and pathophysiology of liver inflammation, damage and repair. *J Physiol Pharmacol* 2008; 59 (Suppl. 1): 107-117.
6. Streba LA, Vere CC, Rogoveanu I, Streba CT. Nonalcoholic fatty liver disease, metabolic risk factors, and hepatocellular carcinoma: an open question. *World J Gastroenterol* 2015; 21: 4103-4110.
7. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; 19: 329-338.
8. Santulli G. Angiopoietin-like proteins: a comprehensive look. *Front Endocrinol* (*Lausanne*) 2014; 5: 4. doi: 10.3389/fendo.2014.00004
9. Fu Z, Yao F, Abou-Samra AB, Zhang R. Lipasin, thermoregulated in brown fat, is a novel but atypical member of the angiopoietin-like protein family. *Biochem Biophys Res Commun* 2013; 430: 1126-1131.
10. Li S, Liu D, Li L, *et al.* Circulating betatrophin in patients with type 2 diabetes: a meta-analysis. *J Diabetes Res* 2016; 2016: 6194750. doi: 10.1155/2016/6194750
11. Hu W, Shao X, Guo D, *et al.* Relationship of serum betatrophin with nonalcoholic fatty liver in a Chinese population. *PLoS One* 2017; 12: e0170758. doi: 10.1371/journal.pone.0170758
12. Arias-Loste MT, Garcia-Unzueta MT, Llerena S, *et al.* Plasma betatrophin levels in patients with liver cirrhosis. *World J Gastroenterol* 2015; 21: 10662-10668.
13. Monzavi N, Zargar SJ, Gheibi N, Azad M, Rahmani B. Angiopoietin-like protein 8 (betatrophin) may inhibit hepatocellular carcinoma through suppressing of the Wnt signaling pathway. *Iran J Basic Med Sci* 2019; 22: 1166-1171.
14. Bostrom P, Wu J, Jedrychowski MP, *et al.* A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012; 481: 463-468.
15. Chen JQ, Huang YY, Gusdon AM, Qu S. Irisin: a new molecular marker and target in metabolic disorder. *Lipids Health Dis* 2015; 14: 2. doi: 10.1186/1476-511X-14-2
16. Choi ES, Kim MK, Song MK, *et al.* Association between serum irisin levels and non-alcoholic fatty liver disease in health screen examinees. *PLoS One* 2014; 9: e110680. doi: 10.1371/journal.pone.0110680
17. Waluga M, Kukla M, Kotulski R, *et al.* Omentin, vaspin and irisin in chronic liver diseases. *J Physiol Pharmacol* 2019; 70: 277-285.
18. Roca-Rivada A, Castelao C, Senin LL, *et al.* FNDC5/irisin is not only a myokine but also an adipokine. *PLoS One* 2013; 8: e60563. doi: 10.1371/journal.pone.0060563
19. Lindor KD, Kowdley KV, Harrison ME, American College of Gastroenterology. ACG clinical guideline: primary sclerosing cholangitis. *Am J Gastroenterol* 2015; 110: 646-659.
20. Younossi ZM, Bernstein D, Shiffman ML, *et al.* Diagnosis and management of primary biliary cholangitis. *Am J Gastroenterol* 2019; 114: 48-63.
21. Czaja AJ. Diagnosis and management of autoimmune hepatitis: current status and future directions. *Gut Liver* 2016; 10: 177-203.
22. World Health Organisation. Physical status: The use and interpretation of anthropometry: report of a world health organization (WHO) expert committee. Geneva, World Health Organization 1995.
23. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Geneva, World Health Organization 2006.
24. Gaggini M, Cabiati M, Del Turco S, *et al.* Increased FNDC5/Irisin expression in human hepatocellular carcinoma. *Peptides* 2017; 88: 62-66.
25. Shi G, Tang N, Qiu J, *et al.* Irisin stimulates cell proliferation and invasion by targeting the PI3K/AKT pathway in human hepatocellular carcinoma. *Biochem Biophys Res Commun* 2017; 493: 585-591.
26. Lv J, Pan Y, Li X, *et al.* Study on the distribution and elimination of the new hormone irisin in vivo: new discoveries regarding irisin. *Horm Metab Res* 2015; 47: 591-595.
27. Zhang J, Ke M, Ren Y, *et al.* Serum irisin predicts posthepatectomy complications in patients with hepatocellular carcinoma. *Dis Markers* 2019; 2019: 9850191. doi: 101155/2019/9850191
28. Aydin S, Kuloglu T, Ozercan MR, *et al.* Irisin immunohistochemistry in gastrointestinal system cancers. *Biotech Histochem* 2016; 91: 242-250.
29. Provatopoulou X, Georgiou GP, Kalogera E, *et al.* Serum irisin levels are lower in patients with breast cancer: association with disease diagnosis and tumor characteristics. *BMC Cancer* 2015; 15: 898. doi: 10.1186/s12885-015-1898-1
30. Gannon NP, Vaughan RA, Garcia-Smith R, Bisoffi M, Trujillo KA. Effects of the exercise-inducible myokine irisin on malignant and non-malignant breast epithelial cell behavior in vitro. *Int J Cancer* 2015; 136: 197-202.
31. Kong G, Jiang Y, Sun X, *et al.* Irisin reverses the IL-6 induced epithelial-mesenchymal transition in osteosarcoma cell migration and invasion through the STAT3/Snail signaling pathway. *Oncol Rep* 2017; 38: 2647-2656.
32. Shao L, Li H, Chen J, *et al.* Irisin suppresses the migration, proliferation, and invasion of lung cancer cells via inhibition

of epithelial-to-mesenchymal transition. *Biochem Biophys Res Commun* 2017; 485: 598-605.
33. van Zijl F, Zulehner G, Petz M, *et al.* Epithelial to mesenchymal transition in hepatocellular carcinoma. *Future Oncol* 2009; 5: 1169-1179.
34. Fu XT, Dai Z, Song K, *et al.* Macrophage-secreted IL-8 induces epithelial-mesenchymal transition in hepatocellular carcinoma cells by activating the JAK2/STAT3/Snail pathway. *Int J Oncol* 2015; 46: 587-596.
35. Liu L, Dai Y, Chen J, *et al.* Maelstrom promotes hepatocellular carcinoma metastasis by inducing epithelial-mesenchymal transition by way of Akt/GSK-3β/Snail signaling. *Hepatology* 2014; 59: 531-543.
36. Mazur-Bialy AI, Kozlowska K, Pochec E, Bilski J, Brzozowski T. Myokine irisin-induced protection against oxidative stress in vitro. Involvement of heme oxygenase-1 and antioxidazing enzymes superoxide dismutase-2 and glutathione peroxidase. *J Physiol Pharmacol* 2018; 69 (Suppl. 1): 117-125.
37. Polyzos SA, Anastasilakis AD, Efstathiadou ZA, *et al.* Irisin in metabolic diseases. *Endocrine* 2018; 59: 260-274.
38. Mazur-Bialy AI, Bilski J, Pochec E, Brzozowski T. New insight into the direct anti-inflammatory activity of a myokine irisin against proinflammatory activation of adipocytes. Implication of exercise in obesity. *J Physiol Pharmacol* 2017; 68: 243-251.
39. Du XL, Jiang WX, Lv ZT. Lower circulating irisin level in patients with diabetes mellitus: a systematic review and meta-analysis. *Horm Metab Res* 2016; 48: 644-652.
40. Crujeiras AB, Zulet MA, Lopez-Legarrea P, *et al.* Association between circulating irisin levels and the promotion of insulin resistance during the weight maintenance period after a dietary weight-lowering program in obese patients. *Metabolism* 2014; 63: 520-531.
41. Park KH, Zaichenko L, Brinkoetter M, *et al.* Circulating irisin in relation to insulin resistance and the metabolic syndrome. *J Clin Endocrinol Metab* 2013; 98: 4899-4907.
42. Pons F, Varela F, Llovet JM. Staging systems in hepatocellular carcinoma. *HPB* (*Oxford*) 2005; 7: 35-41.
43. Meza-Junco J, Montano-Loza AJ, Baracos VE, *et al.* Sarcopenia as a prognostic index of nutritional status in concurrent cirrhosis and hepatocellular carcinoma. *J Clin Gastroenterol* 2013; 47: 861-870.
44. Peng H, Wang Q, Lou T, *et al.* Myokine mediated muscle-kidney crosstalk suppresses metabolic reprogramming and fibrosis in damaged kidneys. *Nat Commun* 2017; 8: 1493. doi: 10.1038/s41467-017-01646-6
45. Aydin S, Kuloglu T, Aydin S, *et al.* A comprehensive immunohistochemical examination of the distribution of the fat-burning protein irisin in biological tissues. *Peptides* 2014; 61: 130-136.
46. Aydin S, Kuloglu T, Aydin S, *et al.* Cardiac, skeletal muscle and serum irisin responses to with or without water exercise in young and old male rats: cardiac muscle produces more irisin than skeletal muscle. *Peptides* 2014; 52: 68-73.
47. Zhang Y, Zheng L, Huang K. A new way to regulate inflammation: selective autophagic degradation of IKKγ mediated by ANGPTL8. *Cell Stress* 2018; 2: 66-68.
48. Liao Z, Wu X, Song Y, *et al.* Angiopoietin-like protein 8 expression and association with extracellular matrix metabolism and inflammation during intervertebral disc degeneration. *J Cell Mol Med* 2019; 23: 5737-5750.
49. Li H, Li JR, Huang MH, *et al.* Bicyclol attenuates liver inflammation induced by infection of hepatitis C virus via repressing ROS-mediated activation of MAPK/NF-κB signaling pathway. *Front Pharmacol* 2018; 9: 1438. doi: 10.3389/fphar.2018.01438
50. Xie Z, Xiao Z, Wang F. Hepatitis C virus nonstructural 5A protein (HCV-NS5A) inhibits hepatocyte apoptosis through the NF-κB/miR-503/bcl-2 pathway. *Mol Cells* 2017; 40: 202-210.
51. Jiang X, Kanda T, Wu S, *et al.* Hepatitis C virus nonstructural protein 5A inhibits MG132-induced apoptosis of hepatocytes in line with NF-κB-nuclear translocation. *PLoS One* 2015; 2015: e0131973. doi: 10.1371/journal.pone.0131973
52. Eser Karlidag G, Arslan Solmaz O. Are adropin, apelin, elabela, asprosin and betatrophin biomarkers for chronic hepatitis and staging of fibrosis? *Biotech Histochem* 2020; 95: 152-159.
53. Davis GL, Dempster J, Meler JD, *et al.* Hepatocellular carcinoma: management of an increasingly common problem. *Proc Bayl Univ Med Cent* 2008; 21: 266-280.
54. Chang SC, Yang WV. Hyperglycemia, tumorigenesis, and chronic inflammation. *Crit Rev Oncol Hematol* 2016; 108: 146-153.
55. Xu L, Xiaocong W, Pujun G. Diabetes mellitus and risk of hepatocellular carcinoma. *BioMed Res Int* 2012; 28: 109-122.
56. Lu P, Chen X, Zhang Z, *et al.* Insulin upregulates betatrophin expression via PI3K/Akt pathway. *Sci Rep* 2017; 7: 5594. doi: 10.1038/s41598-017-06052-y
57. Wang H, Du L, Wu T, *et al.* Circulating betatrophin is associated with insulin resistance in humans: cross-sectional and interventional studies in vivo and in vitro. *Oncotarget* 2017; 8: 96604-96614.
58. Gomez-Ambrosi J, Pascual E, Catalan V, *et al.* Circulating betatrophin concentrations are decreased in human obesity and type 2 diabetes. *J Clin Endocrinol Metab* 2014; 99: E2004-E2009.
59. Hwang YC, Jeon WS, Park YC, Youn BS. The ratio of skeletal muscle mass to visceral fat area is amain determinant linking circulating irisin to metabolic phenotype. *Cardiovasc Diabetol* 2016; 15: 9. doi: 10.1186/s12933-015-0319-8

Received: January 5, 2020
Accepted: February 28,2020

Author's address: Assoc. Prof. Michał Kukla, Department of Endoscopy, University Hospital in Cracow, 2 Jakubowskiego Street, 30-688 Cracow, Poland.
E-mail: kuklamich@poczta.onet.pl