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## OMENTIN, VASPIN AND IRISIN IN CHRONIC LIVER DISEASES

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Omentin and vaspin are adipokines potentially considered in the development of liver pathology. Irisin is new myokine potentially participating in energy processes in the organisms. The aim of this study was to evaluate the plasma concentration of these cytokines and the relationships of them with selected parameters of laboratory tests and of histopathological changes in selected chronic liver diseases: non-alcoholic fatty liver diseases (NAFLD), primary biliary cholangitis (PBC) and alcoholic cirrhosis (AC). The plasma concentration of omentin was the highest in AC group and the lowest in control group (CG). Irisin plasma concentration was the highest in CG and the lowest in AC. Mean vaspin concentrations did not differ significantly between groups. Among many laboratory parameters, only in the AC group positive relationships were found between omentin concentration and bilirubin, as well as glucose, and negative between omentin level and the number of platelets and erythrocytes; there was a positive relationship between the concentration of vaspin and bilirubin, as well as negative between vaspin level and the number of erythrocytes or hematocrit value in this group. INR value had positive correlation with vaspin concentration and negative with irisin level in NAFLD group. No significant dependences between the concentrations of explored cytokines and laboratory tests were found in PBC group. It was found the positive correlation between the plasma concentration of irisin and fibrosis as well as inflammation in PBC group. The negative correlation between irisin level and inflammation in NAFLD was also showed. Omentin can be considered as an indicator for predicting inflammation, steatosis and balloon degeneration in NAFLD and PBC. Summarizing, it is unclear but possible that explored cytokines have some relationships with certain features of liver damage and development of chronic diseases of this organ.

**Key words:** *non-alcoholic fatty liver disease, primary biliary cholangitis, alcoholic cirrhosis, liver fibrosis, homeostasis model assessment-insulin resistance, omentin, vaspin, irisin*

### INTRODUCTION

Progression of chronic liver diseases, regardless of etiology, leads to progressive damage, cirrhosis and failure of this organ. Primary biliary cholangitis (PBC), which represents the chronic autoimmune disease, and non-alcoholic fatty liver disease (NAFLD) - the most common metabolic illness, as well as some toxins (like alcohol) also lead to cirrhosis. Despite fundamental etiopathogenetic differences between PBC, NAFLD and toxic cirrhosis (AC), it is interesting to explore potential common links between them. From this point of view some cytokines are interesting.

Omentin is the protein discharged by cells of stroma vascular fraction (SVF) of visceral adipose tissue (VAT). This adipokine facilitates insulin-stimulated transport of glucose and phosphorylation of serine-threonine protein kinase Act - main signal transmitter in the 3-kinase pathway of phosphatidylinositol (PI3K), and plays an important role in the regulation of cell growth, metabolism, survival and proliferation cells

processes (1). Some studies suggest, that the concentration of omentin in adipose tissue is decreased in obese persons and in patients with insulin resistance (2).

Several studies on the relationship between omentin and liver diseases have been published. For example Yilmaz *et al.* showed increased plasma level of this adipokine in patients with balloon degeneration of hepatocytes and death of liver cells (3). However, the importance of omentin in progression of liver diseases is not enough explored.

VAT provides the vaspin - natural inhibitor of serine protease. The administration of human vaspin to mice with developed obesity improves the tolerance of glucose and insulin sensitivity (4). It was suggested, that this adipokine is the metabolic regulator of fat store in the body (5, 6). Vaspin belongs to group of 'good' adipokines. However, its level in obese persons with diabetes was paradoxically increased (7). Some studies suggested the relationship between the concentration of vaspin and type of obesity, percentage content of adipose tissue, fasting insulin concentration, insulin resistance and waist/hip

ratio (6). It is possible, that induction of vaspin expression is a countervailing effect of obesity, insulin resistance or type 2 diabetes mellitus (T2DM). The results of many studies are inconsistent. In some studies the vaspin concentration was higher in men with metabolic syndrome (MS) (8), but in other was not changed or even lower (9). The importance of vaspin in liver diseases progression is unclear.

Irisin is the cytokine produced by muscles after physical exercise. An increase of oxygen consumption, loss of body mass, improve of glucose tolerance and decrease of insulin secretion are the results of irisin activity (10). This cytokine is primarily myokine, however it is recognized also as adipokine identified in adipose tissue near skeletal muscle of humans. Irisin is also synthesized in many other tissues (11).

It was suggested, that the activation of brown adipose tissue (BAT) and thermogenic genes is the core task of this cytokine (12). Some studies showed higher plasma concentration of this myokine in obese persons (13, 14). Irisin can also be an indicator of insulin resistance or MS development (15). However, the studies exploring the role of irisin in metabolic disease, like T2DM or NAFLD are inconclusive. Moreover, results of irisin assays could be dependent on agents (antibodies) producer, because the differences from this reason are large. There are serious concerns about the available assays that measure these molecules, especially irisin (16). Zhang *et al.* proved the lower plasma levels of irisin in obese patients with NAFLD (17). Oppositely, Rizk *et al.* and Choi *et al.* found significantly higher plasma levels of irisin in patients with MS and liver steatosis, accompanied by an increase of aminotransferases activity (18, 19). However, irisin levels was higher in patients with mild, comparing to moderate or severe disease (19).

Summarizing, the importance of omentin, vaspin and irisin in the pathogenesis and development of PBC, NAFLD and cirrhosis is not clear and there are a lot of conflicting results.

The aim of the study is to determine whether the relationship exists between the plasma concentration of selected cytokines (omentin, vaspin and irisin) and some laboratory parameters or histopathological changes in three chronic liver diseases: PBC, NAFLD and AC.

## PATIENTS AND METHODS

### Patients

The study was carried out in accordance with the guidelines of the World Medical Association. The participants were informed in detail about the purpose and course of the experiment before the start of the study and gave their conscious written consent to participate in it. The research was approved by the Bioethics Commission of Medical University of Silesia in Katowice (KNW/0022/KB1/31/18).

The study involved 100 humans, including 75 patients, divided into 3 groups: 1) primary biliary cholangitis (PBC) - 20 patients, 2) nonalcoholic fatty liver disease (NAFLD) - 25 patients, and 3) alcoholic cirrhosis (AC) - 30 patients, aged 20 to 75 years, and 25 healthy volunteers who represented control group (CG).

The exclusion criteria included the presence of inflammatory, infectious diseases (bacterial as well as viral and fungal), systemic, cancer, hormonal, metabolic (other than obesity, hyperlipidemia and T2DM, which were acceptable in NAFLD group), rheumatological, advanced circulatory system diseases including its insufficiency, coagulation system disorders, including thrombocytopenia, with the exception of AC group, where low number of platelets and coagulation

disorders were agreeable. Alcohol abuse patients were excluded from the study (women: over 10 g/day - 1 drink/day, men: over 20 g/day - 2 drinks/day), except for AC group, in which alcohol abuse was included, but abstinence within 6 months before the study was confirmed by patient and family history, psychological assessment and laboratory results. The exclusion criteria also included lack of informed and voluntary consent. Pregnancy was also an exclusion criterion.

Good health in volunteers included in the study group was determined on the basis of anamnesis, physical examination, laboratory tests and abdominal ultrasound examination. The volunteers had no complaints at the time of participation in the study as well as serious diseases in the past; they had a constant body mass and a good appetite.

All healthy volunteers gave informed written consent to participate in the study.

In all subjects anthropometric measurements were performed: height, body weight, body mass index (BMI).

Clinical diagnosis of PBC was based on laboratory tests, including increased activity of cholestasis enzymes - ALP and GGTP, increased bilirubin concentration, positive test for antimitochondrial antibodies (AMA-M2) and abdominal ultrasound, which showed normal width and shape of bile ducts. The final diagnosis was based on biopsy of the liver. Patients from this group didn't use any medications (including ursodeoxycholic acid (UDCA)) for at least one months before including into the study.

Clinical diagnosis of NAFLD was based on abdominal ultrasound and laboratory results. Diagnosis of NAFLD based on ultrasound includes: 1) parenchymal hyperechogenicity, 2) intensified attenuation, 3) poorly visible vessels, 4) focal hyposteatosis.

The following diagnostic categories for NAFLD have been utilized: not NAFLD (< 5% steatosis); NAFL, not NASH (> 5% steatosis, with or without lobular and portal inflammation) and definite steatohepatitis (all criteria present, including steatosis, hepatocellular ballooning, and lobular inflammation).

Liver biopsy was performed in all patients in whom NAFLD was suspected. Qualification for biopsy was based on above mentioned features in abdominal ultrasound and elevated serum aminotransferases activity during hospitalization and/or before admission (within one month) to the ward. The patients who fulfilled above criteria for NAFLD diagnosis, which are in accordance with AASLD guidelines (20), were included to the study as NAFLD group. The patients from this group did not use any medicines.

In the AC group the diagnosis was based on anamnesis (alcohol consumption as above mentioned), physical examination in which typical signs of cirrhosis were found: vascular spiders, palm erythema, abdominal enlargement due to ascites and/or splenomegaly, thinning of subcutaneous tissue of limbs and laboratory tests, where low concentration of total protein (6 g/dL) and albumins (< 3.5 g/dL) as well as elevated INR value (> 1.2) were demonstrated. The result of abdominal ultrasound with characteristic liver image and symptoms of portal hypertension, or eventually endoscopic examination of the upper gastrointestinal tract was also taken into consideration. The severity of liver disease was determined on the basis of the Child-Pugh scale and according to the model for end-stage liver disease scale (MELD).

Most of patients with AC received treatment for ascites or edemas of the lower limbs or both (furosemide and spironolactone - 15 patients, spironolactone - 10 patients). Medicines reducing portal pressure and the risk of esophageal varices bleeding were used in most patients with AC (carvedilol: 12.5 – 25 mg daily in 8 patients, propranolol: 20 – 60 mg daily in 16 patients).

### Histopathological examination of the liver

Liver biopsy was done using standard procedure in all patients with PBC and NAFLD groups. In the AC group, just as in healthy volunteers, liver biopsy was not performed. The obtained liver samples were stained with hematoxylin and eosin, and for collagen fibers according to the azan method. The liver samples were examined independently by two experienced pathologists and histopathological evaluation was carried out according to Kleiner's scoring system (21) and Batts-Ludwig scoring system (22).

Kleiner's score assesses: 1. steatosis grade according to evaluation of parenchymal involvement by steatosis: grade 0 < 5%, grade 1 = 5 – 33%, grade 2 = 33 – 66%, grade 3 > 66%; lobular inflammation grade with overall assessment of all inflammatory foci: grade 0 - no foci, grade 1: < 2 foci per 200 × field, grade 2: 2 – 4 foci per 200 × field, grade 3: > 4 foci per 200 × field; ballooning hepatocytes: grade 0: none, grade 1: few balloon cells, grade 2: many cells/prominent ballooning; fibrosis: grade 0: none, grade 1: perisinusoidal or periportal (1A - mild, zone 3, perisinusoidal; 1B - moderate, zone 3, perisinusoidal; 1C - portal/periportal), grade 2: perisinusoidal and portal/periportal, grade 3: bridging fibrosis, 4: cirrhosis.

Diagnosis of NAFLD is based on the presence of three hallmarks: steatosis, inflammation and ballooning hepatocytes.

Batt-Ludwig score assesses inflammation: grade 1: chronic hepatitis with minimal activity, grade 2: chronic hepatitis with mild activity, grade 3: chronic hepatitis with marked activity, grade 4: chronic hepatitis with marked activity and bridging or multiacinar necrosis; fibrosis: stage 0: no fibrosis, stage 1: fibrous portal expansion, stage 2: few bridges or septa, grade 3: numerous bridges or septa, stage 4: cirrhosis.

### Blood collection and serologic assays

In all fasting subjects a 5 ml blood sample was taken from the elbow vein. This sample was centrifuged for 15 minutes at 2500 revolutions per minute, then the serum was divided into 2 parts and frozen to –70°C, where it was stored in the freezer until laboratory tests were done.

Routine laboratory tests were carried out, determining blood morphology (assumed laboratory standards: WBC 4.0 – 10.0 10<sup>3</sup>/μL, PLT 130 – 400 10<sup>3</sup>/μL and for men: RBC 4.2 – 5.7 10<sup>6</sup>/μL; HGB 13.5 – 16.5 g/dl, for women: RBC 3.7 – 5.0 10<sup>6</sup>/μL; HGB 11.5 – 15.0 g/dl, aspartate aminotransferases (AST) activity in plasma with upper limit of norm (ULN) = 45 U/L in men, and 34 U/L in women, for alanine aminotransferase (ALT), with ULN = 35 U/L in men and 31 U/L in women, alkaline phosphatase activity (ALP) with the scope of the standard 38 – 126 U/L, γ-glutamyltranspeptidase (GTP) with ULN < 55 U/L, bilirubin concentration (norm: 0.30 – 20 mg/dL), total cholesterol (ULN < 200 mg/dL), LDL (ULN < 135 mg/dL, HDL (norm: 40 – 150 mg/dL), triglycerides (ULN < 150 mg/dL), creatinine (norm: 0.66 – 1.09 mg/dl), glucose (norm: 74 – 106 mg/dL), CRP (norm: < 5.0 mg/dL), PT (norm: 9.4 – 12.5 s), INR (norm 0.8 – 1.2).

The value of non-invasive hepatic fibrosis tests was calculated according to the following formulae (23):

$$\text{AAR} = \text{AST}/\text{ALT}$$

$$\text{FIB-4 (fibrosis 4 score) index} = \frac{\text{age (years)} \times \text{AST}}{\text{number of platelets} \times \sqrt{\text{ALT}}}$$

APRI (AST to platelet ratio index) = [(AST/ULN/number of platelets (× 10<sup>9</sup>)) × 100

All participants had performed an oral glucose tolerance test to detect T2DM or impaired glucose tolerance. Insulin resistance (IR) was determined according to a homeostatic model for

calculating insulin resistance (HOMA-IR) in accordance with the formula (24):

$$\text{HOMA IR} = \text{fasting insulin concentration (mUI/L)} \times \text{fasting glucose concentration (mg/dL)}/405.$$

Insulin concentration was measured using Diametra Insulin EIA Kit, Catalogue number DKO076; Diametra S.r.l. headquarters: via Garibaldi, Foligno, PG, Italy, sensitivity 0.25 μIU/m.

The second serum sample obtained from the blood taken from the subjects was used to determine the concentration of omentin, irisin and vaspin.

Serum omentin-1 concentrations were determined in duplicate samples using the immunoenzymatic method and commercially available Human Omentin-1 ELISA Kit, BioVendor Laboratorni Medicina a.s., Brno, Czech Republic, with sensitivity 0.5 ng/ml, intra-assay error 3.7% and inter-assay error 4.6%. This method included determination of omentin-1 molecule of full length.

Serum levels of irisin were determined using the Irisin Elisa Kit, BioVendor - Laboratorni Medicina a.s., Brno, Czech Republic, with sensitivity 1 ng/ml, intra-assay error 6% and inter-assay error 8%.

Serum vaspin concentration was also determined by the immune-enzymatic method using the commercial Vaspin ELISA Kit, BioVendor – Laboratorni Medicina a.s., Brno, Czech Republic, with sensitivity 0.01 ng/ml, intra-assay error 7.6% and inter-assay error 7.7%.

### Statistical analysis

The type of distribution of individual values of continuous variables was determined using Shapiro-Wilk test. In the case of a normal distribution, the analysis of variance was used for a detailed comparative analysis of continuous variables of the obtained results. The Kruskal-Wallis test and the median test were used if the distribution of a continuous variable differed from normal. *Post-hoc* tests (Tukey's RIR test) were used to determine significant relationships after the analysis of variance. In the case of discrete variables, the results obtained were compared using quadrant independence test and Fisher's exact test if the number of groups was small.

Correlations between variables were examined using Spearman's rang correlation test. Logistic regression analysis was used to study potential predictors for selected binary variables. The level of statistical significance, at which differences were considered statistically significant, was assumed for P < 0.05.

All calculations were performed using Statistica PL version 13.0 (StatSoft Inc., Tulsa, OK, USA).

## RESULTS

### Demographic and anthropometric data

Demographic and anthropometric data of survey participants of the study are presented in *Table 1*.

### Concentrations of omentin, vaspin and irisin

Statistical analysis showed, that the plasma concentration of omentin (*Table 2*) was the highest in AC and the lowest in CG. *Post-hoc* test indicated, that in the PBC group omentin is no different comparing to NAFLD group. Mean vaspin concentrations did not differ significantly between groups.

The plasma concentration of irisin was the lowest in AC group and the highest in CG; moreover, in CG the irisin concentration

Table 1. Demographic and anthropometric data.

|                                | PBC                             | NAFLD                           | AC                             | CG                              | P      |
|--------------------------------|---------------------------------|---------------------------------|--------------------------------|---------------------------------|--------|
| <b>Number of participants</b>  | 20                              | 25                              | 30                             | 25                              |        |
| <b>Sex (W/M)#</b>              | 20/0<br>(100/0%)                | 13/12<br>(52/48%)               | 8/22<br>(26.7/73.3%)           | 14/11<br>(56/44%)               | < 0.01 |
| <b>Age (years)^</b>            | 57 ± 7,5<br>(41 – 65)           | 31 ± 10<br>(20 – 60)            | 49.5 ± 14<br>(35 – 65)         | 42 ± 15<br>(26 – 65)            | < 0.01 |
| <b>Body mass (kg)*</b>         | 65.5 ± 8.62<br>(50 – 84)        | 94.08 ± 13.72<br>(61 – 123)     | 77.91 ± 20.19<br>(44 – 128)    | 62.56 ± 11.89<br>(46 – 76)      | < 0.01 |
| <b>Height (m)*</b>             | 1.60 ± 0.04<br>(1.54 – 1.7)     | 1.73 ± 0.1<br>(1.54 – 1.86)     | 1.71 ± 0.1<br>(1.53 – 1.9)     | 1.69 ± 0.07<br>(1.53 – 1.82)    | < 0.01 |
| <b>BMI (kg/m<sup>2</sup>)^</b> | 25.37 ± 4.54<br>(20.81 – 32.41) | 31.14 ± 6.07<br>(18.42 – 51.86) | 26.21 ± 5.67<br>(17.4 – 36.33) | 22.15 ± 0.83<br>(19.65 – 23.46) | < 0.01 |

Abbreviations: AC, alcoholic cirrhosis; BMI, body mass index; CG, control group; M, men; NAFLD, nonalcoholic fatty liver disease; PBC, primary biliary cholangitis; NAFLD, nonalcoholic fatty liver disease.

^ median and interquartile range (Kruskal Wallis test); \* mean and standard deviation (ANOVA); # Chi<sup>2</sup>.

Table 2. Concentrations of omentin, vaspin and irisin.

|                         | PBC   | NAFLD   | AC   | CG                              | P      |
|-------------------------|---|---|--|---------------------------------|--------|
| <b>Omentin (ng/ml)^</b> | 408.8 ± 155.1 <sup>**#</sup><br>(207.4 – 808.2) | 266.6 ± 82.56 <sup>*S</sup><br>(63.1 – 511.3) | 1054.5 ± 430.8 <sup>***S</sup><br>(579.7 – 2208.9) | 114.5 ± 95.88<br>(57.3 – 176.8) | < 0.01 |
| <b>Vaspin (ng/ml)^</b>  | 0.25 ± 0.1<br>(0.12 – 0.54)                     | 0.28 ± 0.154<br>(0.1 – 1.26)                  | 0.27 ± 0.26<br>(0.093 – 0.84)                      | 0.26 ± 0.05<br>(0.185 – 0.32)   | NS     |
| <b>Irisin (µg/ml)^</b>  | 5.82 ± 2.41 <sup>**#</sup><br>(4.1 – 15.04)     | 4.98 ± 2.017 <sup>**</sup><br>(1.712 – 11.38) | 3.13 ± 1.96 <sup>**#</sup><br>(1.712 – 11.38)      | 29.67 ± 19.9<br>(12.87 – 75.04) | < 0.01 |

Abbreviations: AC, alcoholic cirrhosis; CG, control group; NAFLD, nonalcoholic fatty liver disease; NS, non significant; PBC, primary biliary cholangitis.

^ median and interquartile range; IQR (Kruskal Wallis test);

\* P < 0.05, \*\* P < 0.01 - comparison of patients groups to control group;

# P < 0.01 - comparison between PBC and AC for omentin and irisin;

S P < 0.01 - comparison between NAFLD and AC for omentin.

was significantly higher than in all other groups. Significant differences were found between the PBC and AC groups, whereas between PBC and NAFLD, as well as between AC and NAFLD the differences were not statistically significant (Table 2).

#### Selected laboratory parameters in three groups of patients

Statistical analysis of laboratory parameters in all patient groups showed significant differences in all of them except CRP concentration, ALT activity and HOMA-IR index. *Post-hoc* analysis showed that AC group significantly differed from the remaining groups of patients with regard to the number of platelets, leucocytes, bilirubin concentration, AST activity, PT, INR value, as well as APRI and FIB4 indexes. *Post-hoc* analysis also showed the highest value of AAR index in the AC group and the lowest in NAFLD. The activity of GGT was significantly the lowest in NAFLD group, but between PBC and AC groups the mean values weren't different (Table 3).

In any of the study groups of patients (NAFLD, PBC and AC) correlations between the concentration of irisin, omentin and vaspin were not found (intragroup calculation). Also, there were no significant relationships between the concentrations of cytokines and any laboratory parameters in the PBC group.

In the NAFLD group significant, negative correlation between INR and irisin concentration (R = -0.418, P < 0.05), as

well as positive correlation between INR and vaspin concentration was observed (R = 0.503, P < 0.05).

We assayed the concentration of total cholesterol (mean 201 ± 22 mg%), HDL cholesterol fraction (mean 49 ± 7 mg%), and LDL cholesterol fraction (mean 112 ± 18 mg%), as well triglycerides concentration (mean 214 ± 62 mg%) in NAFLD group. There were no significant relationships between cytokines and these parameters.

In AC group the plasma omentin concentration was positively correlated with bilirubin concentration (R = 0.502, P < 0.01), and glucose concentration (R = 0.390, P < 0.05). The omentin concentration showed negative correlation with PLT (R = -0.379, P < 0.05) and RBC (R = -0.451, P < 0.05). It was found also positive correlation between vaspin level and BIL (R = 0.411, P < 0.05), and negative correlation between vaspin and RBC (R = -0.500, P < 0.01), as well as between vaspin and HC (R = -0.475, P < 0.01).

However, the relationship between the cytokines concentrations and MELD or Child-Pugh scales, as well as BMI were not observed in this group.

#### The results of histopathological examination

The results of histopathological changes in patients of groups PBC and NAFLD are shown in Table 4.

Table 3. Selected laboratory parameters in three groups of patients.

| Variable                                     | PBC                               | NAFLD  | AC  | P      |
|--|-----------------------------------|--|---|--------|
| <b>WBC</b><br>(thous./ $\mu$ l) <sup>^</sup> | 6.64 $\pm$ 1.65<br>(4.34 – 9.92)  | 7.1 $\pm$ 1.9<br>(3.7 – 13)                  | 5.5 $\pm$ 2.29 <sup>a</sup><br>(2.71 – 11.2)  | < 0.01 |
| <b>RBC</b><br>(mill./ $\mu$ l) <sup>*</sup>  | 4.47 $\pm$ 0.42<br>(3.42 – 5.45)  | 4.94 $\pm$ 0.35<br>(4.3 – 5.6)               | 3.7 $\pm$ 0.64<br>(2.76 – 5.26)               | < 0.01 |
| <b>HGB</b><br>(g/dl) <sup>*</sup>            | 13.63 $\pm$ 1.32<br>(11.5 – 17.1) | 15.02 $\pm$ 1.08<br>(13.3 – 17.7)            | 11.85 $\pm$ 1.79<br>(8.4 – 15.1)              | < 0.01 |
| <b>HC</b><br>(%) <sup>*</sup>                | 40.43 $\pm$ 4<br>(31.8 – 49.1)    | 43.25 $\pm$ 2.7<br>(38 – 48)                 | 35.02 $\pm$ 4.47<br>(26.6 – 43.1)             | < 0.01 |
| <b>PLT</b><br>(thous./ $\mu$ l) <sup>^</sup> | 272.5 $\pm$ 102.5<br>(157 – 387)  | 264 $\pm$ 59<br>(150 – 371)                  | 97.11 $\pm$ 33 <sup>a</sup><br>(41 – 230)     | < 0.01 |
| <b>CRP</b><br>(mg/l) <sup>^</sup>            | 4.18 $\pm$ 2.94<br>(0.9 – 8.11)   | 3.3 $\pm$ 2.5<br>(0.6 – 11.9)                | 3.43 $\pm$ 2.87<br>(0.93 – 10.82)             | NS     |
| <b>ALT</b><br>(U/l) <sup>^</sup>             | 55.5 $\pm$ 38<br>(20 – 132)       | 67 $\pm$ 64<br>(10 – 153)                    | 53 $\pm$ 20<br>(32 – 123)                     | NS     |
| <b>AST</b><br>(U/l) <sup>^</sup>             | 48.5 $\pm$ 26.5<br>(24 – 113)     | 41 $\pm$ 25<br>(12 – 82)                     | 64 $\pm$ 22 <sup>a</sup><br>(45 – 124)        | < 0.01 |
| <b>Bilirubin</b><br>(mg/dl) <sup>^</sup>     | 0.98 $\pm$ 0.56<br>(0.55 – 5)     | 1.3 $\pm$ 0.5<br>(0.8 – 3.5)                 | 3.28 $\pm$ 2.32 <sup>a</sup><br>(0.63 – 7.8)  | < 0.01 |
| <b>GGTP</b><br>(U/l) <sup>^</sup>            | 263 $\pm$ 203<br>(43 – 909)       | 71 $\pm$ 61<br>(11 – 555)                    | 162 $\pm$ 95<br>(79 – 325)                    | < 0.01 |
| <b>Creatinine</b><br>(mg/dl) <sup>^</sup>    | 0.89 $\pm$ 0.2<br>(0.72 – 1.13)   | 1 $\pm$ 0.14<br>(0.8 – 1.4)                  | 0.98 $\pm$ 0.22<br>(0.7 – 2.17)               | < 0.05 |
| <b>Glucose</b><br>(mg/dl) <sup>^</sup>       | 96 $\pm$ 12<br>(80 – 150)         | 105 $\pm$ 29<br>(82 – 235)                   | 87 $\pm$ 29<br>(67 – 132)                     | < 0.01 |
| <b>PT</b><br>(s) <sup>^</sup>                | 11.2 $\pm$ 0.65<br>(10.3 – 13.6)  | 11.8 $\pm$ 2<br>(10.6 – 13.8)                | 15.7 $\pm$ 3.6 <sup>a</sup><br>(12 – 44)      | < 0.01 |
| <b>INR</b> <sup>^</sup>                      | 1 $\pm$ 0.06<br>(0.91 – 1.2)      | 1.04 $\pm$ 0.1<br>(0.94 – 1.22)              | 1.42 $\pm$ 0.3 <sup>a</sup><br>(1.06 – 2.19)  | < 0.01 |
| <b>APRI</b> <sup>^</sup>                     | 0.54 $\pm$ 0.31<br>(0.22 – 2.32)  | 0.47 $\pm$ 0.25<br>(0.12 – 1.31)             | 1.93 $\pm$ 1.48 <sup>a</sup><br>(0.76 – 4.56) | < 0.01 |
| <b>FIB4</b> <sup>^</sup>                     | 67 $\pm$ 56<br>(17 – 422)         | 40 $\pm$ 62<br>(2.83 – 271)                  | 267 $\pm$ 279 <sup>a</sup><br>(70 – 692)      | < 0.01 |
| <b>AAR</b> <sup>^</sup>                      | 0.96 $\pm$ 0.26<br>(0.62 – 1.32)  | 0.65 $\pm$ 0.27 <sup>b</sup><br>(0.42 – 1.2) | 1.2 $\pm$ 0.2 <sup>cd</sup><br>(1.01 – 1.47)  | < 0.01 |
| <b>HOMA-IR</b> <sup>^</sup>                  | 3.38 $\pm$ 2.35<br>(1.2 – 13.06)  | 4.73 $\pm$ 4.49<br>(1.54 – 19.75)            | 3.68 $\pm$ 2.92<br>(1.3 – 15.52)              | NS     |

**Abbreviations:** AAR, aspartate aminotransferase and alanine aminotransferase ratio; ALT, alanine aminotransferase; APRI – AST to platelet ratio index; AST, aspartate aminotransferase; CRP, C-reactive protein; FIB4, Fibrinogen 4 Score; GGTP, gamma-glutamyltranspeptidase; HC, hematocrite; HGB, hemoglobin; HOMA-IR, homeostasis model assessment - insulin resistance; INR, international normalized ratio; NAFLD, nonalcoholic fatty liver disease; PBC, primary biliary cholangitis; PLT, platelets; PT, prothrombin time; RBC, red blood cells; WBC, white blood cells.

<sup>^</sup> median and interquartile range IQR (Test Kruskal Wallis test);

<sup>\*</sup> mean and standard deviation (ANOVA);

<sup>a</sup> significant difference between AC and other groups

<sup>b</sup> significant difference between NAFLD and PBC groups

<sup>c</sup> significant difference between PBC and AC groups

<sup>d</sup> significant difference between NAFLD and AC groups.

Statistical analysis was performed between the cytokines and liver histopathological changes: fibrosis, inflammation, steatosis, balloon degeneration and in two groups of patients: PBC and NAFLD.

Irisin had significant positive correlation with the stage of fibrosis ( $r = 0.545$ ,  $P < 0.05$ ) and grade of inflammation ( $r = 0.515$ ,  $P < 0.05$ ) in PBC group, but negative correlation ( $r = -0.402$ ,  $P < 0.05$ ) with the grade of inflammation in NAFLD group.

Significant relationships between histopathological features and omentin or vaspin plasma concentrations were not found.

Exploring the importance of cytokines as the predictors of histopathological changes of the liver and taking into account together all the patients in whom the liver biopsy was done (groups PBC and NAFLD), positive correlations between omentin concentration and stage of fibrosis ( $R = 0.326$ ,  $P < 0.05$ ), as well as grade of inflammation ( $R = 0.456$ ,  $P < 0.01$ ), and

Table 4. Results of histopathological examination.

|       | Number of patients | Fibrosis  |     |     |     |     | Inflammation |                      |    |     |    |
|-------|--------------------|-----------|-----|-----|-----|-----|--------------|----------------------|----|-----|----|
|       |                    | 0         | I   | II  | III | IV  | 0            | I                    | II | III | IV |
| PBC   | 20                 | 1         | 12  | 4   | 3   | 0   | 0            | 2                    | 10 | 5   | 3  |
| NAFLD | 25                 | 13        | 8   | 2   | 2   | 0   | 7            | 9                    | 3  | 4   | 2  |
|       |                    | Steatosis |     |     |     |     |              | Balloon degeneration |    |     |    |
|       |                    | 0%        | 10% | 20% | 30% | 40% | 50%          | >60%                 | 0  | 1   | 2  |
| PBC   | 20                 | 16        | 1   | 1   | 1   | 1   | 0            | 0                    | 10 | 10  | 0  |
| NAFLD | 25                 | 0         | 3   | 3   | 4   | 7   | 7            | 1                    | 1  | 16  | 8  |

0, I, II, III, IV, stages of fibrosis, grades of inflammation; % percent of steatosis; balloon degeneration: 0 - absent, 1 - mild, 2 - severe. Abbreviations: NAFLD, nonalcoholic fatty liver disease; PBC, primary biliary cholangitis.

negative correlation between omentin concentration and grade of steatosis ( $R = -0.325$ ,  $P < 0.05$ ), as well as balloon degeneration ( $R = -0.295$ ,  $P = 0.05$ ) were found. The positive correlation between omentin level and AAR ( $R = 0.435$ ,  $P < 0.01$ ) was also showed.

#### DISCUSSION

Three groups of patients with NAFLD, PBC and AC as well as the group of healthy volunteers were included to the study, and three cytokines (two of them are adipokines and one is mainly myokine) were explored.

The highest concentration of omentin was found in AC group and the lowest in CG. This concentration was not statistically different between PBC and NAFLD groups. Similar results were shown in the Eisinger *et al.* study (25), where the concentrations of omentin were increased in patients with cirrhosis (without portal hypertension), especially in portal vein comparing to hepatic veins and systemic circulation, because of metabolism of this adipokine by the liver. Cirrhosis reduces the ability of this adipokine metabolism, which is the reason of its higher serum concentration (25).

Other study (14) showed higher plasma concentration of omentin in cirrhotic patients without portal thrombosis comparing to subjects with it.

The positive correlation between omentin and bilirubin concentration in AC group could indicate, that the concentration of this adipokine is proportional to the severity of the disease. Also the negative correlation between omentin concentration and the number of platelets as well as erythrocytes in AC group showed in the study suggested, that this adipokine is higher in most advanced liver damage. Notwithstanding of the above results, any relationships between the plasma concentration of omentin and the severity of the disease, measured according to Child - Pugh or MELD scale, was not showed, similarly as in other studies (25, 26).

There was not relationship between the concentration of omentin and BMI in any of the explored group. Herder *et al.* found negative correlation between omentin concentration and BMI in obese patients (2).

We did not found the relationship between omentin concentration and the elements of lipidogram: total cholesterol, its HDL, LDL fractions and TG concentration in NAFLD group, similarly as in Elsaid *et al.* study (27). In opposite, Montazerifar *et al.* found positive correlation between omentin and HDL cholesterol plasma concentration (28) in NAFLD group. Herder

*et al.* found positive correlation between omentin and HDL concentration and negative between omentin and TG concentration (2).

Generally known tendency to intolerance of glucose in cirrhotic patients could explain the weak, but positive correlation between omentin and glucose in our study.

There was no relationship between plasma omentin concentration and histopathological characteristics in PBC or NAFLD group. This results are in agreement with the previous study, where fibrosis in the liver of obese patients with NAFLD did not have influence on plasma omentin concentration, though it was associated with higher glucose concentration and HOMA-IR value (29). However, other research proved the positive correlation between omentin concentration and balloon degeneration of hepatocytes in patients with NAFLD (3).

We explored the relationship between the omentin concentration and the histopathological characteristics in patients submitted liver biopsy independently of disease (calculation for PBC and NAFLD groups together). It was found, that the concentration of omentin correlated positively with the grade of inflammation and stage of fibrosis, whereas negatively with the grade of steatosis and hepatocytes balloon degeneration. However, this results should be considered with caution, because this combination of the different groups definitely decreases the scientific value of this results.

The correlations between serum omentin level concentration and laboratory markers of fibrosis like APRI, FIB-4, HOMA-IR and AAR in any of independent group of patients were not found.

Irisin plasma concentration was the highest in CG, the lowest in AC group, and between PBC and NAFLD the difference was not significant. Zahng *et al.* (17) and Polyzos *et al.* (30) showed that plasma concentration of irisin was lower in NAFLD group than in control healthy subjects. In opposite, in the study of Choi *et al.*, the level of irisin was significantly higher in patients with NAFLD (19).

The relationship between plasma irisin concentration and BMI in any group was not found. Pardo *et al.* (13), and Shoukry *et al.* (14) showed higher irisin concentrations in obese adults.

Any relationships between irisin plasma concentration and laboratory parameters were not found, except INR. The negative correlation between it and irisin was observed in NAFLD group. It is possible, that even mild liver damage has the weak impact on irisin plasma level. However, it could also be a coincidence. Zahng *et al.* (17) found negative correlation between plasma irisin concentration and aminotransferases activity which was not showed in our study.

There was positive correlation between irisin plasma concentration and the grade of inflammation as well as the stage of fibrosis in PBC group, and negative correlation between irisin and severity of inflammation in NAFLD group. Polyzos *et al.* (30), oppositely proved, that irisin level positively correlated with severity of periportal inflammation, and its concentration had a trend to increase according to the grade of inflammation as well as stage of fibrosis in NAFLD patients. These opposite results obtained by Polyzos in NAFLD patients could suggest, that the relationship between irisin and liver histopathological lesions depends on severity of them. In less advanced lesions correlation could be negative, but in moderate - positive. In our NAFLD group mild lesions dominated. In PBC group, moderate histopathological lesions brought positive correlations with irisin plasma concentration. However, the reason of these variability is obscure, because in AC group, where the histopathological lesions are the most advanced, the irisin concentration was the lowest. In this point the results are similar like in Polyzos study. Nevertheless, it is likely that other factors have the impact on obtained results.

The exercises and physical effort have the most established impact on the production and plasma level of irisin. In overweight and obese patients the muscle mass is relatively decreased (31). NAFLD group was obese and it could be the additional reason of decreased irisin concentration. Probably AC group has the lowest physical activity (not measured, however it is well known that cirrhotic patients have small activity) and the muscle mass is small. Thus, the plasma concentration of irisin can be the result of variability of muscle mass and physical activity, and only partially, if any, the consequence of liver histopathological lesions.

Explored groups did not differ with regard to plasma vaspin concentrations. These results are in agreement with Montazerifar *et al.* study (28). Oppositely, other authors confirmed increased vaspin levels in NAFLD group (32), NASH (nonalcoholic steatohepatitis) group (33) (however canceled after multifactorial analysis corrected by parameters of glucose and lipid profile), and higher vaspin mRNA level assessed in liver of morbidly obese women with NAFLD (34). Positive correlation between plasma vaspin concentration and INR in NAFLD group as well as with bilirubin in AC group, and negative correlation with number of erythrocytes as well as hematocrit value suggests, that vaspin level depends on some laboratory signs of liver damage severity.

However, the relationship between vaspin concentration and the grades of liver steatosis, inflammation or balloon degeneration was not found. Oppositely, Aktas *et al.*, showed positive correlation between vaspin and stage of fibrosis, suggesting, that vaspin could be the independent indicator of fibrosis (32). In the other study (35) vaspin level was decreased in patients only without or with mild fibrosis, but not with more advanced fibrosis. Vaspin plasma concentration had also positive correlation with the grade of steatosis (35), hepatocyte balloon degeneration and aminotransferases activity, which was not proved in this study.

The relationship between vaspin concentration and MELD index or Child-Pugh scale was not found, which is in agreement with other previous research (26). Thus, vaspin seems to not be good indicator of liver histopathological lesions or severity of liver disease, but has the trend to be dependent on some laboratory parameters. Probably, the larger group with more advanced liver lesions is required for discover these relationships.

As was mentioned above, vaspin is the natural serine protease inhibitor. On the other hand, sorafenib - approved drug-based treatment for terminal primary liver cancer - is a small multi-kinase inhibitor. It was showed that sorafenib suppressed

neoplastic proliferation and resultant metastasis in malignant liver cancer cells (36). Sorafenib blocks tumor growth in advanced HCC through activating hepatocellular endoplasmic reticulum (ER) stress (36). It is the subject of interest if waspin (as serine protease inhibitor) could affect proliferation of the cells in chronic liver disease. It should be explored in the future studies. Moreover, some mechanisms of sorafenib could be probably used in other therapeutic agents and employed in the treatment of benign but progressive chronic liver diseases.

It should be emphasized, that our patients have not clinical and laboratory symptoms of infection. It is important issue, because infection could affect clinically state and the level of some molecules. It was showed (37), that the levels of endocan (newly recognized biomarker of sepsis), procalcitonin, CRP and TNF- $\alpha$  were significantly higher in cirrhotic patients with clinically overt infection. The degree of endothelial cell injury induced by a systematic inflammatory response is a pathologic process that could modify the course of advanced liver diseases, among them cirrhosis (37). Endocan and procalcitonin, but also proinflammatory, as well as profibrotic cytokines should be considered as the biomarkers of advancement of liver diseases, especially cirrhosis.

Many factors, both beneficial and unfavorable can affect the course of liver disease. It was proved that short term treatment with probiotic (VSL#3) administration in patients with liver cirrhosis led to modulation of plasma levels of several molecules and compounds (MIP-3 $\alpha$ /CCL20, nitric oxide, big-endothelin, thromboxane B<sub>2</sub>, myeloperoxidase) (38). However, the grade of encephalopathy remained unaffected in the patients with liver cirrhosis in this study. It is not clear if probiotics could affect irisin, vaspin and omentin in chronic liver disease and it will be the subject of interest in the future studies.

#### *Limitations of the study*

This study was performed on relatively small number of patients because of difficulties in recruiting patients with newly diagnosed PBC, without treatment. The number of patients included into each group and the control group should be comparable; thus, we didn't include more patients into the NAFLD, AC and control group as well. Therefore, we consider this study as a preliminary analysis.

Anthropometric measurements and gender differed significantly between groups in the study. PBC is relatively more frequent in women over 50 years old. AC is more frequent in men, at any age. Patients with NAFLD are usually overweight or obese and the number of male and female were comparable. Quantitative distribution by gender and age in each group approximately mirrored it in general population and patients included into the study represented the population's prevalence of diseases. It is the reason, that the groups are not comparable in terms of anthropometric parameters and age. It should be emphasized, that the presence of ascites, edema, protein disorders and decreasing of muscle mass have the impact on anthropometric parameters in AC group, which is an additional reason for the incomparability of groups.

Summarizing, our study indicate that omentin, among many other factors, probably could participate in the pathophysiology of liver diseases. The highest concentration of it in AC group, and its relationship with some laboratory parameters typical for severe liver damage suggests potential involvement of it in more advanced liver diseases. The trend to dependence of omentin on stage of fibrosis and grade of inflammation only in NAFLD and PBC is unclear and required future studies.

Irisin is very interesting, probably 'good' myokine, which was the lowest in AC. However, positive correlation of it with grade of inflammation in PBC group but negative in NAFLD, as

well as positive with the stage of fibrosis only in NAFLD, was unclear and required following studies on larger groups of patients with more advanced histopathological lesions.

Vaspin should be considered as an inappropriate indicator of liver damage, because only few relationship in PBC and in AC group was proved. Other conflicting results require future studies.

Conflict of interests: None declared.

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