INTRODUCTION

Nonalcoholic steatohepatitis (NASH) has become one of the most common liver diseases, affecting both adults and children in the United States as well as many other Western countries. It is a progressive disease characterized by pathologic findings resembling those of alcoholic hepatitis despite the absence of significant alcohol consumption (1). NASH is part of the spectrum of liver damage named nonalcoholic fatty liver disease (NAFLD), ranging from simple fatty liver, which is generally nonprogressive, to advanced fibrosis and cirrhosis (2, 3).

Although in most cases NAFLD follows benign natural history, recent studies have suggested that about 10-20% of patients will progress to cirrhosis during an approximately 14-years period (4, 5). Moreover, the risk of development of hepatocellular carcinoma in NASH-related cirrhosis is comparable to the one complicating hepatitis C infection (6). NAFLD and NASH are strongly associated with obesity, insulin resistance (with or without type 2 diabetes mellitus), dyslipidemia, and hypertension (7, 15). About 90% of patients with NAFLD have one or more characteristic features of metabolic syndrome (6).

Numerous studies have suggested that adipohormones (adiponectin, resistin, leptin, TNF-α and IL-6) are involved in the pathogenesis of many acute and chronic liver diseases. The aim of this study was to evaluate the plasma concentrations of adiponectin, resistin, leptin, TNF-α, and IL-6 in patients with NASH, as well as their correlation with the pathologic parameters. Serum concentration of leptin, adiponectin, resistin, insulin, TNF-α, IL-6 were measured with ELISA method. Liver biopsies were obtained from 18 (age 42.55±21 years) patients. NASH has been classified according to Dixon score. The control group was represented by 16 non-obese subjects. Mean serum concentration of adiponectin in patients with NASH was significantly lower than in healthy subjects (4.87±1.96 vs. 8.33±4.56 ng/ml; p<0.05). Mean serum levels of TNF-α in patients with NASH were significantly higher than in controls (34.2±19.7 vs. 20.7±15.5 ng/ml; p<0.05). In patients with more advanced inflammation (grade 2-3) and fibrosis (stage 2) in pathology, serum concentration of leptin was significantly higher than in patients with steatosis and less advanced inflammation (grade 1) and fibrosis (stage 1) (median 8.94 vs. 16.2 ng/ml; p<0.05). No significant differences of serum concentration of others adipohormones between these two groups of patients were stated. Moreover, we observed the correlation in serum levels (examined group vs controls) between: resistin and TNF-α (r = 0.62; p<0.05), adiponectin and IL-6 (r = -0.60; p<0.05) and leptin and insulin (r = -0.51; p<0.05). In conclusion, based on our study we speculate that changes of adipohormones levels may be markers of NASH and the serum level of leptin can be associated with more advanced form of NASH.

Key words: nonalcoholic steatohepatitis, leptin, resistin, adiponectin, tumor necrosis factor α, interleukin 6, insulin resistance
skeletal muscles (17). Resistin is up-regulated by proinflammatory cytokines including TNF-α, IL-6, IL-1β (11, 17). In turn, resistin through NF-κB activation, promotes synthesis of TNF-α, IL-6 and another proinflammatory agents (11).

Leptin is responsible for reduction of food intake and increases energy expenditure (12, 18, 19). However, obese persons have increased leptin levels (16, 20, 21) with reduced leptin receptor expression, resulting in leptin resistance (5, 12). Apart from its effects on energy homeostasis leptin is implicated in prevention of lipid accumulation in nonadipose tissue (18) and regulation of immune system (polarizes T-helper cytokine production toward proinflammatory phenotype). In addition, it is also involved in fibrogenesis (profibrogenic action) and modification of insulin sensitivity in muscles and liver (12, 20, 22).

A number of study have demonstrated enhanced TNF-α expression in patients with NASH (23). TNF-α is an adipokine with a well-known role in antagonizing the effects of adiponectin (suppresses the transcription of adiponectin in adipocytes (10) and contributes to insulin resistance (5, 13). Recent observations suggest that it is also involved in the metabolic syndrome and progression of NAFLD (5, 20).

Besides TNF-α, IL-6 is other proinflammatory cytokine produced by adipose tissue (10). The circulating level of IL-6 increases in obese subjects (24) and decreases in parallel with weight loss and improvement in insulin resistance (10). Moreover, IL-6 impairs insulin signaling in hepatocytes, resulting in increased hepatic gluconeogenesis, followed by hyperglycemia and compensatory hyperinsulinemia (9, 25).

High prevalence of NAFLD and different progression for patients with simple steatosis and steatohepatitis, make important to identify patients with NASH, particularly those at risk for advanced fibrosis (8, 26). Currently liver biopsy is the only definitive diagnostic tool and gold standard to distinguish patients with NASH, as well as their correlation with the severity of liver disease. The aim of this study was to evaluate the plasma concentrations of adiponectin, resistin, leptin, TNF-α and IL-6 in patients with NASH, as well as their correlation with the severity of liver disease.

MATERIAL AND METHODS

The study group comprised 18 patients with biopsy-proven NASH (median age 42.55±21; 16 men and 2 women). 11 (61%) patients have arterial hypertension, 3 (16.7%) suffer from diabetes mellitus and 3 (16.7%) were affected with coronary heart disease. Patients who consumed more than 20 g of alcohol per day, with coexisting viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson disease, hemochromatosis, biliary obstruction and drug-induced liver disease were excluded. 16 non-obese, healthy volunteers were recruited as controls. Informed written consent was obtained from every patient. The study protocol was approved by the Ethics Committee of the Medical University of Lodz.

Liver biopsy specimens were stained with hematoxylin and eosin, and according to Van Gieson. Additionally, periodic acid-Schiff (PAS) and Masson trichrome stains were made. Grading and staging of NASH followed the criteria of Dixon et al. (28). Nonalcoholic steatohepatitis was defined as steatosis accompanied with the two of the following: inflammatory foci with mononuclear cells and/or neutrophils, ballooning degeneration of hepatocytes (with or without Mallory bodies) and pericellular fibrosis (28).

Blood samples have been obtained in the morning after 12 hours fasting from all examined subjects. In all patients body mass index (BMI) value has been calculated. Blood serum was obtained after 30 minutes clotting and centrifugation at 2000 rpm for 15 minutes at 4°C. Serum was removed and stored frozen at 80°C. Leptin, resistin and adiponectin concentration were measured with ELISA (R&D Systems, USA). The ELISA tests for all investigated adipohormones had been performed at the same time in all patients.

Statistical analysis

To perform statistical analysis STATISTICA version 5.0 PL software was used. The data are presented as mean±SD. To test the distribution of results the Kolmogorov-Smirnoff test has been performed at levels 0.05. Comparisons between the groups were performed with Student t-test or Mann-Whitney U test. Pearson correlation between investigated parameters in examined groups has been used. For all tests a P value <0.05 was considered as statistically significant.

RESULTS

Mean serum concentration of adiponectin was significantly lower in patients with NASH than in controls (4.87±1.96 vs. 8.33±4.56 ng/ml; P<0.05). In examined group the serum levels of TNF-α were significantly higher than in healthy subjects (34.2±19.7 vs. 20.7±15.5 ng/ml; P<0.05). Serum concentration of leptin, resistin and IL-6 showed no significant differences between examined and control group, and are summarized in Table 1.

### Table 1. Serum concentration of adipokines and insulin in examined and control group.

<table>
<thead>
<tr>
<th></th>
<th>Experimental</th>
<th>Controls</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>n=18</td>
<td>n=16</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>12.9±13.25</td>
<td>14.82±12.92</td>
<td>0.633</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>4.87±1.95</td>
<td>8.33±4.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>22.94±6.3</td>
<td>25.78±26.02</td>
<td>0.165</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>16.41±8.07</td>
<td>17.87±16.04</td>
<td>0.425</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>34.2±18.88</td>
<td>20.70±10.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.03±3.4</td>
<td>4.23±2.12</td>
<td>0.403</td>
</tr>
</tbody>
</table>

Mann-Whitney U test
In patients with more advanced inflammation (grade 2-3) and fibrosis (stage 2) (n=7) mean serum concentration of leptin was significantly higher than in patients with steatosis and less advanced inflammation (grade 1) and fibrosis (stage 1) (n=11), (16.2 vs. 8.94 ng/ml; P< 0.05). No significant differences of levels the others adipohormones depending on histological parameters were found.

Moreover, we observed the positive correlation between resistin and TNF-α (r = 0.62; P<0.05) (Fig. 1), negative correlation between adiponectin and IL-6 ( r = -0.60; P<0.05) (Fig. 2) and leptin and insulin (r =-0.51; P<0.05) (Fig. 3).

**DISCUSSION**

The pathogenesis of NAFLD/NASH and, in particular, the mechanisms responsible for liver injury and disease progression remain still incompletely understood (9). Recent studies have focused on the adipokines, bioactive proteins secreted by adipose tissue, including leptin, resistin, adiponectin, tumor necrosis factor α and interleukin 6 (10, 11, 25, 29). Recently, adipokines influence on insulin resistance (central factor in the development and progression of NAFLD) and inflammation, have been investigated (23, 30). Increasing evidence indicates that they might play important roles in the NASH pathogenesis (8, 29).

A number of studies have demonstrated the association between hypo-adiponectinemia and NAFLD (29). In our study we observed significantly lower serum concentration of adiponectin in patients with NASH than in healthy subjects. Our finding are in accordance with the recent report by Hui et al. (3), Musso et al. (2) and Shimada et al. (1). They reported that serum adiponectin level was significantly lower in patients with NASH than in the control group. Moreover, Hui et al. (3) observed that lower serum adiponectin level in NASH patients was associated with more extensive necroinflammation. Similarly, Musso et al. (2) reported negative correlation between serum adiponectin and liver steatosis, necroinflammation and fibrosis. In our study we did not observe relationship between serum adiponectin level and histologic features what is in contradictory with two previous reports. On the other hand, Wong et al. (29) and Bugianesi et al. (31) did not find the correlation between serum adiponectin concentration and the disease severity, what is in agreement with our data. An explanation for this differences between the studies could be that Musso et al. (2) enrolled only non-obese, non-diabetic patients with NASH, while in our investigated group we observed diabetes mellitus in 3 patients and all patients had BMI ≥25 kg/m². Furthermore, in the Hui et al. (3) study 20% of the NASH patients had stage 4 fibrosis. Our study did not include the patients with liver cirrhosis.

In the present study we observed the negative correlation between adiponectin and IL-6. Adiponectin level is correlating inversely with circulating levels of TNF-α and IL-6 because both these cytokines inhibit adiponectin messenger RNA in adipose tissue (30). On the other hand, adiponectin induces its anti-inflammatory properties by suppression of IL-6 (23). Studies of Ota et al. (25) support the idea that excessive production of IL-6 versus the defective production of adiponectin may provide a link between insulin resistance and inflammation in NASH.

Our study also demonstrates higher TNF-α serum levels in patients with NASH than in control, what is in agreement with Jarrar et al. study (8). In the latter, TNF-α levels significantly increased in simple steatosis compared to obese controls, and even higher in NASH (8). Similarly, Crespo et al. (32) and Wai-Sun Wong et al. (29) showed increased expression of TNF-α and its type 1 receptor in patients with NASH compared with patients with simple steatosis.

**Fig. 1.** Correlation between resistin and TNF-α serum levels in examined group vs. controls; r = 0.62

**Fig. 2.** Correlation between adiponectin and IL-6 serum levels in examined group vs. controls; r = -0.60

**Fig. 3 Correlation between leptin and insulin serum levels in examined group vs. controls; r = -0.51**

The role of resistin in the pathogenesis of NASH is not clear. Jarrar et al. (8) did not find statistically significant correlation between serum resistin levels in patients with NASH, simple steatosis and obese controls. Similarly, in our study we did not observed relationship between serum resistin concentration in patients with NASH and controls. On the other hand, Pagano et al. (33) observed that increased resistin levels in NAFLD patients were related to the histological severity of the disease.

Another interesting issue in our study was the correlation between resistin and TNF-α levels. This observation is in agreement with data showing that resistin is up-regulated by
proinflammatory cytokines including TNF-α, IL-6, IL-1β (11, 17). In turn it promotes the synthesis of these cytokines, by NF-κB activation (11). Moreover, Yagmur et al. (17) reported the correlation between serum TNF-α level (which was one of the parameters indicating chronic inflammatory condition) and serum resistin in patients with chronic liver diseases (chronic hepatitis B or C virus infection, alcohol, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis).

We did not observe significant differences in serum leptin levels between patients with NASH and controls. Our data correspond with those of Chalasani et al. (34), Musso et al. (2) and Angulo et al. (35), where no differences between serum leptin levels in patients with NASH and controls were found. On the other hand, Chitturi et al. (18) found that leptin levels were significantly higher in NASH patients than in controls. However, we noted significantly higher serum concentration of leptin in patients with more advanced inflammation and fibrosis than in patients with less advanced inflammation and fibrosis, which is in agreement with Angulo et al. (35) data showing the correlation between serum leptin levels and fibrosis severity.

Another interesting issue in our research is that we have observed the negative correlation between the concentration of leptin and insulin. It might indicate an inhibiting role of leptin on secretion of insulin by the β-cells. Due to the presence of leptin receptor on the surface of the β cells it is possible that leptin has influence on insulin secretion (36). It is reported that leptin is able to stimulate, restrain an even have no effect on the secretion of insulin by the β-cells (37). It has also been suggested that insulin has a direct influence on the synthesis and secretion of leptin (38). Due to the uncertain information about the concentration of leptin in patients with NASH, future research is needed in order to explain the role of leptin in the pathogenesis of development and progression of NAFL (39).

Based on our study we speculate that changes of adipokines levels provide the additional tool for NASH detection. Our data also suggest that serum leptin level may be associated with more advanced form of NASH. The possible role of all mentioned adipokines in the pathogenesis and progression of non alcoholic steatohepatitis needs to be confirmed in further investigations.

Conflict of interest statement: None declared.

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