Correlations between anthropometric and serologic elements of metabolic syndrome and histopathologic features of nonalcoholic fatty liver disease

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Abstract

Aim of the study: Studying the correlation between elements of metabolic syndrome and histological changes of the liver in nonalcoholic fatty liver disease.

Patients and Methods: Thirty-nine patients with nonalcoholic fatty liver disease were included in our study. Inclusion criteria were: presence of liver steatosis on ultrasound in patients with waist circumference over 94 cm in men and over 80 cm in women and with serologic elements of metabolic syndrome. Exclusion criteria were: chronic viral hepatitis, autoimmune hepatitis, Wilson disease, hemochromatosis, regular alcohol consumption. Body mass index, waist circumference, fasting plasma glucose, serum triglyceride and cholesterol levels and serum ALT were determined. On liver biopsy specimens, performed in each patient, the NASH score, representing the sum of fibrosis, steatosis, lobular inflammation and ballooning, was calculated.

Results: Necroinflammation was mild in 15 patients, medium in 19 patients and severe in five patients. Mild fibrosis was present in four cases, medium in 14 cases, severe in six, and two patients were diagnosed with cirrhosis. We found statistically significant correlation between waist circumference and the grade of histological activity, the presence of diabetes and both fibrosis grade and histological activity, and the serum ALT and histological activity.

Conclusions: Noninvasive assessment of the severity of hepatic histological changes in nonalcoholic fatty liver disease could be made by anthropometric parameters or by serologic components of metabolic syndrome, but it is not an accurate method to identify patients with high-risk for disease progression. These noninvasive parameters cannot replace liver biopsy.

Keywords: nonalcoholic fatty liver disease, metabolic syndrome, liver histology, body mass index, waist circumference.

Introduction

Metabolic syndrome or insulin resistance syndrome appears in overweight patients, because of visceral adipose tissue accumulation. It represents a multiple cardiovascular risk factor, nonalcoholic fatty liver disease being present in most patients with metabolic syndrome.

ATPIII (The National Cholesterol Education Program’s Adult Treatment Panel III report) defines metabolic syndrome as a multiple cardiovascular risk factor. Components of metabolic syndrome are central obesity, atherogenic dyslipidemia, elevated blood pressure, insulin resistance and/or impaired glucose tolerance, proinflammatory state, prothrombotic state. Proinflammatory and prothrombotic state are new in the definition of metabolic syndrome. Proinflammatory state is recognized by elevations of C-reactive protein, caused by obesity, inflammatory cytokines being released by the adipose tissue. Prothrombotic state is characterized by increased plasma plasminogen activator inhibitor (PAI)-1 and fibrinogen levels. Fibrinogen, an acute-phase protein, rises as a response to high-levels of cytokines [1].

Nonalcoholic fatty liver disease (NAFLD) and its more severe form, nonalcoholic steatohepatitis (NASH) are relatively new concepts in gastroenterology. The importance of NAFLD was underestimated for a long time, but researches revealed the evolutive potential of this liver condition. Today, NAFLD is considered the main cause of cryptogenic cirrhosis [2], and it can lead to development of hepatocellular carcinoma [3–5].

NAFLD is regarded as the liver manifestation of metabolic syndrome; its pathogenesis is related to insulin resistance, which plays a central role in the development of this hepatic disorder. The pathogenesis of NAFLD has been described based on a “two-hit model”. Insulin resistance promotes the transport of fatty acids from adipose tissue to the liver and their storage in the hepatocytes, as a first hit [6]. The steatotic liver becomes vulnerable to other aggressions. The second hit is represented by the harmful effect of certain aggressive factors: oxidative stress and cytokines, mainly TNF-α, leading to the exacerbation of insulin resistance, increased oxidative stress and dysfunction of hepatocyte organelles, resulting in an inflammatory process associated with hepatocellular degeneration and development of liver fibrosis [7]. In addition to this mechanism, it has been highlighted the pathogenic role of intestinal bacterial overgrowth, which may increase hepatic oxidative stress by two mechanisms: increase of

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endogenous ethanol production and release of bacterial lipopolysaccharides.

Histological findings are identical to those seen in alcoholic liver disease [8, 9], characterized by predominantly macrovesicular hepatic steatosis, that occurs in overweight, diabetic individuals in the absence of alcohol consumption in amounts considered harmful to the liver, justifying the name of nonalcoholic steatohepatitis. NAFLD has a wide range of presentation, from simple liver steatosis to steatosis associated with severe necroinflammation and fibrosis (Figures 1 and 2). Severe fibrosis increases the chance of cirrhosis development [10]. The NASH score is a histological scoring system for NAFLD, designed after the model of chronic viral hepatitis histological scoring. This score is based on the quantification of activity on a scale of 0–3 (including steatosis, ballooning and inflammation) and fibrosis on a scale from 0 to 4, stage 4 being equivalent with liver cirrhosis [11] (Tables 1 and 2).

### Table 1 – Global activity grade for nonalcoholic steatohepatitis

<table>
<thead>
<tr>
<th>Grade, grade</th>
<th>Steatosis</th>
<th>Ballooning</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild, grade 1</td>
<td>1–2</td>
<td>Minimal</td>
<td>Lobular: 1–2</td>
</tr>
<tr>
<td>Moderate, grade 2</td>
<td>2–3</td>
<td>Present:</td>
<td>Lobular: 2</td>
</tr>
<tr>
<td>Severe, grade 3</td>
<td>3</td>
<td>Marked:</td>
<td>Lobular: 3</td>
</tr>
</tbody>
</table>

1Steatosis grade: 1 = ≤33%; 2 = >33%≤66%; 3 = >66%.

### Table 2 – Fibrosis score for nonalcoholic steatohepatitis

<table>
<thead>
<tr>
<th>Stage</th>
<th>Zone 3:</th>
<th>Portal based fibrosis</th>
<th>Bridging fibrosis</th>
<th>Cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Perisinusoidal fibrosis, focal or extensive</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>As above</td>
<td>Portal and/or perportal fibrosis, focal or extensive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Bridging septa</td>
<td>Bridging septa</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>+/+; zone 3 may be incorporated into septa and no longer identified</td>
<td>Portal tracts may be replaced or incorporated into septa</td>
<td>Extensive</td>
<td>+</td>
</tr>
</tbody>
</table>

The prevalence of NAFLD and nonalcoholic steatohepatitis is high, the number of cases increases in the occidental world with the prevalence of metabolic syndrome. In the United States, NAFLD has a prevalence of 17–33%, 1/3 of the cases being severe, with advanced fibrosis and necroinflammation. In Europe, the prevalence is at least 22%. Approximately 75% of all patients with type 2 diabetes and obesity develop some form of NAFLD/steatohepatitis [12]. Since this is a very common liver disease, implicitly the number of patients affected is high. It is particularly worrying the growing prevalence of obesity among children, therefore nonalcoholic fatty liver has become an important pediatric liver disease [13].

The aim of our study was to determine the correlation between some elements of the metabolic syndrome (body mass index, waist circumference, fasting plasma glucose, serum triglycerides and serum cholesterol value) and histological changes in the steatotic liver (severity of steatosis, grade of necroinflammatory activity and the grade of fibrosis). We tried to find those noninvasive parameters that can be useful in assessing the severity of liver disease in patients suffering of NAFLD.

### Patients and Methods

In our study were included 39 patients with metabolic syndrome, diagnosed on the IDF criteria, all of them with hepatic steatosis, evidenced by abdominal ultrasound.

Inclusion criteria were:
- waist circumference over 94 cm in men and over 80 cm in women, equivalent to abdominal obesity by the IDF criteria;
- hepatic steatosis revealed at ultrasound.

Exclusion criteria were:
- evidence of chronic viral hepatitis: HBs antigen or anti-HCV positivity;
- elevated erythrocyte sedimentation rate, positive antinuclear antibodies and high gamma-globulin level for autoimmune hepatitis;
- transferring saturation >50% for hemochromatosis;
- low serum ceruloplasmin levels for Wilson disease;
- regular consumption of alcohol in quantities above 20 mL/day in men and more than 10 mL/day in women;
- medication with drugs known for their steatosis: inducing side effect (Amiodarone, steroids, Tamoxifen).

We determined in every patient the body mass index (BMI), which provides a reliable indicator of body fatness for most people. Overweight was considered if BMI was between 25–30. BMI over 30 was considered obesity. Since BMI can vary regardless of obesity grade, according to each person’s individual muscle mass, we determined in parallel the waist circumference also, which has a better correlation with insulin resistance than BMI.

Among laboratory parameters, we determined the following:
- fasting plasma glucose: depending on its value, we divided patients into three groups – those with normal fasting blood glucose up to 100 mg/dL, prediabetes if fasting blood glucose was between 100–126 mg/dL, and diabetes if fasting blood glucose was over 126 mg/dL or the patient was treated for diabetes;
- serum ALT, considered normal up to 31 U/L;
- serum total cholesterol, normal below 200 mg/dL;
- serum triglycerides, normal below 150 mg/dL.

All 39 patients underwent liver biopsy, after a written consent. Liver biopsy samples all had at least five complete portal tracts, allowing a proper grading and staging. We used the Hematoxylin–Eosin (HE) stain for an initial evaluation of overall lobular architecture. Masson’s trichrome stain was used to highlight the extension and grade of fibrosis. This latter dye-complex stains collagen fibers blue.

The assessment of liver histology was made by the same pathologist in all patients. NASH score was calculated in each case.

Statistical processing of data has been performed using Graph Pad Prism and SPSS programs. Analysis of relationship between two variables and the intensity
of this relationship was performed using parametric Pearson correlation coefficient, representing graphic correlation with the dispersion diagram. A relationship between the average number of variables was determined using ANOVA. We applied chi-square test to determine whether there is any statistically significant correlation between different parameters or not.

Results

Distribution by sex and age of the 39 patients was the following: 20 women aged between 36 and 75 years (mean age 57.1 years) and 19 men aged between 38 and 75 years (mean age 51.1 years).

Body mass index ranged from 26.5 to 34; the mean body mass index was 29.7. Relative to gender, women had the body mass index between 26.5–34 (mean 29.77) and men between 26.5–34.9 (mean 29.68).

The number of overweight patients, with body mass index up to 30 was 21, representing 53.8% of cases, and of those with obesity with a body mass index over 30 was 18 (46.2% of cases). Waist circumference ranged from 86 cm to 118 cm in women (mean 91 cm) and from 100 cm to 120 cm in men (mean 103.9 cm). Overweight women had waist circumference between 86–101 cm and obese women between 98–118 cm. Overweight men’s waist circumference ranged from 91 to 107 cm, and that of the obese from 101 to 120 cm.

Number of patients with normal fasting plasma glucose (without any treatment for diabetes) was nine (23%), fasting glucose was between 100–126 mg% in 18 (46.1%) patients, the remaining 12 (30.7%) patients were known with diabetes or had a fasting glucose over 126 mg%.

Hyperlipidemia was present in most patients, 22 (56.4%) of them had their triglyceride levels over 150 mg%, 27 (69.2%) patients had a total cholesterol over 200 mg%, 17 (43%) patients had mixed hyperlipidemia with both types of serum lipids elevated, and in four (10%) cases serum cholesterol and triglyceride levels were normal; under specific treatment, all of them being previously diagnosed with mixed hyperlipidemia.

Hepatic cytolysis syndrome, evidenced by elevated ALT values, was present in 17 (43.5%) patients.

In terms of pathology, necroinflammatory activity was minimal in 20 patients, moderate in 15 patients and severe in five patients. Fibrosis was grade 0 in 1 patient, minimal (grade 1) in nine patients, moderate (grade 2) in 15 patients, advanced (grade 3) in 12 cases, and two patients were diagnosed with cirrhosis (fibrosis grade 4).

Analyzing the correlation between body mass index, waist circumference and liver histology, we obtained the following results:

• The grade of histological activity had no statistically significant correlation with the mean body mass index \(p=0.38\).

• Mean waist circumference was 99.4 cm in the group of grade 1 histological activity, 104 cm in the group of grade 2, and 106.6 cm in patients with grade 3 histological activity. We applied Pearson’s correlation \(r=0.31\). The correlation was weak but statistically significant \(p=0.05\) (Figure 3).

Regarding fasting plasma glucose, we found statistically significant difference between the mean blood glucose levels in the three groups of histological activity \(p=0.06\). Mean fasting plasma glucose level was proportional with the histological activity grade, increasing from 99 mg% corresponding to histological activity grade 1 to 126 mg% in the group of grade 3 histological activity.

We applied the Pearson correlation, we had a weak positive correlation \(r=0.35\) and a statistically significant \(p\)-value \(0.02\) between fasting glucose and histological activity grade (Figure 4).

Dividing patients into three groups, with normal glucose levels, with values of prediabetes and diabetes respectively, and applying chi-square test, we found a statistically significant correlation \(p=0.014\) (Table 3).

Table 3 – Distribution of cases after fasting plasma glucose and histological activity grade

<table>
<thead>
<tr>
<th>Activity</th>
<th>No. of cases</th>
<th>Normal plasma glucose</th>
<th>Prediabetes</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>10</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

We can see that five of the eight (62.5%) patients with diabetes had grade 2 histological activity and three (37.5%) patients had grade 3 histological activity. Of the 15 patients with normal fasting plasma glucose, most had grade 1 histological activity (66.7%). We represented data as a figure, illustrating the distribution of cases according to fasting plasma glucose value and the three grades of histological activity (Figure 5).

Serum triglyceride levels had a positive, but statistically insignificant correlation with the grade of histological activity. Mean triglyceride level in patients with grade 3 activity was much higher (223 mg%) than the mean value in the group with grade 1 and 2 histological activity (164, respectively 161 mg%).

Serum cholesterol levels had also positive, but statistically insignificant correlation with the grade of histological activity, mean value of cholesterol increasing proportionally with the activity grade.

Mean ALT value was proportional with the histological activity, it has been elevated (above the maximum normal level) in the group with grade 3 histological activity (49.4 U/mL). There was a positive, statistically significant correlation \(r=0.35, p=0.02\) between transaminase values and grade of histological activity (Figure 6).

Regarding fibrosis, we found no statistically significant correlation between the grade of fibrosis and body mass index. Waist circumference had no statistically significant correlation with fibrosis grade either. The mean values of body mass index and waist circumference were very similar in the four groups of fibrosis grade.

The correlation between fasting plasma glucose levels and the grade of fibrosis, although positive, was not statistically significant \(p=0.37\). Given that the mean value of fasting plasma glucose was proportional with the fibrosis grade, we divided patients according to fasting plasma glucose value in three groups, with normal blood glucose, with values of prediabetes, and with diabetes respectively.
We applied chi-square test, obtaining a statistically significant correlation with the grade of fibrosis ($p=0.032$) (Table 4).

**Table 4 – Distribution of cases after fasting plasma glucose and fibrosis grade**

<table>
<thead>
<tr>
<th>Fibrosis</th>
<th>No. of cases</th>
<th>Normal fasting glucose</th>
<th>Prediabetes</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>8</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>5</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>2</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Six of the eight patients with diabetes (75%) had moderate or advanced fibrosis (grade 2 and over). Of the 15 patients with normal blood glucose, most (80%) had mild or moderate fibrosis (grade 1 and 2 fibrosis). Values of prediabetes we met especially in the intermediate fibrosis grade group, but there was also one case of cirrhosis with prediabetes plasma glucose value.

Serum triglycerides and cholesterol had positive correlation with the grade of fibrosis, but both correlations were found statistically insignificant.

Mean values of serum ALT did have no correlation
with the grade of fibrosis; however, both patients with fibrosis grade 4 had elevated ALT values.

**Discussion**

Nonalcoholic fatty liver disease is a big challenge for clinicians, because the lack of diagnostic methods those are reliable, accurate, reproducible, and easy to perform providing proper information about hepatic necroinflammation and fibrosis grade. Selection of patients with risk for disease progression is important. The most accurate diagnostic method for this is histological scoring of liver fibrosis and necroinflammation, the “gold standard of the grading of hepatic inflammation and the staging of hepatic fibrosis”. Liver tissue can be obtained by percutaneous liver biopsy, an invasive procedure with potential complications. Noninvasive and accurate screening tests that can replace liver biopsy are required to select those patients who need special medical approach to control their liver disease and to prevent the development of end stage liver disease [14]. Since development of NAFLD is closely linked to metabolic syndrome, many researchers tried to find out if there is any correlation between some elements of the metabolic syndrome and the severity of liver disease. There are many studies in literature, which focused on the correlation between the severity of histological changes in NAFLD and patient’s anthropometric data and laboratory parameters: fasting blood glucose, triglycerides, cholesterol, transaminases. Also, a strong relation between NAFLD and cardiovascular risk has been found.

The least studied were anthropometric data. Results are inconsistent. Ratziu V et al. have found that obesity is a risk factor for development of severe liver fibrosis [15]. Cheung O et al. concluded that the grade of abdominal obesity correlates with hepatic necroinflammatory activity [16]. Uslusoy HS et al. found no statistically significant correlation between body mass index, waist circumference and severity of necroinflammatory activity, respectively the fibrosis grade [17]. In our group of patients with nonalcoholic fatty liver, we found that body mass index had a positive, but statistically insignificant correlation with the histological changes (grade of fibrosis, respectively necroinflammatory activity). We also found that waist circumference had statistically significant correlation with the grade of necroinflammatory activity.

Metabolic disorders characteristic for metabolic syndrome (diabetes or impaired glucose tolerance, hypercholesterolemia and hypertriglyceridemia) are considered factors that may influence to some extent liver histology in NAFLD. Studies published in literature reached different, somehow contradictory conclusions. Singh DK et al. consider that serum cholesterol level is an independent predictive factor for the severity of histological lesions in non-alcoholic fatty liver [18]. After Rodriguez-Hernández H et al., diabetes and serum ALT are the factors that correlate best with histological changes [19]. Assy N et al. found that the main risk factors for severe steatosis were diabetes and hypertriglyceridemia [20]. Alkhouri N et al. found strong correlation between the severity of histological changes and atherogenic lipid profile [1]. In our study, we found no statistically significant correlation between hyperlipidemia (both elevated serum cholesterol and hypertriglyceridemia) and histological changes characteristic for nonalcoholic steatohepatitis. Fasting blood glucose and the presence or absence of diabetes were found to be potential predictors for liver disease severity, they had a positive correlation with fibrosis grade and histological activity.

We found several studies about the relationship of serum ALT with histological changes of the liver in NAFLD. Singh DK et al. [18] and Uslusoy HS et al. [17] found statistically significant correlation between serum ALT values and the histological activity grade in nonalcoholic steatohepatitis. Other authors state that a patient might have advanced liver damage and normal serum ALT levels, so the serological markers of hepatic cytolysis are not helpful in selecting cases of severe nonalcoholic steatohepatitis [21]. Our study shows statistically significant correlation between serum ALT and histological activity grade ($p=0.014$) and the lack of statistically significant correlation between the serum ALT and the grade of fibrosis.

**Conclusions**

The severity of histological changes (necroinflammation and fibrosis) that characterize NAFLD cannot be assessed with appropriate sensitivity on anthropometric or serological parameters defining metabolic syndrome. Although there are statistically significant correlations between waist circumference and the grade of necroinflammatory activity, the presence of diabetes and grade of fibrosis and necroinflammatory activity or serum ALT value and the grade of necroinflammatory activity, these correlations were found on relatively small numbers of patients. These parameters could be useful for selecting those patients with high-risk for developing severe, progressive forms of nonalcoholic steatohepatitis, but they cannot substitute percutaneous liver biopsy or serological markers for fibrosis.

**References**


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