Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to the accumulation of fat, mainly triglycerides, in hepatocytes that exceeds 5% of the liver weight [1]. NAFLD can be primary or secondary, depending on the cause [2]. Primary NAFLD results from insulin resistance and thus frequently occurs as part of the metabolic syndrome (MS).

The MS according to the new International Diabetes Federation definition (2004) [3] includes central obesity plus any two of the following factors: raised triglyceride level, reduced high-density lipoprotein cholesterol (HDL-C), raised blood pressure or fasting plasma glucose (FBG). NAFLD covers a wide spectrum of liver pathology: from steatosis alone, via the necroinflammatory lesions of non-alcoholic steatohepatitis (NASH) to cirrhosis and liver cancer [4, 5]. The analogies between the pathophysiology of NAFLD/NASH and the MS point to the fact that the spectrum of fatty liver is the hepatic expression of the MS itself [6].

The diagnosis of NAFLD requires the exclusion of alcohol abuse (a daily intake > 20g in female and > 30g in male) and other etiologies as the cause of the liver disease [1, 7]. NAFLD is emerging as the most common chronic liver condition in the Western world, affecting 20-40% of the general population [2, 8-11]. The true prevalence of NAFLD and its different stages has been incompletely defined.

The aims of this study were: (1) to estimate the prevalence of NAFLD, in a prospective way using liver ultrasonography in hospitalized patients in the 3rd Medical Clinic, Cluj-Napoca; (2) to measure the prevalence of the MS, based on predefined IDF consensus criteria, in NAFLD patients and (3) to assess the independent factors associated with NAFLD.

Material and method

Study population. From November 2006 to April 2007, 3,005 patients who were hospitalized in the 3rd Medical Unit, Cluj-Napoca, were consecutively observed. The variety of reasons/symptoms for hospital admission included internal diseases and the gastrointestinal spectrum, according to
the specificity of our medical unit. All patients underwent a complete clinical and anthropometric evaluation, and an ultrasound scan of the liver.

One experienced physician performed the abdominal ultrasonography (M.L.), and the presence of fatty liver was defined as the increased echogenicity of the hepatic parenchyma and posterior attenuation.

The diagnostic of NAFLD was established by ultrasonography followed by the exclusion of the secondary causes of hepatic steatosis: (1) alcohol intake of 30 g/day or more for males and 20 g/day or more for females, (2) Wilson disease, intestinal bypass surgery, glutenic enteropathy, (3) ingestion of drugs known to produce hepatic steatosis including methotrexate, tamoxifen, amiodarone, nucleoside analogues, (4) a positive serology for hepatitis B or C virus, (5) a history of another known liver disease.

The subjects were interviewed to obtain their history of hypertension, diabetes mellitus, dyslipidemia, myocardial infarction, stroke, as well as alcohol consumption and smoking.

The five components of the MS were searched in all patients, and subjects having 3 or more of the following criteria were labeled as MS: central obesity (waist circumference ≥ 94 cm for men and ≥ 80 cm for women) plus any two of the following four factors: (1) triglyceride levels > 150 mg/dl or current use of fibrates; HDL-cholesterol < 40 mg/dl (men) and < 50 mg/dl (women); (3) arterial pressure ≥ 130/85 mmHg or pharmacologically treated; (4) fasting glucose ≥ 100 mg/dl (3).

Each patient was submitted to an abdominal ultrasound examination with a Honda HS 2000 device, using a 3.5 MHz convex probe.

Body weight was measured in light clothing and without shoes to the nearest half kilogram. Height and waist circumference was measured to the nearest half centimeter. Waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest.

NIH defined body mass index (BMI) (weight in kilograms divided by the square of height in meters) was considered as obesity (BMI ≥ 30), overweight (25 ≤ BMI ≤ 29.9) and normal (BMI < 25) (12). Men and women with waist circumference of at least 94 and 80 cm, respectively, were considered to have central obesity.

Blood pressure was measured using a standard mercury sphygmomanometer after the subject had been seated for at least 10 minutes.

The laboratory evaluation included measurement of the fasting blood glucose, fasting serum triglycerides, high-density lipoprotein cholesterol (HDL-C) levels, alaninaminotransferase (ALT) and aspartate aminotransferase (AST). Serum glucose, triglycerides, ALT, AST and HDL-C were measured by enzymatic colorimetric methods, using an automatic analyzer Konelab 30i with Diagnosticum Rt. reagents (Hungary) and Thermo Electron Co. (Finland) reagents (for HDL-C). Viral markers HBsAg and anti-HCV were assessed using third-generation enzyme-linked immunosorbent assay (ELISA) tests from Sanofi-Pasteur.

According to the results of the ultrasound scan of the liver, a number of 98 patients diagnosed with hepatic steatosis in whom the secondary causes of hepatic disease were excluded underwent a percutaneous liver biopsy; and insulin resistance was established by homeostasis model assessment (HOMA-IR). (These data will be analyzed in a future report).

The Ethics Committee of the University of Medicine and Pharmacy Iuliu Hatieganu, Cluj-Napoca, approved this study, and informed consent was obtained from each subject.

Statistical analysis

Statistical analysis was performed using MedCalc for Windows, version 9.3.2.0 (MedCalc Software, Mariakerke, Belgium). The difference between groups with or without NAFLD was compared using an unpaired t test. The χ² test was used to compare the prevalence data. The odds ratio (OR), the 95% confidence intervals (CI), and p values were calculated. A p value < 0.05 was considered significant. Data in text and tables are reported as mean ± SD. Variables used in the univariate comparison between groups were: age, central obesity, hypertriglyceridemia, low HDL-C, hypertension and high glucose. When significant differences between groups were observed, multiple logistic regression analysis (in one single step), adjusted for age, was used to examine the independent factors on NAFLD.

Results

The prevalence of fatty liver, obtained by ultrasound examination, was 20% (604 cases) in the whole group and it was higher in the overweight and obese group than in the normal-weight group (32.17% vs. 7.33%, p < 0.001).

There were significant differences between the subjects with or without NAFLD in the variables including: sex (female sex was predominant), age, BMI, waist circumference, systolic and diastolic blood pressure, fasting blood glucose, HDL-C, triglycerides and transaminase values (Table I).

In patients with NAFLD the prevalence of positive criteria for the MS was variable, ranging from 39.9% (low HDL-C) to 88.41% (central obesity) (Fig. 1). In decreasing order the following parameters were found: central obesity (88.41%), high glucose (including impaired fasting glucose level and diabetes mellitus) (68.87%), hypertension (62.58%) and hypertriglyceridemia (55.79%) and low HDL-C (39.90%).

The cumulative positive diagnostic criteria for the MS (3 or more criteria) were found in 61.09% cases with NAFLD, at least one risk factor was found in 88.41% of cases and in 14.07% all 5 criteria were fulfilled (Fig.2).

The prevalence of the metabolic disorders according to the presence of NAFLD and BMI criteria is shown in tables II and III.

In the normal weight group, the prevalence of central obesity and hypertriglyceridemia was higher in the subjects with a fatty liver than in those without it. In the overweight
Prevalence and risk factors of non-alcoholic fatty liver disease

The values for OR of the metabolic disorders are shown in Table IV. The OR in the overweight group were higher than those in the normal weight group.

**Multiple logistic regression of metabolic factors independently associated with NAFLD**

According to multiple logistic regression analysis, central obesity and hypertriglyceridemia were independently associated with NAFLD in all groups. Hypertension was

**Table I. Clinical and laboratory data of the patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 3005)</th>
<th>NAFLD absent (n = 2401)</th>
<th>NAFLD present (n = 604)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, %</td>
<td>1656 (55.1%)</td>
<td>1318 (54.89%)</td>
<td>338 (55.96%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age, years</td>
<td>54.29 ± 13.45</td>
<td>53.91 ± 13.93</td>
<td>55.85 ± 11.01</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.74 ± 5.58</td>
<td>25.79 ± 4.84</td>
<td>30.56 ± 6.17</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>92.63 ± 21.15</td>
<td>90.16 ± 21.46</td>
<td>102.46 ± 16.55</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>125.78 ± 21.65</td>
<td>124.29 ± 21.76</td>
<td>131.71 ± 20.11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>77.28 ± 17.86</td>
<td>76.27 ± 11.77</td>
<td>81.30 ± 32.07</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FBG level, mg/dl</td>
<td>112.90 ± 39.65</td>
<td>110.82 ± 37.12</td>
<td>120.31 ± 46.88</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL-C level, mg/dl</td>
<td>52.37 ± 19</td>
<td>53.11 ± 13.78</td>
<td>49.90 ± 15.92</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TG level, mg/dl</td>
<td>148 ± 0.56</td>
<td>137.64 ± 0.55</td>
<td>185.01 ± 0.52</td>
<td>0.0001</td>
</tr>
<tr>
<td>AST level U/L</td>
<td>44.6 ± 70.15</td>
<td>49.93 ± 77.50</td>
<td>25.76 ± 25.17</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ALT level U/L</td>
<td>51.15 ± 70.02</td>
<td>56.16 ± 75.78</td>
<td>33.38 ± 39.08</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Abbrev: BMI (body mass index); SBP (systolic blood pressure); DBP, (diastolic blood pressure); FBG (fasting blood glucose); HDL-C (high density lipoprotein cholesterol); NAFLD (nonalcoholic liver disease); TG (triglycerides); ALT (alanine aminotransferase); AST (aspartate aminotransferase).

**Table II. Prevalence of metabolic disorders***

<table>
<thead>
<tr>
<th>Disorders</th>
<th>NAFLD absent Total(n=3005)</th>
<th>NAFLD present Total(n=3005)</th>
<th>p value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese (by IMC)</td>
<td>401 (16.70%)</td>
<td>322 (53.31%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Central obesity</td>
<td>1383 (57.66%)</td>
<td>534 (88.41%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>708 (33.61%)</td>
<td>337 (56.16%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Low HDL-C</td>
<td>591 (30.28 %)</td>
<td>230 (38.78%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1058 (44.06%)</td>
<td>378 (62.58%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>High glucose</td>
<td>1287 (60.45%)</td>
<td>415 (69.39%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>1011 (42.10%)</td>
<td>369 (61.09%)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*** Data are expressed as number (percentage) of subjects.
** Data are compared with t-test and χ² test.

**Table III. Prevalence of metabolic disorders according to BMI**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal weight* n =1460</th>
<th>Overweight and obese* n =1545</th>
<th>p value</th>
<th>Overweight and obese* n =1545</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central obesity</td>
<td>467 (34.54%)</td>
<td>59 (55.14%)</td>
<td>&lt; 0.001</td>
<td>916 (87.40%)</td>
<td>475 (95.57%)</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>291 (26.82%)</td>
<td>46 (43.80%)</td>
<td>0.001</td>
<td>417 (40.84%)</td>
<td>291 (58.78%)</td>
</tr>
<tr>
<td>Low HDL-C</td>
<td>249 (26.07%)</td>
<td>30 (30.3%)</td>
<td>0.43</td>
<td>342 (34.30%)</td>
<td>200 (40.48%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>481 (35.57%)</td>
<td>45 (42.02%)</td>
<td>0.21</td>
<td>577 (55.05%)</td>
<td>333 (67%)</td>
</tr>
<tr>
<td>High glucose</td>
<td>602 (54.13%)</td>
<td>60 (56.6%)</td>
<td>0.70</td>
<td>685 (67.35%)</td>
<td>355 (72.15%)</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>418 (31.12%)</td>
<td>55 (47%)</td>
<td>&lt; 0.001</td>
<td>593 (56.04%)</td>
<td>314 (64.47%)</td>
</tr>
</tbody>
</table>

* The normal-weight group had BMI less than 25, overweight and obese group had BMI ≥ 25.

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**Fig 1.** Prevalence of one or more criteria of the metabolic syndrome in patients with NAFLD.

**Fig 2.** Prevalence of positive criteria for the metabolic syndrome in patients with NAFLD.
found to be significant only for the whole group and the overweight (Table V).

**Discussion**

The prevalence of NAFLD may range widely as a function of varying definitions, selection and diagnostic criteria, countries or ethnicity.

The most consistent data regarding NAFLD prevalence are those collected in the general population studies [13-21]. These population surveys have defined NAFLD by biochemical criteria (increased serum aminotransferases and/or alkaline phosphatase and gamma-glutamyl transpeptidase) or by hepatic ultrasound. Among the populational studies the most representative are the Third National and Nutritional Examination Survey (NHANES III), USA and the Dionysus study from Italy. Based on these studies, the prevalence of NAFLD is between 9% and 33.5%, and it is higher in obese people (range 56-86%) than in lean subjects 16% [22].

In the present hospital-based study, the overall prevalence of NAFLD was 20%. The data range between limits observed in the general population. According to BMI, these range from 7.33% in the normal weight group and 32.1% in the overweight and obese group.

The large number of patients (3,005) represents a relevant component of the insulin resistance syndrome [29]. The site and cause of the insulin resistance is unknown, a large association may be partly related to the increased prevalence of insulin resistance in older people [4].

The higher prevalence with increasing age may be explained by the higher prevalence of obesity and the altered glucose regulation with advancing age [2, 28]. In addition, this association may be partly related to the increased prevalence of insulin resistance in older people [4].

The amionotransferase levels were significantly higher in the group without NAFLD. Viral and alcohol induced liver diseases are included in the “non-NAFLD” group. The etiological profile of these chronic liver disorders was the objective of a multicenter study performed in 2001. The main etiological factor of chronic hepatitis and liver cirrhosis was viral infection (48.3%) followed by alcoholic etiology (19.5%) [27].

The predominant risk factor for NAFLD appears to be insulin resistance related to the MS. Although the initial site and cause of the insulin resistance is unknown, a large number of studies established that fatty liver is the hepatic component of the insulin resistance syndrome [29].

The present study highlights the association of hepatic pathology, according to the specificity of our medical unit.

In the present study the diagnosis of NAFLD was based on the ultrasound examination and exclusion of known etiologic factors responsible for liver disease. Hepatic ultrasonography has a good sensitivity and specificity (89% and 93%) in detecting steatosis comparing with the gold standard, liver histology [23]. However, there are two important limitations regarding ultrasonographical studies: this method cannot identify fatty infiltration below 30% and is not able to distinguish between fatty liver and NASH [24]. Despite these limitations, our study involved a large number of patients.

The prevalence of steatosis in our study tends to be higher in females. In most of the published series men outnumber women [25, 26]. In our study this might be a result from a selection bias. In a survey recently conducted (2001) on a hospital-based population from our unit, we found an increased prevalence of women with chronic hepatitis (53.10 %) [27]. In Romania, current statistics referring to the population number and demographic structure, report a higher percentage in women than in men (52.1% women and 47.9% men) (Romanian National Institute of Statistics, 2006)

* Definitions of normal-weight and overweight group: see Table III

| Table IV. Odds ratio for metabolic disorders in patients with NAFLD compared with those without* |
|---|---|---|
| Factor | Normal-weight group | Overweight, obese group |
| Central obesity | 1.80 (1.16-2.78) | 3.01 (1.81-5.00) |
| Hypertriglyceridemia | 1.91 (1.22-2.97) | 1.97 (1.56-2.49) |
| Hypertension | – | 1.46 (1.15-1.85) |
| Low HDL-C | – | – |
| High glucose | – | – |

* * Data are expressed as OR (95% confidence interval)
steatosis with features of the MS.

Central obesity is the mandatory condition for the MS. In our study patients with NAFLD had in increased percentage central obesity, even in the presence of normal weight (according to BMI) (Table III). Furthermore, central obesity was found as an independent risk factor for NAFLD in all group patients (Table V), having the highest OR value (Table IV). The central obesity phenotype is associated with increasing visceral fat and increased insulin resistance [1, 30-34].

Over recent years, rates of overweight and obesity have escalated rapidly in many parts of the world to epidemic proportions, reflecting increased consumption of energy dense diets high in fat and sugars, associated with declining levels of physical activity. Few countries in the European region report obesity rates below 10%, according to data from International Diabetes Federation 2003 [35]. The prevalence of obesity in the general population in Romania was reported to be higher (25%) in a study performed in the same year [36]. In our study based on the hospital population we found a prevalence of 16.7% in patients without NAFLD and of 53.31% in patients with NAFLD.

Identification of subjects with MS is mandatory, because these patients are also at higher risk of cardiovascular disease, particularly in the presence of diabetes [37, 38].

In the present study, MS was found in 61.09% of patients with NAFLD (increasing with BMI). The prevalence of the MS, as defined by the ATP III criteria [39], in the NHANES III study [21], in the United States was 22% in the general population. In our study we found a more than three-fold increase of these values in patients with NAFLD, owing to restrictive criteria established by IDF consensus. It is possible that the adherence to the IDF criteria for diagnosing MS, especially the waist circumference could be too restrictive compared to the WHO criteria [40] or ATP III criteria [39] adopted in most studies analysing the MS. This and the fact that the study is based on hospital population might have overestimated the MS prevalence.

The presence of one component of the metabolic risk factor imposes the evaluation for other risk factors.

In addition to obesity, diabetes may be a particularly important risk factor. In our study, the next in frequency risk factor for NAFLD was “high levels of glucose” (including raised fasting plasma glucose and type 2 diabetes). This factor was not significantly associated with the presence of NAFLD in any group studied (Table III). A recent study has confirmed that in NAFLD patients, diabetes is an independent predictor for cirrhosis and liver-related deaths. The reason might be additional oxidative stress [29].

The world diabetes prevalence has been estimated to 5.1% [35]. In Europe the prevalence is higher (7.9%). Among European countries, in Romania a percentage of 9.3% has been reported by the IDF (2003) [36]. Diabetes mellitus and milder forms of glucose intolerance (particularly raised fasting plasma glucose) can now be found in almost every population in the world and without effective prevention, diabetes will likely continue to increase globally.

Hypertriglyceridemia and hypertension were the next two factors independently associated with the presence of NAFLD, especially in the overweight group (Table V).

Central obesity, hypertension and hyperlipidemia have been repeatedly reported in NAFLD, and their simultaneous presence significantly increased the risk of more severe liver disease. This association was partly maintained also in the normal-weight group and in overweight and obese groups.

Metabolic disorders such as NAFLD, diabetes and MS are chronic diseases in which genetically susceptible individuals are exposed to an imbalance between energy needs and energy (food) intake. Concerted action by regional societies and public health authorities are needed to fight against over-nutrition as a cause of liver disease [22, 41, 42].

In conclusion, in our study the prevalence of NAFLD in hospitalized patients has been set alongside the limits observed in the general population. The prevalence of the metabolic syndrome increased with increasing BMI. Central obesity and triglyceride level were independently associated with NAFLD in all studied groups.

Acknowledgment

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Conflicts of interest

None to declare.

References


