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Non-alcoholic fatty liver disease – clinical and histopathological aspects

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Abstract

Introduction: We conducted a retrospective study on patients who were hospitalized in the Emergency County Hospital of Craiova, Romania, between 2009–2014. We selected 75 patients out of 248 cases of fatty liver disease who underwent liver biopsies performed during surgical procedures for various diagnoses. *Patients and Methods*: We analyzed the patients' data recorded in examination charts: anthropometric parameters [height, weight, body mass index (BMI), abdominal circumference], metabolic lab tests (blood glucose, lipid profile), liver destruction enzymes, imaging examinations (abdominal ultrasound). The pathological study was performed on specimens directly after sampling as well as after staining. *Results*: After analyzing the results of the histological examination, we grouped our studied patients according to the degree of the liver steatosis: 21 (28%) cases with mild steatosis, 46 (61.33%) cases with moderate disease and eight (10.66%) cases with severe steatosis. The necrotic-inflammatory activity was mild in 28 (37.33%) cases, moderate in 36 (48%) cases and severe in 11 (14.66%) cases. Most of the studied patients exhibited septal fibrosis (45 cases – 60%) and porto-portal and porto-central bridging fibrosis (21 cases – 28%). Septal fibrosis and cirrhosis were recorded in four (5.33%) and five (6.66%) cases, respectively. There was a significant correlation between the degree of the hepatic steatosis, the degree of obesity (as expressed by BMI) and the waist circumference (as a measure of central obesity) – $p<0.001$. *Conclusions*: The non-alcoholic fatty liver disease (NAFLD) was found to be significantly associated with waist circumference, BMI, triglycerides. The liver enzymes are not considered to be sensitive or specific for diagnosing NAFLD. Concerning the association between the steatosis and fibrosis, in our study the septal fibrosis was associate with mild steatosis in most of the cases. Moderate steatosis was mostly associated with septal fibrosis as well as porto-portal and porto-central fibrosis. Severe steatosis was correlated with both porto-portal and porto-central fibrosis and cirrhosis in the majority of cases.

Keywords: non-alcoholic fatty liver disease, obesity, steatosis, fibrosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered a major public health problem. It includes a series of clinical-pathological conditions that occur in the absence of alcohol consumption, characterized by histological alterations, ranging from simple steatosis to steatohepatitis, fibrosis and cirrhosis [1–3].

There is a significant epidemiological, biological, pathogenic and socio-economic impact of NAFLD that is closely connected to other conditions, such as obesity, type 2 diabetes mellitus and dyslipidemia. There is also an alarming increase in the obesity prevalence worldwide, especially in the strong developed countries that is very well documented through numerous epidemiological studies. Recent data reveal concerning aspects reflecting the continuous increase in the NAFLD prevalence in correlation with the obesity epidemics [4–7].

The prevalence of NAFLD among obese subjects has been reported in a variety of studies, according to its definition. Using sonographic surveys in obese subjects, the prevalence of NAFLD has been documented at 57.5–60% in the East [4, 5] and 75.8% in the West [6]. However, the prevalence of the combination of sonographic fatty liver and elevated aminotransferases among the obese has been estimated to be approximately 20% [8]. Despite the absolute certainty of the relationship between obesity and NAFLD, not all obese individuals develop NAFLD. In addition, central adiposity appears to be a more powerful predictor than simple obesity [9, 10].

The term NAFLD includes a spectrum of histologically-defined liver disorders. The disease can progress from macro-vesicular lipid accumulation in the hepatocytes (termed steatosis) to non-alcoholic steatohepatitis (NASH; steatohepatitis in the presence of inflammatory infiltrate possibly with some fibrosis) to outright fibrosis, cirrhosis and even hepatocellular carcinoma. A combination of environmental and genetic factors determines the individual risk for NAFLD's development and progression, nutrition being the most significant modifiable environmental risk factor. The pathogenesis of NAFLD was

initially envisaged as a "two-hit process" [7] with fat accumulation in hepatocytes viewed as the primary insult and increased oxidative stress leading to inflammation being the second "hit" in the progression to NASH and fibrosis. However, at cellular level mechanisms influencing disease progression are clearly multifactorial and dependent on numerous genetic and environmental interactions.

The existence of an association between obesity and insulin resistance has been theorized on many occasions, and insulin resistance is also considered to be a primary risk factor for NAFLD [11, 12]. Although obesity is generally regarded as the principal cause of insulin resistance, not all obese people develop this condition. In view of the reports of the *European Group for the Study of Insulin Resistance*, insulin resistance is found only in 26% of the obese individuals [13]. Moreover, the insulin resistance, regardless of the degree of obesity, exacerbates the risk of coronary heart disease and type 2 diabetes, sharing the risk factors of NAFLD [14].

Although this unanimous recognition in the establishment/diagnosis of the NAFLD subtypes, its severity and disease progression assessment as well as the certifying of the liver fibrosis was given to the liver biopsy together with the pathological examination, some disadvantages and problems still remain in clinical practice. The liver biopsy has intra- and inter-observatory variability, a considerable error rate, high cost; it is an invasive method, reduced adherence of the patient, major risk of complications [15–18]. There are no minimal histological criteria sets in the diagnosis of the NAFLD subtypes. Problems still exists, related to the fact that the liver histology spectrum in NAFLD is very similar to the one in the chronic liver disease that is related to alcohol-consumption. There is no consensus regarding the indications of liver biopsy in NAFLD [19].

Although the NAFLD/NASH definition is based on histological terminology, there is no consensus regarding the mandatory alterations required for diagnosis [18].

There is to date no consensus on the specificity and necessity of each histological alteration existence. There are also variations in the description of the same element by various pathologists, thus making the issue even more complicated [19].

Aim

The aim of our study was the identification of the spectrum of histological alterations in the non-alcoholic steatosis and the correlation with various clinical and biological findings.

Patients and Methods

We conducted a retrospective study on patients who were hospitalized in the Emergency County Hospital of Craiova, Romania, between 2009 and 2014.

We selected 248 cases of fatty liver disease out of 463 liver biopsies performed during surgical procedures for various diagnoses. We excluded 215 cases because of the existence of various liver tumors.

Histories of alcohol consumption were assessed according to the declared type of alcoholic beverage consumed, the frequency of alcohol consumption per week, and the amount drunk per day. Subjects consuming less than 30 g of alcohol per day were considered to be non-drinkers [20, 21]. The other 90 were considered alcohol consumers, therefore, were excluded from the research.

Other exclusion criteria that we applied: 18 patients had a history of viral hepatitis (B, C or B+D), 20 patients had a body mass index (BMI) lower than 25 kg/m^2, while 23 had a history of cardiovascular diseases, 14 had diabetes mellitus and eight patients were undergoing hepatotoxic medication (methotrexate, amiodarone, corticotherapy, chemotherapy, hormonal therapy).

In the end, we retained a number of 75 patients that were investigated as following.

The pathological study was performed as the specimens were fixated immediately after sampling in 10% neutral formaldehyde for a duration of six to 24 hours according to specimen's size.

The fixated fragments were processed in the classical histological technique of paraffin inclusion that allows performing slices of 3–5 μm in order to analyze the cellular and tissue details.

Several techniques were used for the staining of the histological specimens. For basic histological examination, the Hematoxylin–Eosin (HE) and van Gieson stainings were used.

The results of the HE staining consist of blue-violet staining of the cells' nuclei according to the contained Hematoxylin, pink colored cytoplasm, fair pink slightly coloration of the collagen fibers and no staining for the elastin and reticulin fibers.

The results of the van Gieson staining show black-violet staining the nuclei, yellow staining of the cytoplasm, and red coloration of the collagen fibers.

In quantifying steatosis, we assessed steatosis invasion in the liver tissue: absent – 0; mild – less than 1/3; moderate – between 1/3 and 2/3; severe – more than 2/3 [22].

The fibrosis staging was assessed by the following criteria: stage 1 – zone 3 pericellular fibrosis (focal or extensive); stage 2 – zone 3 pericellular fibrosis (focal or extensive) plus portal fibrosis; stage 3 – bridging fibrosis (focal or extensive); stage 4 – cirrhosis ± pericellular fibrosis areas.

Subsequently, we analyzed the patient examination charts recording the following data: anthropometric parameters (height, weight, BMI, abdominal circumference); metabolic lab tests (blood glucose, lipid profile); liver destruction enzymes; imaging examinations (abdominal ultrasound).

The BMI was calculated according to the height divided to the square of the body weight (kg/m^2).

The liver ultrasound was performed through epigastric as well as intercostal approach (transversal and longitudinal sections) and we recorded the dimensions of the left and right liver lobe, its echogenicity and structure, diaphragm visibility, posterior attenuation, blood vessels' appearance, the portal vein system and the spleen [23, 24].

The acquired results in this study were processed and analyzed using the statistics program SPSS.

Then we performed the statistical processing of their data, researching the correlation between the patients'

anthropometric data, lipid profile and liver function, the ultrasound and histological liver appearance. The significance tests lower than 0.001 indicate very strong correlations.

Standard deviation (SD) is used for data which are "normally distributed" to provide information on how much the data vary around their mean. SD indicates how much a set of values is spread around the average. A range of one SD above and below the mean (abbreviated to ± 1 SD) includes 68.2% of the values.

Results

After analyzing the results of the histological examination, we grouped our studied patients according to the degree of the liver steatosis: 21 (28%) cases with mild steatosis, 46 (61.33%) cases with moderate disease and eight (10.66%) cases with severe steatosis (Table 1).

Table 1 – ***Steatosis degrees***

Steatosis degree	No. of cases (percent)
Mild	21 (28%)
Moderate	46 (61.33%)
Severe	8 (10.66%)

Steatosis was characterized by the accumulation of lipid drops in the hepatocyte; the non-alcoholic steatosis was macrovesicular and the nucleus was pushed to the cell periphery (Figure 1).

Type 2 steatosis consisted of steatosis and intralobular inflammatory infiltration (Figure 2). In type 3, we could observe granulo-vacuolar degeneration at hepatocyte level on a liver steatosis background (Figure 3).

Type 4 non-alcoholic liver steatosis consisted of ballooning degeneration and fibrosis with evolution towards liver cirrhosis and failure (Figure 4).

The isolated alteration of some liver cells varied from minor aspects like the ballooning generation to acidophilic necrosis and Councilman bodies formation.

Necrosis and apoptosis coexisted in some areas, leading to liver cells death. Necrosis occurred as a result of losing the barrier function of the cell membrane followed by lysis. Apoptosis consisted of the fragmentation of the cellular DNA and of the cell itself, maintaining the cell membrane and cytoplasmic organites intact. Apoptosis manifested as Councilman bodies or small cellular fragments surrounded by a membrane, many of these being already phagocytosed by the neighboring cells or by macrophages.

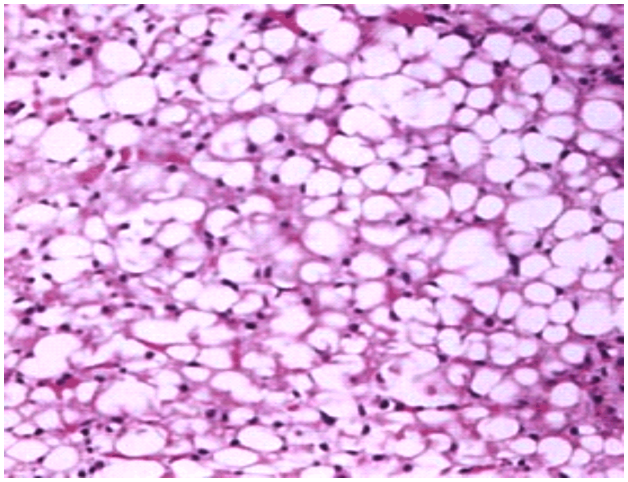

Figure 1 – ***Simple liver steatosis (HE staining, ×200).***

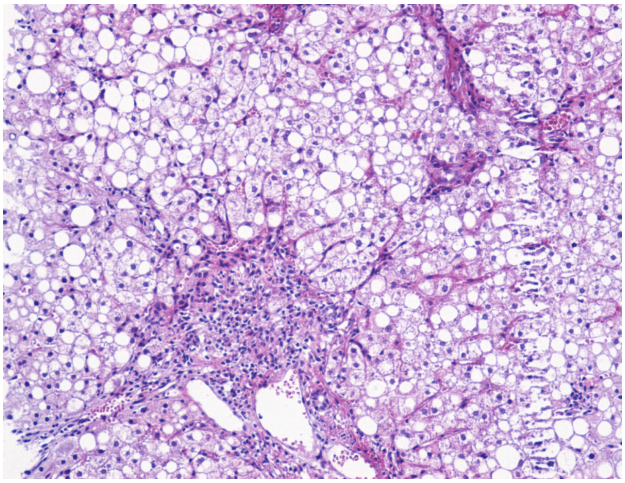

Figure 2 – ***Liver steatosis and intralobular inflammation (HE staining, ×200).***

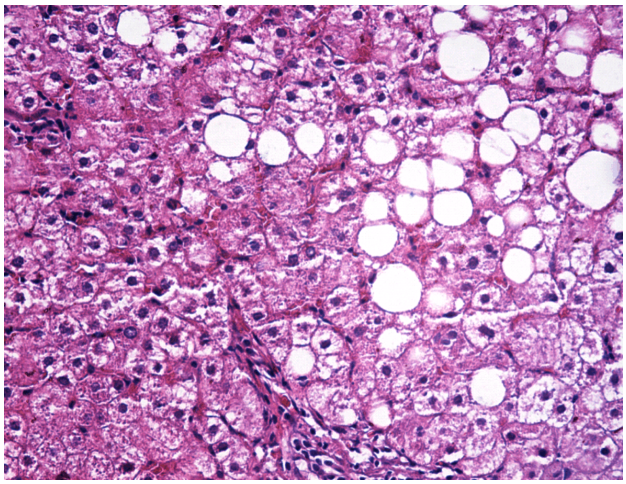

Figure 3 – ***Liver steatosis – hepatocytes with granular-vacuolar degeneration (HE staining, ×200).***

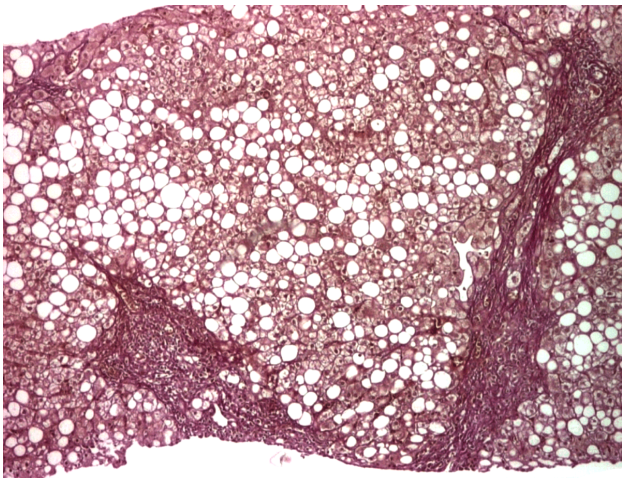

Figure 4 – ***Severe steatosis with porto-portal fibrosis and necrotic-inflammatory activity (van Gieson staining, ×100).***

Liver cells necrosis was found either focal or in spots, affecting one cell or a small group of cells. Another instance was that of confluent necrosis that affected larger liver areas where we could notice necrosis areas containing conjunctive tissue and inflammatory cells, including macrophages and cellular residue.

The inflammatory cellular infiltrate consisted mainly of lymphocytes, together with plasma cells, histiocytes and occasionally lymph-granulocyte infiltrate. The infiltrate was most often seen in the port spaces, as well as intralobular, as small foci, in spots, often around altered hepatocytes or forming intrasinusoid chains. In the port spaces, that were enlarged, the lymphocytes may form lymphoid aggregates.

The confluent necrosis was characteristic to aggressive forms of chronic hepatitis and involved hepatocytes locate very close to the port spaces or the fibrous septa – piecemeal necrosis – or the hepatocyte chords situated between the nearby vascular structural complexes – bridging necrosis.

In our study, the necrotic-inflammatory activity was mild in 28 (37.33%) cases (Figure 5), moderate in 36 (48%) cases and severe in 11 (14.66%) cases (Figure 6; Table 2).

Table 2 – *The necrotic-inflammatory activity in the non-alcoholic liver steatosis*

Necrotic-inflammatory activity	No. of cases (percent)
A1 – Mild	28 (37.33%)
A2 – Moderate	36 (48%)
A3 – Severe	11 (14.66%)

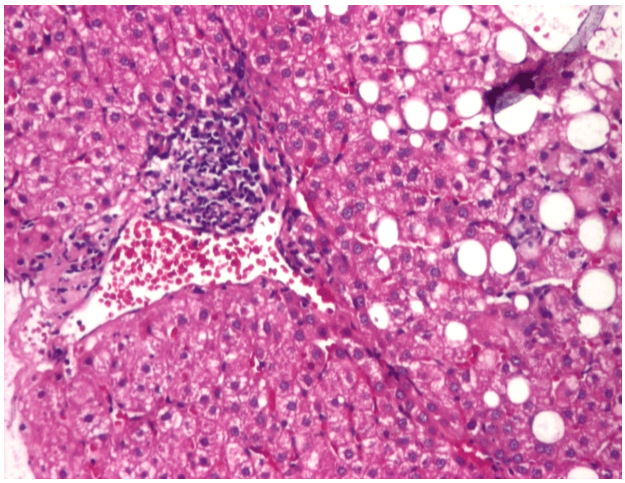

Figure 5 – *Perivascular intralobular inflammation (HE staining, ×200).*

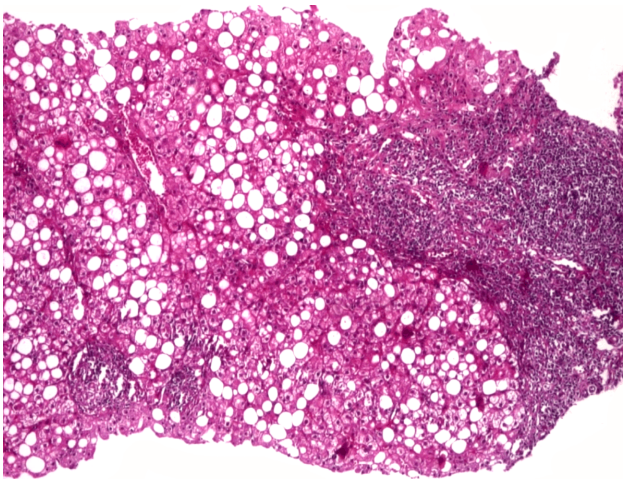

Figure 6 – *Severe necrotic-inflammatory activity (HE staining, ×100).*

Fibrosis

Fibrosis initially appeared in the centrolobular region and was characterized by its pericellular and even perivascular disposition. In evolution, fibrosis affected the septa and port spaces. In severe cases, fibrosis can develop in obvious bridges and cirrhotic nodules and pseudonodules formation.

Most of the studied patients exhibited septal fibrosis (45 cases – 60%) (Figure 7) and porto-portal and porto-central bridging fibrosis (21 cases – 28%). Septal fibrosis and cirrhosis were recorded in four (5.33%) and five (6.66%) cases, respectively (Figure 8; Table 3).

We have shown the correlations between steatosis, the necrotic-inflammatory activity and fibrosis in Tables 4 and 5.

Regarding the association between the steatosis and fibrosis, the septal fibrosis (F2) was associated with mild steatosis (S1) in most of the cases. Moderate steatosis (S2) was mostly associated with septal fibrosis (F2) as well as porto-portal and porto-central fibrosis. Severe steatosis (S3) was correlated with both porto-portal and porto-central fibrosis (F3) and cirrhosis (F4) in the majority of cases.

We have shown the clinical and biological parameters of the patients in Table 6.

There was a significant correlation between the degree of the hepatic steatosis, the degree of obesity (as expressed by BMI) and the waist circumference (as a measure of central obesity) – $p<0.001$.

A significant number of NAFLD patients had normal liver enzymes (mild steatosis, some of the moderate steatosis patients), while in the more advanced stages of steatosis 37 (49.3%) of the patients had elevated alanine aminotranferase (ALT).

The patients with mild steatosis showed a lower serum triglycerides level compared to the ones with fibrosis (161±7.1 *versus* 225.75±8.2 mg/dL).

Table 3 – *Fibrosis in the non-alcoholic steatosis*

Stage	No. of cases (percent)
F1 – Portal	4 (60%)
F2 – Septal	45 (28%)
F3 – Porto-portal and porto-central	21 (5.33%)
F4 – Cirrhosis	5 (6.66%)

Table 4 – *The necrotic-inflammatory activity*

Steatosis	Mild	Moderate	Severe	Total (percent)
A1	8	17	3	28 (37.33%)
A2	11	21	4	36 (48%)
A3	2	8	1	11 (14.66%)
Total	21 (28%)	46 (61.33%)	8 (10.66%)	75 (100%)

Table 5 – ***The correlation between steatosis and fibrosis***

Stage	S1	S2	S3	Total (percent)
F1	1	2	1	4 (5.33%)
F2	28	15	2	45 (60%)
F3	8	10	3	21 (28%)
F4	0	2	3	5 (6.66%)
Total	37 (49.33%)	29 (38.66%)	9 (12%)	75 (100%)

Table 6 – ***The clinical and biological parameters of the patients***

Parameter	Mild steatosis (*n*=21)	Moderate steatosis (*n*=46)	Severe steatosis (*n*=8)
**BMI [kg/m^2]*	28±0.2	29.5±0.12	33.8±2
**Waist circumference [cm]*	94±0.4	106±0.3	117.12±3
Cholesterol [mg/dL]	203±2.6	206.3±2.9	208.25±15.4
HDL-cholesterol [mg/dL]	50.8±0.5	48.6±0.2	39.87±0.8
**Triglycerides [mg/dL]*	161±7.1	194±5.6	225.75±8.2
AST [UI/L]	26±0.6	36±1	52.25±4.5
ALT [UI/L]	32±0.8	54.5±2	67.12±5
Fasting glucose [mg/dL]	95.5±0.7	97±0.5	98±0.81

BMI: Body mass index; HDL: High-density lipoprotein; AST: Aspartate aminotranferase; ALT: Alanine aminotranferase. *Correlation is significant at the 0.001 level.

After the analysis of the previous data, the results of the ultrasound imaging were evaluated according to parameters that are described below.

Considering the recorded data, we performed a semi-quantitative staging of the liver steatosis:

- grade 1 (mild steatosis) – less than 1/3 of the liver is affected, minor attenuation and visible diaphragm: 19 patients;
- grade 2 (moderate steatosis) – less than 1/2 of the liver is affected, obvious posterior attenuation and dimmed diaphragm: 49 patients;
- grade 3 (severe steatosis) – more than 1/2 of the liver is affected, posterior diaphragm not visible: seven patients.

The results are similar to those we obtained after the pathological examination, although it is recognized that both methods may exhibit interobservational differences.

Discussion

This retrospective study tried to emphasize the significance of certain biological parameters that are considered as risk factors for NAFLD as a diagnostic alternative to liver biopsy.

Inflammation and fibrosis evaluation represent key elements in investigating the liver diseases. Compared to the fibrosis evaluation, made on distinct architectural alterations that can be precisely revealed through additional staining, the inflammatory infiltrate is more subjective, leading to inter-observation variations.

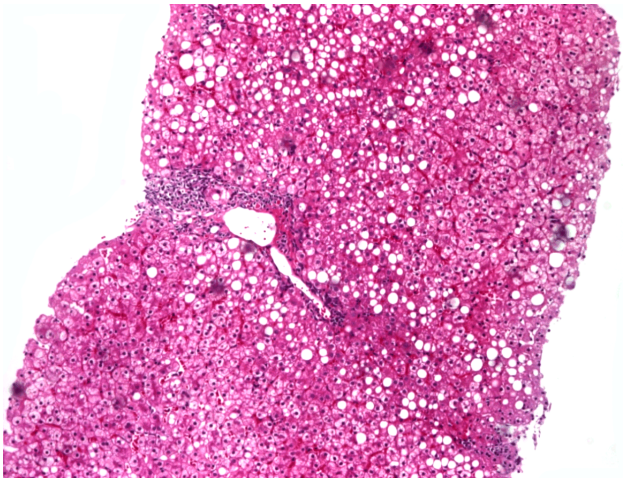

Figure 7 – ***Pericellular and septal fibrosis (HE staining, ×100).***

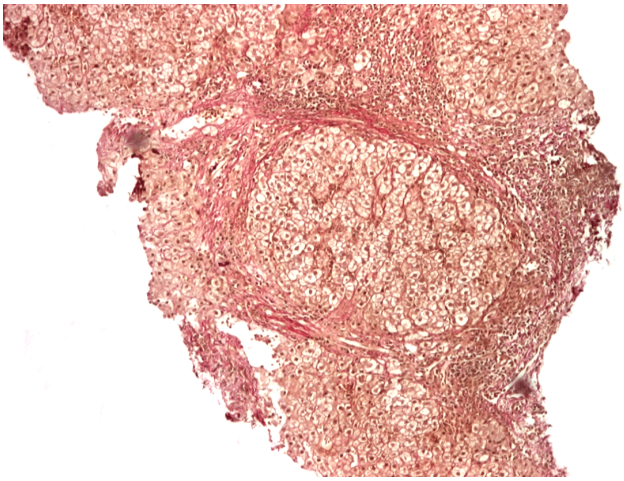

Figure 8 – ***Liver cirrhosis – cirrhotic nodule (van Gieson staining, ×40).***

The term piece-meal necrosis was stated for the first time by Popper [25]. In order to describe the immunological aspects of the necrosis of a group of hepatocytes situated near the port spaces. It can be defined as a necrosis, a disappearance of the hepatocytes at the separation limit between the liver parenchyma and the conjunctive, mesenchymal structures. These necrosis areas are infiltrate by numerous lymphocytes and other inflammatory cells and can be readily recognized on the histological cuts by the fading of the lobular limitant, by irregular contour of the parenchyma-mesenchyme interface.

Fibrogenesis is a dynamic process characterized by the synthesis of constituents of the extracellular matrix that is a glycoprotein (collagen, elastin, fibronectin, laminin) and proteoglycans complex organized in a three-dimensional network. Fibrogenesis is a non-specific mechanism dependent on the duration and intensity of the liver aggression. It is characterized by the accumulation of collagen and other proteins of the extracellular matrix and their organization in insoluble complexes leading to the destruction of the liver architecture. The process has more stages, from portal fibrosis to septal and bridging fibrosis and cirrhosis.

During the fibrogenesis process, the capillarization of the liver sinusoid vessels manifests that affects the nutrition exchanges between the sinusoid blood and the hepatocytes. Thus, the quality and quantity of the extracellular matrix is altered; actually, the normal extracellular matrix (the basal membrane) is transformed into a dense network of fibers that is more resistant to enzymatic degradation [26].

NAFLD is characterized as defining element by

steatosis, meaning the presence of fat representing a minimum of 5% of liver weight or by the presence of lipids in at least 5% of the hepatocytes in the optical microscopy. The disposition of fat is mainly macrovesicular, one can observe one single lipid drop pushing the nucleus to the periphery.

The criteria for steatosis evaluation are not well established in the literature [27]. Moreover, the steatosis quantities recognized as abnormal are not known and their assessment seems difficult. A study based on necroptic examination suggested that "low" quantities steatosis might be present normally in the healthy liver parenchyma, an observation that increases with aging.

The generally accepted normal value of 5% for liver steatosis is based on the measurement of the lipid content, but in literature, the value required for the steatosis diagnosis varies from "any quantity" to 15 to 30% [28]. An opposing opinion argues that even this small amount cannot be considered normal by the use of modern imaging techniques to detect the absence of steatosis in healthy subjects.

The clinical features, the physical findings, liver tests and hepatic ultrasound – none of these can differentiate patients with simple fatty liver from those with non-alcoholic steatohepatitis.

Among patients with NAFLD, 90% are overweight (BMI of at least 25 kg/m^2), and 50% are obese (BMI ≥30 kg/m^2). In moderate obesity, the regional distribution of body fat seems to be an important indicator of metabolic and cardiovascular changes, since there are inconstant correlations between BMI and the above changes [29, 30].

BMI has two major limitations: first, it does discriminate between muscle mass and fat mass and second, it does not provide information on body fat location.

In our study, 26 patients were obese (BMI>30 kg/m^2), representing 34.66%, while the rest were overweight (BMI>25 kg/m^2).

Measuring of waist circumference (WC) is used in clinical practice to determine the degree of central obesity. The diagnosis criteria of metabolic syndrome are represented by values that exceed the established limits according to gender and geographical distribution. Studies showed that abdominal circumference is a better indicator of visceral body fat than the body mass index, especially in Caucasians and African Americans, the abdominal obesity being a risk factor of NAFLD even in subjects with normal BMI [31].

This study reveals that waist circumference as a measure of central obesity is a risk factor which is independent of the overall obesity as indicated by the body mass index. It now appears that central obesity may be at least as important as obesity in terms of the development of hepatic steatosis. Three population-based epidemiological studies have concluded that elevated alanine aminotransferase activity is profoundly and independently associated with the waist circumference [32–34]. Thus, central obesity has been demonstrated to be associated with sonographic fatty liver, as well as degree of steatosis, upon liver biopsy [35–37].

Liver function tests: the main laboratory anomalies present in NAFLD are the increase of aminotransferases. These are moderately increased, usually 1.5–4 times normal value, increases of up to 10×N being possible, but rare. Usually, alanine aminotransferase (GPT, ALT) has higher values than aspartate aminotransferase (GOT, AST), the AST/ALT ratio being less than 1 in 65–90% of cases; in advanced stages of disease (severe fibrosis or even cirrhosis) ratio values less than 1 are possible, but never more than 2. The increase of values of transaminases is not always correlated to liver disease. The level of transaminases is fluctuant, in 78% of patients being normal at a given moment in the evolution of the disease. However, 20% of patients constantly present elevated transaminases on repeated checks. In several surveys, the increased ALT value proved to be an independent prediction parameter of NASH and advanced fibrosis [38–40].

In our study, the liver enzymes did not correlate with the extent and degree of the steatosis (p<0.34 and p<0.68, respectively). Actually, the liver enzymes are not considered to be sensitive or specific for diagnosing NAFLD, and a significant number of NAFLD patients may have normal liver enzymes. The accuracy of the combination of elevated liver enzymes and radiographic techniques, as a surrogate for the detection of fatty liver disease, needs to be evaluated.

The dyslipidemic triad [namely elevated triglycerides and LDL (low-density lipoprotein)-cholesterol and low HDL (high-density lipoprotein)-cholesterol] is commonly associated with overweight (in particular with central adiposity) [41, 42].

Dyslipidemia in NAFLD is characterized by increased plasma triglycerides (in about 20–80% of patients) and low HDL-cholesterol and the value of triglycerides/HDL-cholesterol ratio higher than 3 is a marker of insulin-resistance. In our study, this ratio showed values more than 3 in most of the patients.

Transabdominal ultrasound identifies steatosis only when it exceeds 20–30%, but it does not differentiate it from fibrosis. Fatty liver is a big liver, with increased echogenicity (big, white and bright liver), with pseudodilations of venous type, with posterior attenuation, and in case of cirrhosis with ultrasound signs of portal hypertension [43, 44].

There are still some debatable issues regarding the diagnosis of NAFLD, such as the lack of well-defined clinical and biological criteria capable of accurately confirming the NAFLD subtypes or lower accuracy of the imaging methods in the diagnosis of NAFLD subtypes. These methods (liver ultrasound, computed tomography, magnetic resonance imaging), although useful in the diagnosing of liver steatosis, cannot quantify its grade, cannot distinguish between steatosis and steatohepatitis, or identify the presence or severity of fibrosis.

Currently, the mechanisms underlying the observed association of insulin resistance with NAFLD remains poorly understood. It can be assumed that excess portal adipose tissue increases the influx of free fatty acids through the portal vein to the liver, possibly resulting in the accumulation of hepatic fat [28]. Insulin resistance is the basis of hepatic steatosis, and that fat-accumulated hepatocytes are predisposed to injury by endotoxins or other oxidative stresses [7]. However, screening methods for insulin resistance and the definition of insulin resistance have not, thus far, been established in clinical practice.

Due to the fact that our study was retrospective in nature, we could not perform a routine evaluation of the serum insulin and of the HOMA-IR (homeostatic model assessment of insulin resistance) index [45] in order to calculate the insulin resistance, while previous studies [36, 45–47] indicate a profound association between NAFLD and increasing insulin resistance. It is our intention to approach these aspects in our further research.

Conclusions

NAFLD was found to be significantly associated with waist circumference, BMI, triglycerides. The liver enzymes are not considered to be sensitive or specific for diagnosing NAFLD. In our retrospective study, most of the cases showed moderate liver steatosis, moderate necrotic-inflammatory activity, septal fibrosis as well as porto-portal and porto-central bridging fibrosis. Concerning the association between the steatosis and fibrosis, in our study, the septal fibrosis was associate with mild steatosis in most of the cases. Moderate steatosis was mostly associated with septal fibrosis as well as porto-portal and porto-central fibrosis. Severe steatosis was correlated with both porto-portal and porto-central fibrosis in the majority of cases. Additional studies are needed to validate other non-invasive diagnostic methods in order to replace liver biopsy or at least to decrease its indications in NAFLD. The identification and management of the group with risk of progression of the disease remains a challenge in the clinical practice.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Mihaela Popescu and Sergiu-Marian Cazacu equally contributed to the manuscript and share main authorship.

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