

Association between liver histology, carotid ultrasonography and retinal vascular changes in patients with nonalcoholic fatty liver disease (NAFLD)

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Abstract

Introduction: The prevalence of nonalcoholic fatty liver disease (NAFLD) is increasing all over the world. NAFLD has been demonstrated to be associated with carotid artery atherosclerosis, evaluated using the intima-media thickness (IMT). In this article, we focused on the association between NAFLD, carotid parameters such as: intima-media thickness (IMT), pulsatility index (PI) and resistivity index (RI) as markers of subclinical atherosclerosis and the presence of retinal vascular disorders. **Patients and Methods:** We compared carotid IMT, pulsatility and resistivity index evaluated by ultrasonography, in 10 patients with histological-proven NAFLD and retinal vascular changes (retinophotographies). **Results:** The degree of hepatic steatosis, necroinflammation and fibrosis in NAFLD patients was strongly associated with the value of carotid IMT and also with PI and RI. Moreover, there seems to be a connection between the degree of NAFLD and the retinal vascular changes in patients with carotid atherosclerosis. **Conclusions:** These results suggest that the severity of liver histopathological lesions among NAFLD patients is strongly associated with carotid parameters: IMT, PI, RI and also with retinal vascular changes. Further controlled studies are needed to confirm the results.

Keywords: nonalcoholic fatty liver disease, intima media thickness, pulsatility index, resistivity index, retinal vascular disorders.

Introduction

Nonalcoholic fatty liver disease (NAFLD), considered nowadays the hepatic manifestation of the metabolic syndrome (MS) [1], is characterized by an important storage of lipids in hepatocytes affecting patients with negative history of alcohol consumption [2].

Usually, the diagnosis of NAFLD is based on a combination of laboratory tests and ultrasonographic modifications [2, 3]. Liver ultrasonography results correlates well with histological modification of fatty infiltration, but they are not sensitive enough to detect liver inflammation or fibrosis. The liver biopsy is the examination that can best establish the diagnosis of NAFLD [2–4]. Histologically, perivenular regions of the liver parenchyma are mostly affected and include steatosis, steatohepatitis and perisinusoidal or pericellular fibrosis in different degrees that might progress to cirrhosis [4].

Nowadays, it is poorly understood the manner by which liver findings in NAFLD patients could be associated with the progression of atherosclerosis. The pathogenetic mechanism might include: endothelial dysfunction, oxidative stress, inflammation, inflammatory cytokines, and lipid and glucose metabolism disorder [5].

Cross-sectional studies have demonstrated an important increase in carotid artery intima-media thickness

(IMT), early marker of atherosclerosis that is normally evaluated by ultrasounds, in patients with NAFLD [5–9].

Doppler ultrasonography helps in calculating the pulsatility index (PI), which is an expression of the vascular resistance distal to the examined artery. Resistivity index (RI), a hemodynamic parameter that can be determined by Doppler sonography, shows local wall extensibility and the related vascular resistance. It has been proven that both PI and RI of the common carotid artery may also be surrogate markers of atherosclerosis [10, 11].

Internal carotid artery delivers blood to the retina, therefore atherosclerosis related pathologies of this artery may have direct effect on retinal circulation and may coexist with retinal arteriosclerosis. Central retinal artery occlusion (CRAO) and branch retinal artery occlusion (BRAO) are most common expression of carotid atherosclerosis. The most frequent mechanism of CRAO and BRAO is embolism, usually with the origin in a plaque of the carotid artery [12]. Generalized or localized at the level of ophthalmic or central retinal vein, arteriosclerosis is the most common cause for retinal vein occlusion occurrence [13].

In this article, we investigated the associations between liver histology, carotid ultrasonography para-

meters (IMT, PI and RI) and retinal vascular changes in patients with NAFLD.

☞ Patients and Methods

Ten patients with NAFLD and retinal vascular changes have been included in our study, five men and five women, mean age 41.8 ± 3.3 (min. 36, max. 45, standard deviation 3.3). They were admitted in the Department of Gastroenterology of the Emergency County Hospital of Craiova, between 2010–2011, for increased liver enzymes or fatty liver infiltration detected by ultrasonography.

All patients provided written informed consent prior to enrollment in the study. The approval of the ethic committees of the Emergency County Hospital of Craiova and of the University of Medicine and Pharmacy of Craiova was obtained.

Inclusion criteria consisted of: chronically elevated liver enzymes, hepatic steatosis detected by ultrasonography and histological examination after liver biopsy, alcohol intake <20 g/day confirmed by at least one family member, no evidence for other causes of chronic liver disease (viral hepatitis, autoimmune liver disease, $\alpha 1$ -antitrypsin deficiency, Wilson's disease, hemochromatosis or hepatotoxic drugs consumption).

Blood measurements were determined by standard laboratory procedures: alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyl-transferase (GGT), glucose, cholesterol, triglycerides, creatinine. Liver function tests were determined before the liver biopsy.

Dyslipidemia was defined by the presence of elevated cholesterol (>200 mg/dL) and/or triglycerides (>150 mg/dL or active lipid-lowering therapy) [14].

Blood pressure was measured according to standard protocol with a standard mercury sphygmomanometer. BMI was calculated as weight in kilograms divided by height in square meters. Waist circumference was measured at the level of umbilicus.

A percutaneous liver biopsy was performed in all patients by senior operators.

Liver biopsies were analyzed and classified by an experienced pathologist (VC) blinded to patients' clinical results, based on the Matteoni classification [15].

Steatosis was graded according to its severity based on the extent of involved parenchyma:

- grade 1: $<33\%$ of hepatocytes affected;
- grade 2: $33\text{--}66\%$ of hepatocytes affected;
- grade 3: $>66\%$ of hepatocytes affected.

Nonalcoholic steatohepatitis (NASH) was associated with the presence of steatosis, lobular inflammation and hepatocellular ballooning or steatosis plus any stage of fibrosis. There are four stages of fibrosis: stage 1, zonal three-perivenular, perisinusoidal, or pericellular fibrosis; stage 2, same with focal or extensive periportal fibrosis; stage 3, bridging fibrosis, focal or extensive; stage 4, cirrhosis.

All subjects underwent ultrasonography of the carotid artery, with determination of intima-media thickness, pulsatility index and resistivity index. The measurements were performed using an Aloka Prosound $\alpha 7$ machine.

The retinal vascular changes were evaluated using retinal photography obtained with a non-mydratic Topcon retinal camera and they were classified based on the Keith–Wagener–Barker system [16].

☞ Results

The most important clinical and biochemical characteristics of NAFLD patients are described in Table 1. The levels of liver enzymes were increased in this group of patients. Dyslipidemia was present in all NAFLD patients. There were no significant values regarding creatinine or glucose levels.

Table 1 – Clinical and laboratory characteristics of NAFLD patients

Age [years]	41.8 ± 3.3
BMI [kg/m^2]	27.7 ± 2
Waist circumference [cm]	95 ± 5
Systolic blood pressure [mmHg]	120 ± 13.7
Diastolic blood pressure [mmHg]	70.5 ± 6.35
Total cholesterol [mg/dL]	211 ± 28
Triglyceride [mg/dL]	221 ± 53
Glucose [mg/dL]	86 ± 6
ASAT [IU/L]	144 ± 23
ALT [IU/L]	156 ± 26
γ -GT [IU/L]	53 ± 19
Creatinine [mg/dL]	0.8 ± 0.3

Data is expressed as mean \pm standard deviation.

The histopathological assessment of liver biopsies showed: mild steatosis (grade 1) in two patients (Figure 1), moderate steatosis (grade 2), described in three patients (Figure 2) and severe steatosis (grade 3), present in liver biopsies of five NAFLD patients (Figure 3). Steatosis alone was described in two patients, the rest of eight patients had associated steatosis plus: lobular inflammation and hepatocellular ballooning or fibrosis (NASH). No differences between men and women were detected.

The lowest value of carotid IMT and also of pulsatility and resistivity index was described in patients with mild steatosis, intermediate values in patients with steatosis plus lobular inflammation and hepatocellular ballooning, and highest in patients with steatosis plus fibrosis (Table 2).

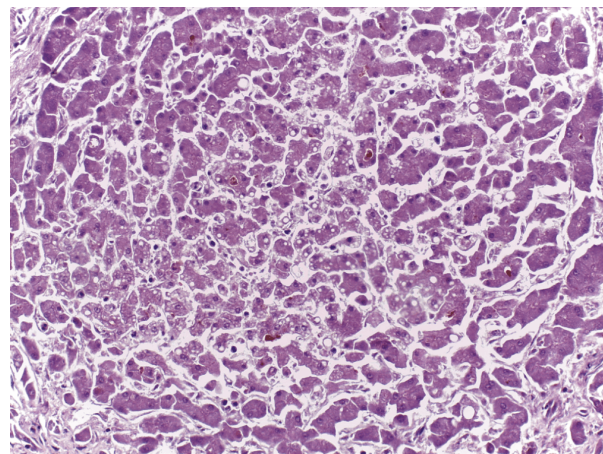


Figure 1 – Nonalcoholic fatty liver disease (NAFLD) with mild steatosis, mainly microvesicular (HE stain, $\times 200$).

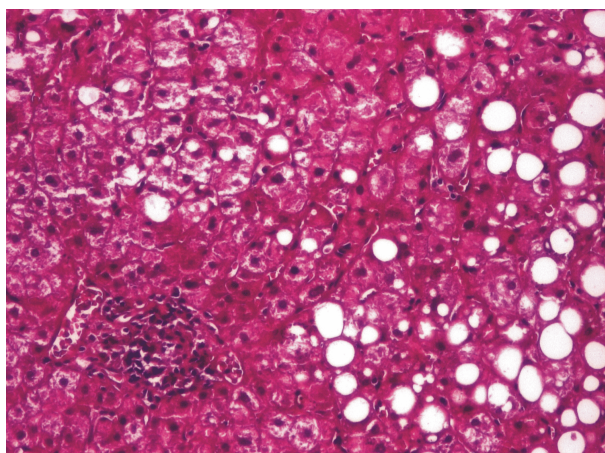


Figure 2 – Moderate steatosis with reduced intra-lobular infiltrate of inflammatory cells and hepatocytes with degeneration (HE stain, ×200).

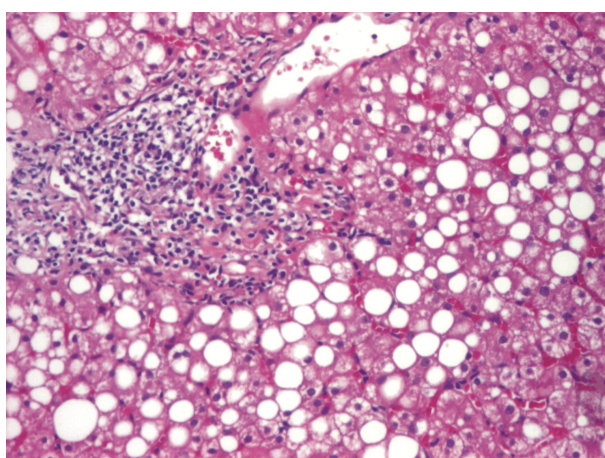


Figure 3 – Nonalcoholic steatohepatitis (NASH). In addition to macrovesicular severe steatosis there is an intralobular infiltrate of inflammatory cells (HE stain, ×200).

Table 2 – Values of carotid IMT based on the type of liver histology in NAFLD patients

Histological assessment	N	Carotid IMT [mm]	Pulsatility Index [mm]	Resistivity Index [mm]
Simple steatosis	2	0.92±0.03	1.51±0.03	0.67±0.03
Steatohepatitis (NASH)	8	1.17±0.07	1.62±0.06	0.74±0.05
Steatosis				
Grade 1 (mild)	2	0.92±0.03	1.51±0.03	0.67±0.03
Grade 2 (moderate)	3	1.14±0.02	1.59±0.06	0.70±0.06
Grade 3 (severe)	5	1.21±0.02	1.62±0.05	0.73±0.05
Necroinflammation				
Grade 1	2	1.03±0.03	1.55±0.03	0.68±0.03
Grade 2	3	1.19±0.01	1.61±0.05	0.72±0.06
Grade 3	3	1.24±0.02	1.64±0.05	0.75±0.05
Fibrosis				
Grade 1	3	1.06±0.02	1.60±0.04	0.74±0.05
Grade 2	3	1.20±0.01	1.63±0.06	0.76±0.05
Grade 3	2	1.28±0.03	1.79±0.03	0.84±0.03

Data is expressed as mean ± standard deviation.

The degree of internal carotid artery stenosis in NAFLD patients was less than 25%.

In the same time, the type of histological lesions in NAFLD is strongly associated with the severity of retinal vascular changes. Patients with liver histology of

mild steatosis and lowest values of carotid IMT, PI and RI have only mild narrowing of the retinal arterioles, which corresponds to stage 1 from Keith–Wagener–Barker classification. In patients with moderate steatosis and intermediate levels of carotid IMT, PI and RI, moderate narrowing of the arterioles, exaggeration of the light reflex and arteriovenous crossing changes were described, which corresponds to stage 2 from Keith–Wagener–Barker classification.

Histological lesions of marked steatosis and highest levels of carotid IMT, PI and RI were associated with marked narrowing and irregularity of retinal arterioles–copper wire or silver-wire arterioles and arteriovenous nicking characterized by narrowing of retinal veins at arteriovenous crossing sites. This corresponds to stage 3 from the Keith–Wagener–Barker classification. Two patients with liver biopsies of marked steatosis and increased carotid IMT presented vascular complications like retinal vascular occlusions: central retinal artery occlusion (CRAO – Figure 4) and central retinal vein occlusion (CRVO – Figure 5).

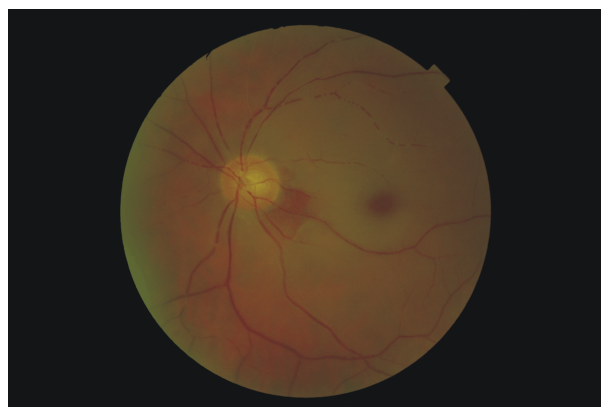


Figure 4 – Retinophotography of the left eye. Central retinal artery occlusion (CRAO): ischemic retinal whitening of the posterior pole, box-caring of blood flow in the retinal vessels and cherry-red spot appearance of fovea in a patient with marked steatosis and high level of carotid IMT.

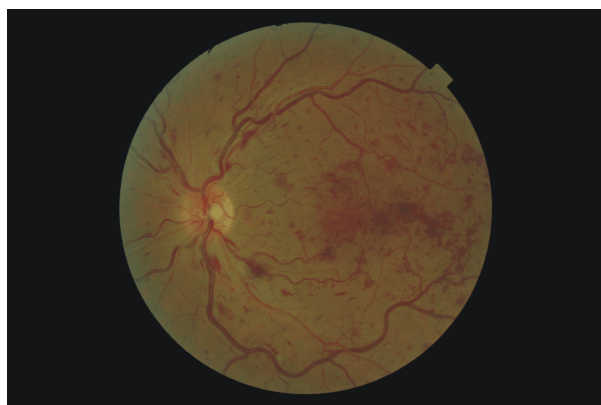


Figure 5 – Retinophotography of the left eye. Central retinal vein occlusion (CRVO): diffuse retinal hemorrhages, optic nerve head edema, dilated and tortuous veins in a patient with marked steatosis and high level of carotid IMT.

Discussion

In hepatology practice, NAFLD is considered the

most frequent cause of increased liver enzymes in the asymptomatic patients and the main cause of cryptogenic cirrhosis in developed countries [17].

NAFLD represents a group of conditions associated with negative alcohol intake, histologically characterized by simple liver steatosis, usually asymptomatic or non-alcoholic steatohepatitis (NASH) which includes the presence of: apoptosis, inflammation and fibrosis that might progress to cirrhosis [17].

A strong association between NAFLD and each component of the metabolic syndrome, including: dyslipidemia, arterial hypertension, central obesity and type II diabetes mellitus has been lately demonstrated [17].

Nonalcoholic fatty liver disease (NAFLD) is considered nowadays the hepatic expression of metabolic syndrome [1, 17] and it is also a major health problem in developed countries, with a prevalence of 20% to 40% in the general population, higher in obese patients (57.5–74%) [18–20].

Our study included ten patients with NAFLD and retinal vascular changes.

Blood measurements were determined by standard laboratory procedures. The levels of liver enzymes were increased in this group of patients. There were no significant values regarding creatinine, glucose levels and blood pressure.

Dyslipidemia was present in all NAFLD patients.

An association between increased carotid IMT, evaluated by ultrasonography and NAFLD, diagnosed using abdominal ultrasonography and liver biopsy was demonstrated in previous case-control and cross-sectional studies [21–27].

Abdominal ultrasonography results correlate well with different types of histological lesions in NAFLD but are not enough sensitive to describe the stage of steatosis, inflammation or fibrosis [4].

Although its use is limited in patients with non-progressive fatty liver diseases, the liver biopsy followed by histological interpretation remains the ‘gold standard’ for diagnosing NAFLD and establishing the grade of fibrosis and disease severity [28–31].

Our study shows that carotid IMT, PI and RI evaluated by ultrasonography are increased in patients with biopsy-proven NAFLD, well correlated with the severity of histological lesions. The lowest value of carotid IMT, IP and also IR was described in patients with mild steatosis, intermediate values in patients with steatosis plus lobular inflammation and hepatocellular ballooning. Patients with biopsy-proven steatosis plus fibrosis have the highest values of carotid IMT, IP and IR.

The measured flow velocity by Doppler ultrasonography helps in calculating the pulsatility index (PI) and it was proven to increase with DM, old age, hypertension, intracranial hypertension, vascular dementia, and small artery disease.

The PI is an expression of the vascular resistance distal to the examined artery and it has been proven that PI and resistivity index (RI) of the common carotid artery may also be surrogate markers of atherosclerosis.

RI, a hemodynamic parameter that can be easily determined by Doppler sonography, shows local wall

extensibility and the related vascular resistance [4, 30]. A correlation has been established between increasing RI values and arteriosclerosis risk factors and clinical outcome [10, 11, 32].

Nowadays, it is poorly understood the manner by which liver findings in NAFLD patients could be associated with the progression of atherosclerosis. The pathogenetic mechanism might include endothelial dysfunction, oxidative stress, inflammation, inflammatory cytokines, and lipid and glucose metabolism disorder [5].

Several studies have been demonstrated that insulin resistance has an important implication in the clinical results of NAFLD patients [2, 8, 9] and that the advanced forms of NAFLD stimulates an increasing of insulin-resistance and dyslipidemia. This way the progression of atherosclerosis is accelerated. In our study, dyslipidemia is present in all patients, although none of the patients has insulin-resistance.

Other prospective studies showed that a high level of liver enzymes is strongly associated with producing metabolic syndrome [33, 34]. Increased oxidative stress and subclinical inflammation, considered to be causal factors in the progression from simple steatosis to more advanced forms of NAFLD may represent a possible atherogenic mechanism linking NAFLD and carotid IMT [2, 9].

Another possible mechanism linking NAFLD and carotid IMT could be the decreased plasma levels of adiponectin, an adipose-secreted cytokine with anti-atherogenic properties. It has been shown that hypo-adiponectinemia closely correlates to NAFLD in obese individuals, unrelated to insulin resistance and other metabolic syndrome components [35, 36].

NAFLD could be associated to accelerated atherogenesis through the presence of abnormal lipoprotein metabolism. Hepatic apolipoprotein B-100 synthesis, implicated in hepatic VLDL formation and in hepatocyte lipid export, is reduced in NAFLD [37]. Also, perturbation of VLDL was described and can lead to increased levels of atherogenic triglyceride- and cholesterol-rich remnant particles [2, 8, 9]. An increasing value of small dense LDL particles might be present in NAFLD patients and could stimulate atherogenesis [38].

Internal carotid artery delivers blood to the eye, therefore arteriosclerosis related pathologies of this artery may have direct effect on retinal circulation and may coexist with retinal arteriosclerosis.

The most frequent cause of CRAO is embolism, usually with the origin in a plaque of the carotid artery [12]. Arteriosclerosis is the cause for the majority of retinal vein occlusion [13]. Patients with retinal occlusive disease have presented additional metabolic and hematologic abnormalities: arterial systemic hypertension, atherosclerosis, diabetes mellitus, dyslipidemia such as found in cardiovascular disease. Occlusions of retinal arterial and venous circulation are frequently related to severe visual loss and also to critical cerebrovascular and cardiovascular events. Systemic treatment is needed in these cases [39, 40].

In a meta-analysis, it has been demonstrated that the risk factors for atherosclerosis might be also implicated

in retinal vein occlusion pathogenesis [41]. A retrospective study showed that disorders in lipoprotein metabolism might contribute to the etiology of retinal vascular occlusions. Increased levels of Lp(a) and LDL-C are both risk factors for cardiovascular diseases and for retinal artery occlusion or retinal vein occlusion. Elevated levels of LDL-TG may be involved in retinal vascular occlusion pathogenesis [42].

In our study, the type of histological lesions in NAFLD patients is strongly associated with the value of carotid IMT, pulsatility index, resistivity index and also with the presence of retinal vascular changes. Patients with a liver histology of mild steatosis and lowest values of carotid IMT, PI and RI have only mild-to-moderate narrowing of the retinal arterioles; patients with moderate steatosis and intermediate levels of carotid IMT, PI and RI have moderate narrowing of the arterioles, local and/or generalized, exaggeration of the light reflex and arterial-venous crossing changes.

Patients with histological lesions of marked steatosis and highest values for carotid IMT, PI and RI have marked narrowing and irregularity of retinal arterioles (copper wire or silver-wire arterioles), arteriovenous nicking (narrowing of retinal veins at arteriovenous crossing sites) or retinal vascular occlusions (central retinal artery occlusion or central retinal vein occlusion).

One of the limitations of our study could be the reduced number of patients; further studies on larger groups are needed to confirm these results.

Conclusions

This study demonstrates that patients with biopsy-proven NAFLD have an increased value of carotid hemodynamic parameters: IMT, early marker for sub-clinical atherosclerosis and also of pulsatility and resistivity index. The type of liver histological lesions in NAFLD patients (hepatic steatosis, inflammation and fibrosis) was strongly associated with the value of carotid IMT, IP and IR. It is also connected to the type of retinal vascular changes (narrowing of the arterioles, exaggeration of the light reflex, arteriovenous crossing changes, central retinal artery occlusion, central retinal vein occlusion).

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