DOCTORAL THESIS

STUDY OF RETINAL AND CAROTID VASCULAR DISORDERS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

-THESIS RESUME-

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Keywords: steatosis, intima media-thickness, pulsatility index, resistivity index, retinal vascular changes.
**Introduction**

Non-alcoholic 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 correlate 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]. 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 artherosclerosis. 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].
Aim and objectives

The aim of this study is to evaluate, in a non-invasive manner, the retinal and carotid vascular changes of patients with NAFLD and to establish if there is a correlation between these changes and the hepatic disease evolution, invasively evaluated by liver biopsy.

The objectives of the study are:

* **Clinical and biochemical assessment;**
* **Imagistical assessment:**
  - Abdominal ultrasonography;
  - Carotid ultrasonography;
  - Retinal photography.
* **Histopathological assessment of liver biopsies.**
* **Establishing a correlation between the results of the investigations.**
* **The impact of retinal and carotid vascular modifications assessment upon improving the outcome of the disease by adequate therapy in NAFLD patients.**

Patients and Methods

Eighty-five patients with NAFLD and retinal vascular changes have been included in our study, 48 women and 37 men, mean age 45.5±5.15 (min. 36, max. 54, standard deviation 5.15). They were admitted in the Department of Gastroenterology of the Emergency County Hospital of Craiova, between 2010–2012, 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, α1-antitripsin deficiency, Wilson’s disease, hemochromatosis or hepatotoxic drugs).

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

Liver biopsies were analyzed and classified by an experienced pathologist blinded to patients’ clinical results, based on the Matteoni and Brunt et al classification [14].

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

▪ grade 1 (mild): <33% of hepatocytes affected;
▪ grad 2 (moderate): 33–66% of hepatocytes affected;
▪ grade 3 (severe): >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.

All subjects underwent ultrasonography of the carotid artery, with determination of intima-media thickness, pulsatility index and resistivity index.

The retinal vascular changes were evaluated using retinal photography obtained with a non-mydriatic Topcon retinal camera and they were classified based on the Keith–Wagener–Barker system [15].

〈Results〉

A) Clinical results

The most important clinical and biochemical characteristics of NAFLD patients are described in Table 1.

<table>
<thead>
<tr>
<th>Table 1 – Clinical and laboratory characteristics of NAFLD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
</tr>
<tr>
<td>Waist circumference [cm]</td>
</tr>
<tr>
<td>Systolic blood pressure [mmHg]</td>
</tr>
<tr>
<td>Diastolic blood pressure [mmHg]</td>
</tr>
<tr>
<td>Total cholesterol [mg/dL]</td>
</tr>
<tr>
<td>Triglyceride [mg/dL]</td>
</tr>
<tr>
<td>Glucose [mg/dL]</td>
</tr>
<tr>
<td>Test</td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>ASAT [IU/L]</td>
</tr>
<tr>
<td>ALT [IU/L]</td>
</tr>
<tr>
<td>γ-GT [IU/L]</td>
</tr>
<tr>
<td>Creatinine [mg/dL]</td>
</tr>
</tbody>
</table>

Values are expressed as means ± Standard Deviation

B) Histopathological results

The histopathological assessment of liver biopsies showed:
- mild steatosis (grade 1) in 14 patients- 16.47%
- moderate steatosis (grade 2), described in 32 patients- 37.64%
- severe steatosis (grade 3), in liver biopsies of 39 NAFLD patients- 45.88 %.

Steatosis alone was described in 14 patients, the rest of the patients had associated steatosis plus: lobular inflammation and hepatocellular ballooning or fibrosis (NASH). No differences between men and women were detected.

C) Imagistical results

Carotid ultrasonography showed that the lowest value of carotid IMT and also of pulsatility and resistivity index was described in patients with mild steatosis (Table 2).

<table>
<thead>
<tr>
<th>Histological assessment</th>
<th>N</th>
<th>Carotid IMT [mm]</th>
<th>Pulsatility Index</th>
<th>Resistivity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Steatosis (grade 1)</td>
<td>14</td>
<td>0.91±0.01</td>
<td>1.50±0.03</td>
<td>0.65±0.03</td>
</tr>
</tbody>
</table>

Table 2 – Values of carotid IMT, IP and IR in patients with liver histology of mild steatosis. Data is expressed as mean ± Standard Deviation
Intermediate values were described in patients with steatosis plus lobular inflammation and hepatocellular ballooning (Table 3).

<table>
<thead>
<tr>
<th>Histological assessment</th>
<th>Număr pacienți</th>
<th>Valoarea IMT(mm)</th>
<th>Valoarea IP</th>
<th>Valoarea IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate steatosis+lobular inflammation+ hepatocellular ballooning (NASH)</td>
<td>32</td>
<td>1,14±0,01</td>
<td>1,59±0,03</td>
<td>0,70±0,03</td>
</tr>
<tr>
<td>Necroinflamaţion:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- grade I</td>
<td>9</td>
<td>1,02±0,01</td>
<td>1,53±0,03</td>
<td>0,66±0,03</td>
</tr>
<tr>
<td>- grade II</td>
<td>11</td>
<td>1,16±0,01</td>
<td>1,59±0,03</td>
<td>0,70±0,03</td>
</tr>
<tr>
<td>- grade III</td>
<td>12</td>
<td>1,22±0,01</td>
<td>1,62±0,03</td>
<td>0,73±0,03</td>
</tr>
</tbody>
</table>

Table 3 – Values of carotid IMT, IP and IR in patients with liver histology of moderate steatosis, lobular inflammation and hepatocellular ballooning = NASH. Data is expressed as mean ± Standard Deviation.

Highest values of carotid parameters were calculated in patients with steatosis plus fibrosis (Table 4).

<table>
<thead>
<tr>
<th>Histological assessment</th>
<th>Număr pacienți</th>
<th>Valoarea IMT(mm)</th>
<th>Valoarea IP</th>
<th>Valoarea IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe steatosis + Fibrosis (NASH)</td>
<td>39</td>
<td>1,21±0,01</td>
<td>1,62±0,03</td>
<td>0,73±0,03</td>
</tr>
</tbody>
</table>
Fibrosis:
- grade I | 10 | 1,04±0,01 | 1,58±0,03 | 0,74±0,03
- grade II | 14 | 1,19±0,01 | 1,62±0,03 | 0,75±0,03
- grade III | 15 | 1,26±0,01 | 1,74±0,03 | 0,83±0,03

|

Table 4– Values of carotid IMT, IP and IR in patients with liver histology of severe steatosis and fibrosis (NASH). Data is expressed as mean ± Standard Deviation

The type of histological lesions in NAFLD is strongly associated, in the same time 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.

14 patients with liver biopsies of marked steatosis and increased carotid IMT presented vascular complications like retinal vascular occlusions: 9 patients- central retinal artery occlusion (CRAO) and 5 patients- central retinal vein occlusion (CRVO).
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 [16].

Non-alcoholic fatty liver disease (NAFLD) is considered nowadays the hepatic expression of metabolic syndrome [1, 16] 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%) [17].

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 [18–22].

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 [23, 24].

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 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. A correlation has been established between increasing RI values and arteriosclerosis risk factors and clinical outcome [25].

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.

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 [26].

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 [27].

Also, perturbation of VLDL was described and can lead to increased levels of atherogenic triglyceride and cholesterol-rich remnant particles [2, 8, 9].

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 [12,28,29].

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.

**Conclusion**

This study demonstrates that patients with biopsy-proven NAFLD have an increased value of carotid hemodynamic parameters, IMT, early marker for subclinical 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).

Patients with histological lesions of marked steatosis and highest values for carotid IMT, PI and RI have an increased risk for the occurrence of retinal vascular occlusion.

In our study, patients with NAFLD have presented additional metabolic and hematologic abnormalities: arterial systemic hypertension, diabetes mellitus, dyslipidemia and atherosclerosis, such as found in cardiovascular, cerebrovascular and retinal vascular disease.

Retinal photography and carotid ultrasonography should be performed in all patients with NAFLD in order to evaluate the severity of the disease and the prognostic.

**References**


