Non-alcoholic Fatty Liver Disease, as a Possible Independent Risk Factor for Obliterative Arteriopathy

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Introduction: Non-alcoholic fatty liver disease, the hepatic manifestation of metabolic syndrome is the most common liver disease in civilized countries. There are a large number of studies which have demonstrated that cardiovascular diseases have a higher prevalence in those who suffer from non-alcoholic fatty liver disease. Direct causality is still largely debated, most components of metabolic syndrome being present in the case of non-alcoholic fatty liver disease too, which are well known risk factors for atherosclerosis also. The aim of our study was to find out the relations between non-alcoholic fatty liver disease and peripheral arterial disease (PAD).

Material and methods: Our retrospective study included 176 patients with PAD and 175 controls. We recorded the stages of PAD, risk factors and associated cardiovascular conditions.

Results: Our results show a significantly higher prevalence of PAD in patients suffering from non-alcoholic fatty liver disease. We also found that the association of non-alcoholic fatty liver disease and obesity or hypertension or hypertrygliceridaemia increase the risk of symptomatic PAD.

Conclusions: The prevalence of peripheral arterial disease is higher in patients with non-alcoholic fatty liver compared to the control group. Studies demonstrating the role of non-alcoholic fatty liver as an independent risk factor for this particular form of atherosclerosis are needed.

Keywords: non-alcoholic fatty liver disease, peripheral arterial disease, risk factor

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Introduction

Peripheral arterial disease (PAD) is a manifestation of atherosclerosis like coronary heart disease, as well as cerebrovascular disease. The main risk factors for peripheral arterial disease are smoking, dyslipidaemia, hypertension and diabetes. Disease prevalence is much higher in men and increases with age. Stadialization of PAD is made after Leriche-Fontaine which is a clinical staging, useful in assessing the functional reserve of a limb's circulation. The majority of cases are subclinical or in stage II after Leriche-Fontaine, but some patients reach advanced stages of disease with critical limb ischemia. Leriche-Fontaine stages III and IV are predictive factors for poor life expectancy, death occurring after a cardiovascular catastrophe [1].

Non-alcoholic fatty liver disease (NAFLD) is a hepatic condition which has become "epidemic" in recent decades, with the boom in the prevalence of metabolic syndrome in civilized countries [2]. The pathogenesis of NAFLD is linked to insulin resistance [3]. It can be found in about 20 to 30% of adults in Western countries and in over 70% of those with type 2 diabetes. The importance of this liver disease cannot be underestimated, because some forms are aggressive, with progressive evolution causing terminal liver disease (end-stage liver disease) or liver cancer. Non-alcoholic steatohepatitis is the severe form of NAFLD, being the leading cause of cryptogenic cirrhosis [4].

Since the risk factors for PAD are components of metabolic syndrome except for smoking, we wonder if NAFLD, as the hepatic manifestation of metabolic syndrome, has any relation with PAD. There are studies in the literature that argue that NAFLD is an independent risk factor for cardiovascular disease besides the elements of metabolic syndrome [5], so a causal relationship could exist with PAD, too.

Material and methods

We enrolled in our study 176 patients with PAD, hospitalized in our Clinic from January 1, 2011 to June 30, 2011. We recorded in each case the age and sex of the patient, stage of PAD, presence and type of diabetes (impaired glucose tolerance, type 2 diabetes on oral antidiabetics, insulindependent type 2 diabetes), the presence of clinically significant atherosclerosis localized at other vascular territories – upper limbs, carotid artery, heart (three forms of cardiac ischemia – silent, angina or arrhythmias), previous stroke, hypertension, presence and type of hyperlypidaemia (hypercholesterolemia, hypertriglyceridemia) and presence or absence of NAFLD.

The control group consisted of 175 patients hospitalized for different conditions in our clinic in the same period. The control group had the same gender distribution and mean age as the study group. Patients were randomly selected, the main exclusion criteria was the presence of PAD. The other exclusion criteria were similar with the study group (see below).

The presence of PAD was confirmed by peripheral arterial Doppler examination, we measured the pressures in the anterior and posterior tibial arteries, measured values being reported to the systolic blood pressure value of the brachial artery. All patients were in clinically manifest stages of the disease (stage II, III, IV). Upper extremity arterial involvement was diagnosed by the same method and that of the carotid arteries by Doppler-duplex examination.

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Every investigation mentioned above was performed in each patient, regardless of symptoms.

Waist circumference was measured in each patient. Metabolic syndrome was considered in case of waist circumference over 94 cm in men and over 80 cm in women, equivalent to abdominal obesity plus any two of diabetes type 2/ impaired glucose tolerance, hypertriglyceridemia, hypercholesterolemia, arterial hypertension.

Impaired fasting glucose has been considered if fasting blood sugar was between 100–125 mg% (5.6– 6.9 mmol/l) and impaired glucose tolerance, if glucose levels were between 140 and 199 mg/dL (7.8–11.0 mmol) two hours after 75 g oral glucose ingestion. Overt diabetes was considered if fasting glucose was over 126 mg% on two different measurements.

Hyperlipidaemia was diagnosed based on history, in those patients who had treatment for some lipid abnormality and based on laboratory findings, serum triglycerides over 150 mg/dl and/or cholesterol over 200 mg/dl and/or HDL below 40 mg% in men and below 50 mg% in women.

The presence of systemic arterial hypertension was confirmed in patients who had been already treated for elevated blood pressure values or in those with values above 140/90 mmHg measured on two separate occasions.

A body mass index between 25–30 was equivalent with overweight, or obesity, if it was over 30.

Ischemic heart disease was divided into three subgroups, as follows: painful form (angina or myocardial infarction), silent form, diagnosed on the basis of ischemic ECG changes and arrhythmic form.

The diagnosis of NAFLD was based on positive and negative criteria [6,7]. Positive criteria were represented by:

- presence of metabolic syndrome, diagnosed according to the new IDF (International Diabetes Federation) criteria;
- hepatic steatosis, screened by abdominal ultrasound. Although ultrasonography has only a sensitivity of 66–70% for the diagnosis of liver steatosis, it has a high specificity, around 95–97%. Its sensitivity increases over 95% at a grade of steatosis over 30%. Including only patients with at least 30% steatosis increases sensitivity to 90% [8]. It was impossible to perform other and more precise diagnostic procedures, mainly for financial reasons. To reduce the subjectivity of the method, two independent sonographers were involved in the diagnostic procedure. The diagnosis of liver steatosis was accepted if both of the investigators independently made this statement.

Exclusion criteria were the following:

- regular alcohol consumption (over 20 g/day for men and 10 g/day for women) for alcoholic liver disease;
- evidence of chronic viral hepatitis – HBs antigen or antiHCV positivity;
- elevated erythrocyte sedimentation rate, positive antinuclear antibodies and high gammaglobulin levels for autoimmune hepatitis;
- transferrin saturation above 50% for haemochromatosis;
- low serum coeruloplasmin levels for Wilson's disease;
- previous treatment with drugs like amiodarone, tamoxifenum, steroids for drug induced liver steatosis;
- parenteral nutrition or severe weight loss;
- type 1 diabetes and peripheral arterial disease, because of the early and high cardiovascular risk represented by this disease.

Exclusion criteria were applied to all patients included in our study and to the control group too.

Statistical analysis was performed using GraphPad Prism and SPSS programs. To analyze the relationship between two variables and intensity of this relationship we used Pearson's correlation test, representing a graphical correlation with the dispersion diagram. A link between the means of variables was determined using ANOVA. We applied the chi-square test to determine the existence of statistically significant relationships between different parameters.

Results

The study included 176 patients with PAD and 175 patients without PAD as the control group, the total number of patients being 351. In the PAD group there were 136 men (77.3%) and 40 women (22.7%). NAFLD was present in 44.9% of cases (79 patients) and absent in 55.1% (97 patients). In the control group there were only 45 patients with NAFLD (25.7%) and 132 (74.3%) without.

The distribution of patients according to gender and the presence or absence of NAFLD is summarized in Table I and that of the control group in Table II.

The prevalence of PAD in the NAFLD and in the non-NAFLD group respectively, was the following:

- 63.7% in the NAFLD group (79 patients out of 124);
- 42.7% in the non-NAFLD group (97 patients out of 227).

OR was 2.3, which means that there is a 2.3 times greater probability for patients with NAFLD to develop PAD, compared with patients without NAFLD (p=0.0003).

The distribution of patients according to the stage of PAD and gender was the following:

- Stage II – 60 patients (34.1%), 13 women, 47 men;
- Stage III – 72 patients (40.9%), 10 women, 62 men;
- Stage IV – 44 patients (25%), 17 women, 27 men.

The distribution of patients according to the presence or absence of diabetes, hyperlipidaemia, upper limb arterial involvement, atherosclerotic carotid artery stenosis, the three forms of ischemic heart disease, the presence of stroke in anamnesis, the presence of obesity and that of high blood pressure is presented in Table III. The distribu-

Table I. Gender distribution of patients with arteriopathy according to the presence or absence of non-alcoholic fatty liver disease

Sex	Without NAFLD No. of cases	With NAFLD No. of cases
Female	25 (25.7%)	15 (19%)
Male	72 (74.3%)	64 (81%)
Total	97 (100%)	79 (100%)

Table II. Gender distribution of the control group according to the presence or absence of non-alcoholic fatty liver disease

Sex	Without NAFLD No. of cases	With NAFLD No. of cases
Female	25 (20.2%)	15 (35.7%)
Male	99 (79.8%)	37 (64.3%)
Total	124 (100%)	42 (100%)

tion according to the same conditions in the control group is presented in Table IV.

The distribution of the three clinical stages of peripheral arterial disease is presented in Table V, according to the presence or absence of NAFLD.

Statistical data processing was done separately for different stages of arterial disease.

The prevalence of type 2 diabetes, obesity and arterial hypertension had statistically significant correlation with the presence of NAFLD.

The prevalence of NAFLD in patients with diabetes, obesity and hypertension was as follows:

- 57.9% of patients with type 2 diabetes had NAFLD (55 patients out of 95), p=0.002;
- 97.6% of patients with obesity suffered from NAFLD (95 out of 97 patients, most of them with type 2 diabetes), p=0.0001;
- 50.4% of patients with arterial hypertension had NAFLD (69 patients out of 137), versus 25.6% of those without hypertension (10 patients out of 39), p=0.01.

Regarding coronary heart disease, we had 149 patients with different forms of ischemic heart disease. NAFLD was present in 45.6% of the cases (68 patients out of 149). We found that the presence of NAFLD does not increase the risk of PAD in patients with coronary heart disease.

After dividing patients according to the stage of PAD and the presence or absence of arterial hypertension, we analyzed the correlation of NAFLD with arterial hypertension. We found a statistically significant correlation between NAFLD and arterial hypertension in stage II of PAD. In this group, 29 out of the 31 patients with NAFLD were hypertensive. Odds ratio was 6.52, with a confidence interval ranging from 1.27 to 33.45. This means that hypertension is an associated risk factor for PAD in patients with NAFLD.

In PAD stage III and IV the odds ratio was above 1, confidence interval including 1. In stages III and IV of PAD, the association of NAFLD with hypertension does not represent a higher risk for PAD than arterial hypertension alone.

In patients with NAFLD and hypertriglyceridemia, we found a statistically significant correlation between these two conditions in stage III PAD. 73% (14 out of 19) of patients with stage III PAD and hypertriglyceridaemia were with NAFLD, p=0.007. In case of hypertrigliceridaemia the presence of NAFLD conferes an additional risk for PAD. Odds ratio was 4.62, with a confidence interval between 1.4–14.7.

There were 60 patients with stage II PAD, 31 of them were found with NAFLD, but only 9 had hypertriglyceridemia too. In patients with stage IV PAD, the distribution of the 44 cases was the following: 14 patients with NAFLD, 15 with hypertriglyceridaemia. Only 5 people with NAFLD had also hypertriglyceridaemia.

Hypercholesterolemia was present in 54% of cases (95 patients out of 176). 63.9% of patients with hypercholesterolemia had NAFLD (62 patients). We could not demonstrate any statistically significant correlation between the presence or absence of NAFLD and PAD in patients with hypercholesterolemia.

In overweight and obese patients the risk of PAD was higher in those with NAFLD, regardless of the stage of

Table III. Distribution of cases according to the presence or absence of associated conditions in patients with obliterative artheriopathy

Condition associated to PAD	Present No. of cases	Absent No. of cases
Diabetes	95 (54%)	81 (46%)
Hypertriglyceridaemia	55 (31.2%)	121 (68.8%)
Hypercholesterolaemia	95 (54%)	81 (46%)
Obesity	37 (21%)	139 (79%)
Hypertension	137 (77.8%)	39 (22.2%)
Upper limb arteriopathy	11 (6.2%)	165 (93.8%)
Carotid artery atherosclerosis	52 (29.5%)	124 (70.5%)
Ischemic heart disease/myocardial infarction or angina	45 (25.6%)	131 (75.4%)
Silent cardiac ischemia	80 (45.5%)	96 (54.5%)
Ischemic heart disease – arrythmias	24 (13.6%)	154 (86.4%)
Ischemic heart disease – total	142 (80.7%)	35 (19.3%)
Stroke	16 (9%)	160 (91%)

Table IV. Distribution of cases in the control group according to the presence or absence of associated conditions

Condition	Present No. of cases	Absent No. of cases
Diabetes	51 (29.1%)	124 (70.9%)
Hypertriglyceridaemia	58 (33.1%)	117 (66.9%)
Hypercholesterolemia/ Low HDL	99 (56.6%)	76 (43.4%)
Obesity/ overweight	63 (36%)	112 (64%)
Hypertension	73 (41.7%)	102 (58.3%)
Carotid artery atherosclerosis	26 (14.8%)	149 (85.2%)
Ischemic heart disease/ total	124 (70.8%)	48 (69.2%)
Stroke	9 (5.1%)	166 (94.9%)

Table V. Distribution of cases according to the stage of arteriopathy and presence or absence of NAFLD

Stage of arteriopathy	NAFLD present No. of cases	NAFLD absent No. of cases
II.	31 (39.3%)	29 (29.9%)
III.	34 (43%)	38 (39.1%)
IV.	14 (17.7%)	30 (31%)
Total	79 (100%)	97 (100%)

PAD. Table VI summarizes the distribution of patients according to the presence of NAFLD and obesity. The estimated risk for a patient with obesity and NAFLD to develop PAD was found to be statistically significant (Table VII).

Table VI. Distribution of cases of PAD according to the stage of PAD and the presence of hepatic steatosis and obesity

Stage of arteriopathy	Steatosis	Obesity		Total
		Present	Absent	
II.	Absent	28	1	29
	Present	19	12	31
	% within obesity	40.4%	92.3%	51.7%
	Total (n)	47	13	60
III.	Absent	38	0	38
	Present	18	16	34
	% within obesity	32.1%	100.0%	47.2%
	Total (n)	56	16	72
IV.	Absent	29	1	30
	Present	7	7	14
	% within obesity	19.4%	87.5%	31.8%
	Total (n)	36	8	44

Discussions

There are many epidemiological studies which found an increased prevalence of cardiovascular disease in patients with different forms of non-alcoholic fatty liver disease. Ruttmann et al. studied mortality causes in patients with NAFLD. They found that cardiovascular disease was on the second place after cancer [9]. Huang Y et al. found that patients with NAFLD had a higher carotid intima-media thickness independent from conventional cardiovascular disease risk factors and metabolic syndrome [10].

Alkhouri et al. demonstrated strong correlation between the severity of liver damage and increased cardiovascular risk, and atherogenic lipid profile, respectively [11]. New evidence suggests that NAFLD increases the risk of cardiovascular disease independently of metabolic syndrome [12,13,14,15]. Epidemiological studies of Ruttmann et al. demonstrated that the presence of cardiovascular disease is associated with elevated liver enzymes [9].

Our study focused on patients with PAD. Since we did not find any other study in the medical literature assessing the potential role of NAFLD as an additional risk factor for this peripheral vascular condition, we could not compare our results with others.

We found the following:

1. Epidemiological aspects are consistent with those from the literature [1,5]
   - Predominance of male gender – 77.3% of patients with PAD were men.
   - Hypertension was present in 77.6% of patients, as a major risk factor for atherosclerosis.
   - Obesity was present only in 21% of patients, which is apparently surprising. It can be explained by the fact that most patients included in the study were heavy smokers. Smoking is an independent risk factor for PAD. The study included also many patients with PAD in advanced stages – III and IV. These patients suffer from chronic pain that can lead to weight loss because of lack of appetite and depression.
   - 66.4% of patients were with critical limb ischemia (stages III and IV). This high prevalence of severe cases is due to the fact that our Clinic has an angiologic profile, we admit these patients to hospital in order to find an urgent solution for limb salvage.
2. Odds ratio was statistically significant for PAD in patients with NAFLD, which could be an independent risk factor for PAD. The atherogenic effect of metabolic syndrome and NAFLD on limb arteries may not completely overlap.
3. Regarding the relation between obesity, NAFLD and PAD, our results highlight the role of non-alcoholic fatty liver as an independent risk factor for PAD in obese patients. There is a statistically significant relation between the presence of obesity and NAFLD in every stage of PAD. The chance for an obese patient with NAFLD to develop PAD is significantly higher than that of a person with obesity and without NAFLD.
4. Regarding arterial hypertension, we found that the association of hypertension with NAFLD significantly increases the likelihood of stage II PAD .
5. The prevalence of hypertriglyceridemia was low in our group of patients. One explanation could be that we excluded those patients who consume alcohol on a regular basis. Our study didn't include patients with alcohol induced hypertriglyceridemia. We found that the presence of NAFLD significantly increases the risk of PAD in those few patients with hypertriglyceridemia.

Table VII. The estimated risk for different stages PAD in the presence of obesity and hepatic steatosis

PAD stage		Value	Confidence interval	
			Lower	Upper
2	Odds Ratio for steatosis (0/1)	17.684	2.119	147.558
3	For cohort obesity = 0	1.889	1.376	2.593
4	Odds Ratio for steatosis (0/1)	29.000	3.050	275.694

The results of this study suggest the possibility that the presence of NAFLD could be an additional risk factor for

atherosclerosis and in particular for PAD. NAFLD occurs in people who possess multiple cardiovascular risk factors, components of the metabolic syndrome that underlies liver disease [16,17]. Studies in the literature have hypothesized that NAFLD could have an additional atherogenic role, especially its aggressive histological forms, through the release of inflammatory mediators from the damaged liver into the circulation [18,19].

Conclusions

As our results suggest, the relationship between non-alcoholic fatty liver disease and peripheral arterial disease is undeniable, but direct causality has not been demonstrated. The effects of metabolic syndrome and NAFLD on PAD may not completely overlap. Be it direct causal relationship or not, if one diagnoses a patient with non-alcoholic fatty liver disease, one should have to screen for atherosclerosis of different vascular territories and should introduce primary and secondary cardiovascular preventive measures.

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