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Research Article

Symptomatic and asymptomatic significant carotid artery disease in type 2 diabetes patients with non-alcoholic steatohepatitis

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Abstract. Background: Type 2 diabetes mellitus (DMT2) is considered also an inflammatory disease. NAFLD, in its various forms, is a component of the metabolic syndrome (MetS) associated with abdominal obesity, impaired glucose tolerance / diabetes, dyslipidemia, hypertension; all these represent atherogenic risk factors. Objective: The purpose of this study was to assess the presence of significant carotid artery stenosis (symptomatic or asymptomatic) in type 2 diabetes mellitus patients with steatohepatitis, compared to diabetic patients without evidence of fatty liver (NAFLD) nor NASH phenotype negative. Materials and methods: This cross-sectional study included 107 consecutive diabetic patients and 110 controls; ultrasound and a panel of serological biomarkers were used as non-invasive means to diagnose NASH. The controls were diabetic patients without criteria for fatty liver disease. Carotid ultrasonography was performed along with a set biochemical tests (hepatic liver tests, fasting plasma glucose, HbA1c, a panel of lipid components, hs-CRP, and a panel of serological biomarkers for NASH phenotype). Results: 30.84% of the total DMT2 with steatohepatitis, with long duration of the disease and poor metabolic control, had significant carotid stenosis (both symptomatic and asymptomatic) vs. controls (10%), $p<0.001$. The prevalence of non-stenotic carotid atheromatosis was not significantly different in the two groups of diabetic patients (61.68% vs. 42.73%, $p>0.05$). Carotid atherosclerosis was present in 99 patients (92.52%) from the DMT2 and steatohepatitis group and in 58 subjects (52.73%) from the control group. We also found high levels of hs-CRP in the DMT2 patients with steatohepatitis, and extended carotid atherosclerosis vs. controls. Conclusions: In type 2 diabetic patients with steatohepatitis, the prevalence of CAD is higher than in controls, and the severe atherosclerotic lesions are more frequent in this high-risk group of patients. High levels of hs-CRP are linked to the severity of atherosclerotic lesions, and this situation requires an intensive and multifactorial management of the global cardiovascular risk.

Key Words: type 2 diabetes mellitus, non-alcoholic steatohepatitis, carotid artery disease, cardiovascular risk factors, systemic inflammation.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a highly prevalent clinical and histological condition (Gaggini et al 2013) characterized by fatty infiltration of hepatocytes, in the absence of significant alcohol consumption (Ludwig et al 1980). It is strongly associated with several atherosclerotic risk factors, all of which are components of the metabolic syndrome (MetS): impaired glucose tolerance / type 2 diabetes mellitus, obesity, dyslipidemia, hypertension (Marchesini et al 2001). NAFLD was shown to be related to insulin resistance syndrome (Goto et al 1995; Ikai et al 1995). This association is present in diabetic as well as in nondiabetic patients. NAFLD comprises a wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis (NASH) and cirrhosis (Angulo 2002; McCullough 2002).

Due to its strong association with components of MetS, all representing risk factors for atherosclerosis, it has been suggested that NAFLD may involve an independent cardiovascular risk. Several studies (Targher et al 2006; Brea et al 2005) have demonstrated an association between carotid atherosclerosis and NAFLD by evaluating intima-media thickness (IMT), thus suggesting that the addition of NAFLD to the other components of the metabolic syndrome may represent an independent atherogenetic risk (Hamaguchi et al 2008). Oxidative stress and subclinical systemic inflammation were incriminated in this pathogenetic link (Gastaldelli et al 2007; Yki-Jarvinen et al 2005).
The purpose of this study was to assess the presence of significant carotid artery stenosis – symptomatic or asymptomatic – in type 2 diabetes mellitus patients with steatohepatitis, compared to diabetic patients without evidence of fatty liver (NAFLD) nor NASH phenotype negative.

Table 1. Clinical and biochemical characteristics of diabetic patients with NASH and control subjects

Variable		NASH diabetic patients	Control subjects	p
Sex	Males	52	68	-
	Females	55	42	-
Age (years)		62.3±3.1	61.7±2.2	0.1
Weight (kg)		88.9±12.8	84.5±6.4	0.07
BMI (kg/m^2)		34.8±6.5	28.8±7.9	0.042
Waist circumference (cm)		108±18.5	94.9±7.5	0.037
Systolic blood pressure (mm Hg)		153±10.5	145±8.9	0.076
Diastolic blood pressure (mm Hg)		102±3.8	88±4.1	0.023
Medium blood pressure (MBP)		131±6.8	116±5.4	0.031
HbA1c (%)		7.9±3.4	7.1±1.6	0.02
Fasting plasma glucose (mg/dl)		132±45	121±52	0.11
Triglycerides (mg/dl)		287±138	188±89	0.024
Total cholesterol (mg/dl)		264.2±103.2	239.4±55.1	0.09
HDL cholesterol (mg/dl)		32.6±7	43.8±9.1	0.03
LDL cholesterol (mg/dl)		148±12	129±32	0.07
Disease duration (years)		13±3	11±6	0.1
Sedentary behavior (no. of subjects) (%)		89 (83.17%)	23 (20.9%)	0.015
Smoking status (no. of subjects) (%)		35 (32.71%)	28 (25.45%)	0.1
Alanine aminotransferase (ALAT) (units/ml)		88.2±35.1	38.4±12.9	0.001
Aspartate aminotransferase AST (IU/l)		39.2±10.2	22.4±14.8	0.08
High-sensitive PCR (hs CRP)		12.1±8.42	7.3±3.52	0.022
Fibrinogen (mg/dl)		489.82±108.9	304.72±51.60	0.029

Materials and methods

Subjects

In this study were included 107 consecutive diabetic patients with NASH phenotype positive (Grigorescu et al 2012). They were selected from an outpatient pool at N. Stancioiu Heart Institute, Cluj-Napoca, during 2012-2014.

One of the conditions to participate in this study was the duration of diabetes, longer than 10 years. Based on a questionnaire, the subjects with alcohol consumption (> 20 g/day) were excluded from the study. Similarly, patients with viral B or C hepatitis, autoimmune hepatitis, and hemochromatosis or other chronic liver disease were also excluded. The diagnostic of NAFLD was established based on liver function tests with increased liver enzymes, and ultrasonographical criteria (Qayyum et al 2009) of evidence for fatty liver infiltration. An experienced radiology operator assessed the patients using an Aloka SSD4000 unit. For the non-invasive diagnostic of NASH phenotype positive, we used a panel of biomarkers (e.g., IL-6, adiponectin, cytokeratin-18) (Grigorescu et al 2012).

The control group consisted of 110 diabetic patients, matched for sex and age, with absence of fatty liver (NAFLD) as confirmed by abdominal ultrasonography (with normal liver). All participants signed an informed consent form, and the study protocol followed the ethical guidelines of Helsinki Declaration, with the approval of the local Ethics Committee.

Clinical and laboratory measurements

All patients were evaluated for components of the metabolic syndrome. Body mass index (BMI) was computed as the weight [kg] divided by the square of height [m2]. Overweight was defined as a BMI ≥ 25 kg/m2, while obesity was defined when BMI ≥ 30 kg/m2. Waist circumference (in cm) was measured after expiration at the midpoint between the lower rib and the iliac crest. Hip circumference (in cm) was obtained at the widest point between hip and buttock. Blood pressure was measured with a random-zero sfygmomanometer, bilateral, twice for each patient, after 10 minutes rest. Hypertension was defined as an average systolic blood pressure of 140 mmHg, and an average diastolic blood pressure of 90 mmHg (Manchia et al 2007). After an overnight 12-hour fasting, flebotomy was performed and the following parameters were measured according to local laboratory methods: liver function tests (AST, ALT, GGT) and other biochemical blood parameters, including the biomarker panel for NASH phenotype.

Carotid ultrasound

A General Electrics Vivid S6 apparatus, equipped with a 9 mHz multi-frequency linear transducer was used for B mode carotid ultrasound. An experienced ultrasonographer blinded to the patients status, scanned longitudinally the right and left carotid arteries, and recorded the digital images for offline assessment. Each subject was assessed in supine positon.

Table 2. Type of carotid atherosclerosis in the study group vs. controls

Type of carotid atherosclerosis	DM T2+ NASH	DMT2	p
Number of patients	107	110	-
Significant carotid atherosclerosis symptomatic	13 (12.15%)	2 (1.82%)	0.025
Significant carotid atherosclerosis asymptomatic	21 (19.63%)	9 (8.18%)	0.035
Carotid unobstructive atherosclerosis	66 (61.68%)	47 (42.73%)	0.001
Total carotid atherosclerosis	99 (92.52%)	58 (52.73%)	0.001
Normal carotid	7 (6.54%)	52 (47.27%)	0.001

Table 3. Mean values of high-sensitive C reactive protein

Variable	NASH + DM	Controls	p
Symtomatic CAD	15.1±2.5	10.8±1.6	0.045
Asymptomatic CAD	11.1±3.4	7.5±1.2	0.03
Nonstenosant atheromatosis	8.1±1.7	7.7±1.9	0.06
Normal ultrasonographical carotid	6.2±1.5	6.6±1.3	0.055

The variability of ultrasonographic measurement was assessed by performing two measurements in 10 volunteers, over one week period.

A carotid plaque was defined as a focal thickening ≥ 1.2 mm, at the level of the carotid artery.

Significant carotid stenosis (≥ 60%) were detected based a peak systolic velocity (PSV), diastolic velocity (EDV), and carotid index defined as the peak systolic velocity in the internal carotid artery / peak velocity in the common carotid artery. For a good sensitivity and specificity, the following thresholds were used for a significant stenosis: PSV>200 cm/s, EDV>140 cm/s, and a carotid index>4.5 (Van Bortel 2005).

Statistical analysis

To compare the patient and control groups, we used the t-test or Mann-Whitney U-test when appropriate, for continuous variables; and we performed a χ^2 test for the categorical variables. Coefficients are expressed as mean and standard deviation (SD) for continuous variables, and as frequency (%) for the categorical variables. A value vas considered statistically significant for $p<0.05$.

Results

The patients in the two groups did not differ significantly in terms of age and gender. The diabetic subjects with NASH had a significantly higher BMI and waist circumference ($p < 0.05$). Although the fasting glucose was similar in both groups, patients with steatohepatitis had a significantly poorer long term glycemic control. Also the levels of triglycerides were higher, whereas the level of HDL cholesterol was significantly lower, suggesting a more pro-atherogenic dyslipidemia status in these patients.

Patients in both groups had a carotid ultrasound. A significantly higher proportion of diabetic patients with steatohepatitis (30.84%) had significant carotid stenosis both symptomatic and asymptomatic compared to controls (10%), with $p<0.001$. From among the 13 patients with symptomatic carotid artery disease, 10 had a transient ischemic attack in the last 6 months, whereas 3 had a stroke in the last 12 months in the carotid artery.

The prevalence of non-stenotic carotid atheromatosis was not significantly different in the diabetic patients with steatohepatitis (61.68%) versus controls without NASH (42.73%), $p>0.05$). Overall, significantly fewer patients with steatohepatitis (6.54%) had a normal carotid ultrasonography versus controls (47.27%), $p<0.001$.

Carotid atherosclerosis was found in 99 patients (92.52%) from the study patient group, and in 58 subjects (52.73%) from the control group (Table 2).

We have also assessed the mean values of high-sensitive C reactive protein (hsCRP) as a marker of subclinical inflammation; we found the highest values in the diabetic patients with NASH, who had significant symptomatic carotid artery diseaese (Table 3), suggesting that these patients may be at risk for symptomatic atherosclerotic disease, in other vascular territories.

Discussion

This case control study assessed the frequency of significant atherosclerotic carotid artery disease in two groups of diabetic patients, recognized with high cardiovascular atherosclerotic risk. Given that NAFLD represents a component of MetS, and that type 2 diabetes is frequently associated with NAFLD, large prospective studies compared diabetic patients with those without NAFLD, reporting the highest value for the subclinical atherosclerosis in the diabetic patients, specifically with steatohepatitis (Temelkova-Kurktschev et al 2000; Tuomilehto et al 1998; Targher et al 2005). From the large number of subjects with NAFLD, this particular subgroup of patients seems to have the highest risk for cardiovascular events.

The elevation of serum liver enzymes, used in our study as a surrogate marker for steatohepatitis, has been proven by other studies (Angulo 2002; Targher et al 2005) to be strongly associated with an increased incidence of cardiovascular disease, both in diabetic and nondiabetic patients.

Marchesini et al (2003) reported that diabetic patients with ultranonographically-diagnosed NAFLD had moderate to increased risks for cardiovascular events (Marchesini et al 2001), while Targher et al. (2007) demonstrated that NAFLD is independently associated with an increased incidence of cardiovascular events for this group of patients. Some other studies highlighted an

independent relationship between hepatosteatosis and the risk of carotid atherosclerosis (Volzke et al 2005); in contrast, some authors (McKimmie et al 2008) have failed to demonstrate this association between carotid atherosclerosis and type 2 diabetes patients with NAFLD diagnosed by computer tomography. In the same vein, another study using ultrasound to detect hepatosteatosis in asymptomatic type 2 diabetic patients, found non-significant carotid atherosclerosis (Poanta et al 2011).

In our study, we selected a subgroup of patients with long duration diabetes (>10 years), with poor metabolic control, and used ultrasonography as well as a panel of biochemical markers to determine the NASH phenotype of fatty liver disease, and to assess the carotid atherosclerotic lesions; this was necessary since it was the purpose of the study, and because it was required due to neurological manifestations in some diabetic patients, or to determine the known vascular atherosclerotic lesions in this type of high-risk patients.

We found a high prevalence of carotid atherosclerosis in diabetic patients with steatohepatitis, compared to controls (92.5% vs. 52.73%,). Moreover, we found significant high prevalence of significant atherosclerotic lesions in the carotid artery in this group of patients vs. controls (30.84% vs. 10%).

The results of our study suggest that in this selected subgroup of diabetic patients with non-alcoholic steatohepatitis, the frequency and severity of carotid atherosclerotic disease (CAD) might be unexpectedly high, but these patients with diabetes are known as a high-risk class for cardiovascular disease and events; this is explained by the presence of atherosclerotic risk factors: abdominal obesity, diastolic and medium blood pressure, atherogenic dyslipidemia – hypertriglyceridemia, low level of HDL cholesterol – and subclinical systemic inflammation – hsCRP. Also, the presence of steatohepatitis in the group with high prevalence of carotid atherosclerotic disease may increase the severity of atherogenesis through various pathogenetic pathways such as an intensified systemic and hepatic inflammation, imbalance of the oxidative stress, and metabolic abnormalities (Kampoli et al 2011).

In the absence of liver biopsy for a histological diagnosis of steatohepatitis, hepatic ultrasonography combined with elevated liver function tests and a panel of serological biomarkers for NASH phenotype (adiponectin, IL-6, cytokeratin-18) ensured reliability to diagnose steatohepatitis in diabetic patients. The presence of hepatic inflammation is associated with high risk of vascular atherosclerosis and cardiovascular events, and requires an active screening to diagnose the atherosclerotic lesions.

Conclusions

In conclusion, this study suggests that in patients with type 2 diabetes and fatty liver disease with hepatic and systemic subclinical inflammation, the carotid atherosclerosis is more prevalent and more severe. In type 2 diabetic patients in this study, the fatty liver disease in severe forms is a key factor associated with the severity of the atherosclerotic process. Furthermore, our data underscores the importance of evaluating cardiovascular risk in NAFLD diabetic patients, and of active screening for CAD. This subgroup of high-risk patients should also be targeted for intensive and multifactorial management of cardiovascular risk factors.

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