

Association between fatty liver disease and carotid atherosclerosis in patients with uncomplicated type 2 diabetes mellitus

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) is a clinic-pathological syndrome closely associated with obesity, dyslipidemia, diabetes and atherosclerosis. Some authors suggest that NAFLD is, in fact, another component of the metabolic syndrome. **Aim:** To determine the prevalence of NAFLD in diabetes mellitus (DM) patients, and to evaluate the carotid artery status in these patients. **Methods:** Fifty six patients with uncomplicated type 2 DM were enrolled. Hepatic steatosis (HS) and carotid atherosclerosis (intima-media thickness - IMT) were evaluated by ultrasonography. Plasma liver function tests and other biochemical blood measurements were determined. **Results:** HS was found in 38 patients (67.8%) with DM. Subjects with HS had higher values for body mass index, diastolic blood pressure, mean blood pressure and triglycerides, and lower HDL cholesterol concentration, but there were no differences regarding IMT between DM patients with or without HS. Behavioral variables (smoking, diet, and sedentarism), fasting plasma glucose, and LDL cholesterol levels, also, did not significantly differ between subjects with and without HS. **Conclusion:** DM patients with HS in our study showed a cluster of cardiovascular risk factors but non-significant carotid atherosclerosis. The detection of HS by abdominal ultrasound should alert to the existence of a higher cardiovascular risk, but in DM this is still under discussion, the results being still unconfirmed.

Keywords: nonalcoholic fatty liver disease, diabetes mellitus, intima-media thickness, ultrasonography

Rezumat

Premise: Steatohepatita nonalcoolică este o condiție clinico-patologică puternic asociată cu obezitatea, dislipidemia, diabetul zaharat (DZ) și ateroscleroza. Unii autori sugerează că SHNA ar fi, de fapt, o altă componentă a sindromului metabolic. **Scop:** De a determina prevalența SHNA la pacienții cu DZ și de a evalua statusul carotidelor la acești pacienți. **Metode:** Cincizeci și șase de pacienți cu DZ tip 2 necomplicat au fost înrolați în studiu. Au fost evaluate ecografic steatoza hepatică (SH) și ateroscleroza carotidiană (cu ajutorul grosimii intimă-medie – GIM). Au fost măsurate, de asemenea, testele hepatice și alte constante biochimice. **Rezultate:** SH a fost diagnosticată la 38 de pacienți (67,8%). Subiecții cu SH au avut valori mai mari ale indicelui de masă corporală, tensiunii arteriale diastolice și a celei medii, ale trigliceridelor, și valori mai mici ale HDL-colesterolului. Nu s-au găsit diferențe semnificative ale GIM la pacienții cu SH versus cei fără SH. Fumatul, obiceiurile alimentare, sedentarismul, glicemia a jeun și LDL-colesterolul nu au avut diferențe semnificative la cele două subgrupuri de pacienți. **Concluzii:** Pacienții diabetici cu SH, în lotul nostru, a avut un cluster de factori de risc cardiovasculari, dar ateroscleroza carotidiană nu a fost semnificativ diferită la pacienții cu sau fără SH. Detectia SH prin ecografie abdominală este un semnal de alarmă pentru riscul cardiovascular crescut, însă la pacienții cu DZ discuția rămâne deschisă, rezultatele fiind încă contradictorii.

Cuvinte cheie: steatohepatita nonalcoolică, diabet zaharat, grosimea intimă-medie, ecografie

Received 05.06.2011 Accepted 01.07.2011

Med Ultrason

2011, Vol. 13, No 3, 215-219

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Introduction

For a long time, fatty liver disease or hepatic steatosis (HS) was considered as a benign manifestation. However, recent data indicate a wide spectrum of clinical and pathological manifestations that subjects with nonalcoholic hepatic steatosis develop, termed as nonalcoholic fatty liver disease (NAFLD) [1-3]. NAFLD is a form of

liver disease resembling the histological changes of alcoholic liver disease, but found in subjects who do not abuse alcohol. Subjects have often associated metabolic conditions such as insulin resistance, overweight, obesity, dyslipidemia, and diabetes [3-6].

Carotid intima-media thickness (IMT) is a known marker for early atherosclerosis, and its progression. IMT is increased in subjects with several risk factors and is a predictor of cardiovascular events and end-organ damage. The first clinical manifestation of cardiovascular disease often arises in a stage of well-advanced atherosclerosis. However, arterial vessel wall changes occur during a long subclinical phase characterized by gradual thickening of intima-media. IMT of large peripheral arteries, especially carotid, can be assessed by B-mode ultrasound in a relatively simple way [7].

Cholesterol intake, body mass index and smoking are significantly related to the annual progression of carotid IMT [7,8]. Of all the traditional risk factors, hypertension appears to have the greatest effect on IMT.

Accordingly, the metabolic syndrome (MS), including also NAFLD, can be considered the link to the presence of vascular diseases in patients with NAFLD. In NHANES III, the authors demonstrated that the presence of MS was associated with increased risk of myocardial infarction, stroke or both [9-11]. The association between NAFLD and carotid IMT was discussed in previous studies as a possible direct relationship between atherosclerosis and hepatic steatosis [11]. However, in subjects with type 2 diabetes, the link between fatty liver and atherosclerosis is less clear, and different studies give conflicting results [12-14].

We examined the prevalence of NAFLD in a group of patients with uncomplicated type 2 DM, and whether NAFLD associates with carotid atherosclerosis.

Material and methods

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. All the patients signed the informed consent.

Carotid atherosclerosis, cardiovascular risk factors, and the presence of HS were analyzed in 56 patients (34 males and 22 females) under 65 years old with uncomplicated DM admitted in our clinic. Diabetes was defined as self-reported physician diagnosis of diabetes or according to the WHO and guidelines definition [18]. A control group of 52 subjects, age and sex matched, was also analyzed.

The patients that reported more than 20 g/day alcohol drinking were excluded from the study. Hepatic steatosis (HS) and carotid IMT were diagnosed by ultrasound examination. Plasma liver function tests (e.g. aspartate aminotransferase-ASAT, and alanine aminotransferase-

ALAT) and other biochemical blood measurements were determined.

Ultrasound examination of the liver was performed after 12 hours fasting. Each subject was examined in the supine and left lateral positions during quiet inspiration and asked to stop breathing during inspiration. The presence or absence and grading of fatty infiltration of the liver were recorded.

Hepatic steatosis was defined as the presence of an ultrasonographic pattern of: parenchymal brightness (from normal to severe increased), liver-kidney contrast (absent = 0/present = 1), deep beam attenuation (diaphragm bright and clear = 0/diaphragm blurred = 1) and bright vessels wall into parenchyma (present = 0/absent = 1) [15]. The final findings were classified as normal liver, and intermediate, moderate or severe HS.

In all subjects, carotid IMT was measured by high-resolution real-time B mode ultrasonography with a 7.5-MHz linear transducer (Aloka Prosound Alpha 10). Each subject was examined in the supine position. The carotid arteries were investigated bilaterally in longitudinal scans. The examination included sections of approximately 2–3 cm of common carotid artery just below the carotid bulb. IMT was defined as the distance between the leading edge of the first echogenic line (lumen-intima interface) and the second echogenic line (media-adventitia interface) of the far wall. A carotid plaque was defined as a focal thickening ≥ 1.2 mm at the level of carotid artery; none of the study participants had clinically relevant carotid stenosis (i.e. $\geq 60\%$). The variability of ultrasonographic measurement was assessed by performing two measurements in 15 volunteers over a one week period. The reproducibility of IMT measurement was 10% [16].

Overweight was defined as a BMI ≥ 25 kg/m². Systolic and diastolic blood pressure was measured and hypertension was defined as an average systolic blood pressure 140 mmHg, an average diastolic blood pressure 90 mmHg, or self-reported use of antihypertensive medication [17].

Statistical analysis. Data on quantitative characteristics are expressed as mean \pm standard deviation (SD). Data on qualitative characteristics are expressed as percentage values or absolute numbers as indicated. Participants were divided into two groups according to the presence or absence of hepatic steatosis. Comparisons between groups were made using ANOVA (continuous data) and χ^2 -test (nominal data). A p value under 0.05 was considered statistically significant.

Results

Demographic data of the study group are shown in the table I.

Table 1: baseline characteristics of DM patients with and without hepatic steatosis

	Patients with HS 38 (67.8%)	Patients without HS 18 (32.2%)
Age (years)	59.4 ± 3.5	61.5 ± 2.9
Male/female	19/12	15/10
IMT (mm)	1.09 ± 0.77	0.98 ± 0.68
BMI (kg/m ²)*	33.2 ± 6	28.1 ± 9.2
DBP (mmHg)*	105 ± 4.4	91 ± 8.2
SBP (mmHg)	166 ± 10.1	148 ± 9.9
MBP (mmHg)*	134 ± 8	120 ± 7.7
TC (mg/dl)	240 ± 128	228 ± 117
TGL (mg/dl)*	388 ± 107	202 ± 98
HDLc (mg/dl)*	38 ± 25	55 ± 16
LDLc (mg/dl)	131 ± 18	127 ± 25
FPG (mg %)	120 ± 28	104 ± 19
HbA _{1c} (%)	7.9 ± 2.2	7.5 ± 2.1
Disease duration (for DM) (years)	13 ± 4	11 ± 6
Sedentary behavior (yes/no)	31 (95%)	16 (89%)
Smoking (current)	27 (71%)	12 (67%)

* p<0.05

HS = hepatic steatosis, IMT = intima media thickness, BMI = body mass index, DBP = diastolic blood pressure, SBP = systolic blood pressure, MBP = mean blood pressure, TC = total cholesterol, TGL = triglycerides, HDLc = high density lipoprotein, LDLc = low density lipoprotein, FPG = fasting plasma glucose.

Thirty-eight out to fifty six patients had HS (67.8%). Patients with HS had higher values for body mass index, borderline significant ($p = 0.045$), diastolic and mean blood pressure and triglycerides ($p = 0.020$, $p = 0.028$, respectively), and lower HDL cholesterol concentration ($p = 0.04$). There was a strong correlation between liver enzymes and HS ($r = 0.71$ for ALAT and $r = 0.59$ for ASAT), but 18 of the patients with HS had normal levels of ASAT.

Behavioral variables (smoking, diet, and sedentarism), fasting plasma glucose, HbA_{1c}, and LDL cholesterol levels did not significantly differ between subjects

with and without HS. Hepatic steatosis did not correlate with IMT ($r = -0.03$; $p = 0.75$) in our study, but there is a positive, although non-significant correlation, between grade of HS and IMT value ($r = 0.28$, $p = 0.06$).

There is a mild positive correlation between IMT and triglycerides levels ($r = 0.54$, $r = 0.04$), total cholesterol ($r = 0.049$, $p = 0.039$) and a negative but non-significant correlation with HDL c levels ($r = -0.18$, $p = 0.02$).

Measures of glucose or HbA_{1c} did not correlate with IMT.

Older age and disease duration were independent predictors of an increased IMT ($r = 0.44$, $p = 0.03$; $r = 0.41$, $p = 0.029$, respectively), but not of the presence of HS.

Discussions

In our study, we could not demonstrate the correlation between HS and IMT in uncomplicated DM patients; this is in concordance with other studies in which the correlation between HS and IMT was non-significant [13], as opposed to non-diabetic patients and patients with metabolic syndrome, where this connection was well established [12,14].

Increased carotid IMT is a mirror of atherosclerotic burden and a predictor of subsequent events [10]. Carotid IMT measurement is more and more frequently used in clinical trials to follow the harmful effects of risk factors on vessel walls and, more importantly, the effect of treating risk factors that cause reduction or prevent the progression of the IMT, paralleled by a decrease in cardiovascular risk and events [10,11]. Therefore, IMT measurements may be used in addition to classical risk factors of individual risk assessment. Measurement of carotid IMT could influence a clinician to intervene with medication and to use more aggressive treatments of risk factors in primary prevention, and in patients with atherosclerotic disease in whom there is evidence of extension of atherosclerosis on carotid arteries.

Fatty liver infiltration can be determined by many different methods. Although the direct measurement of hepatic fat using a biopsy is considered the "gold standard," its use is limited due to risks involved (as it is an invasive method) and the availability of a very small sample of which, in the case of inhomogeneous fat distribution, may not provide an accurate estimate [3,15]. Ultrasound, computerized tomography (CT), magnetic resonance imaging (MRI), and ¹H magnetic resonance spectroscopy (¹H MRS) are noninvasive and should be used instead. However, the best method for frequent, repetitive, and highly specific estimation of hepatic fat in vivo seems to be localized ¹H MRS [19]. This fact is the main limitation of our study since our method for determining fatty

liver infiltration was ultrasound, which is known to be an operator dependent technique. Another limitation of the study was the small number of patients

In our study patients with HS showed a cluster of cardiovascular risk factors, but non-significant carotid atherosclerosis. In recent studies, it seems that subjects with ultrasound-diagnosed steatosis have an increased incidence of cardiovascular events that is independent of components of the metabolic syndrome [20, 21]. In diabetic patients, the presence of hepatic steatosis was associated with elevated serum triglycerides, LDL c, and reduced HDL which is also evidenced in our study (with one exception LDL c) [22]. However, almost all of the studies in which independent associations between fatty liver and atherosclerosis were found in non-diabetic subjects did not adequately evaluate the impact of visceral adipose tissue and insulin sensitivity [14]. Moreover, in the majority of these studies, the diagnosis of fatty liver was based on ultrasonography.

For patients with type 2 DM, there are conflicting conclusions [12]. Targher *et al* [12] showed that fatty liver evaluated by patient history and liver ultrasound was associated with a higher prevalence of cardiovascular disease in people with type 2 DM. A limitation of this study was that the diagnosis of NAFLD was based only on ultrasonography, like in our study, a method that allows the detection of steatosis only when fat on liver biopsy exceeds 33% [23].

In contrast, McKimmie *et al* [13] demonstrated that fatty liver evaluated by computed tomography was not associated with carotid IMT in people with type 2 diabetes mellitus. In this study, the authors suggested that hepatic steatosis was less likely to be a direct mediator of subclinical cardiovascular disease and may instead be an epiphenomenon [13]. Our study, although using ultrasound, is in agreement with this work.

In **conclusion**, this study suggests that in patients with type 2 DM, fatty liver is not associated with carotid IMT, maybe due to more complex mechanisms involved. In a diabetic population, it seems that fatty liver is not a determinant factor associated with cardiovascular disease expressed by IMT. Future studies will have to analyze these conflicting data.

Conflict of interest: none

Acknowledgement

Research supported by the CNCSIS project number 1277 of the Romanian Ministry of Education and Research.

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