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Non-alcoholic fatty liver disease per se is not associated with carotid atherosclerosis

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ABSTRACT

Nonalcoholic fatty liver disease associated with increased risk of cardiovascular mortality. Carotid artery intima media thickness is a reliable index of subclinical atherosclerosis. There are contradictory reports about relationship between nonalcoholic fatty liver disease and carotid atherosclerosis. *In this study, we aimed to investigate carotid artery intima media thickness levels and its association with histological findings in biopsy proven nonalcoholic fatty liver disease.*

113 male patients and 57 healthy male controls were enrolled. *Patients and control groups mean ages were 31.7± 5.7 and 30.1± 5.5 respectively.* Body mass index, Waist circumference, glucose, lipids and insulin levels, and HOMA-IR were determined. Carotid atherosclerosis evaluated by carotid artery intima media thickness using high resolution carotid ultrasonography. Carotid artery intima media thickness levels were higher in patient group than the control group. This difference was disappeared when the findings were adjusted according to the body mass index, waist circumference, glucose and lipid levels, and HOMA-IR indexes. In addition, no significant association was observed between carotid artery intima media thickness and liver histology. However, there was a significant association between age and carotid artery intima media thickness in patients and controls.

Our study does not support an association between carotid atherosclerosis and nonalcoholic fatty liver disease. Otherwise, it can be speculated that a longer exposure to the disease is required to determine significant differences in carotid atherosclerosis.

Key words: *Carotid intima media thickness, Nonalcoholic fatty liver disease, Steatohepatitis.*

ÖZET

Alkolik olmayan yağlı karaciğer hastalığı aslında Karotid ateroskleroz ile ilişkili değildir

Alkolik olmayan yağlı karaciğer hastalığı artmış kardivasküler mortalite ile ilişkilidir. Karotid arter intima medya kalınlığı subklinik aterosklerozun güvenilir bir göstergesidir. Alkolik olmayan yağlı karaciğer hastalığı ve karotid ateroskleroz ilişkisi hakkında çelişkili raporlar mevcuttur. Bu çalışmada, biyopsi ile tanı konmuş alkolik olmayan yağlı karaciğer hastalarında karotid arter intima medya kalınlık düzeylerini ve bu düzeylerin histolojik bulgularla ilişkisini araştırmayı amaçladık.

113 erkek hasta ve 57 sağlıklı kontrol çalışmaya dahil edildi. *Olgu ve kontrol gruplarının ortalama yaşları sırası ile 31.7± 5.7 ve 30.1± 5.5 idi.* Olgu ve kontrol gruplarının vücut kitle indeksi, bel çevresi, glukoz, lipid, insulin düzeyleri ve HOMA-IR indeksleri belirlendi. Karotid ateroskleroz, yüksek çözünürlüklü karotid ultrasonoğrafi yöntemi ile karotid intima-medya kalınlığı belirlenerek değerlendirildi.

Karotid intima medya kalınlık düzeyleri, hasta grubunda kontrol grubuna göre daha yüksekti. Bu fark, bulgular vücut kitle indeksi, bel çevresi, glukoz, lipid düzeyleri ve HOMA-IR indeksine göre ayarlandığında ortadan kalkıyordu. Ek olarak, karotid intima medya kalınlığı ile karaciğer histolojisi arasında istatistiksel anlamlı ilişki saptanmadı. Ancak, hasta ve kontrol gruplarında yaş ile karotid intima medya kalınlığı arasında istatistiksel anlamlı ilişki vardı.

Çalışmamız, alkolik olmayan yağlı karaciğer hastalığı ile karotid ateroskleroz arasındaki ilişkiyi desteklememektedir. Diğer yandan, bu hastalıkta karotid arterde anlamlı aterosklerotik değişiklikler olabilmesi için hastalığa uzun süre maruziyetin gerekliliğinden bahsedilebilir.

Anahtar kelimeler: *Alkolik olmayan yağlı karaciğer hastalığı, Karotid İntima medya kalınlığı, Steatohepatit.*

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is increasingly diagnosed worldwide and considered to be the commonest liver disorder in clinical practice (1). It comprises a spectrum of conditions ranging from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH). Simple steatosis is benign, whereas NASH is characterized by hepatocyte injury, inflammation and fibrosis which can lead to cirrhosis, liver failure and hepatocellular carcinoma (2).

There is increasing evidence for an association between NAFLD and an increased risk of cardiovascular morbidity and mortality (3). The association between NAFLD and cardiovascular risk factors can largely explain the higher risk of cardiovascular disease (CVD) among people with NAFLD. Recent case–control studies and cross-sectional studies have reported the increased risk of carotid atherosclerosis among patients with NAFLD (4). Possible biological mechanisms linking the NAFLD to atherosclerosis development include endothelial dysfunction, oxidative stress, inflammation, and abnormal lipid and glucose metabolism. However, these mechanisms are also closely related to other risk factors for atherosclerosis. Therefore, it is unclear whether NAFLD contributes to the development of atherosclerosis directly (5).

We designed cross-sectional, case-control study and aimed to investigate carotid atherosclerosis as assessed by carotid artery intima media thickness (cIMT), a well known marker of atherosclerosis in a large cohort of biopsy proven patients with NAFLD and also to search the association of cIMT with histopathological findings. In order to prevent any interference of confounding factors for endothelial dysfunction or atherosclerosis, we studied a specifically selected group having no additional disorders such as hypertension, diabetes mellitus (DM) or morbid obesity.

Material and Methods

Study population

A total of 113 male subjects with biopsy proven NAFLD (33 with SS alone and 80 with NASH) referred to the outpatient clinic were enrolled the present study. Inclusion criteria were; persistently (at least 6 months) elevated aminotransferases, ultrasonographic presence of bright liver without any other liver or biliary tract disease, liver histology compatible with a diagnosis of NASH or SS. Exclusion criteria were; a history of alcohol consumption >40 g/wk, as assessed by a detailed interview extended to family members, morbid obesity (BMI ≥40 kg/m^2), hypertension, positive blood markers of viral, autoimmune, or celiac disease, abnormal copper metabolism or thyroid function tests, a diagnosis of DM, total cholesterol (TC) ≥250 mg/dL, triglycerides (TG) ≥400 mg/dL, exposure to occupational hepatotoxins or drugs known to be steatogenic or to affect glucose and lipid metabolism. The control group, recruited from hospital staff members and relatives, consisted of 57 apparently healthy male volunteers with normal liver ultrasonography and normal liver function tests who were matched for age and gender. The study was approved by the local ethics committee of our center and all participants gave their consent to study, which was conducted according to the Helsinki Declaration.

Clinical and Laboratory Assessments

All participants provided a medical history and underwent a clinical examination. The weight and height of the participants were measured with a calibrated scale after the patients had removed their shoes and any heavy clothing. Body mass index (BMI) was computed as body *weight (in kilograms)/ height^2 (in meters)*. Waist circumference (WC) was measured as the mid-point between the lower costal margin and the level of the anterior superior iliac crests.

For biochemical analyses, all blood samples were collected from an antecubital vein, between 08.00 and 09.00 a.m. after an overnight fasting. The samples were centrifuged for 15 minutes at 4000 rpm, aliquoted and immediately frozen at -80 ºC for analyses until examination. All samples were run in the same assay. Fasting plasma glucose (FPG), TC, TG, and high-density lipoprotein cholesterol (HDL-C) levels were measured by the enzymatic colorimetric method with Olympus AU2700 auto analyzer using reagents from Olympus Diagnostics, (GmbH, Hamburg, Germany). Low density lipoprotein cholesterol (LDL-C) was calculated by Fridewald's formula (6). *The triglyserid levels above 400 mg/dL was excluded from the analysis.*

The serum basal insulin level was measured in duplicate by the chemiluminescence's method using reagents from Roche Diagnostics (Mannheim, Germany). Insulin resistance was calculated by modified homeostasis model assessment of insulin resistance (HOMA-IR), with the following formula: HOMA-IR = fasting plasma insulin (μU/ml) X fasting plasma glucose (mg/dl)/405. Homeostasis model assessment of insulin resistance was originally reported by Matthews et al. [7] and this index has been shown to be well correlated with the results of the euglycemic–hyperinsulinemic clamp method to determine insulin resistance (8, 9).

Liver Histology

An experienced hepatopathologist blinded to subjects' details scored liver biopsy specimens using the semiquantitative classification of Kleiner et al. (10). Briefly, the severity of steatosis was graded on the basis of the extent of involved parenchyma: grade 1, <33% of hepatocytes affected; grade 2, 33–66% of hepatocytes affected; and grade 3, >66% of hepatocytes affected. NASH was defined as the presence of steatosis plus lobular inflammation plus hepatocellular ballooning or steatosis plus any stage of fibrosis. The stages of fibrosis were graded as follows: stage 1, zona three perivenular, perisinusoidal, or pericellular fibrosis; stage 2, as above with focal or extensive periportal fibrosis; stage 3, bridging fibrosis, focal or extensive; and stage 4, cirrhosis.

Ultrasound Examination of the Carotid Artery

Carotid artery intima media thickness was measured with ultrasonography by a single trained operator who was blind to clinical characteristics of participants. *cIMT* measurements were made bilaterally at the level of the common carotid artery far wall and always in stenotic-free segments. For each subject, three measurements on both sides were performed, i.e., the anterior, lateral, and posterior projection of the near and far wall. All readings were then averaged. 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., ≥60%).

Statistical Analysis

SPSS 15.0 (Chi., Il., USA) was used for statistical analysis. Results are reported as the mean ± standard deviation (SD) and median (min–max). Kolmogorov–Smirnov test was used to determine the distribution characteristics of variables, and Levene's test was used to evaluate the equality of variance. *Differences between groups were tested for significance independent samples t test or Mann–Whitney U test, as appropriate*. The relationship between variables was analyzed by Spearman's rho correlation. Differences and correlations were considered significant at $p < 0.05$.

Results

The baseline characteristics of participants are shown. (Table I). *Age was similar in two groups*. BMI, WC, FPG, TC, LDL-C, TG, insulin and HOMA-IR levels were higher and, HDL-C levels were lower in NAFLD group than the control group.

Subjects with NAFLD had significantly greater cIMT measurements than the healthy controls. However, the differences about the cIMT levels were disappeared when the findings were adjusted according to the BMI, WC, glucose, lipids and HOMA-IR indexes. In subgroup analysis, there were no significant differences regarding the BMI, FPG, TC, TG, HDL-C, LDL-C, insulin, HOMA-IR and cIMT levels in subjects with NASH and SS.

We also searched for the association of cIMT with histopathological findings and found no significant relationships between these parameters. On the other hand, there was a significant association between age and cIMT in subjects with NAFLD and healthy controls ($r=0.37$, $p= 0.01$).

Because of the well known relationship of cIMT with metabolic syndrome (MetS), we wanted to search the possible relationship of these parameters with MetS in subjects with NAFLD. But, according to the National Cholesterol Education Program (NCEP) (11) only 8 subjects had MetS, so we couldn't analyze this relationship.

Discussion

Our study has shown that cIMT measurements are not different in male patients with biopsy proven

Table I. Comparison of some characteristics and laboratory findings between NAFLD and control group.

	NAFLD group (n= 113)	*Control group (n= 57)*	*p*
Age (year)	31.7± 5.7	30.1± 5.5	0.261†
BMI (kg/m²)	28.3 ± 3.2	23.8 ± 2.5	<0.001†
WC (cm)	97.9±7.1	86.6±6.5	<0.001†
Glucose (mg/dl)	92.1 ±12.5	80.0+9.3	<0.001†
TC (mg/dl)	204.2± 41.2	177.7± 27.8	<0.001†
TG (mg/dL)	196.3± 118.8	120.1± 54.4	<0.001‡
HDL-C (mg/dL)	41.8± 6.6	46.0± 7.9	0.001†
LDL-C (mg/dL)	121.7± 36.5	108.3± 27.1	0.027†
ALT (IU/L)	99.0± 44.3	21.5± 9.7	<0.001†
AST (IU/L)	47.3± 20.5	22.3± 5.1	<0.001†
GGT (IU/L)	71.2±54.3	26.1±17.4	<0.001†
Insulin (mU/mL)	15.1± 9.5	7.6± 3.6	<0.001‡
HOMA-IR	3.5± 2.4	1.5 ± 0.8	<0.001†
cIMT (mm)	0.60± 0.11	0.54± 0.08	0.005†
Histology			
Fat score (1–3) (%)	55/28/17	-	-
Necroinflammation score (0–3) (%)	8/72/20/0	-	-
Fibrosis stage (0–4) (%)	35/57/6/2/0	-	-

Data is presented as the mean ± SD

NAFLD, non alcoholic fatty liver disease; ***BMI,*** body mass index; ***WC,*** waist circumference; ***TC,*** total cholesterol; ***TG,*** triglyceride; ***HDL-C,*** high-density lipoprotein-cholesterol; ***LDL-C,*** low-density lipoprotein-cholesterol; ***ALT,*** alanine aminotransferase; ***AST,*** aspartate aminotransferase; ***GGT,*** gamma-glutamyl transpeptidase; ***HOMA-IR,*** homeostasis model assessment -insulin resistance; ***cIMT,*** carotid intima-media thickness.

† t test; ‡ Mann Whitney U test

NAFLD who have no DM, hypertension and morbid obesity when compared to healthy male controls. In addition, there was no significant difference regarding the cIMT levels in subjects with NASH and SS. Moreover, no significant relationship was observed between cIMT and liver histology. The abnormalities in glucose metabolism and blood pressure that can potentially affect carotid atherosclerosis are frequently accompanied by NAFLD. Therefore, we think that including only the subjects free from these confounding factors is an important feature of the present investigation.

Carotid artery intima media thickness is a reliable index of subclinical atherosclerosis and epidemiologic studies have demonstrated that there is a significant association between cIMT and CVD. Large body of evidence has shown that NAFLD is an independent predictor of CVD as estimated by the Framingham risk score (12). But, there are also contradictory reports about the relationship between NAFLD and CVD (13, 14). As far as the association between liver histology and cIMT is concerned, Francanzani et al. reported no difference in cIMT levels between patients with SS and those with NASH, even if these latter had higher cIMT values than controls (15). They also had lower HDL and higher triglycerides, fasting glucose, insulin resistance, and frequency of MetS than controls. On the other hand, Targher et al. measured cIMT in 85 patients with biopsy-proven NAFLD and in 160 age-, gender-, and BMI-matched healthy controls. In their study, cIMT was associated with the degree of steatosis, inflammation and fibrosis among NAFLD patients and the severity of histological features was an independent predictor of cIMT after adjustment for confounders (16). In the present study, we did not find any differences regarding cIMT levels between subjects with NAFLD and healthy controls when the findings were adjusted according to BMI, WC, glucose and lipid levels, and HOMA-IR indexes. In addition, no significant difference was found regarding the cIMT levels in subjects with NASH and SS. However, there was a significant association between age and cIMT in subjects with NAFLD and healthy controls.

We suggest some possible explanations for the lack of association between NAFLD and cIMT observed in the present study. Firstly, most of the studies that investigate the association of cIMT with NAFLD were performed in subjects with ultrasonographically diagnosed fatty liver (3). *Although it's well known correlation with the histological findings of fatty infiltration, liver ultrasonography is not sufficiently sensitive to detect liver inflammation and fibrosis* (17). Secondly, when the above mentioned studies were analyzed separately, it can be seen that some of the patients with NAFLD had metabolic confounders like morbid obesity, DM and hypertension. In addition, some part of these subjects was also using the medications related to these metabolic problems (3). It has been reported that cIMT levels may be affected from these metabolic risk factors and also medications (18). So, we think that some of the previous findings regarding the cIMT in NAFLD might be affected from these confounders. Thirdly, mean age of the participants in the present study was younger than the subjects in the previous studies that report the link between cIMT and NAFLD. It is well known that there is a strong and independent relationship between age and cIMT. Moreover, longitudinal studies found that differences as small as 0.1 mm of cIMT could be found after 20 years and over of follow up, when a combination of adverse risk factors clustered together (19). So, age may be an important determinant for the development of atherosclerosis in NAFLD and a longer exposure time to fatty liver may be required to develop higher cIMT. Thus, a recent study reported no association between cIMT and NAFLD in children and adolescents (20, 21). Lastly, cIMT may not reflect all the components of the CVD risk and other parameters such as arterial stiffness and flow-mediated dilation may be more useful indexes of early vascular changes. Hence, in a population of biopsy proven NAFLD, Senturk et al. have shown that patients with NASH had significantly worse endothelial dysfunction as measured with brachial artery flow mediated dilatation, compared with patients with SS and healthy subjects (22). In light of these data, we suggest that NAFLD may not have a direct impact on carotid atherosclerosis and it may contribute to CVD by acting in concert with metabolic abnormalities. In agreement with this hypothesis, a recent cross-sectional study found NAFLD to be associated with cIMT only in people with the MetS (23).

There are two limitations of the present study. Firstly, though the sample size and the strict inclusion criteria, the findings obtained are not representative for all subjects with NAFLD. But as mentioned above in details, we think that the design of our study was

a requirement for the goals to achieve. Secondly, the cross-sectional nature of the study prevents any definitive causal inference.

In conclusion, our study does not support the existence of an association between cIMT and NAFLD, suggesting that other metabolic abnormalities might act in concert with NAFLD to promote atherosclerosis. Otherwise, it can be speculated that a longer exposure to the disease is required to determine significant differences in endothelial dysfunction and carotid atherosclerosis. However, waiting for prospective and interventional studies in order to definitely determine the nature of the relationship NAFLD/CVD, an overall assessment of the CVD risk, and the aggressive management of the atherosclerotic risk factors, seems mandatory in all NAFLD patients.

Conflict of interest: None.

References

1. Radu C, Grigorescu M, Crisan D, Lupsor M, Constantin D, Dina L. Prevalence and associated risk factors of non-alcoholic fatty liver disease in hospitalized patients. J Gastrointestin Liver Dis 2008;17(3): 255-260.
2. Portincasa P, Grattagliano I, Palmieri VO, Palasciano G. The emerging problem of nonalcoholic steatohepatitis (NASH). Rom J Gastroenterol. 2005; 14(1): 43-51.
3. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: A systematic review. J Hepatol 2008; 49: 600-607.
4. Edens MA, Kuipers F, Stolk RP. Non-alcoholic fatty liver disease is associated with cardiovascular disease risk markers. Obes Rev 2009; 10: 412-419.
5. Perseghin G. The role of non-alcoholic fatty liver disease in cardiovascular disease. Dig Dis 2010; 28: 210-213.
6. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
7. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.
8. Bonora E, Targher G, Alberiche M, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000; 23: 57-63.
9. Diagnosis and Classification of Diabetes Mellitus. ADA position statements. Diabetes Care 2010; 33: 62-69.
10. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005; 41: 1313-1321.
11. Grundy SM, Cleeman JI, Merz CN, et al. National Heart, Lung, and Blood Institute; American College of Cardiology Foundation; American Heart Association. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 2004; 110: 227-239.
12. Vallance P, Leiper J. Cardiovascular biology of the asymmetric dimethylarginine: Dimethylarginine dimethylaminohydrolase pathway. Arterioscler Thromb Vasc Biol 2004; 24: 1023-1030.
13. Sung KC, Ryan MC, Wilson AM. The severity of nonalcoholic fatty liver disease is associated with increased cardiovascular risk in a large cohort of non-obese Asian subjects. Atherosclerosis 2009; 203: 581-586.
14. Petit JM, Guiu B, Terriat B, et al. Nonalcoholic fatty liver is not associated with carotid intima-media thickness in type 2 diabetic patients. J Clin Endocrinol Metab 2009; 94: 4103-4106.
15. Fracanzani AL, Burdick L, Raselli S, et al. Carotid artery intima-media thickness in nonalcoholic fatty liver disease. Am J Med 2008; 121: 72-78.
16. Targher G, Bertolini L, Padovani R, et al. Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. Diabetes Care 2006; 29: 1325-1330.
17. Lupsor M, Badea R, Stefanescu H, et al. Performance of unidimensional transient elastography in staging non-alcoholic steatohepatitis. J Gastrointestin Liver Dis 2010; 19(1): 53-60.
18. Lorenz MW, Markus HS, Bots ML, et al. Prediction of clinical cardiovascular events with carotid intima-media thickness: A systematic review and meta-analysis. Circulation 2007; 115: 459-467.
19. McKimmie RL, Daniel KR, Carr JJ, et al. Hepatic steatosis and subclinical cardiovascular disease in a cohort enriched for type 2 diabetes: The Diabetes Heart Study. Am J Gastroenterol 2008; 103: 3029-3035.
20. Chen W, Srinivasan SR, Li S, et al. Metabolic syndrome variables at low levels in childhood are beneficially associated with adulthood cardiovascular risk: The Bogalusa Heart Study. Diabetes Care 2005; 28: 126-131.
21. Manco M, Bedogni G, Monti L, et al. Intima-media thickness and liver histology in obese children and adolescents with non-alcoholic fatty liver disease. Atherosclerosis 2010; 209: 463-468.
22. Senturk O, Kocaman O, Hulagu S et al. Endothelial dysfunction in Turkish patients with non-alcoholic fatty liver disease. Intern Med J 2008; 38: 183-189.
23. Kim HC, Kim DJ, Huh KB. Association between nonalcoholic fatty liver disease and carotid intima-media thickness according to the presence of metabolic syndrome. Atherosclerosis 2009; 204: 521-525.