

Effect of non-alcoholic fatty liver disease on carotid artery intima-media thickness as a risk factor for atherosclerosis

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ABSTRACT

Aim: This study aimed to evaluate the effect of NAFLD on CIMT as a risk factor for atherosclerosis.

Background: The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide due to rise of obesity and diabetes mellitus (DM) prevalence. Non-invasive assessment of carotid intima-media thickness (CIMT) by high-resolution carotid B-mode ultrasonography is widely used for determining the atherosclerosis.

Patients and methods: In this case-control setting, 151 subjects were categorized in three groups: group I including 49 patients with NAFLD and DM; group II including 50 non-diabetic NAFLD patients; and the control including 52 normal subjects as group III. The right and left CIMTs and its maximum reading (CIMT_{max}) were measured by a skilled sonographer blind to the groups. The sonographic grading of the NAFLD was determined in group I and II.

Results: Median CIMT_{max} was significantly higher in group I comparing with group II and control group ($p < 0.001$). This difference between group I and group II was not significant after adjusting for age and history of hypertension and hyperlipidemia ($p = 0.089$). After controlling the confounders, there was statistical significant between group I and group II with the control group ($p < 0.05$). There was no significant difference in median maximal thickness of intima-media in the carotid of group I compare to group II in patients with and without elevated liver enzymes (in both groups, 0.6 mm, $p = 0.402$).

Conclusion: Based on our findings, there is a significant association between the presence of NAFLD and atherosclerosis. This association was independent to the DM presence. The grade of NAFLD and elevated liver function tests had no effect on severity of atherosclerosis.

Keywords: Fatty Liver, Carotid Arteries, Atherosclerosis, Diabetes mellitus.

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Introduction

Diagnosis of non-alcoholic fatty liver (NAFLD), as a cause of progress towards the end stage of liver disease, is increasing (1). This disease represents a spectrum of clinico-pathological conditions that is determined with

macrovesicular steatosis in the absence of alcohol consumption. The disease includes clinical, laboratory and pathological conditions ranged from mild steatosis to liver diseases such as non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and eventually hepatocellular carcinoma (1-3). With increasing urbanization and behavioral changes such as decreased physical activity, fat-high-energy diet and increased occurrence of diabetes mellitus type II, its prevalence has

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increased in the Asian region (1-3). Prevalence of the disease was estimated between 7 and 40 percent in different populations, and in a country like Japan it has been 3-20 folds within the past 20 years (4). Increased body fat accompanied with diabetes mellitus, hypertension or changes in lipid metabolism, which is considered a separate disease as "metabolic syndrome" increases risk of cardiovascular diseases (5). Due to association between NAFLD with metabolic syndrome and also metabolic syndrome with cardiovascular diseases, many studies were performed regarding this relationship. All studies have found significant correlation between them. Now the question is NAFLD itself is a predisposing factor for atherosclerosis and cardiovascular diseases or not? Carotid intima-media thickness (CIMT) is a standard method for evaluation of early general atherosclerosis (6). Studies conducted in this area suggest a correlation between the carotid intima-media thickness and NAFLD (7-10). However, there are no studies to evaluate correlation of intensity of steatosis, sonographic grade of fatty liver and increased liver enzymes with increasing of carotid intima-media thickness. This study was conducted to evaluate the correlation between these factors and carotid intima-media thickness.

Patients and Methods

In this case-control study, 151 individuals were studied in three groups: 49 diabetic patients with fatty liver (case group I); 50 non-diabetic individuals with fatty liver (case group II); 52 non-diabetic individuals without fatty liver (control group). This study was done in the gastroenterology ward of Sheykh-Al-raees clinic, Tabriz, Iran, between December 2008 and July 2010. An expert sonographer confirmed the presence of fatty liver. A skilled sonographer measured the right and left CIMTs and its maximum reading (CIMT_{max}) blind to the groups.. CIMT was measured by high-resolution

ultrasound B-mode with a 7 MHz transducer device ALOKA ProSound SSD 3500 plus in common carotid artery, internal carotid and carotid bulb in each group. One centimeter below the bifurcation of common carotid artery was measured. Thickness of vessel from the edge between intima and the lumen vessels to media-adventitia was evaluated in a region without plaque. Mean of maximum values of right and left side was calculated. The maximum values of both sides were considered as maximum. Simultaneously, the transducer of 3.5 MHz evaluated liver echogenicity and size, in order to determine the presence of fatty liver. The grading was done according to these criteria: grade I (mild): mild increase in the fine echoes of hepatic parenchyma with normal visualization of the diaphragm and intrahepatic vessel borders; grade II (moderate): moderate diffuse increase in fine echoes with mild impaired visualization of the intrahepatic vessels and diaphragm, grade III (severe): marked increase in fine echoes with poor or non-visualization of the intrahepatic vessel borders, diaphragm and posterior portion of the right lobe of the liver (11). According to liver enzyme (AST, ALT) status, patients of the first and second group were divided into two groups, with and without increased liver enzymes, and CIMT also was compared between two groups.

Ethical committee of Tabriz University of Medical Sciences approved this project.

Age, sex, family history of premature CHD, walking time per week, height, weight, body mass index (BMI), waist circumference, fasting blood glucose, HDL, triglyceride, AST, ALT, ALP, right, left and maximum value of CIMT, history of smoking, history of hypertension, obesity (BMI>30), history of hyperlipidemia, regular daily walking, increased liver function tests (case groups I and II) and grading of fatty liver (case group I and II) were evaluated.

Statistical analysis

Data were expressed as mean \pm standard deviation for numeric data and frequency (percentage) for categorical data. TM SPSS statistical program version used was 15. Quantitative variables were compared by Kruskal-Wallis test, Mann-Whitney U, One-way ANOVA and Tukey post hoc test; qualitative variables by chi-square test or Fisher exact test using SPSS. In Mann-Whitney U test without using post hoc test, $p < 0.05$ was considered significant. While, in Mann-Whitney U test with using Post hoc test, $p < 0.017$ was considered significant using Bonferroni equivalent. General Linear Model (GLM) was used to assess the difference of CIMT (R, L Max) values between three groups adjusted for traditional risk factors as the multivariate analysis.

Results

Diabetic and non-diabetic patients with fatty liver

Table 1 presents quantitative variables between two groups. Mean age of diabetic patients with fatty liver is significantly higher than non-diabetic patients with fatty liver ($p = 0.001$). Mean height of non-diabetic individuals with fatty liver is significantly higher than the diabetics ($p = 0.027$). The median fasting blood sugar of diabetic patients with fatty liver was significantly higher than non-diabetic subjects with fatty liver ($p < 0.001$). In other cases there was no statistical difference.

Table 2 presents qualitative variables between two groups. Median of right CIMT in diabetic patients with fatty liver was significantly higher than non-diabetic individuals with fatty liver ($p = 0.008$). Also, median of left CIMT was significantly higher in diabetic patients with fatty liver than right CIMT in non-diabetic individuals with fatty liver ($p = 0.010$). The median of maximum CIMT in diabetic patients with fatty liver was significantly higher than right CIMT in non-diabetic individuals with fatty liver ($p = 0.005$). Within traditional risk factors only

frequency of patients with a history of hypertension or hyperlipidemia were significantly higher in diabetic patients with fatty liver ($p = 0.007$ and $p = 0.002$, respectively). In multivariate analysis, the mean of maximum CIMT showed no significant difference after controlling variables with significant differences between groups, including age, history of hypertension and hyperlipidemia ($p = 0.089$).

Diabetic patients with fatty liver and non-diabetic subjects without fatty liver

As you see in table 1, the mean age of diabetic patients with fatty liver was significantly higher than non-diabetic subjects without fatty liver ($p = 0.001$). The mean height of non-diabetic individuals without fatty liver disease was significantly higher than diabetic people with fatty liver ($p = 0.007$). The mean weight of diabetic patients with fatty liver was significantly higher than non-diabetic people without fatty liver ($p < 0.001$). The median BMI and waist of diabetic patients with fatty liver were significantly higher than non-diabetic subjects without fatty liver (both $p < 0.001$). The median of fasting blood sugar and triglyceride levels in diabetic patients with fatty liver were significantly higher than non-diabetic individuals without fatty liver (both $p < 0.001$). While, mean serum value of HDL in non-diabetic individuals without fatty liver was significantly higher than diabetic patients with fatty liver ($p = 0.003$).

The median of right CIMT and maximum CIMT in diabetic patients with fatty liver was significantly higher than non-diabetic subjects without fatty liver (both $p < 0.001$). Qualitative variables of two groups were summarized in table 2. Accordingly, the percentage of smokers in non-diabetic subjects without fatty liver was significantly higher than the percentage of smokers in diabetic group with fatty liver ($p = 0.016$). Percent of individuals with a history of hypertension was significantly higher in diabetic patients with fatty liver ($p < 0.001$).

Table 1. Comparison of quantitative data among three groups

	Diabetics with fatty liver (n=49)	Non- diabetics with fatty liver (n=50)	Non- diabetics without fatty liver (n=52)	p-value
Age (years)	(50)50.1±9.9 [29-76]	(41.5)43.3±10.9 [24-69]	(44)43.1±6.2 [25-55]	<0.001
walking time per week (min/day)	(30)36.2±17.5 [15-60]	(30)45.8±31.5 [10-12]	-	0.346
Height (Cm)	(158)161.4±9.6 [146-184]	(166.5)166.9±12 [145-190]	(168)8±167.8 [149-185]	0.002
Weight (Kg)	(74)78.8±17.3 [58-124]	(82)82.1±10 [60-101]	(68)7.5±66.6 [50-80]	<0.001
Body mass index (Kg/m ³)	(29.3)30.2±5.5 [22-50.5]	(28.8)29.5±3.6 [23.5-38]	(24)23.8±1.8 [19.9-30.3]	<0.001*
Waist circumference (Cm)	(104)103.5±11.7 [77-130]	(102)103.9±7.9 [90-120]	(85.5)84±6 [74-101]	<0.001*
FBS (mg/dL)	(137)143±54.6 [88-399]	(95.5)94.8±16.4 [60-134]	(86)85.7±10.1 [68-100]	<0.001*
HDL (mg/dL)	(43)45.3±16.9 [27.4-135]	(44)43.7±8 [27-63]	(54)52.5±7.3 [41-71]	<0.001
TG (mg/dL)	(180)205±97.4 [37-518]	(180.5)200.8±89.9 [46-461]	(112)115.2±33.8 [57-213]	<0.001*
AST (U/L)	(25)27.8±14.1 [11-70]	(23.5)27.8±12.9 [9-60]	-	0.993
ALT (U/L)	(27)33.4±18.9 [10-85]	(31.5)40.7±31.6 [11-202]	-	0.17
ALP (U/L)	(162)171.2±72.4 [14-380]	(178)191.6±75.5 [74-465]	-	0.172
Ultrasonographic grading of fatty liver	(1)1.5±0.7 [1-3]	(1)1.3±0.5 [1-3]	-	0.13

Data were presented as median † standard deviation [range]; * Non- parametric tests; ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, FBS: Fasting blood glucose, HDL: High density lipoprotein, TG: Triglyceride

Table 2. Comparison of qualitative data among three groups

	Diabetics with fatty liver (n=49)	Non- diabetics with fatty liver (n=50)	Non- diabetics without fatty liver (n=52)	p-value
Sex				
Male	17 (34.7)*	16 (32)	21 (40.4)	0.665
Female	32 (65.3)	34 (68)	31 (59.6)	
Smoking	0	3 (6)	6 (11.5)	0.05
Hypertension	21 (42.9)	9 (18)	0	<0.001
Obesity	15 (30.6)	9 (18)	1 (1.9)	0.001
Hyperlipidemia	29 (59.2)	14 (28)	0	<0.001
Daily walking	13 (26.5)	12 (24)	0	<0.001
Elevated liver enzymes	12 (24.5)	19 (38)	0	0.147

* Number (percent)

Frequency of obese individuals and history of hyperlipidemia was significantly higher in diabetic patients with fatty liver (both p<0.001). Percentage of subjects with regular walking was significantly higher in diabetic patients with fatty liver (p<0.001). In other cases there were no significant differences. Mean maximum CIMT was significantly and independently

higher in diabetic patients with fatty liver, after controlling for variables with significant differences between two groups, including age, history of hypertension, history of hyperlipidemia, regular daily walking, smoking and obesity (p=0.018).

Non-diabetic subjects with and without fatty liver

Quantitative variables in two groups were summarized in Table 1. According to this result, the median of BMI in non-diabetic subjects with fatty liver was significantly higher than non-diabetic subjects without fatty liver ($p < 0.001$). The median of waist in non-diabetic subjects with fatty liver was significantly higher than non-diabetic subjects without fatty liver ($p < 0.001$).

The median fasting blood sugar and triglyceride was significantly higher in non-diabetic individuals with fatty liver compared with non-diabetic individuals without fatty liver ($p = 0.002$ and $p < 0.001$ respectively). Mean serum levels of HDL in non-diabetic individuals without fatty liver was significantly higher than non-diabetic subjects with fatty liver ($p < 0.001$) (table 1).

Median of right CIMT, left CIMT and maximum CIMT was significantly higher in non-diabetic patients with fatty liver compared with non-diabetic patients without fatty liver ($p < 0.001$).

Table 3. Comparison of carotid intima-media thickness among patients with non-alcoholic fatty liver according to liver enzyme status

CIMT	Increased liver enzyme (n= 31)	Normal liver enzyme (n= 68)	p-value univariate	p-value multivariate
Right	0.53±0.12	0.58±0.14	0.093	0.957
Left	0.56±0.13	0.58±0.13	0.473	0.824
Maximum	0.57±0.13	0.59±0.14	0.402	0.742

Data were presented as mean ± standard deviation; CIMT: Carotid intima-media thickness; multivariate: Adjusted for sex, smoking, hypertension, obesity, hyperlipidemia, daily walking, elevated liver enzymes

Qualitative variables of two groups are summarized in table 2. Accordingly, the incidence of individuals with a history of high blood pressure was significantly higher in non-diabetic subjects with fatty liver ($p = 0.001$). On the other hand, the prevalence of obese non-diabetic subjects with fatty liver was significantly higher ($p = 0.007$). The frequency of individuals with a history of

hyperlipidemia was significantly higher in non-diabetic subjects with fatty liver ($p < 0.001$). Also the prevalence of regular walking was significantly higher in non-diabetic subjects with fatty liver ($p < 0.001$). In other cases there was no significant difference.

Mean maximum CIMT was significantly and independently higher in non-diabetic patients with fatty liver, after controlling for variables with significant differences between groups, including FBS, history of hypertension, obesity, hyperlipidemia, walking regularly and HDL ($p = 0.043$). Mean CIMT had no significant difference between two groups with and without elevated liver enzymes (table 3). There were no significant differences between mean values of CIMT in different groups of fatty liver according to ultrasonographic findings (table 4).

Table 4. Comparison of carotid intima-media thickness among patients with non-alcoholic fatty liver according to ultrasonographic grading †

CIMT	Grade I (n= 63)	Grade II (n= 29)	Grade III (n= 7)
Right	(0.5) 0.56±0.14	(0.5) 0.56±0.1	(0.6) 0.62±0.13
Left	(0.5) 0.57±0.14	(0.5) 0.57±0.11	(0.6) 0.62±0.11
Maximum	(0.5) 0.58±0.15	(0.6) 0.58±0.12	(0.6) 0.64±0.13

† Multivariate and univariate p-value was not significant in studied groups. Data were presented as median ± standard deviation; *Non-parametric test CIMT: Carotid intima-media thickness; multivariate: Adjusted for sex, smoking, hypertension, obesity, hyperlipidemia, daily walking, elevated liver enzymes

Comparison between the three groups

As seen in table 5, univariate analysis showed fatty liver has significant impact on CIMT (R, L, Max), while after entering known risk factors in a multivariate analysis, fatty liver was effective only on CIMT (Max).

Table 5. Comparison of carotid intima-media thickness among three groups

CIMT	Diabetics with fatty liver (n=49)	Non-diabetics with fatty liver (n=50)	Non-diabetics without fatty liver (n=52)	p-value [#]	p-value ^{\$}
Right	(0.6) 0.61±0.15 [0.4-1.1]	(0.5) 0.53±0.11 [0.3-0.9]	(0.4) 0.45±0.13 [0.3-0.8]	<0.001*	0.064
Left	(0.6) 0.61±0.13 [0.4-0.9]	(0.5) 0.54±0.11 [0.3-1]	(0.4) 0.44±0.12 [0.3-0.8]	0.004*	0.106
Max	(0.6) 0.63±0.15 [0.4-1.1]	(0.5) 0.54±0.11 [0.3-1]	(0.4) 0.45±0.13 [0.3-0.8]	<0.001*	0.047

Data were presented as median ± standard deviation [range]; * Non-parametric tests; CIMT: carotid intima-media thickness #; Univariate \$: Multivariate: Adjusted for sex, smoking, hypertension, obesity, hyperlipidemia, daily walking, elevated liver enzymes

Discussion

This study was conducted to compare the thickness of the intima-carotid (CIMT) in diabetic and non-diabetic patients with non-alcoholic fatty liver compared to healthy subjects. Our finding showed that median CIMT in diabetic patients with fatty liver was significantly higher than non-diabetic with fatty liver and healthy subjects. However, after controlling for confounding factors, the differences between two groups of diabetics and non-diabetics with fatty liver were not statistically significant. Median CIMT in the healthy group was significantly and independently less than two other groups. Several studies have been performed to evaluate CIMT in these patients, but different findings have been found.

In a meta-analysis, Sookoian et al. (2008) evaluated the relationship between CIMT and non-alcoholic fatty liver by summarizing 7 studies, including 1427 patients and 2070 healthy individuals. They concluded that there was strong correlation between NAFLD and atherosclerosis (increased CIMT) (12). In another study, Targher et al. (2007) showed CIMT is higher in patients with NAFLD compared with healthy subjects (13). Fracanzani et al. (2008) evaluated CIMT values in 125 patients with NAFLD and 250

healthy individuals. In this study, the mean CIMT was significantly higher among NAFLD patients (14). Our results are compatible with the results of mentioned studies. We found only one study to assess CIMT values in diabetic patients with fatty liver. Petit et al. (2009) studied on 101 diabetic patients with fatty liver showed that the mean CIMT had no significant difference between patients with and without steatosis. They finally concluded that there is no significant relationship between CIMT and NAFLD among this group of diabetic patients (15) and that is in contrast with our results. One of the major limitations of Petit et al. study was lack of healthy or non-diabetic control. To our information, our study is the first research, which evaluated simultaneously CIMT values among three groups of diabetic and non-diabetic patients with NAFLD and healthy control.

Non-alcoholic fatty liver, diabetes mellitus, obesity, hyperlipidemia and metabolic syndrome create a complex situation in which the effects of different parameters on each other and ultimately increasing effects of other variables increases significantly risk of atherosclerosis. Therefore, evaluation of each variable regardless of the role of other factors is not reasonable (16). According to results of the current study, the risk of atherosclerosis is already increased in patients with fatty liver, after controlling soft intervening parameters. This emphasizes the importance of NAFLD in developing atherogenesis, even in comparison diabetes mellitus.

On the other hand, CIMT results in these two groups and healthy individuals, reveals the role of fatty liver in this area. Previous studies demonstrated obviously increased risk of cardiovascular-related mortality in patients with non-alcoholic fatty liver (17, 18). Our findings are compatible with the results of these studies. Therefore, prompt treatment and preventive interventions can considerably reduce the risk of atherosclerosis (19). In this regard, we

recommended performing interventional studies with long-term follow up (20).

Various important mechanisms that have been proposed in this area included: vascular endothelial dysfunction, increased intensity of oxidative stress, increased inflammation and derangement in lipoproteins metabolism (14, 16). We compared the CIMT values in diabetic and non-diabetic patients with fatty liver and elevated or normal liver enzymes. No significant differences were observed between these two groups. Also, there was no significant association between CIMT and different grade of fatty liver, based on ultrasonographic scoring. To this point, few studies assessed the relationship between CIMT status and liver enzyme and also severity of NAFLD. Petit et al. (2009) found no significant association between increasing liver enzymes and grading of fatty liver among diabetic patients. As previously mentioned, they also didn't find any significant association between CIMT and severity of liver involvement (15). Fracanzani et al. (2008) have also concluded that even mild degrees of fatty liver are associated with high risk of vascular atherosclerosis (14). In the same way Manco et al. (2010) didn't report significant association between CIMT and severity of NAFLD (21) that is compatible with our study. On the other hand, Targher et al. (2007) detected a significant association between CIMT and histologic findings of fatty liver (13). In another study, Schindhelm et al. (2007) showed increased liver enzymes in patients with NAFLD might be associated with severity of atherosclerosis (19). Due to these controversies, further investigations in this area can achieve definitive results.

The results of the present study showed a strong association between non-alcoholic fatty liver and increased CIMT. On the other hand, this association is not influenced by the severity of fatty liver and increasing liver enzymes. Therefore, it is recommended immediately to treat patients with NAFLD in order to prevent

atherosclerotic complications. These interventions should be also considered in the early stages of fatty liver disease.

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