

Original Article

Evaluation of relationship between Grading of fatty liver and severity of Atherosclerotic finding

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Abstract: Purpose: Nonalcoholic fatty liver disease (NAFLD) is a feature of metabolic syndrome and has a high prevalence in the general population. Objective: To determine the severity of atherosclerotic finding in various grade of NAFLD. Material and Methods: We examined 250 consecutive patients with various grade of NAFLD and 85 control subjects matched for age, gender, to determine carotid intima-media thickness (CIMT) in each groups. Results: The mean CIMT in patients with G1, G2 and G3 NAFLD were 0.78 ± 0.15 mm, 0.82 ± 0.11 mm and 0.85 ± 0.16 mm respectively. The differences in CIMT between the various grades of NAFLD were statistically significant ($p=0.01$). Conclusion: According to our study results the presence of NAFLD; severity of atherosclerosis will be higher parallel with severity of fatty liver infiltration. Therefore, Patients with NAFLD especially high-grade fatty liver may be candidate to be investigated for the presence CAD.

Keywords: Fatty liver, ultrasonography, atherosclerosis

Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive accumulation of fat in the liver cells [1, 2] and is the most common cause of liver disease affecting western countries [3]. NAFLD is strongly associated with cardiovascular risk factors such as dyslipidemia, Type 2 Diabetes Mellitus, insulin resistance and obesity [4].

Patients with NAFLD exhibit a higher mortality rate compare to general population [5]. Previous report revealed that the mortality rates of coronary heart disease in patients with NAFLD are equal to those related to cirrhosis [6].

In recent years some case-control studies have shown a relationship between NAFLD and presence of early manifestation of atherosclerosis by CIMT measurement [4, 7, 8].

To our best knowledge there is no published data regarding the association of NAFLD grade and severity of subclinical atherosclerosis. The

aim of the present study is to evaluate the relationship between grading of NAFLD and severity of subclinical atherosclerosis.

Materials and methods

We examined 250 Consecutive patients with ultrasonographically confirmed NAFLD and 85 age and sex matched Control group with normal parenchymal liver echogenicity for determination of CIMT and presence of carotid atherosclerotic plaque.

The patients were referred to the Radiology department of Training University Hospital for measurement of CIMT from Gastroenterology outpatient clinic after abdominal ultrasonography was performed, due to non-specific abdominal discomfort, and diagnosis of fatty liver was made according to ultrasonography examination. One radiologist performed all of the abdominal sonograms.

After basic history taking, subjects with heart disease, Diabetes Mellitus, acute or chronic

liver disease, acute or chronic kidney disease, any malignancy, alcohol consumption, pregnancy, liver masses, abnormal copper metabolism or thyroid function test, history of any medication with adverse effects on liver or history of cigarette smoking were excluded. Only Hepatitis B surface antigen (HBS Ag) and hepatitis C antibody (HCV Ab) negative patients were enrolled. Thirty-five patients with NAFLD were excluded from study because they fail to meet the inclusion criteria for the study.

Hypertension was diagnosed by history for value $>140/90$ mmHg or when subjects were taking antihypertensive drugs. The Body Mass Index (BMI) was calculated by dividing weight in kilograms by height in meters squared.

Blood samples were collected in the morning, after an overnight fasting. Blood was tested for Fasting level of glucose (Normal range <126 mg/dl), Triglycerides (Normal range <200 mg/dl), Total cholesterol (Normal range <240 mg/dl), High-density lipoproteins, C- Reactive proteins, mean platelet volume, Serum transaminases (ALT: alanine aminotransferase, AST: aspartate aminotransferase). The entire blood samples were tested in the same laboratory.

High resolutions B mode ultrasonographies of both common and internal carotid arteries were performed with an ultrasound machine (Siemens, sonoline G40, Germany) equipped with a 10 MHz linear array transducer. Patients were examined in the supine position with the head tilted backwards. After the carotid arteries were located by transverse scans, the probe was rotated 90° to obtain and record a longitudinal image of common carotid arteries.

The maximum CIMT was measured at the posterior wall of the common carotid artery, 2 cm before the bifurcation, as the distance between first and second echogenic lines of anterior and posterior arterial walls. The image was focused on posterior wall of common carotid artery and gain settings were used to optimize image quality. Measurement was performed vertical to arterial wall for accurate measurement of CIMT. Three measurements of CIMT were taken at each site and the average measurement was used.

All of the sonograms of CIMT measurement

were obtained by another radiologist blinded to the results of abdominal sonography and clinical and laboratory data of cases and control subjects.

One experienced Radiologist in Doppler evaluation of extra cranial vessels, who had no prior knowledge of the patients' clinical and laboratory data, obtained ultrasonographic examinations.

Abdominal ultrasound scanning was performed in all participants by another trained radiologist, who was blind to all clinical and laboratory data of patients, using a Toshiba Nemio 30 scanner (Toshiba CO. Ltd, Tokyo, Japan) with a 3.5-MHz linear transducer. US examinations were performed after 8 to 12 hours fasting. Each subject was examined in the supine and 60° left lateral positions during quiet inspiration. The presence or absence and grading of fatty infiltration of the liver were recorded. Grade 0 of fatty infiltration was considered to be the normal liver echogenicity. In grade 1 (mild) fatty infiltration, echogenicity was slightly increased, with normal visualization of the diaphragm and the intrahepatic vessel borders. The grade 2 (moderate) of fatty infiltration was established when echogenicity was moderately increased, with slightly impaired visualization of the diaphragm or intrahepatic vessels. In grade 3 (severe) of fatty infiltration, echogenicity was markedly increased with poor or visualization of the diaphragm, the intrahepatic vessels, and the posterior portion of the right lobe.

The board's institutional review board of the University approved the study, and all the participants provided written informed consent.

Secondary data analysis was performed using spss.16 software. Statistical significance was set at two-sided p -value ≤ 0.05 . Results are reported as the mean $\pm$ standard deviation (SD) or n (%) for continuous variables and as frequencies for categorical variables. In comparison of patients and controls data One-way ANOVA and t-test for quantitative variables, and made statistical significance by chi-squared test for qualitative variables. The relationship for continuous variables was examined by Pearson's correlation coefficients and categorical variables by Spearman correlation analysis. Thereafter, One-way ANOVA analysis was utilized to compare different variables (such as

IMT, FBS, TG, Total cholesterol, HDL, LDL, ALT, AST, MPV and BMI) with grading's of fatty liver. Multivariate modeling was done by linear regression analysis.

Results

We examined 250 patients with NAFLD (139 male and 111 female) and 85 controls (46 male and 39 female). Because of the study design, case and control subjects were almost identical in terms of gender and age. The mean age of patients with NAFLD was 46.5 ± 10.5 years and 44.8 ± 14.1 in controls ($p=0.078$). The fatty liver was grade (G) 1 in 101 patients, grade 2 in 85 patients, and grade 3 in 64 patients.

Patients with NAFLD showed increased mean CIMT (0.81 ± 0.14 mm) when compared to control subjects (0.58 ± 0.15 mm). This difference was statistically significant ($p=0.001$). The mean CIMT in patients with G1, G2 and G3 NAFLD were 0.78 ± 0.15 mm, 0.82 ± 0.11 mm and 0.85 ± 0.16 mm respectively. The differences in CIMT between the various grades of NAFLD were statistically significant ($p=0.01$).

Patients with NAFLD had a higher frequency of high blood pressure compare normal subjects (40% vs. 15.2%). This difference was statistically significant ($p=0.001$). The mean Fasting Blood Glucose was higher in patients with NAFLD (117.16) compare to normal subjects (90.77) ($p=0.001$) but the difference in serum FBS between grade 1, 2 and 3 NAFLD was not statistically significant. The level of AST ($p=0.006$), ALT, MPV (Mean platelet volume) and CRP was higher in patients with NAFLD compare to control group ($p=0.001$) but between these factor only MPV was statistically significant in patients with grade 3 NAFLD compare to grade 1 and 2.

In patients with NAFLD serum levels of Triglycerides and Total cholesterol was higher ($p=0.001$) and HDL Cholesterol was lower ($p=0.001$) compared to control subjects. The differences in serum TG, TC and between grade 1, 2 and 3 fatty liver was not statistically significant but patients with grade 3 NAFLD had statistically significant lower serum HDL compare to grade 1 and 2 NAFLD ($P=0.001$).

The patients with NAFLD had a statistically sig-

nificant higher BMI compare to controls (29.88 ± 3.88 kg/m² vs. 25.29 ± 4.19 kg/m²; $P=0.001$). The differences in BMI between the various grades of NAFLD were statistically significant ($p=0.001$).

Most of the risk factors related to metabolic syndrome (BMI, Hypertension, Glucose level, and low HDL cholesterol) except for hypertriglyceridemia were significantly higher when the grade of NAFLD was increased (**Table 1** and **2**).

Furthermore, after adjustment for multiple confounding factors such as Hypertension, Diabetes Mellitus, Hypertriglyceridemia, Hypercholesterolemia and hyperglycemia in both patients and controls with NAFLD, the mean value of CIMT in patients with NAFLD was significantly higher compare to control subjects (0.76 ± 0.09 mm vs. 0.56 ± 0.13 mm; $P=0.001$). This shows that the presence of NAFLD was associated with abnormal CIMT independently from other atherogenic risk factors.

In Pearson analysis there was a strong positive correlation between CIMT and MPV, FBS, TG, Total cholesterol, BMI and age and there was a strong negative correlation between HDL cholesterol and CIMT.

Table 2 shows the correlation between CIMT and multiple confounding factors.

In multivariate linear regression analysis, the presence of elevated CRP ($p \leq 0.006$), HTN ($p=0.005$) and combination of HTN and elevated CRP are the independent factors affecting the CIMT in patients with NAFLD ($p=0.003$).

Discussion

Nonalcoholic fatty liver disease (NAFLD) is defined as an excessive accumulation of fat in hepatocytes and has a range of pathologic condition from simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. Prevalence of NAFLD in adult population is 20 to 30% in the general population in western countries [9] and in Asian -pacific countries is now 12% to 24% [10], and its prevalence in obese or diabetic patients increases up to 70 to 90% [9].

NAFLD is strongly associated with cardiovascular risk factors such as obesity, dyslipidemia, Type 2 Diabetes Mellitus and insulin resistance

Relationship of fatty liver and atherosclerosis

Table 1. Shows that the main clinical, ultrasonographic and the laboratory data of the patients with different grade of fatty liver.

Variable	Grade 1 NAFLD (n=101)	Grade 2 NAFLD (n=84)	Grade 3 NAFLD (n=65)	P-Value
Sex (male)	57	43	33	0.9
Age (years)	45.89±9.64	48.07±10.95	45.75±1.77	0.27
BMI (kg/m ²)	28.79±2.99	29.71±4.10	31.77±4.18	0.001
ALT (mlu/dl)	31.33±47.15	31.08±19.09	26.95±19.37	0.67
AST (mlu/dl)	35.06±27.13	34.55±24.61	42.92±26.74	0.12
TG (mg/dl)	182.57±79.92	192.41±85.85	194.98±102.45	0.62
TC (mg/dl)	200.97±49.59	208.27±46.04	217.23±53.83	0.12
HDL (mg/dl)	41.68±7.88	44.06±8.97	36.22±7.09	0.001
MPV(fl)	10.05±1.04	10.23±1.07	11.58±5.20	0.002
CRP (+)	30	17	12	0.11
HTN (+)	34	38	40	0.004
FBS (mg/dl)	103.18±40.80	124.28±69.89	129.68±72.48	0.01
CIMT (mm)	0.78±0.15	0.82±0.11	0.85±0.16	0.01

BMI, body mass index; TG, triglycerides; HDL, high-density lipoprotein; CRP, C-reactive protein; HTN, hypertension; FBS, fasting blood glucose; IMT, intima-media thickness; MPV, mean platelet volume; ALT, alanine aminotransferase; AST, aspartate aminotransferase; NAFLD, nonalcoholic fatty liver disease; TC, total cholesterol.

Table 2. Shows correlation of main clinical and laboratory data's of patients with different grads of NAFLD with carotid intima media thickness.

Variable		TG	TC	FBS	MPV	HDL	Age	BMI
CIMT	r	0.36	0.195	0.471	0.18	-0.327	0.449	0.3
	P value	0.0001	0.0001	0.0001	0.001	0.0001	0.0001	0.0001

[4]. In the previous published data the mortality rates of coronary heart disease in patients with NAFLD are equal to those related to cirrhosis [6]. Recently NAFLD is considered as hepatic manifestation of Metabolic Syndrome [11] and we well known that Metabolic syndrome is the "Tsunami" of cardiovascular risk factor.

To understand the true pathophysiologic basis of developing NAFLD we still have a phenomenon similar to Chicken and Egg: Some author believe that NAFLD can produce insulin resistance [12] and other claim that excessive accumulation of triglyceride in hepatocytes, due to insulin resistance, is the first step and oxidative stress reactions, resulting from mitochondrial fatty acids oxidation, and inflammatory cytokine expression and adipocytokines are the other potential factors which cause hepatocyte injury, inflammation and fibrosis [13].

One of the main explanations for the relationship between atherosclerosis and NAFLD is that, there is a significant relationship between

atherosclerosis and inflammation [14].

Some authors demonstrated that the inflammatory response was more prominent in metabolic syndrome and even in patients with NAFLD [15, 16].

The main risk factors associated with NAFLD are increased bodyweight, diabetes mellitus, hyperlipidemia, and metabolic syndrome [4].

In our study most of the patients with NAFLD had one or multiple cardiovascular risk factor. Previous studies revealed that approximately 90% of patients with NAFLD have ≥ 1 characteristic feature of metabolic syndrome and about 33% have the complete diagnosis [17, 18]. This strong association stimulated the concept that it may have a potential role in the development and progression of atherosclerosis.

The outcome in patients with NAFLD is more frequently dependent on cardiovascular events than the progression of liver disease [19-21].

Our study showed that patients with high grade of fatty liver have more severe atherosclerotic finding than low grade fatty liver. Difference in CIMT in patients with grade 3 fatty liver in comparison with lower grade of fatty liver is statistically significant but the difference between grade 1 and grade 3 is only 0.08 mm that in clinical practice is smaller than that be measurable by traditional CIMT measurement. In conclusion we recommend another study with sample volume larger than our study and measurement of CIMT by using new software for more accurate measurement of CIMT.

Previous investigation revealed that there is a strong relationship between CIMT and risk of myocardial and cerebral infarction, and CIMT can effectively identify patients at high risk for coronary artery disease (CAD) [22, 23]. The patients with mean CIMT over 1.15 mm have a 94% likelihood of having CAD [22].

In our study, in comparison with sex and age - matched control group, the CIMT in patients with NAFLD was significantly higher than normal subjects ($p=0.001$) which is in accordance to some previous studies [24, 25] but in contrast with the study of Volzke et al, which showed that there is no independent association between hepatic steatosis with CIMT [26].

Although NAFLD in most patients is accompanied with the classical risk factors for CIMT and CAD, it could stimulate this concept that the observed relationship between NAFLD and CIMT may actually be due to these atherosclerotic risk factors. However, it seems to be unlikely because multivariate regression analysis with all possible confounding risk factors included in patients with NAFLD demonstrated that NAFLD is a significant independent risk factor for CIMT.

The main limitation of our study is that we established the diagnosis of NAFLD according to ultrasonography appearance of liver and exclusion of known etiologic factors of chronic liver disease. The diagnosis was not confirmed by liver biopsy due to ethical reasons. However ultrasonography is a reliable noninvasive method for diagnosis of fatty liver and has a 89% sensitivity and 93% specificity [27, 28].

Morbidity and mortality from CAD is higher in patients with NAFLD. Therefore, the diagnosis of CAD in patients with NAFLD is critical. According

to our study results the presence of NAFLD was associated with CIMT and severity of atherosclerosis will be higher parallel with severity of fatty liver infiltration. Therefore, Patients with NAFLD especially high-grade fatty liver may be candidate to be investigated for the presence CAD.

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