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Research Article

Association Between Non-Alcoholic Fatty Liver Disease and Subclinical Atherosclerosis: A Study of Carotid Intima-Media Thickness

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is a major contributor to chronic liver disease worldwide. It is associated with metabolic syndrome, central obesity, insulin resistance, hypertension, hyperlipidemia, and cardiovascular risks. Carotid intima-media thickness (CIMT) is a recognized surrogate marker for atherosclerosis and cardiovascular risk. ***Objective***: To evaluate the CIMT in patients diagnosed with NAFLD, highlighting potential pathophysiological correlations and clinical implications. ***Methods***: A cross-sectional study was conducted at Al Kindy Teaching Hospital over 4 months from October 2023 to February 2024. The sample size was 100 patients showing ultrasonic signs of fatty liver changes (44 were males and 56 were females). These patients underwent carotid Doppler ultrasound, and the intima-media thickness was measured. ***Results***: Of the 100 patients, 53 had a CIMT greater than 0.08 cm, 23 had grade I fatty liver, 24 had grade II fatty liver, and 6 had grade III fatty liver. Of the 47 patients with a CIMT < 0.08 cm, 16 had grade II fatty liver and 31 had grade I fatty liver. The results were statistically significant (p=0.015). The logistic regression shows a higher fatty liver stage significantly associated with increased IMT, but BMI is not significantly associated with thickened carotid intima-media. ***Conclusions***: NAFLD patients exhibit a higher propensity for subclinical atherosclerosis, as evidenced by increased CIMT, and its monitoring may serve as a valuable noninvasive tool in cardiovascular risk stratification, especially in metabolically compromised NAFLD populations.

Keywords: Body mass index, Carotid intima-media thickness, Fatty liver disease, Ultrasound.

العلاقة بين مرض الكبد الدهني غير الكحولي وتصلب الشرايين تحت الإكلينيكي: دراسة سمك الوسائط الإنتيماوية للشريان السباتي

الخلاصة

الخلفية: مرض الكبد الدهني غير الكحولي (NAFLD) هو مساهم رئيسي في أمراض الكبد المزمنة في جميع أنحاء العالم. يرتبط بمتلازمة التمثيل الغذائي والسمنة المركزية ومقاومة الأنسولين وارتفاع ضغط الدم وارتفاع شحميات الدم ومخاطر القلب والأوعية الدموية. سماكة الوسائط الشريان السباتي (CIMT) هي علامة بديلة معترف بها لتصلب الشرايين ومخاطر القلب والأوعية الدموية. **الهدف**: تقييم CIMT في المرضى الذين تم تشخيص إصابتهم بمرض الكبد الدهني غير الكحولي، مع تسليط الضوء على الارتباطات الفيزيولوجية المرضية المحتملة والآثار السريرية. **الأساليب**: تم إجراء دراسة مقطعية في مستشفى الكندي التعليمي على مدار 4 أشهر من أكتوبر 2023 إلى فبراير 2024. كان حجم العينة 100 مريض ظهرت عليهم علامات الموجات فوق الصوتية لتغيرات الكبد الدهني (44 من الذكور و 56 من الإناث). خضع هؤلاء المرضى لفحص الموجات فوق الصوتية دوبلر السباتي، وتم قياس سمك الوسائط الانتيمائية. **النتائج**: من بين 100 مريض، كان لدى 53 مريض CIMT أكبر من 0.08 سم، و 23 يعانون من الكبد الدهني من الدرجة الأولى، و 24 يعانون من الكبد الدهني من الدرجة الثانية، و 6 يعانون من الكبد الدهني من الدرجة الثالثة. من بين 47 مريضا يعانون من سم CIMT < 0.08 ، كان 16 يعانون من الكبد الدهني من الدرجة الثانية و 31 يعانون من الكبد الدهني من الدرجة الأولى. كانت النتائج ذات دلالة إحصائية (p=0.015). يظهر الانحدار اللوجستي مرحلة أعلى من الكبد الدهني مرتبطة بشكل كبير بزيادة IMT، لكن مؤشر كتلة الجسم لا يرتبط بشكل كبير بالوسط السميك في الشريان السباتي. **الأستنتاجات**: يظهر مرضى NAFLD ميلا أعلى لتصلب الشرايين تحت الإكلينيكي، كما يتضح من زيادة CIMT، وقد تكون مراقبتها بمثابة أداة قيمة غير جراحية في التقسيم الطبقي لمخاطر القلب والأوعية الدموية، خاصة في مجموعات NAFLD المعرضة للخطر من الناحية الأيضية.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the accumulation of extra fat in hepatocytes that is not related to alcohol consumption [1]. Hepatic accumulation of fat is not uncommon; nonetheless, a fatty liver (steatosis), the initial stage of non-alcoholic fatty liver disease (NAFLD), is identified when the liver's weight exceeds 5–10% fat, which may only be diagnosed during tests carried out for another reason [2,3]. Steatosis may progress to a more serious form of NAFLD called non-alcoholic steatohepatitis (NASH), where the liver has become inflamed. Persistent inflammation can cause fibrosis and the formation of scarred stromal tissue surrounding the hepatocytes and nearby vascular structures, but the hepatic function is still good [4]. Fibrosis may progress to cirrhosis after years of inflammation,

which is the most severe form of NAFLD, where the liver gets smaller in size and becomes scarred and nodular; these changes may be irreversible, as liver failure and even liver cancer may be sequelae [5]. Currently, NAFLD is regarded as one of the leading causes of chronic liver disease in the younger generation in the developed world [6]. Genetic and socioeconomic factors, as well as varying obesity rates, all contribute to the significant regional variations in the prevalence of NAFLD. In the Americas and Southeast Asia, NAFLD prevalence is higher than 40%. It is predicted that if current trends continue, the prevalence of NAFLD will rise dramatically in several parts of the world by 2030 [7]. It has been reported that obesity is a major risk factor for fatty liver infiltration [8]. The primary theory posits that a combination of variables, including insulin resistance, gut microbiota, dietary factors, and genetic and epigenetic factors, causes genetically predisposed individuals to develop NAFLD. But even without any risk factors, some people get non-alcoholic fatty liver disease [9,10]. Although the liver biopsy is the definitive diagnostic tool for confirmation and grading of hepatic steatosis, the high incidence of sampling error, especially in cases of inhomogeneous fatty infiltration, and the risk of complications make this interventional technique not suitable for the majority of cases [11]. Since ultrasound is non-invasive, accessible, affordable, and yields valuable information [12], it is currently the most widely used technique for qualitative evaluation of hepatic steatosis. Increased hepatorenal contrast, liver brightness, deep attenuation, and vascular blurring are typically two characteristics that indicate the presence of hepatic steatosis on abdominal ultrasound [13]. NAFLD has been shown in previous studies to be a separate risk factor for cardiovascular disease and atherosclerosis [14]. The majority of these studies employed carotid artery intima-media thickness (IMT) as a non-invasive indicator of subclinical atherosclerosis [15,16]. Increased CIMT caused by old age, high cholesterol, smoking, high blood pressure, diabetes, obesity, and a sedentary lifestyle, and it is associated with an increased risk of atherosclerotic vascular disease by the formation of plaque that leads to narrowing or blocking of the main arteries that supply the brain, and this will lead to stroke formation [17]. Recent studies suggested that the presence of fatty changes in the abdominal ultrasound should warn us about the presence of increased carotid IMT, which is a sign of atherosclerosis, which is associated with increased risk of MI, stroke, or peripheral vascular disease [18]. The purpose of this study was to measure the CIMT in patients with NAFLD incidentally detected on abdominal ultrasound and consider it as a non-invasive indicator of atherosclerosis.

METHODS

Study design and setting

This is a hospital-based, descriptive, cross-sectional study implemented at Al-Kindy Teaching Hospital for 6 months (October 2023-April 2024), including adult patients with NAFLD after giving written, informed consent.

Inclusion criteria

The study includes adult patients who were referred to undergo abdominal ultrasound for causes not related to recent or previous history of liver disease, who agree to undergo Doppler ultrasound of the carotid vessels.

Exclusion criteria

Patients with a history of liver disease, alcohol consumption, or any type of hepatitis have been excluded from the study.

Study groups

Depending on the abdominal and neck ultrasound findings, the sample was divided into two groups based on CIMT: group 1 included those with high CIMT, while group 2 included subjects with normal CIMT. Demographic and clinical data were compared between patients in both groups ,figure (1).

Sample size calculation

Sample size was estimated by the following formula [21]:

$$N = \frac{Z^2 P (1-P)}{d^2}$$

Where Z=1.96 corresponds to a confidence interval of 95%, with estimated prevalence =0.5, and taking a margin of error d=0.07. So, the estimated sample size is 196. We managed to enroll 200 patients in the current study.

Outcome measurements

Sociodemographic data were collected, including sex, age, BMI, and smoking status, from each patient. All members included are examined by the same radiologist (10 years of experience in abdominal and Doppler neck ultrasound examinations). The GE LOGIC S8 ultrasound machine was used to conduct ultrasound exams, using 4.5 MHZ convex and 9 MHz linear array transducers to examine the abdomen and neck, respectively. During an abdominal ultrasound, the radiologist searches for findings and grades of fatty liver. If present, the radiologist takes permission to examine the patient's neck and measure the carotid arteries' intima-media thickness (IMT). Based on the presence of fatty liver on abdominal ultrasonography, fatty liver disease was diagnosed and graded as follows: Grade 1: The internal fine echoes have increased mildly, and in contrast to the kidney's cortex, the liver appears brighter, but the diaphragm and intrahepatic vessel border visualization is normal. Grade 2: The internal fine echoes have increased

moderately and diffusely. But the visibility of the diaphragm and intrahepatic vessels is somewhat compromised. Grade 3: significant rise in the fine echoes. Intrahepatic vessel borders, the diaphragm, and vessels are poorly or not at all visible [19] (Figure 1). Carotid IMT is considered increased if it is greater than 0.08 cm and normal if it is equal to or less than 0.08 cm [20] (Figure 2).

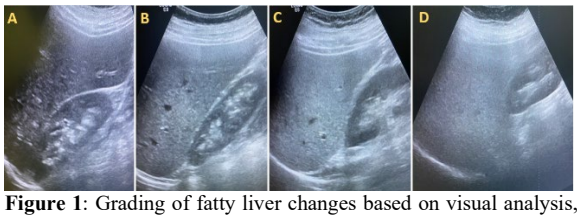

Figure 1: Grading of fatty liver changes based on visual analysis, depending on the internal fine echoes and comparison with renal cortex echogenicity. A) Normal; B) Mild fatty changes; C) Moderate fatty changes; and D) Severe fatty changes.

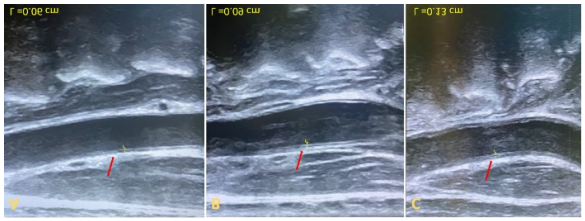

Figure 2: B-mode ultrasound images of the carotid artery show the two thicknesses of the deepest layers of blood arteries (the intima and media) (red arrows) in patients with non-alcoholic fatty liver disease, showing varying thicknesses. A) normal intima–media thickness in a patient with grade I fatty liver changes; B) mildly thickened intima-media in a patient with grade II fatty liver changes; and C) markedly thickened in a patient with grade III fatty liver changes.

Ethical considerations

The study protocol was officially approved by the local Research Ethics Committee of Al-Kindy College of Medicine, University of Baghdad.

Statistical analysis

Data obtained were entered into the computer using the Microsoft Excel program with their statistical analysis and relevant statistical tests, along with the generation of tables using Statistical Package for Social Statistics (SPSS) version 26 software. Data were categorical, presented as numbers and percentages, and analyzed by the X^2 test. Variables showed significant association with increased CIMT were analyzed by binary logistic regression to determine predictors for increased CIMT in patients with NAFLD. Statistically significant association was considered when the *p*-value was less than 0.05.

RESULTS

A total of 200 patients with NAFLD enrolled in the study. The majority were female, with the highest frequency of patients aged fifty years or more. Nearly half of the samples studied were obese and smokers. About the carotid intima thickness, 53% of patients' CIMT was > 0.08 cm and regarded as high CIMT. Grade I NAFLD was reported in 53% of study samples, and 56% of patients had hepatomegaly (Table 1).

Table 1: Demographical and clinical characteristics of patients with NAFLD.

Variable	Result
Gender	
Male	88(44)
Female	112(56)
Age (years)	
21-30	34(17)
31-40	24(12)
41-50	68(34)
≥ 50	74(37)
BMI	
Normal	60(30)
Overweight	44(22)
Obese	96(48)
Fatty Grade	
I	108(54)
II	80(40)
III	12(6)
Liver size	
Normal	88(44)
Enlarged	112(56)
Smoking	
Yes	110(55)
No	90(45)
CIMT	
High	106(53)
normal	94(47)

Values were expressed as frequency and percentage.

Higher CIMT was significantly associated with four factors, including older age, obesity, enlarged liver size, and higher fatty liver grade. Gender of the patients with NAFLD did not significantly correlate with CIMT (p= 0.053) (Table 2). Data reveals a clear trend between liver size and grade of fatty liver in NAFLD patients, as patients with grade III steatosis were exclusively reported in patients with enlarged liver. Otherwise, there was a non-significant difference reported between grade I&II fatty liver and liver size with p= 0.052 (Figure 3).

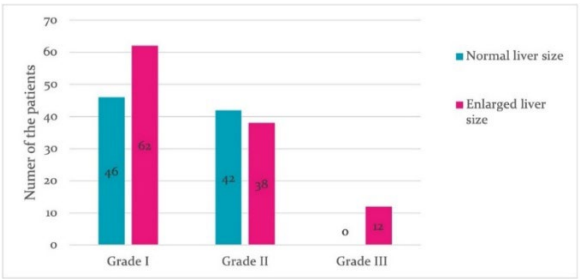

Figure 3: The distribution of liver fatty grades by liver size in patients with NAFLD.

In a binary logistic regression, higher fatty liver stage (OR = 3.214, 95% CI: 1.115–9.259, p = 0.031), increasing age (OR = 2.576, 95% CI: 1.427–4.650, p = 0.002), and history of smoking (OR = 10.032, 95% CI: 1.035–97.267, p = 0.047) were found to be statistically significant predictors of increased carotid intima-media thickness. BMI was not significantly associated with CIMT in this model (p= 0.278) (Table 3).

Table 2: The relation between carotid intima-media thickness (CIMT) and demographical and clinical characteristics of patients with NAFLD

Variables	CIMT		*p*-value
	Normal ≤ 0.08 *n*=94	High >0.08 *n*=106	
Fatty Grade			
I	62 (66.0)	46 (43.4)	
II	32 (34.0)	48 (45.3)	0.015
III	0 (0.0)	12 (11.3)	
BMI			
Normal	42(44.7)	18(17.0)	
Overweight	22 (23.4)	22(20.8)	0.004
Obese	30 (31.9)	66(62.2)	
Age (year)			
21-30	30 (31.9)	4(3.8)	
31-40	20 (21.3)	4(3.8)	0.0003
41-50	14 (14.9)	54(50.9)	
≥ 50	30 (31.9)	44(41.5)	
Liver Size			
Normal	54 (57.4)	34 (32.1)	0.011
Enlarge	40 (42.6)	72 (67.9)	
Gender			
Male	44 (46.9)	44(41.5)	0.548
Female	50 (53.2)	62(58.5)	

Values were expressed as frequency and percentage. CIMT: Carotid intima-media thickness.

Table 3: Logistic regression analysis of the predictors of increased carotid intima-media thickness

Variables	B	Odds Ratio	95% C.I. for EXP(B)	*p*-value
Fatty liver stage	1.167±0.54	3.214	1.115-9.259	0.031
BMI	0.403±0.371	1.496	0.723-3.096	0.278
Age	0.946±0.301	2.576	1.427-4.650	0.002
Smoking (yes)	2.306±1.159	10.032	1.035-97.267	0.047

Values were expressed as mean±SE.

DISCUSSION

Data revealed that more than half of patients with NAFLD (53%) have high CIMT, and those demonstrated a significant association with four clinical factors, including older age, obesity, enlarged liver size, and higher fatty liver grade. However, only four predictors demonstrated higher odds for having high CIMT in adults with NAFLD, including higher fatty liver stage (OR= 3.214, 95% CI: 1.115–9.259, *p*= 0.031), increasing age (OR= 2.576, 95% CI: 1.427–4.650, p = 0.002), and history of smoking (OR= 10.032, 95% CI: 1.035–97.267, *p*= 0.047). These predictors allow clinicians to apply better patient triage, risk stratification, and management strategies. Identifying predictors for atherosclerosis in NAFLD has gained increasing interest recently [22]. This could be attributed to the increasing prevalence of NAFLD, affecting one in 4 adults in some parts of the world, together with mounting evidence that links the disease with coronary artery disease and subclinical atherosclerosis [23]. The diagnostic dilemma relies on the inability to depend on the ordinary inflammatory markers of atherosclerosis (BMI, lipid profile) in NAFLD, together with the asymptomatic nature of the condition. CIMT is a validated surrogate marker for atherosclerosis, as it offers several advantages in at-risk populations [24,25]. The advantage of CIMT over other markers is that it is non-invasive and cost-effective and provides a direct view of vascular changes as it quantifies the actual arterial wall thickness, and it allows tracking the disease progression and regression [26]. In the current study, high CIMT was reported in more than half of the patients with NAFLD, which matches the previously reported frequency in an Indian study [27]. A lower frequency was reported in an Egyptian study where only 44% of NAFLD showed a high CIMT; this discrepancy was attributed to different clinical characteristics of the included patients[28]. A higher CIMT was significantly associated with four factors, including older age, obesity, enlarged liver size, and higher fatty liver grade. These findings align with results by Rasool *et al.* [29], who found that the level of CIMT was higher in patients with NAFLD. In addition, a meta-analysis by Cai *et al.* [30] demonstrated a 0.16 mm increment in CIMT in NAFLD patients with a more than 3-fold increase in risk of carotid plaque. The association of BMI and CIMT in the present study is consistent with results by Riaz *et al.* [31], which showed a statistically significant association between NAFLD and raised CIMT in patients with BMI ≥30 kg/m^2. Sex-based differences had been described in CMIT in NAFLD patients, with previous studies consistently showing that males tend to have higher CIMT; however, the impact of NAFLD on CIMT was more pronounced in females [32,33]. These findings were not demonstrated in the current data, as gender was not significantly associated with CIMT; this discrepancy is attributed to different factors, including genetic and environmental factors, as these may influence vascular reactivity and atherosclerosis development. There is an increasing body of evidence supporting the association of hepatomegaly and high-grade steatosis in NAFLD [34,35]. In the present study, although the association was not statistically significant, probably due low the small sample size, it's noteworthy that all patients with grade III fatty liver exhibit hepatomegaly. This finding suggests a potential trend for increasing liver size in patients with higher grades of steatosis. Similarly, Borai *et al.*, in an Egyptian study involving patients with different grades of steatosis, reported an excessive increase in liver size

with increasing grade of steatosis. Therefore, they conclude that liver size assessed by ultrasound could be of value in differentiating the grade of steatosis [36]. Advancing age was a significant and independent risk factor for high CMT among patients with NAFLD, as each year increment in age of those patients is associated with a 2.5-fold increase in the odds of having high CMT. Chouhan *et al*. [37] reported a significant association of high CMT and NAFLD with increasing age of the patients. These findings are consistent with a growing body of evidence suggesting that vascular structural changes intensify with aging [38].

Study limitations

This study had several limitations, including a single-center setting and small sample size; both limit the generalization of the results. However, the identification of predictors of high CIMT in Iraqi adults with NAFLD was not tried before, so this study can lead to a multicenter study with a large sample size.

Conclusion

This study concluded that more than half of patients with NAFLD develop high CIMT, which is closely related to atherosclerosis. Four predictors associated with increased risk of high CIMT include older age, obesity, smoking, and high grades of fatty liver. Once NAFLD is diagnosed in patients with these factors, screening for CIMT for early diagnosis and management of atherosclerosis.

Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

Supplementary data are available from the corresponding author on request.

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