Original Article

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Radiology Section

A Study of Carotid Atherosclerosis in Subjects with non Alcoholic Fatty Liver Disease

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ABSTRACT

Introduction: Non Alcoholic Fatty Liver Disease (NAFLD) is considered to be the hepatic manifestation of metabolic syndrome, a highly atherogenic condition. However, it is important to determine whether NAFLD is an independent predictor of cardiovascular disease.

Aim: To evaluate any possible association between NAFLD with Carotid Intima-Media Thickness (CIMT) and plaque prevalence, which are proven markers of subclinical atherosclerosis.

Materials and Methods: This cross-sectional study was conducted in the Department of Radiodiagnosis in Mysore Medical College. Total 200 patients between 18-60 years were subjected to abdominal sonography for assessment of presence or absence of fatty liver, followed by evaluation of both carotid arteries for CIMT and plaques. Statistical analysis was performed using Microsoft Excel 2013 and SPSS 20.0 software.

Results: Majority of subjects (64.5%) belonged to age group of 18 to 39 years. Total 121 female and 79 male subjects participated in the study. The prevalence of NAFLD in the study population was 31% among which 56% had Grade I fatty liver. The average maximum and mean CIMT in patients with NAFLD and non-NAFLD were 0.73 ± 0.16mm, 0.69 ± 0.14mm and 0.54 ± 0.14mm, 0.51 ± 0.13mm. The prevalence of plaques in normal population was 2.9% while that in NAFLD patients was 19.3%. Association between NAFLD and CIMT/plaques were statistically significant. As the grade of fatty liver increased the CIMT and incidence of plaques and hepatomegaly also increased.

Conclusion: NAFLD is a strong independent risk factor for atherosclerosis. Incidentally detected fatty liver on ultrasound should act as a trigger for complete cardiovascular risk assessment.

Keywords: Cardiovascular disease Intima-Media thickness, Plaques

INTRODUCTION

NAFLD is characterised by fatty infiltration of hepatocytes in a pattern similar to that seen in alcoholic liver disease. The spectrum of NAFLD ranges histologically from pure steatosis to steatohepatitis, fibrosis, ultimately resulting in cirrhosis. Since the turn of the century, NAFLD has emerged as the leading cause of chronic liver disease. The incidence of NAFLD is on the rise and has reached epidemic proportions [1]. Epidemiologic data reveals a parallel rise in prevalence of obesity, diabetes, NAFLD, and HCC [2]. The study evaluating the awareness of NAFLD in the general population demonstrated that the majority of people (83%) had never come across the term NAFLD, knowledge about NAFLD diagnosis and risk factors was also inadequate among those who had ever heard of it [3].

Recently conducted studies show the importance of NAFLD and its association with dyslipidemia, metabolic syndrome, Type 2 diabetes and obesity has initiated the curiosity to know the possible role of NAFLD in the development of atherosclerotic and cardiovascular disease.

Among the various imaging markers for atherosclerosis, measurement of CIMT using ultrasound is the most routinely employed modality. Not only is CIMT a reliable predictor of atherosclerosis, it can also be used in assessing severity of disease process and for risk stratification. Increased CIMT is associated with greater morbidity and mortality from myocardial infarction and stroke [4].

Inspite of the increased awareness about NAFLD in recent times, many physicians still consider it a trivial incidental finding on ultrasound examination. A possible association between

NAFLD and CIMT may change this outlook and result in long term implications in the future.

In this backdrop, this study is an attempt to investigate the role of NAFLD as an individual risk factor for atherosclerosis by estimating CIMT and plaque prevalence as indirect markers using ultrasound.

MATERIALS AND METHODS

This cross-sectional study was conducted during the period from November 2015-August 2016 in the Department of Radiodiagnosis, Mysore Medical College and Research Institute, Mysuru, India. As ultrasound is a reliable and cost effective diagnostic tool for diagnosing fatty liver, we decided to select the subjects on the basis of ultrasound findings. All the patients above age of 18 years old and below age of 60 years were considered for the study. Subjects found to have fatty liver on ultrasound with history of alcohol, diabetes, hypertension, chronic liver disease and drugs which cause hepaotoxicity and pregnant women were excluded from the study. Finally the patients were divided into non-NAFLD and NAFLD. Total 200 subjects of both sexes between 18-60 years of age were studied. Out of all, 62 subjects of NAFLD and 138 subjects of non-NAFLD were studied. The study was performed with the approval of Institutional ethical committee review board. Written informed consent was obtained from every patient.

Data acquisition: A structured pre-prepared case proforma was used to enter the patient details, clinical history and imaging findings.

Ultrasound examination of the abdomen was performed using high resolution, 3.0–6.6 MHz, curvilinear array transducer of Esoate MyLab 40. Patients with fatty liver were identified as diffuse increase in echogenicity of liver. Patients with fatty liver are graded accordingly as follows [5]:

Grade I: Minimal diffuse increase in the fine echoes. Liver appears bright compared to the cortex of the kidney. Normal visualization of diaphragm and intrahepatic vessel borders.

Grade II: Moderate diffuse increase in the fine echoes. Slightly impaired visualization of the intrahepatic vessels and diaphragm.

Grade III: Marked increase in the fine echoes. Poor or no visualization of intrahepatic vefcssels and diaphragm and poor penetration of the posterior segment of the right lobe of the liver [5].

The liver is measured in the mid hepatic line with a large field of view from the posterior diaphragm to the lower anterior edge. Patient was considered to have hepatomegaly if liver size was ≥16cm with rounded margins.

Next carotid ultrasonography was performed for all patients in the same machine (Esoate MyLab 40) using high resolution, 7.5-12 MHz linear array transducer.

Carotid IMT measurements were made bilaterally at the level of the common carotid artery approximately 1cm proximal to carotid bulb for the wall and always in stenotic-free segments. Three standard measurements were taken and the average of the measurements and also maximum values noted. CIMT is considered to be increased if it is >0.8mm.

The walls of the common carotid arteries, internal carotid arteries and carotid bulbs of both sides were evaluated for determination of presence of carotid plaque, defined as a focal thickening >1.2 mm of the intima-media complex, measured from the media adventitia interface to the intima-lumen interface.

STATISTICAL ANALYSIS

Both descriptive and inferential statistics were employed for data analysis. Results are reported as the mean ± standard deviation, n (%) for continuous variables, and as frequencies for categorical variables. Degree of association between various parameters were assessed by Pearson's Chi square test. Statistical significance was set at a two-sided p-value of <0.05. All the statistical calculations were done through Microsoft Excel 2013 and SPSS for windows (version 20.0).

RESULTS

The age of the patients in this study ranged from 18 to 60 years, with a mean age of 35.7 years. Majority of the patients in this study were in their third or fourth decade of life with 64.5% of patients between 18 and 39 years [Table/Fig-1]. The average age among non-NAFLD patients was 33 ± 10 and among NAFLD patients was 40 ± 10 years. Age had a significant association with presence of NAFLD with a p-value of 0.001 [Table/Fig-2].

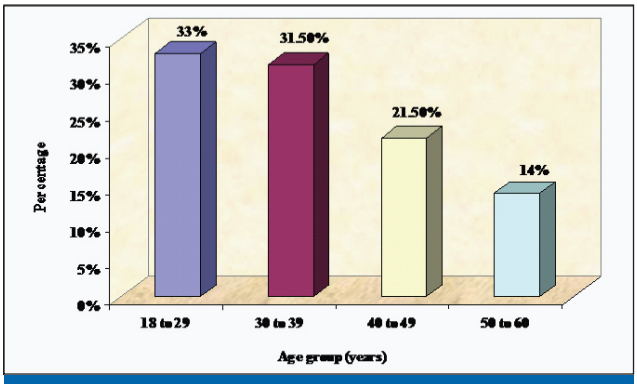
In our study, the majority of the population belonged to female gender (60.5%) [Table/Fig-3]. Among the 121 female patients 40 (33%) had NAFLD. Among the 79 male patients 22 (28%) had NAFLD. There was no significant association between sex and NAFLD with p-value of 0.436 [Table/Fig-2].

Within the study population, 62 patients accounting to around 1/3rd (31%) had NAFLD detected on ultrasound [Table/Fig-4]. Among 62 patients with NAFLD, more than half of the patients (56.5%) belonged to Grade I. About 38.7% of patients fell under category of Grade II. Only three patients (4.8%) had Grade III fatty liver on ultrasound [Table/Fig-5].

About 1/5th of the patients (39) patients had hepatomegaly. Among these 39 patients, 36 patients had fatty liver on ultrasound [Table/Fig-2]. The average size of liver measured in normal patients was 14.4 ± 0.9cm while the mean size of liver among patients with NAFLD was 15.9 ± 0.88cm.The

presence of hepatomegaly is significantly higher in patients with NAFLD compared to normal population. As the grade of fatty liver increased, so did the size of liver and presence of hepatomegaly and this is statistically proven with p-value of <0.001.

The CIMT values of subjects in the study population range from 0.3 to1.3mm.The average maximum and mean CIMT in patients with NAFLD and non-NAFLD were 0.73 ±0.16 mm,



[Table/Fig-1]: Bar diagram showing age distribution of patients.

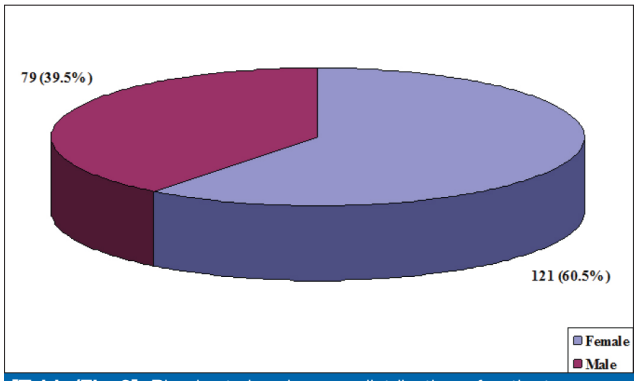
Parameters	non-NAFLD n (%)	NAFLD n (%)	Significance
No. of Subjects,	138 (69%)	62 (31%)	
Age (in years)	33±10	40 ± 10	p <0.001
Female	71 (67%)	40 (33%)	p = 0.436
Size (in cm)	14.4±0.9	15.9±0.88	p <0.001
Max. CIMT (in mm)	0.54 mm ± 0.14	0.73 mm±0.16	p <0.001
Mean CIMT (in mm)	0.51mm ± 0.13	0.69 mm±0.14	p = 0.049
Plaque Prevalence	4 (2.9%)	12 (19.3%)	p <0.001

[Table/Fig-2]: Comparison of various parameters and their level of significance between NAFLD and non-NAFLD groups.

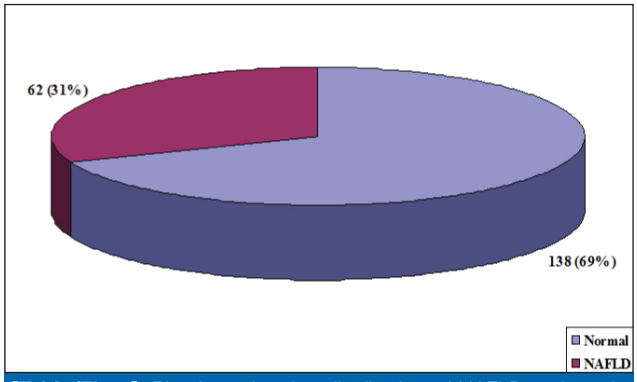
0.69±0.14 mm and 0.54±0.14 mm, 0.51±0.13 mm.

Association between NAFLD and the markers of atherosclerosis i.e., max CIMT, mean CIMT and plaques [Table/Fig-2] were statistically significant. However, the level of significance differed between max and mean CIMT. The association between NAFLD and max CIMT was highly significant with p-value <0.001while that with mean CIMT value was just significant with p-value of 0.049.

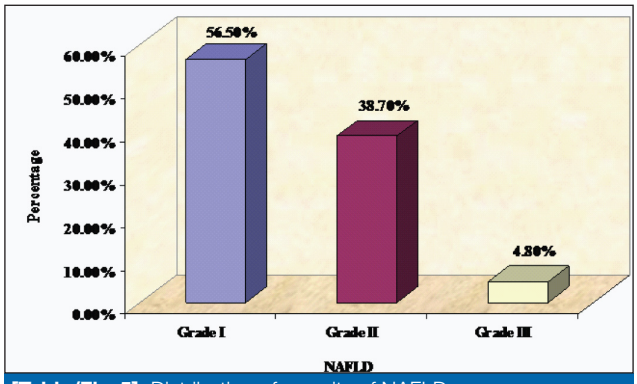
Only 16 patients (8%) among 200 patients had plaques visualized in the carotid system [Table/Fig-6]. Among these 16 patients, 12 had non-alcoholic fatty liver. The prevalence of plaques in normal population was 2.9% while that in NAFLD patients was 19.3%. The max CIMT values and presence of plaques increased progressively with increasing grade of fatty liver and was statistically significant [Table/Fig-7]. Mean



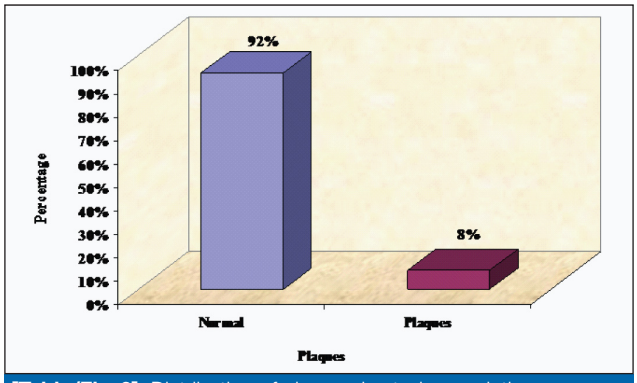
[Table/Fig-3]: Pie chart showing sex distribution of patients.



[Table/Fig-4]: Pie chart showing distribution of NAFLD patients in study population.



[Table/Fig-5]: Distribution of severity of NAFLD.



[Table/Fig-6]: Distribution of plaques in study population.

			GRADE			Total
			Grade I	Grade II	Grade III	
Max CIMT	>0.8mm	Count	8	7	1	23
		% within Max CIMT	34.8%	30.4%	4.3%	100.0%
	<0.8mm	Count	27	17	2	177
		% within Max CIMT	15.3%	9.6%	1.1%	100.0%
Total		Count	35	24	3	200
		% within Max CIMT	17.5%	12.0%	1.5%	100.0%

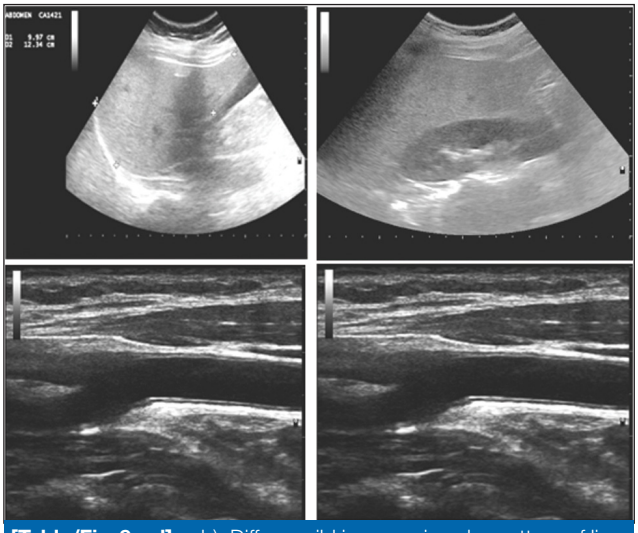
[Table/Fig-7]: Association between grade and Max CIMT.
*p value < 0.001

CIMT values even though it showed a rising trend with raise in severity of fatty liver did not have statistical significance with p-value of 0.127.

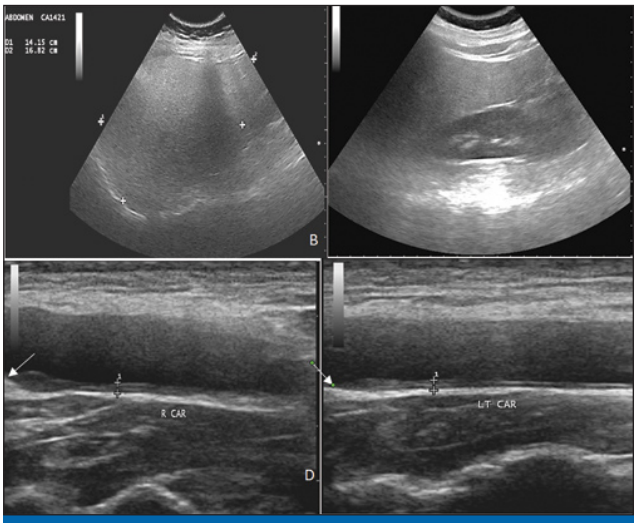
DISCUSSION

On studying the natural history of NAFLD, there was higher mortality in these subset of patients due to cardiovascular disease [1]. Initially, thought to be an epiphenomenon, recent studies have shown that NAFLD is strong risk factor for the development of cardiovascular disease [6]. In the long run, this results in coronary and carotid artery disease with myocardial infarction and stroke as potential complications.

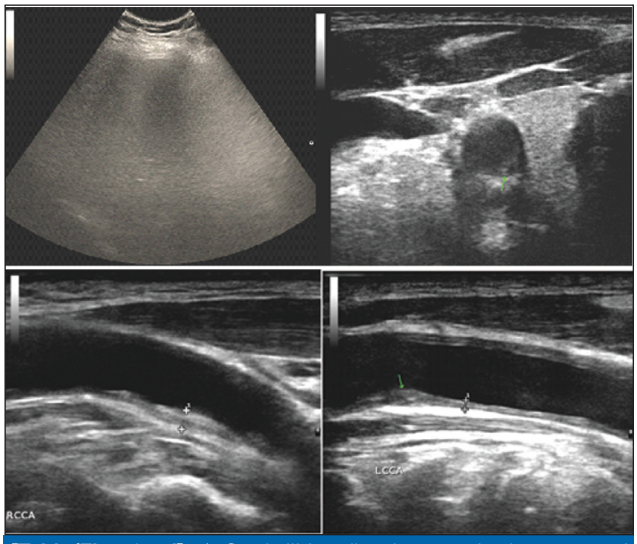
This study was done on 200 non-alcoholic, non-diabetic and non-hypertensive patients between 18 and 60 years of age. [Table/Fig-8-10] shows USG characteristics of various grades of fatty liver along with intima media thickness of CCA. In our study, prevalence of fatty liver increased with advancing age. This correlated well with many large scale



[Table/Fig-8a-d]: a,b): Diffuse mild increase in echo pattern of liver on comparison with renal echogenicity suggestive of Grade I fatty liver; c,d): CCA IMT measures 0.07mm bilaterally.



[Table/Fig-9a-d]: a,b): Grade II fatty liver-Hepatomegaly (mid hepatic liver measurement-16.8cm) and moderately increased echo pattern of liver with slight impairment of visualisation of intrahepatic vessels and blurring of diaphragmatic margin; c,d): Right and left CCA IMT measures 1.0mm and 1.1mm respectively. Atheromatous plaques (arrows) noted in bilateral carotid bulbs.



[Table/Fig-10a-d]: a): Grade III fatty liver-Increased echo pattern of liver with poor visualization of intrahepatic vessels and diaphragm and poor penetration of the posterior segment of the right lobe of the liver; b,c): Axial and longitudinal sections of right CCA showing plaque in posterior wall; d): Left CCA shows diffuse intima-media thickening (IMT – 1.3mm) with a plaque.

epidemiological cohort studies. Kojima S et al., in their cross-sectional/retrospective study in 39151 Asian patients, found the prevalence of fatty liver under 20 years of age to be 7%, which increased to 25.8% in age group of 40-49 [7]. Similar to their study, we found the incidence of fatty liver in less than 30 years and 40-49 years to be 8% and 29% respectively.

Among the 62 patients with NAFLD in the present study, majority were female. This female predominance is partly due to the fact that most of the men had history of alcohol consumption

categorizing them under alcoholic fatty liver disease. In spite of this female predominance, no gender specific association was noted with presence of NAFLD which correlates well with studies by Mohammadi A et al., [8], and Bedogni G et al., [9]. However, most of the literature shows that male gender is an independent risk factor for development of NAFLD. Few examples are studies done by Kojima S et al., [7], Marchesini G et al., [10], Volzke H et al., [11]. They attribute this to the protective role of estrogens in menstruating females. Hence, females tend to have later onset of NAFLD. However, post menopause or with increasing age female sex has no longer a protective effect.

Among 200 subjects, 62 patients had NAFLD. So the prevalence of NAFLD was 31%. The prevalence of NAFLD in the United States in the general population varies from 20-40% and in Europe it is about 29% [12]. Only a hand full of large scale epidemiological studies to identify the prevalence of NAFLD in India has been done. Mohan V et al., analyzed the prevalence of NAFLD in 541 urban south Indian population and found it to be 32% [13]. The prevalence of NAFLD in our study matches well with these studies.

In the early stages, NAFLD can be asymptomatic. The most common physical finding is hepatomegaly, the presence of which varies in different studies; up to 50% in various studies [14] and may rise up to 75%. Fatty liver is also a common cause of hepatomegaly. The size of liver increases and the edges become rounded in NAFLD due to fatty infiltration as well as due to hepatocyte enlargement. In the current study, the prevalence of hepatomegaly among NAFLD patients is 50%, which is statistically significant. Also, the size of liver increases with progressive increase in grade of fatty liver.

In this study, maximum CIMT in patients with NAFLD was significantly higher than in an age matched control group ($p < 0.001$). This correlates well with many case controlled and cross-sectional studies [3,8,12]. These studies showed a relationship between NAFLD and CIMT, an early independent predictor of cardiovascular events. In the study conducted by Brea A et al., the average CIMT values for NAFLD and control group were 0.70 mm and 0.54 mm [15]. These values have been reproduced in this study. The data from this study matches with that done by other Indian authors. Mishra S et al., (SMS Jaipur) in 645 non-diabetic patients found NAFLD as an independent risk factor for atherosclerosis [16]. Kaur M et al., showed that increased CIMT was associated with increased serum cholesterol levels in NAFLD patients [17].

The mean CIMT value, however, had a positive but weak correlation with p-value 0.049. It is unclear whether mean CIMT or maximum CIMT should be used as the primary outcome. Dogan S et al., compared mean CIMT and mean maximum CIMT measurements in fifteen trials with lipid-modifying treatment that had information on both outcome measures [18]. Findings showed that the reported reproducibility was high for both measurements. Trials using mean maximum CIMT progression more often (12 out of 15 studies) paralleled the findings of event trials in contrast to the mean CIMT (11 out of 15 studies), a difference not reaching statistical significance. One favorable point is that information on mean CIMT can be obtained easily in protocols assessing mean maximum CIMT.

The CIMT values and plaques were increased in patients with higher grades of fatty liver. This correlation between CIMT with increasing severity of disease process is similar to study by Kaur M et al., [17]. Kucukazman M et al., also emphasized the positive correlation between grading of fatty liver on ultrasound findings and mean IMT ($r = 0.376$, $p<0.001$) [19].

The presence of plaques in normal and NAFLD subjects were 19.3% and 2.9% in the present study. These values are the same detected in the study by Mishra S et al., (19.2% vs 2.2%)[16]. Other studies done by Targher G et al., [6], Brea A et al., [15] confirm the association of NAFLD with carotid plaques. Volzke H et al., [11] even though found no correlation with CIMT, showed a relationship with presence of more advanced atherosclerotic lesions in carotid arteries like plaques. However, most of these studies had a very high plaque prevalence rate compared to our study. This should be interpreted with caution due to differences in age groups and ethnicity studied.

LIMITATION

There were several limitations to the study. First, there might have been selection bias. Since most of alcoholics in the community are men, there was a predominance of females in our study population. Hence, gender matched controls were not taken.

The grading of fatty liver on ultrasound is a highly subjective one with increased intra and inter observer variations. This might have led to differences in grading. Plaque location, morphology, characterization and severity of stenosis were not evaluated. Since, this study was not a prospective study, follow-up of patients were not taken.

CONCLUSION

Presence of NAFLD is associated with increased CIMT values and prevalence of plaques and is a strong independent risk factor for atherosclerosis. Incidentally detected fatty liver on ultrasound should act as a trigger for search of existence of extrahepatic disease such as silent carotid lesions and warranting complete cardiovascular risk assessment.

Ultrasound screening and surveillance strategies followed by prompt treatment can be recommended in ever growing number of patients with NAFLD to prevent future cardiovascular complications.

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