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Original Research Article

A Study of Clinical, Biochemical and Radiological Profile of Non-Alcoholic Fatty Liver Diseases & its Correlation with Carotid Artery Intimal Media Thickness

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Conflict of interest: Nil

Abstract:

Background and Objectives: Diagnosis of non-alcoholic fatty liver (NAFLD), as a cause of progress towards the end stage of liver disease, is increasing. 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. The present study was conducted at tertiary healthcare center to evaluate the clinical, biochemical profile and prevalence of atherosclerosis by measuring the CIMT in Indian patients with incidentally detected NAFLD and to study its relationship with metabolic syndrome.

Material and Methods: It was an Observational study conducted among outdoor patients and indoor patients admitted at our tertiary care hospital among 100 non-alcoholic individuals, with age >12 years defined as either total abstainers or who consumed <20 g of alcohol per day (confirmed by two family members) and ultrasound showing hyperechoic liver suggestive of fatty liver. Carotid Intima-Media thickness (CIMT) was assessed by trained professional using high resolution B mode ultrasonography system (Phillips HD 11XE) having an electrical linear transducer mid frequency of 7- 12 Mega Hertz.

Results: Total 47% cases were in Grade I, while 43% cases in Grade II and 10% cases were in Grade III. Maximum cases in all grades were aged more than 60 years followed by age group 51-60. The mean CIMT on right side was 0.65 and left side was 0.66.

Conclusion: Grade II and III USG findings were comparatively observed more among advanced age groups as compared to lesser age groups. And the mean CIMT on right side was 0.65 and left side was 0.66.

Keywords: Non-alcoholic fatty liver, Carotid Intima-Media thickness, Liver disease, ultrasound.

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 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.

Hence the present study was conducted at tertiary healthcare centre to evaluate the clinical, biochemical profile and prevalence of atherosclerosis by measuring the CIMT in Indian patients with incidentally detected NAFLD and to study its relationship with metabolic syndrome.

Materials and Method:

- This was a observational study carried out in the department of medicine pacific medical college and hospital udaipur rajasthan.
- A total of 100 study subjects were undertaken to study the clinical biochemical and radiological profile of NASH and its co relation with carotid artery intimal media thickness.
- Detailed history including that of alcohol consumption was taken after informed consent. Anthropometric measurements like weight, height, Body mass index (BMI) and waist-hip ratio were measured. General examination, and Systemic examination was done and findings were notedwith the help of standard, semi-structured, pre-validated case record proforma. Biochemical tests such as fasting blood glucose, post prandial blood glucose, Glycosylated haemoglobin (HbA1c), fasting lipid profile and liver function tests were done.USG Abdomen and MRI PDFF and Fibroscan were performed wherever necessary Electrocardiography (ECG) and Echocardiography (ECHO) was done.
- Carotid Intima-Media thickness (CIMT) was assessed by trained professional usinghigh resolution B mode ultrasonography system (Phillips HD 11XE) having an electrical linear transducer mid frequency of 7- 12 Mega Hertz.Scans were performed on both the right and left Common Carotid Arteries at 3 points-base,mid junction and just before bifurcation. The IMT is measured as the distance from the leading edge of the first echogenic line to the second echogenic line. The first echogenic line represents the luminal intimal interface and the secondline is produced by the collagen containing upper layer of intimal adventitia.At each longitudinal projection determination of IMTs will be conducted at the sideof greatest thickness and at two points 1 cm upstream and 1cm downstream from the side of greatest thickness as described .The mean of a total of six IMT measurements from both sides were used as the representative value for each subject.The data collected was presented in the form of percentages, frequencies and figures such as tables, charts and graphs. Data will be analysed using SPSS software. Correlation between CIMT and NAFLD was measured.

Results

Age distribution: In the present study we assessed the age distribution among the study subjects. We observed that majority of the study subjects belonged to the age group of 51 to 60 years (42%), followed by 41 to 50 years (24%). The mean age of the study subjects was 52.39 ± 10.07 years.

Table 1: Age distribution

Age distribution	Number of subjects	Percentage
Less than 30 years	2	2
31 to 40 years	9	9
41 to 50 years	24	24
51 to 60 years	42	42
More than 60 years	23	23
Total	100	100

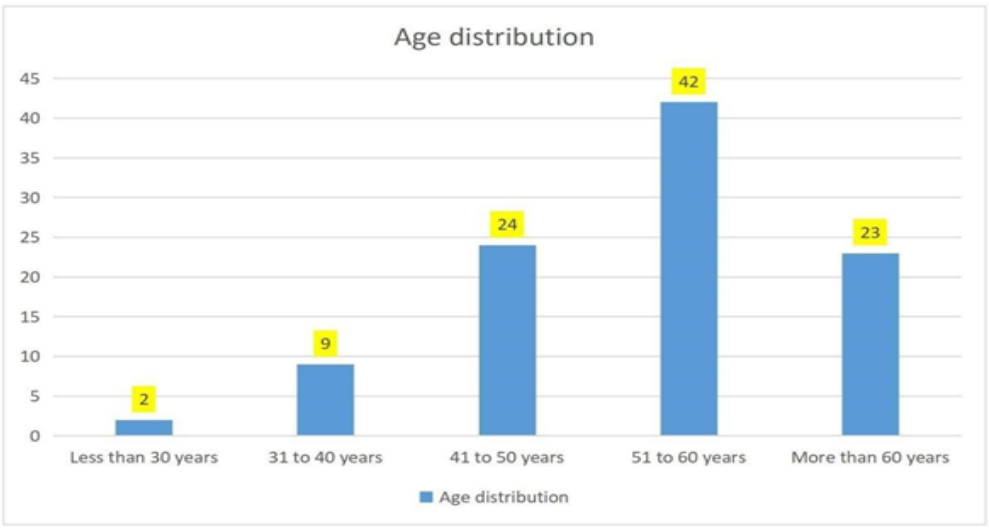

Figure 1: Age distribution

Gender wise distribution

In the current study we assessed the gender wise distribution among the study subjects. We observed that majority were females (57%), followed by 43% were males. The male:female ratio in this study was 1:1.32.

Table 2: Gender wise distribution

Gender wise distribution	Number of subjects	Percentage
Males	43	43
Females	57	57
Total	100	100

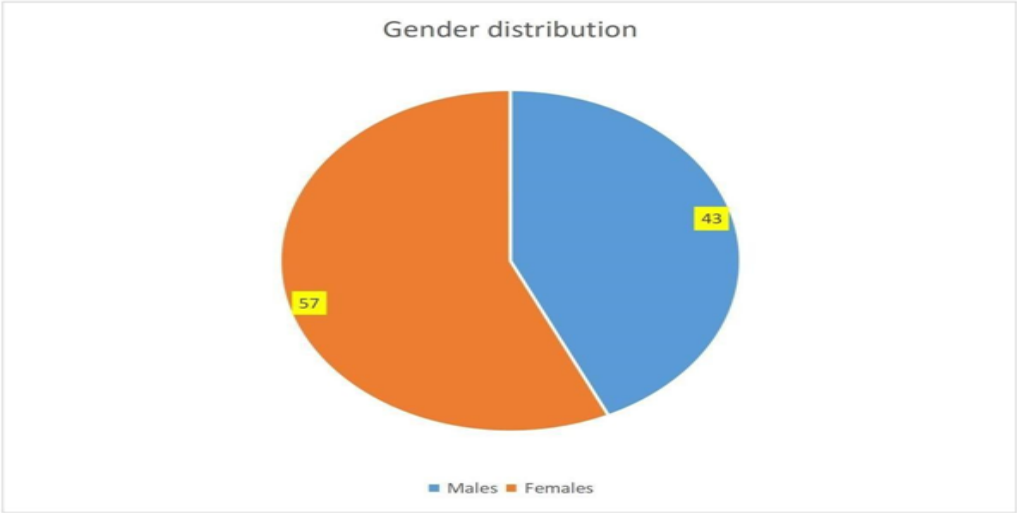

Figure 2: Gender wise distribution

Clinical profile of patients

Table 3: Clinical profile of patients

Parameter	Mean	SD
Height	167.80	5.88
Weight	77.31	8.68
BMI	28.58	2.56
Waist Circumference	87.42	7.97
Pulse Rate	80.92	8.62
Systolic Blood Pressure	130.15	19.73
Diastolic Blood Pressure	75.07	9.27

The mean height of cases in present study was observed to be 167.8 cms, while average weight was noted 77.31 kg, BMI was 28.58 and Waist circumference was
87.42cms. Pulse rate was observed to be 80.92, the mean systolic and diastolic blood pressure was observed to be 130.15 and 75.07 respectively.

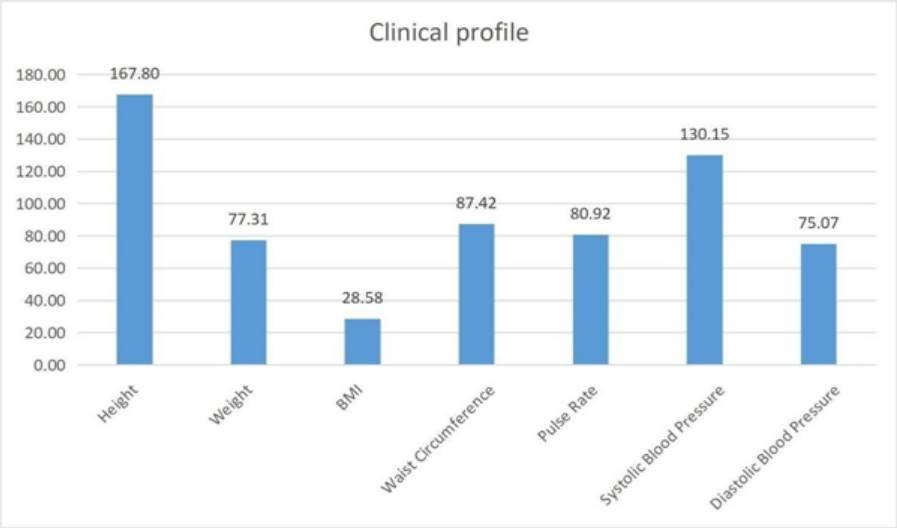

Figure 3: Clinical profile

Biochemical profile

Table 4: Biochemical profile

Parameters	Grade I		Grade II		Grade III		
	Mean	SD	Mean	SD	Mean	SD	p - value
Triglyceride	165.2	52.2	212.2	92.8	224.3	131.2	0.01
Total cholesterol	181.2	38.7	218.4	46.6	252	48.7	0
HDL	44.6	6.6	40.5	5.2	32.9	3.4	0
LDL	103.5	24.4	127.8	27.4	152.1	45.3	0
VLDL	26.1	6.3	27.3	3.4	34.7	7.8	0.001

In the present study triglyceride, total cholesterol, LDL, VLDL significantly increased through Grade I through Grade III. HDL significantly decreased through Grade I through Grade III. It is observed that the mean levels of lipid profile parameters worsens significantly in grade III NAFLD group as compared to grade I NAFLD group.

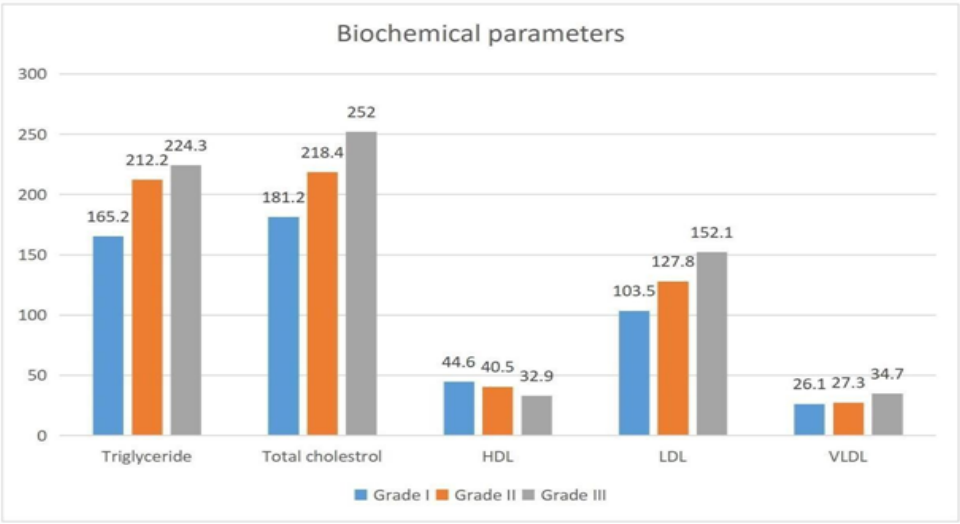

Figure 4: Biochemical profile

Ultrasound findings and comparison with age distribution

Table 5: Ultrasound findings

Age Vs Grades of NAFLD	Grade I	Grade II	Grade III
< 30	0	2	0
31 - 40	4	5	0
41 - 50	7	9	2
51 - 60	11	9	3
> 60	25	18	5
Total	47	43	10

Total 100 cases of Nonalcoholic fatty liver disease were included in this study. They were classified into three grades viz. Grade I, Grade II, Grade III. Total 47% cases were in Grade I, while 43% cases in Grade II and 10% cases were in Grade III. Maximum cases in all grades were aged more than 60 years followed by age group 51-60.

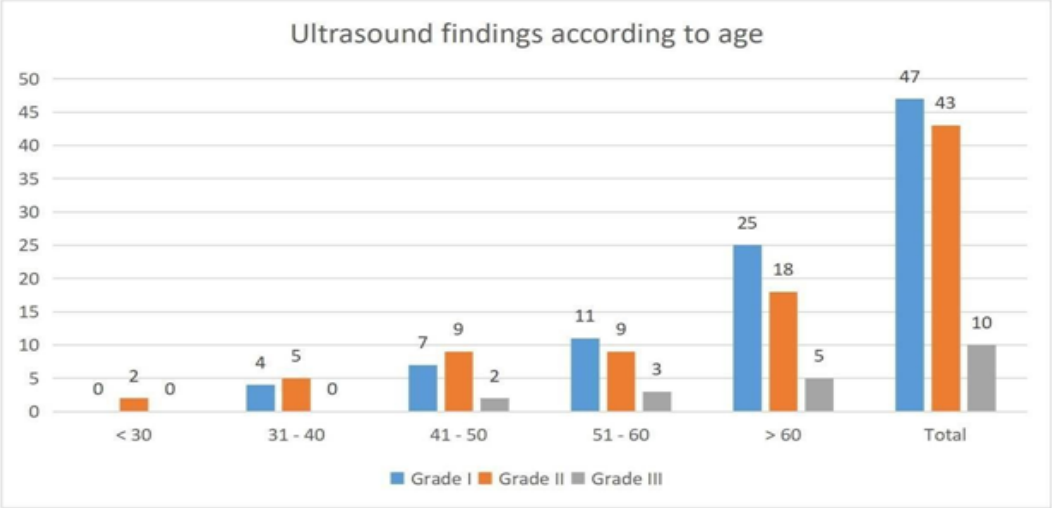

Figure 5: Ultrasound findings

CIMT in NAFLD cases

Table 6: CIMT in NAFLD cases

CIMT	Mean	SD
Right	0.65	0.2
Left	0.66	0.2

The mean CIMT on right side was 0.65 and left side was 0.66.

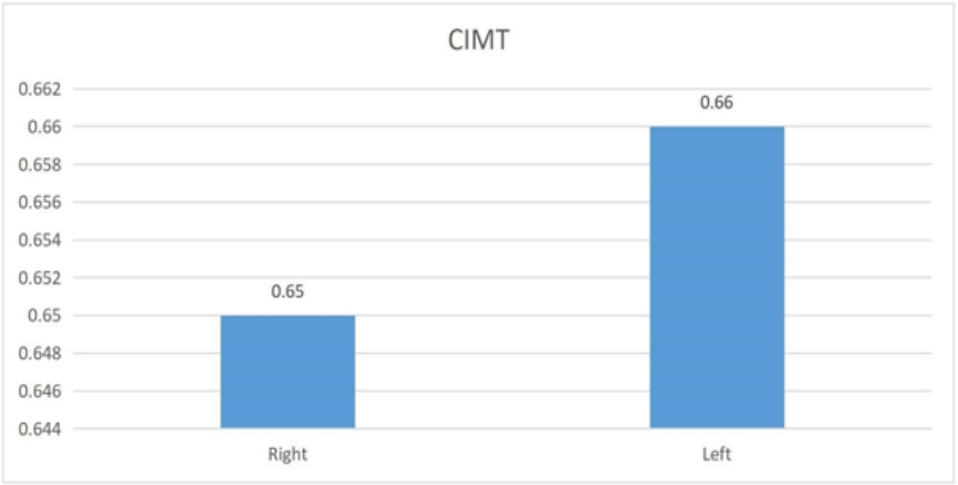

Figure 6: CIMT

Discussion

Non-Alcoholic Fatty Liver Disease (NAFLD) is one of the most common liver diseases reported all over the world. Non-alcoholic fatty liver disease is presented in up to one-third of the general population and is observed in the majority of patients with cardio-metabolic risk factors such as abdominal obesity, type II diabetes, and other metabolic syndrome (MetS) components. Currently, the importance of NAFLD and its relationship with MetS is being increasingly recognized, and this has got increased attention to the possible role of NAFLD in developing cardiovascular diseases. [11]

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. [12]

Additionally, we know that metabolic syndrome includes a group of risk factors such as: abdominal obesity, dyslipidemia, high blood pressure, insulin resistance or glucose intolerance, prothrombotic condition or proinflammatory states. Carotid Intima Media Thickness (CIMT) is a useful tool for detection of sub-clinical atherosclerosis. [13]

Carotid ultrasound is likely to remain among the most widely employed imaging tech niques for the tracking and quantification of subclinical atherosclerosis. The major advantage of CIMT assessment is that it is completely non-invasive and can be repeated as often as required, it is relatively in expensive to perform, the technology is widely available and its methodology is clearly standardized. [14]

The biological mechanisms of accelerated atherosclerosis contributed by NAFLD are still poorly understood. NAFLD itself might act as a stimulus for further increased whole-body insulin resistance and dyslipidemia, leading to accelerated atherosclerosis. Recent prospective studies demonstrated that presence of components of metabolic syndrome plays role in the development of fatty liver disease. [15]

Hence the present study was conducted at tertiary healthcare center to evaluate the clinical, biochemical profile and prevalence of atherosclerosis by measuring the CIMT in Indian patients with incidentally detected NAFLD and to study its relationship with metabolic syndrome. [16]

Demographic variables

Growing age has a correlation with the increased severity of fatty liver disease in this study. In the present study we assessed the age distribution among the study subjects. We observed that majority of the study subjects belonged to the age group of 51 to 60 years (42%), followed by 41 to 50 years (24%). The mean age of the study subjects was 52.39 ± 10.07 years.

In the current study we assessed the gender wise distribution among the study subjects. We observed that majority were females (57%), followed by 43% were males. The male:female ratio in this study was 1:1.32.

Sistani SS et al in their study observed that Forty six percent of the subjects were men and 54% were women, and mean age of fatty liver group (57±10) was almost equal with that of control group (57±8).

Shams F et al in their study observed that the mean age of group A and group B patients were 36.8±12.4 and 46.15±10.3 years, and male:female ratio was 20:29 and 28:24 respectively.85 Grade I fatty liver was more among young age groups whereas grade II fatty liver was higher by increasing age. These findings were similar to the study carried out by Kim.

Clinical profile of patients

The mean height of cases in present study was observed to be 167.8 cms, while average weight was noted 77.31 kg, BMI was 28.58 and Waist circumference was 87.42cms. Pulse rate was observed to be 80.92, the mean systolic and diastolic blood pressure was observed to be 130.15 and 75.07 respectively.

Sistani SS et al in their study observed that Fatty liver group had significantly higher BMI than control group (30.7±5.7 versus 25.7±4.3; p=0.001); significant differences (p=0.001) were observed regarding waist circumference and waist/hip ratio between fatty liver and control groups (103±11 versus 89±10, and 0.93±0.04 versus 0.88±0.04, respectively).

Shalini Kumari et al in their study observed that Body Mass Index, Alanine Transaminase, Aspartate Transaminase, Alkaline Phosphatase, Total Cholesterol, Triglycerides, Low Density Lipoprotein were also found to have statistical significant difference between cases and controls. Age, gender, Blood pressure, Fasting Blood Sugar, HbA1c, Hemoglobin, Total leucocyte Count, Platelet count, Serum Bilirubin, Total protein and Albumin were found to be statistically insignificantly different between cases and controls.

These findings are not concordant with previous result where several other studies showed that NAFLD is commonly associated with visceral obesity, dyslipidemia, insulin resistance, and type 2 diabetes.

Biochemical Profile: In the present study triglyceride, total cholesterol, LDL, VLDL

significantly increased through Grade I through Grade III. HDL significantly decreased through Grade I through Grade III. It is observed that the mean levels of lipid profile parameters worsens significantly in grade III NAFLD group as compared to grade I NAFLD group.

Shalini Kumari et al in their study observed that Body Mass Index, Alanine Transaminase, Aspartate Transaminase, Alkaline Phosphatase, Total Cholesterol, Triglycerides, Low Density Lipoprotein were also found to have statistical significant difference between cases and controls. They concluded that CIMT is increased in NAFLD patients. Increase in CIMT is significantly correlated with increasing grades of NAFLD. Hence CIMT can be used as screening tests in NALFD patients to assess cardiovascular risks.

Shams F et al in their study observed that the serum total cholesterol (mg/dl) was 175.1±41.3 versus 207.3±52.6 (P=0.030) among two groups. Both these parameters were significant. On the other hand mean systolic blood pressure (mm Hg) 127.5±16.1 versus 127.5±16.1 (P=0.836), mean diastolic blood pressure (mm Hg) 81.6±11.6 versus 82.4±8.7 (P=0.7.8), serum triglyceride level (mg/dl) 175.3±106.1 versus 213.4±167.4 (P=0.404) were not significant among groups.

Ultrasound findings and comparison with age distribution

As we stated before ultrasound screening method is a cheap and readily available. NAFLD estimates for up to one third of the total population and in the majority of patients with cardiovascular, metabolic and abdominal obesity, type II diabetes risk factors, can be seen.

In our study out of total 100 cases of Nonalcoholic fatty liver disease included. They were classified into three grades viz. Grade I, Grade II, Grade III. Total 47% cases were in Grade I, while 43% cases in Grade II and 10% cases were in Grade III. Maximum cases in all grades were aged more than 60 years followed by age group 51-60. Grade II and III USG findings were comparatively observed more among advanced age groups as compared to lesser age groups.

CIMT in NAFLD cases

Present study found that the mean CIMT on right side was 0.65 and left side was 0.66. Shalini Kumari et al in their study observed that the mean CIMT in the Case group was 0.86 mm while in Control group was 0.52 mm. There was a significant difference between the 3 Grades of NAFLD in terms of CIMT with a p value of & lt;0.001 with maximum CIMT being in Grade 3 of NAFLD. Their study observed that there was a statistically significant difference between the 2 groups in terms of CIMT with a p value of & lt;0.001. The mean CIMT in the Case group was 0.86 mm while in Control group was 0.52 mm. There was a significant difference between the 3 Grades of NAFLD in terms of CIMT with a p value of & lt;0.001 with maximum CIMT being in Grade 3 of NAFLD.

Our findings revealed NAFLD effect on CIMT was significant. In agreement with this finding, De Andrade et al. (2014) measured CIMT in a cross-sectional study on diabetes patients and showed that CIMT and CVD risk may be higher in those with a family history of type II diabetes. Besides, Nahandi et al. (2014) evaluated the effect of NAFLD on CIMT as a risk factor for atherosclerosis in patients with type II diabetes and reported that there is a significant association between the presence of NAFLD and CIMT and its related atherosclerosis.

Mohammadi et al. (2011) examined patients with confirmed NAFLD for determination of CIMT and presence of carotid atherosclerotic plaque and reported that NAFLD with type II diabetes can be associated with increased CIMT and increased risk of atherosclerosis.

Moreover, Han et al. (2013) studied gender differences in the association between CIMT in healthy individuals and age-related increases in CIMT were correlated with a reduction in cardiac function only in women.

The results of our study are consistent with results of previous studies that are considered CIMT was associated with an increase in NAFLD and suggested it as the marker of early diagnosis of generalized atherosclerosis.[67] This actually means that type II diabetic patients with NAFLD are at greater risk of premature atherosclerosis and Coronary Vessels Disease (CVD).

Studies	Mean Age, y	Mean IMT, mm
ARIC	54	0.63±0.16
Rotterdam Study	70	0.80±0.20
CHS	73	...
Kitamura 2004	66	...
MDCS	57	0.77±0.15
CAPS	50	0.73±0.16
Present study	52.39 ± 10.07 years	Right side=0.65 Left side= 0.66 and

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). In another study, Targher et al. (2007) showed CIMT is higher in patients with NAFLD compared with healthy subjects. Fracanzani et al. (2008) evaluated CIMT values in 125 patients with NAFLD and 250 healthy individuals.

In the study by Nahandi et al., the mean CIMT was significantly higher among NAFLD patients. Their 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 and that is in contrast with Nahandi et al results. One of the major limitations of Petit et al. study was lack of healthy or non-diabetic control.

Shams F et al in their study observed that there was a positive correlation between NAFLD with CIMT. High BMI and raised serum cholesterol has significant role in the development of NAFLD. A large-scale study is recommended to find more accurate cut off of CIMT in the relationship with NAFLD.

Hanafi MG et al in their study observed that the mean right CIMT, left CIMT and overall CIMT in three different study groups of diabetic patients according to various NAFLD grading showed no significant difference, so none of the indicators are related to the severity of liver disease in patients with type II diabetes (Table 3). There was a significant association between gender and only left CIMT and overall CIMT in type II diabetic patients with NAFLD ($p<0.05$), but, no significant association was observed in type II diabetic subjects without NAFLD ($p>0.05$)

However, some degree of variability about the mean CIMT values has been observed among all the published reports that result in a difficult evaluation of the magnitude of the observation. For instance, among the different studies, mean CIMT values in NAFLD patients range from 0.64 ± 0.10 mm to 1.24 ± 0.13 mm.15,19 These results are very much concordant with our study. Targher et al also found that NAFLD was associated with a high cardiovascular risk and, in particular, an increased prevalence of carotid lesions.

Currently available epidemiological data indicate that a value of CIMT at or above 1 mm at any age is associated with a significantly increased risk of myocardial infarction and/or cerebrovascular disease.

There is a positive correlation between NAFLD with CIMT. High BMI and raised serum cholesterol has significant role in the development of NAFLD. Carotid screening for NAFLD might be beneficial for assessment of future atherosclerotic complications.

Conclusion

In conclusion, the study subjects exhibited a mean age of 52.39 ± 10.07 years, with a male-to-female ratio of 1:1.32. The analysis revealed a noteworthy deterioration in the mean levels of lipid profile parameters in the Grade III non-alcoholic fatty liver disease (NAFLD) group compared to the Grade I NAFLD group. Moreover, advanced age groups showed a higher prevalence of Grade II and III ultrasonography (USG) findings compared to their younger counterparts. The mean carotid intima-media thickness (CIMT) was measured at 0.65 on the right side and 0.66 on the left side. These findings collectively underscore the association between age, lipid profile deterioration, NAFLD grade, and USG abnormalities in the studied population.

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