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Original Article

Patients with non-alcoholic fatty liver disease (NAFLD) have an increased risk of atherosclerosis and cardiovascular disease

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is now considered a hepatic component of metabolic syndrome (MS). This condition puts patients with NAFLD at an increased risk of atherosclerosis and cardiovascular disease. This study aimed to assess the prevalence of atherosclerosis and risk of cardiovascular disease in patients with NAFLDand to study its relationship with MS.

Methods: Twenty patients with NAFLD (8 men, mean age 39.90±8.73 years) and 20 age- and gender-matched controls with chronic viral hepatitis (8 men, mean age 39.30±8.21years) were included prospectively in the study. Prevalence of atherosclerosis was studied by measuring the carotid intima-media thickness (CIMT) on carotid ultrasound and by measuring the flow-mediated dilatation% (FMD%) on brachial artery doppler ultrasound. The risk of cardiac events at 10 years (RDCE 10) was estimated by the Prospective Cardiovascular Munster study (PROCAM) score.

Results: The mean CIMT of both the right and left side was significantly higher (0.70±0.11 mm vs. 0.61±0.08 mm) (p=0.007) and FMD% was significantly lower in patients with NAFLD (9.79±3.81%) in comparison to controls (17.02±3.39%) (p<0.0001). The mean PROCAM score was higher in patients with NAFLD (27.50±13.32 vs.20.10±7.75) (p=0.067) with the 10-year risk of acute coronary event being 3.9±6.72% in patients with NAFLD in comparison to 1.44±0.85% in controls (p=0.042). On post hoc analysis, a higher CIMT, PROCAM score and 10-year risk of acute coronary events in patients with NAFLD was dependent on MS.

Conclusion: Patients with NAFLD have an increased risk of atherosclerosis and cardiovascular disease.

KEYWORDS: Non-alcoholic steatohepatitis (NASH), coronary artery disease, diabetes mellitus, hepatitis B virus, hepatitis C virus, non-alcoholic fatty liver disease (NAFLD)

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a broad term which includes patients with simple steatosisas well as non-alcoholic steatohepatitis (NASH).[1]NASH is a recently recognized entity, which histologically simulates alcoholic hepatitis in the absence of alcohol ingestion or intake of <20g/day and has the propensity to progress on tocirrhosis of the liver and hepatocellular carcinoma (HCC).[2–4]

Even though the pathogenesis of NAFLD is not fully understood, it may be considered a two-hit process. The first hit leads to deposition of fat in the liver parenchyma for which

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insulin resistance has been implicated as the major pathogenetic mechanism.[5] The exact mechanisms promoting progressive liver injury are not well defined, although substrates derived from adipose tissue such as free fatty acid (FFA) leading to lipotoxicity, tumour necrosis factor alpha, leptin and adiponectin have been implicated.[6]

Obesity, type 2 diabetes mellitus (T2DM) and hyperlipidaemia are coexisting conditions frequently associated with NAFLD. Because of the strong association with various metabolic abnormalities, NAFLD is now considered a part of spectrum of metabolic syndrome (MS). Patients with MS are at an increased risk for atherosclerosis and cardiovascular disease. In the Third National Health and Nutrition Examination Survey, MS was significantly related to myocardial infarction in either gender.[7] In the Atherosclerosis Risk in Communities (ARIC) study, the subjects with MS were approximately 1.5–2 times more likely to develop coronary artery disease (CAD) than the controls.[8]This puts patients with NAFLD also at an increased risk for atherosclerosis and cardiovascular disease.Presence of atherosclerosis can be assessed non-invasively by an increase in the CIMT (structural atherosclerosis) or by endothelial dysfunction studied by flow-mediated vasodilatation (FMD).[9] Increased risk of cardiovascular disease in a population can be estimated by various scoring systems such asthe Prospective Cardiovascular Munster study (PROCAM) score,[10] Adult Treatment Panel III (ATPIII)[11] or Framingham score.

In India, sparse literature is available on the association of NAFLD with atherosclerosis and cardiovascular events. Studies from the West havesuggested increased CIMT and reduced FMD in patients with NAFLD. It is also suggested that increased risk of atherosclerosis and cardiovascular disease in patients with NAFLD may occur independent of MS.[12–14] Hence,the present study aimed to evaluate the prevalence of atherosclerosis by measuring the CIMT and brachial FMD and assessing the risk of cardiovascular disease by using the PROCAM score in Indian patients with NAFLD and to study its relationship with MS.

Patients and methods

This prospective study was done over a period of one year and included 20 patients with NAFLD (defined as cases) and 20 age- and gender-matched disease controls with chronic hepatitis B or chronic hepatitis C. All patients gave an informed consent and the institute's ethics committee approved the study.

A. Patients (NAFLD group)

Inclusion criteria

1. Non-alcoholic individuals, with age >12 years defined as either total abstainers orwho consumed <20 g of alcohol per day (confirmed by two family members)
2. Raised serum transaminases more than one-and-a-half times the upper limit of normal for at least 3 months
3. Ultrasound showing hyperechoic liver
4. Where available, liver biopsy consistent with NAFLD
5. Negative hepatitis B surface antigen (HBsAg)/antibodies to hepatitis C virus (anti-HCV)
6. Negative autoimmune markers (anti-nuclear antibody, anti-smooth muscle antibody, anti-liver kidney microsomal antibody, anti-mitochondrial antibody)
7. Normal ceruloplasmin/negative Kayser–Fleischer rings
8. Normal iron work-up (serum iron, total iron binding capacity, ferritin, transferrin saturation).

Exclusion criteria

1. Use of drugs such asamiodarone, corticosteroids, tamoxifen, methotrexate or high-dose oestrogens.
2. Jejunoileal bypass or extensive small bowel resection
3. Total parenteral nutrition at the time of liver biopsy
4. Pregnancy
5. Clinical, imaging or liver biopsy features of cirrhosis of liver.

B. Controls (chronic viral hepatitis group)

(a) Chronic hepatitis B

i. Persistent/intermittent elevation in the levels of serum alanine aminotransferase/aspartate aminotransferase (ALT/AST)>1.5 times the normal for >6 months
ii. HBsAg positive for >6 months
iii. Hepatitis B 'e' antigen (HBeAg) positive or HBV DNA >10^4 copies /mL in patients who areHBeAg-negative

(b) Chronic hepatitis C

i. Persistent/intermittent elevation in ALT/AST levels >1.5 times the normal for >6 months
ii. Anti-HCV positive (3rd generation enzyme-linked immunosorbent assay [ELISA])
iii. Positive hepatitis C virus (HCV) RNA

Exclusion criteria: Controls having fatty liver on ultrasound and clinical, imaging or liver biopsy features of cirrhosis of the liver were excluded from the study.

Anthropometry

A body mass index (BMI)(body weight in kg/height in m^2) of >23kg/m^2 but <25kg/m^2 was defined as overweight and a BMI >25 kg/m^2 as obesity. A waist circumference >90 cm in men and >80 cm in women was taken as abnormal.[15,16]

Biochemical work-up

All participants, i.e. both NAFLD cases and controls underwent baseline investigations such aselectrocardiogram (ECG), chest X-ray, complete haemogram, renal function tests, and liver function tests. A fasting plasma glucose (FPG) of >126 mg/dL on more than one occasion or a random plasma glucose of >200 mg/dL in a symptomatic patient or a 2-hour plasma glucose(postprandial glucose [PPG]) of > 200 mg/dL on glucose tolerance test (GTT) was defined as diabetes mellitus. FPG of >110 mg/dL and <126 mg/dL was defined as impaired fasting glucose (IFG) and 2-hour plasma glucose after ingestion of 75 g oral glucose between 140 and 200 mg/dL as impaired glucose tolerance (IGT). Lipid profile was done in all patients and serum cholesterol >200 mg/dL; high-density lipoprotein (HDL) <40 mg/dL in menand <50 mg/dL in women, low-density lipoprotein (LDL) >130 mg/dL; and serum triglycerides (TG) >150 mg/dL was taken as abnormal.[11,17]

Insulin resistance

The Homeostasis Model of Assessment-Insulin Resistance (HOMA-IR) was calculated as the product of fasting insulin (mU/L) and FPG (mmol/L) divided by 22.5. Fasting insulin was measured using Bioline INS IRMA (insulin immunoradiometric assay) kit, Belgium and C peptide by Immunotech IRMA kit, Czech Republic. An absolute value of HOMA-IR >1.64 was taken as abnormal.[18–20]

Metabolic syndrome

The modified ATP III criteria using modified waist circumference (>90 cm in menand >80 cm in women), increased TGs and low HDL cholesterol as defined above, high blood pressure(>130/85mmHg;or on anti-hypertensive drugs), and high fasting blood glucose (FBG) (>110 mg/dL; or a known diabetic) were applied and MS was defined by the presence of three or more of these criteria.[18]

Carotid intima-media thickness (CIMT)

Carotid ultrasounds were performed with 5–12 MHz linear array transducer. Images were focused on the posterior wall of each common carotid artery. A minimum of three measurements of thecommon carotid artery were taken 10mm proximal to the bifurcation to derive mean CIMT, on the left side (CIMT LA) and on the right side (CIMT RA) and average of both the sides (CIMT AV) (**Figure 1**).[21]

Brachial artery flow-mediated dilatation (FMD)

To assess brachial FMD, the diameter of the right brachial artery was measured both at rest and after reactive hyperaemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4 minutes and 30 seconds followed by release.[9]Measurements of arterial diameter were performed at a

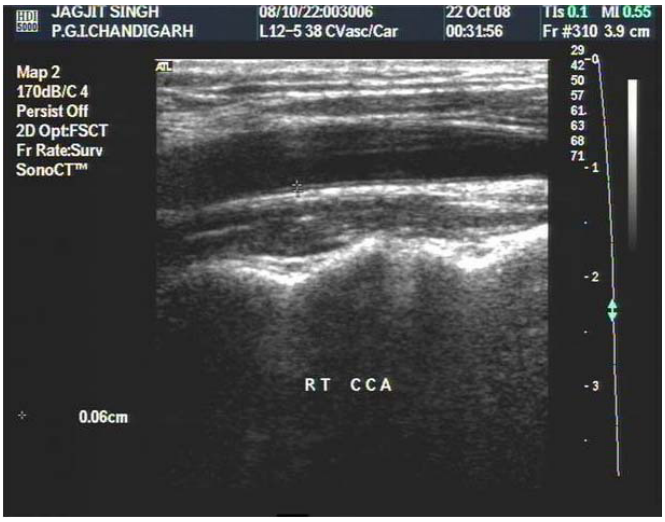

Figure 1: Carotid intima-media thickness (CIMT) measurement in a patient with non-alcoholic fatty liver disease (NAFLD)

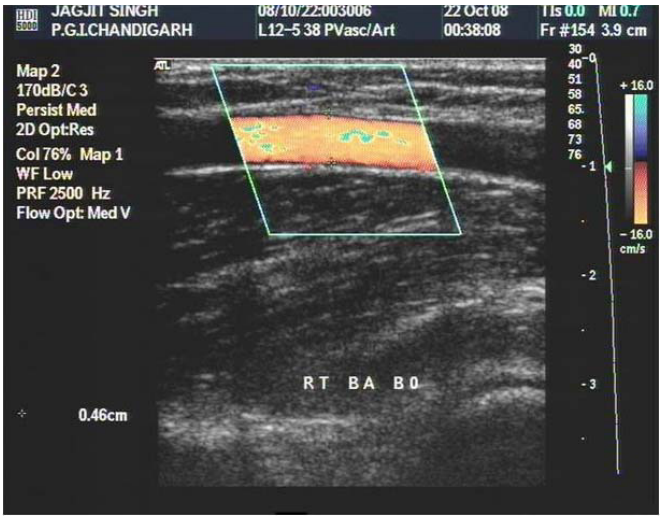

Figure 2: Brachial artery flow-mediated dilatation (FMD) in a patient with non-alcoholic fatty liver disease (NAFLD)

fixed distance from an anatomical marker at rest and at 40, 60 and 80 seconds after the cuff release during systole (**Figure 2**). FMD% was defined as the difference between the brachial artery diameter at rest (BADB) and post-ischaemia (BADAV), divided by BADB (FMD%=BADAV-BADB/BADB). All ultrasounds were analysed by a single reader blinded to the details of the subjects.

PROCAM score

The risk of cardiac events at 10 years (ROCE 10) was estimated by means of the Prospective Cardiovascular Munster study (PROCAM) score.[10]The PROCAM score studies age, blood pressure, diabetes, cigarette smoking, total and low-density cholesterol, TGs and family history of myocardial infarction.

Histopathology

Out of 20 patients with NAFLD, only 9 patients gave their consent for liver biopsy. Grading and staging of liver biopsy was done as per the recommendations by Brunt et al.[22]

Statistical methods

Statistical analysis was performed using SPSS 15.0 software. Variables were expressed as frequencies as well as percentages. Descriptive statistics such as mean, standard deviation (SD) and 95% confidence interval (CI) were calculated for all the continuous variables. Student t test or non-parametric analysis (Mann–Whitney U test) was applied for comparing continuous variables between the cases and controls. Chi-square test was applied for the classified/dichotomous data. The strength of correlation between final outcome variables (CIMT, FMD%, PROCAM score and ROCE 10) and quantitative variables (age, BMI, FBG, ALT, etc.) was determined by Pearson correlation coefficient. Spearman correlation analysis was done to find the correlation between the final outcome variables in both the cases and controls.The final outcome variables were compared among the three study groups (NAFLD patients with MS, without MS and controls) using the Kruskal–Wallis test with subsequent pair-wise post hoc analysis. A 'p' value of <0.05 was taken as statistically significant.

Results

Both the NAFLD and control groups were matched for age and gender (mean age 39.90±8.73 years [cases] vs. 39.30±8.21years [controls]), 8 women in both the groups.

Anthropometric parameters

Thirteen (65%) patients were obese in the NAFLD group compared to 7 (35%) among controls. Five (25%) patients were overweight among the cases compared to 7 (35%) among controls (p=0.127). Thus, only 2 (10%) patients in the NAFLD group and 6 (30%) among controls had normal BMI.Eight (66.7%) out of 12 male patients in the NAFLD group had abnormal waist circumference in comparison to 3 (25%) male controls (p=0.041). Seven (87.5%) out of 8 female patients in the NAFLD group had abnormal waist circumference in comparison to 6 (75%) female controls.

Biochemical parameters

There was no statistically significant difference between the two groups in FBG, PPG, AST, ALT and AST/ALT ratio. Mean ALT was 109.85±56.02 IU/L and 86.70±34.45 IU/L among the cases and controls, respectively (p=0.124). Patients with NAFLD had significantly higher levels of serum LDL, cholesterol, TG and lower HDL levels in comparison to controls.

Insulin resistance, MS and its components

Mean HOMA was 12.11±7.40 in the NAFLD group as compared to 9.82±4.88 amongcontrols with no significant difference (p=0.256).Four (20%) patients among the cases had diabetes in comparison to 2 (10%) patients among controls (p=0.66). Eight (40%) patients among cases were hypertensive in comparison to 1 (5%) among controls(p=0.02). There was no history ofCAD, stroke or peripheral vascular disease in the two groups.High levels of serum TG, levels i.e. >150 mg/dL were present in 12 (60%) cases in comparison to 8(40%) controls (p=0.206). Four men each among the cases and controls had abnormal HDL levels (<40 mg/dL). Seven (87.5%) women among cases had abnormal HDL levels (<50mg/dL) in comparison to 4 (50%) women in the control group (p=0.282).Twelve (60%) patients among cases had MS in comparison to 3 (15%) among controls (p=0.03). In the NAFLD group, more women,i.e. 7 (87.5%) had MS in comparison to 5 (41.6%) men.

Carotid intima-media thickness (CIMT)

The mean CIMT of both the right (CIMT RA)and left side (CIMT LA) and average (CIMT AV)of both the sides were higher among the NAFLD cases in comparison to controls (**Table 1**).

Table 1: Comparison of carotid intima-media thickness (CIMT) among patients with NAFLD and controls

Variable	Mean±SD (mm)	95% CI	p value
Carotid intima-media thickness (left) (CIMT LA)			
• NAFLD	0.72±0.12	0.66–0.78	0.007
• Controls	0.62±0.08	0.58–0.66	
Carotid intima-media thickness (right) (CIMT RA)			
• NAFLD	0.69±0.12	0.63–0.74	0.02
• Controls	0.61±0.08	0.58–0.65	
Carotid intima-media thickness (left+right) (CIMT AV)			
• NAFLD	0.70±0.11	0.65–0.76	0.007
• Controls	0.61±0.08	0.58–0.65	

Table 2: Comparison of brachial artery diameter (baseline), post-ischaemia brachial artery diameter and FMD% among NAFLD cases and controls

Variable	Mean±SD	95% CI	p value
Brachial artery diameter at baseline (BADB)[mm]			
• AFLD	4.15±0.60	3.87–4.43	0.06
• Controls	3.80±0.34	3.64–3.96	
Brachial artery diameter (average) post ischaemia (BADAV) [mm]			
• NAFLD	4.54±0.57	4.27–4.81	0.90
• Controls	4.44±0.33	4.28–4.60	
Flow-mediated dilatation% (FMD%=BADAV-BADB/BADB)			
• NAFLD	9.79±3.81	8.01–11.57	0.000
• Controls	17.02±3.39	15.44–18.61	

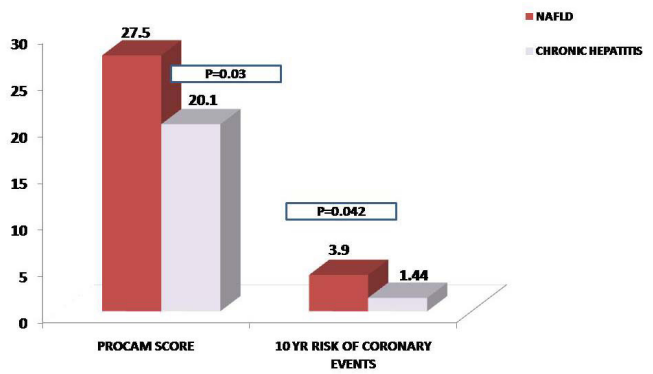

Figure 3: Prospective cardiovascular Munster study (PROCAM) score and 10-year risk of coronary events in patients with non-alcoholic fatty liver disease (NAFLD) and chronic viral hepatitis (Controls)

Flow-mediated dilatation(FMD)

Even though there was no difference in the baseline and post-ischaemia brachial artery diameter, the change in BADAV expressed as FMD% (BADAV-BADB/BADB) was significantly lower in NAFLD cases in comparison to controls (p<0.0001)]suggesting lesser vasodilatation in response to ischaemia in patients with NAFLD (**Table 2**).

PROCAM score

In six (age, LDL, HDL, TG, diabetes and blood pressure) out of eight parameters,the PROCAM score was higher in NAFLD cases in comparison to controls.However, statistically significant difference between the two groups was found for LDL, HDL and blood pressure (p<0.05). Mean of total PROCAM score was higher in cases(27.50±13.32) as compared to controls (20.10±7.75) (**Figure3**).

Risk of acute coronary event

ROCE10 was found to be higher in NAFLD cases (3.9±6.72%) in comparison to controls (1.44±0.85%) (**Figure3**).[3]

Correlation between CIMT, FMD%, PROCAM score and ROCE10

There was a strong inverse correlation between PROCAM score and FMD% (p<0.001).ROCE10 was also found to have strong inverse correlation with FMD% (p<0.001).A positive correlation was found between the PROCAM score and CIMT among cases with NAFLD. ROCE10 was found to have a strong positive correlation with the PROCAM score (p<0.0001) and CIMT.

Comparison of outcome variables in patients of NAFLD with MS, without MS and controls

The final outcomes were compared between patients of NAFLD with MS, without MS and controls. Patients of NAFLD with MS had higher CIMT, PROCAM score, ROCE10 and a lower FMD% in comparison to controls. The PROCAM score was significantly higher in patients with MS (33.33±14.01) in comparison to those without MS ([18.75±5.23] p=0.006).The three groups (NAFLD with MS, without MS and controls) were first compared using the Kruskal–Wallis test (**Table 3**). Since all the three groups showed significant difference, we carried out pair-wise post hoc analysis (**Table 4**). Post hoc analysis showed that increased CIMT and ROCE10 in patients with NAFLD were dependent on the presence of MS (**Table 4**).

Histopathological correlation

Patients with and without liver biopsy were compared for

Table 3: Correlation of CIMT, FMD% and 10-year risk of coronary events with metabolic syndrome (Kruskal–Wallis test)

Variable	n	Mean±SD	95% CI	Kruskal–Wallis test (p value)
CIMT (mm)				
• NAFLD with MS absent	8	0.65±0.08	0.58–0.71	0.010
• NAFLD with MS present		12	0.74±0.13	0.65–0.82
• Controls	20	0.61±0.08	0.58–0.65	
FMD%				
• NAFLD with MS absent	8	8.38±2.99	5.87–10.88	0.000
• NAFLD with MS present	12	10.73±4.11	8.12–13.35	
• Controls	20	17.02±3.39	15.44–18.61	
10-year risk of coronary events				
• NAFLD with MS absent	8	1.15±0.27	0.92–1.37	0.002
• NAFLD with MS present	12	5.72±8.29	0.45–10.99	
• Controls	20	1.44±0.85	1.04–1.84	

CIMT=carotid intima-media thickness; FMD=flow-mediated dilatation; MS=metabolic syndrome

Table 4: Pair-wise correlation of CIMT, FMD% and 10-year risk of coronary events with metabolic syndrome (post hoc analysis)

Variable	Groups	p value
CIMT		
• NAFLD with MS absent	NAFLD with MS present	0.131
• NAFLD with MS absent	Controls	0.639
• NAFLD with MS present	Controls	0.003
FMD%		
• NAFLD with MS absent	NAFLD with MS present	0.324
• NAFLD with MS absent	Controls	0.000
• NAFLD with MS present	Controls	0.000
10-year risk of coronary events		
• NAFLD with MS absent	NAFLD with MS present	0.085
• NAFLD with MS absent	Controls	0.987
• NAFLD with MS present	Controls	0.037

MS=metabolic syndrome; NAFLD=non-alcoholic fatty liver disease; FMD=flow-mediated dilation

baseline parameters such as age, gender, BMI, insulin resistance, AST, waist/hip ratio, systolic blood pressure, lipid profile and FBG.There was statistically no significant difference between patients with and without biopsy. Thus,20patients of NAFLD (with or without liver biopsy) were homogeneous.

All 9 patients who underwent liver biopsy had histological evidence of NASH with presence of steatosis, necroinflammation and fibrosis. Five out of 9 (55%) patients had mild necroinflammation (gradeI) and the remaining4(45%) patients had moderate necroinflammation (gradeII). Seven patients were categorized as having stage I fibrosis and other two having stage II and IV fibrosis, respectively. There was no significant difference in final outcomes (CIMT, FMD%, PROCAM score and ROCE 10) with respect to the grade of necroinflammation. Comparison with respect to fibrosis could not be done because of small number of patients in stage II and IV (one each) fibrosis.

Discussion

In the present study, we compared the presence of atherosclerosis in the form of CIMT and FMD between patients of NAFLD and controls (i.e. chronic hepatitis B/hepatitis C without fatty liver).We found mean CIMT to be significantly higher among patients with NAFLD in comparison to controls. In addition, FMD% was significantly lower in patients with NAFLD as compared to controls. We also studied the risk factors for cardiovascular disease and calculated the ROCE10 using the PROCAM score. We found that the PROCAM score was higher among patients with NAFLD in comparison to controls. Also,ROCE10 was significantly higher in patients with NAFLD as compared to controls.Though FMD% was reduced in patients of NAFLD with and without MS, our post hoc analysis showed that increased CIMT and ROCE10in patients with NAFLD was dependent on the presence of MS (**Table 4**). We also found that CIMTand risk of acute coronary events strongly correlated with the number of MS criteria.

Recently, a lot of data from the Western literature has suggested the increased atherosclerosis and cardiovascular risk in patients with NAFLD but it is still a matter of debate whether NAFLD per se predisposes to these abnormalities or this is all happening because of the presence of metabolic abnormalities or MS. Targheret al. found that patients with NAFLD had greater CIMT (1.14±0.20 vs. 0.82±0.12mm; p<0.001) than controls.[23] They found that MS and its individual components were more frequent in those with NAFLD. The marked difference in CIMT between the groups was slightly weakened after adjustment for MS components. In a cross-sectional study, McKimme et al. evaluated the association between hepatic steatosis and coronary aortic and carotid

artery calcium and CIMT in 623 participants from diabetes heart study. They found a significant association between steatosis and aortic calcium and CIMT which completely disappeared after adjusting for other CVD risk factors including visceral obesity.[24] On the other hand, Volzke and colleagues found that individuals with fatty liver had higher CIMT and more often had carotid plaques than persons without fatty liver (plaque prevalence rate 76.8% vs. 66.6%; p<0.001).[25]This association persisted even after adjustment for confounding factors. Similarly in the study by Brea et al,[14] CIMT was found to be higher in patients with NAFLD than in age- and sex-matched controls (p<0.01). Further, by logistic regression and adjustment for various confounders, the presence of NAFLD was associated with a higher CIMT independently of MS and all its traits.[14]

In our analysis, FMD% which is marker of early atherosclerosis was found to be significantly lower in the NAFLD group (9.7 ±3.81%) in comparison to controls [(17.03±3.39%) p<0.0001], even though there was no difference in FMD% among patients of NAFLD with and without MS. In a study involving 80 patients, Villanova et al.[13] found FMD to be significantly lower in 52 patients (6.33%±5.93%) in comparison to 28 patients without MS [(12.22±5.05%) p<0.001]. The FMD% was also remarkably higher in fatty liver (9.93%) as compared toNASH cases [(4.94%) p=0.01]. Similar to our study, there was a significant difference in BMI and waist circumference between the cases and controls in the above study, thereby suggesting the role of metabolic abnormalities rather than NAFLD per se as the cause of decreased FMD%.

In a recent study from India, Thakur et al. examined the association of subclinical atherosclerosis and endothelial dysfunction in patients with NAFLD.[26] They recruited 40 non-diabetic subjects with NAFLD and 40 apparently healthy controls without NAFLD with similar age, gender and BMI and measured the anthropometric parameters, oral GTT, fasting and 2-hour insulin, lipid profile, C-reactive protein, CIMT and brachial artery FMD. They showed that patients with NAFLD had a higher average and maximum CIMT (0.6±0.12 and 0.684±0.16 mm, respectively, vs. 0.489±0.1 and 0.523±0.1 mm, respectively;p<0.05), and higher prevalence of atherosclerotic plaques (20% vs. 5%,p<0.05) than controls. Significantly higher degree of impairment in FMD was observed in patients with NAFLD than controls. The presence of NAFLD was observed to be an independent predictor of having high average CIMT (OR 4.8; 95% CI: 1.8–12.8), high maximum CIMT (OR 5.4;95% CI: 2.0–14.4) and impaired FMD (OR 11.7; 95% CI: 1.4–96.5) even after adjusting for obesity, MS, insulin resistance and lipid parameters. They concluded that in Asian Indians, NAFLD is significantly associated with subclinical atherosclerosis and endothelial dysfunction independent of obesity and MS.[26] Though our data of increased CIMT and lower FMD% in patients with NAFLD is similar to the results by Thakur et al., our results suggests that increased atherosclerosis and cardiovascular risk in patients with NAFLD is dependent on the presence of MS which is in contrast to the results by Thakur et al.[26] Even though the population cohort of our study and those by Thakur et al. is similar (north Indian patients), the differences between these two studiescould either be related to the small number of patients in our data or to the fact that the increased atherosclerosis and cardiovascular risk in patients with NAFLD is actually dependent on the presence of MS rather than on NAFLD per se.

Increased atherosclerosis has been shown to correlate well with cardiovascular risk factors and severity of coronary atherosclerosis; it reliably predicts the risk of future cardiovascular events.[13,27] We found that the PROCAM score and ROCE10 were higher among patients with NAFLD in comparison to controls and the higher risk was dependent on the presence of MS. Our results are similar to a recent study which found that ROCE10 was moderately increased (p=0.045) in patients with NAFLD calculated according to the Framingham and PROCAM equations.[13]Other studies have also reported increased cardiovascular risk in patients with NAFLD. Kessler et al. found a higher prevalence of NAFLD in patients with myocardial infarction (66% and 50% for women and men, respectively) compared to that found in the general population. NAFLD was also associated with greater severity of CAD. Both these findings were independent of age, gender and BMI.[28] Similarly in a prospective study involving 1221 participants, Hamaguchiet al. found increased incidence of cardiovascular disease (coronary heart disease, ischaemic stroke and cerebral haemorrhage) in 231 patients with NAFLD as comparison to 990 subjects without NAFLD.[29]In fact, a long-term follow-up study of patients with NAFLD and raised liver enzymes, more patients with NASH died of cardiovascular disease rather than liver disease, again suggesting not only a higher prevalence but also higher morbidity and mortality associated with cardiovascular disease in patients with NAFLD in comparison to the general population.[30]

In an Indian study, correlation of NAFLD with CAD and coronary risk factors were studied in a group of persons with type 2 diabetes (with and without NAFLD) in addition to

ultrasonographic measurement of CIMT.[31] They showed that CAD was more prevalent in the NAFLD subgroup (60.5%) compared to the non-NAFLD subgroup (45.2%). The NAFLD subgroup also had a higher prevalence of hypertension, smoking, obesity, central obesity, higher HbAlc, higher TGlevels, lower HDL levels, and higher mean CIMT. Using binary logistic regression analysis, they found that hypertension (p=0.013), LDL cholesterol (p=0.049), microalbuminuria (p=0.034) and NAFLD (p=0.016) were significantly correlated with CAD.[31]In another Indian study, 149 patients with CAD were subjected to routine liver function tests including serum transaminases, enzyme immunoassays for plasminogen activator inhibitor I (PAI-I), C-reactive protein (CRP), and tumour necrosis factor-alpha (TNF-α) and an abdominal ultrasound was done to identify the presence of NAFLD.[32]Patients of CAD with NAFLD had significantly higher liver enzymes and levels of TNF-α and PAI-I were also higher in patients of CAD with NAFLD compared to both womenand men controls ($p<0.1$ and $p<0.05$). Authors suggested that the increased pro-inflammatory status as reflected by serum levels of PAI-I and TNF-α in patients with CAD may be due to the presence of NAFLD which could contribute to the development of cardiovascular disease (CVD).[32]

Ideally, we should have had a control group of patients with NAFLD without MS. Since it was difficult to have patients without any component of MS, we included patients with chronic viral hepatitis (CVH) (chronic hepatitis B [CHB] and chronic hepatitis C [CHC]) as the control group so as to have a group with inflammatory disease of the liver but without steatosis to observe whether the endothelial dysfunction was related to the subclinical inflammation (present in both CVH and NAFLD) or because of the milieu specific to NAFLD. Even though many patients in the control group were overweight or obese and evidence of insulin resistance, absence of steatosis could have decreased the atherosclerosis and cardiovascular risk in these patients. The small number of patients also limits our study but the results suggest that patients with NAFLD have increased atherosclerosis and cardiovascular risk. Further studies with larger sample size are required to establish a link between increased atherosclerosis and cardiovascular risk with MS in patients with NAFLD.

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