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Assessment of Carotid Lesions in Nonalcoholic Fatty Liver Disease Patients

Authors
Sathish Kumar A[1], Sridhar K[2], Srinivas MG[3], Ajith Roni D[4], Srikanth Reddy Y[5], Lalit Nihal[6], Pavan Kumar Reddy Y[7], Panil Kumar K[8], Ram Mohan[9], Pradeep PV[10], Janaki Ram N[11], Mahaboob V Shaik[12], Thankappan KR[13]
[1,2,3,4,5,6,7,11,13]Department of Medical Gastroenterology
[8]Department of Radiology
[9]Department of Pharmacology
[10]Department of Endocrine Surgery
[12]Dept of A.R.C
Corresponding Author
Dr Mahaboob V Shaik
Narayana Medical College & Hospitals, Nellore-524003, Andhrapradesh, INDIA
Email: *drmahaboobvs@gmail.com*

ABSTRACT

Objective: *Assessment of carotid lesions in Nonalcoholic Fatty Liver Disease patients. To know the association of c-IMT with cardiovascular risk factors in NAFLD patients.*

Background: *Nonalcoholic fatty liver disease (NAFLD) affects 10-24% of general population across various countries and in India it varies between 9-32%. Long-term studies revealed that NAFLD patients had an increased mortality rate, with cardiovascular disease being one of the leading causes of death. To this purpose, we evaluated the risk of cardiovascular disease in NAFLD patients using Carotid ultrasound.*

Methods: *50 patients with NAFLD based on USG abdomen were enrolled. Patients were excluded if they were alcoholics or having HBV, HCV, HIV, auto immune, Wilson or having chronic liver disease or pregnant. We measured visceral fat thickness (VFT), subcutaneous fat thickness (SFT), carotid intima media thickness (c-IMT.) and carotid plaque prevalence.*

Results: *A total of 5276 patients were attended to medical gastroenterology OPD during the period of April 2010 to Nov 2011. USG abdomen was done in 826 patients for various reasons, among them 306 (37%) were diagnosed to have fatty liver and 111 (13.5%) out of them were having NAFLD. The Mean age of NAFLD patients was 54.32±9.60 yrs. The mean values of SFT 20.81±4.71 mm, VFT 53.14±17.42 mm and c-IMT 0.86±0.13 mm were higher in NAFLD patients than controls 16.07±3.29, (p=0.000), 40.64±11.46 mm (p= 0.000) and 0.81±0.08 mm, (p <0.05) respectively. Plaques prevalence was also more in NAFLD patients 18/50 (36%) than controls 6/50 (12%) (p-0.009). Among NAFLD patients, the c-IMT was significantly higher in patients with risk factors for cardiovascular disease than those who were not having.*

Conclusion: *The prevalence of NAFLD in our study was 13.5%. C-IMT was significantly higher in the NAFLD group than controls. The relative risk of plaques prevalence was 1.375. Screening of c-IMT might be*

valuable in NAFLD patients, for detecting subclinical atherosclerosis.

Key Words: *Nonalcoholic fatty liver disease, visceral fat thickness, subcutaneous fat thickness, carotid intima media thickness*

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of pathological conditions, ranging from simple steatosis to nonalcoholic steatohepatitis and cirrhosis. It is present in 10-24% of general population across various countries [1,2] and in India it varies between 9-32%.[3] Various studies have proven the association of NAFLD with ageing, sex, obesity, diabetes, hypertension, dyslipidemia, metabolic syndrome (MS) and smoking. Of late, nonalcoholic fatty liver disease has been well thought-out as hepatic manifestation of the metabolic syndrome. Accumulating evidence suggests that cardiovascular disease dictate the outcome in patients with NAFLD more frequently and to a greater extent than does the progression of liver disease. Indeed it is hypothesized that NAFLD is not merely a marker of cardiovascular disease but may also be involved in its pathogenesis. In long-term studies, NAFLD patients had an increased mortality rate, with cardiovascular disease being one of the leading causes of death.[4,5]

Recent studies[6] have shown that NAFLD patients have significantly greater c- IMT than age and sex matched patients without NAFLD, independent of metabolic syndrome. Similar relationship between nonalcoholic fatty liver disease and atherosclerosis has been reported even in apparently healthy men.[7]

Systematic review[8] states that, routine non-invasive screening of c-IMT might be valuable in NAFLD patients, for detecting subclinical atherosclerosis and this could potentially lead to early interventions. Carotid ultrasound is a simple, safe and reproducible way of lumen diameter, intima-media thickness, and the presence and extent of either increased thickness or carotid plaques has been shown to predict myocardial infarction and stroke.[9,10,11,12] To this purpose, we evaluated the risk of cardiovascular disease in NAFLD patients attending to outpatient medical gastroenterology clinics by using carotid ultrasound.

MATERIAL AND METHODS

This study was conducted in the out patient's clinics of Medical Gastroenterology Department, Narayana Medical College, Nellore, during the period of April 2010 to Nov 2011. Institutional ethics committee approved the study and informed consent was obtained from the study participants. A total of 5276 patients attended to Medical Gastroenterology OPD during this period. USG abdomen was done in 826 patients for various reasons, among them 306 patients were diagnosed to have fatty liver and 111 out of them were having NAFLD. From the NAFLD group 50 patients were enrolled into the study. 50 patients who were having normal USG abdomen were

taken as controls. A total of 100 subjects participated in this study.

Patients who were diagnosed as having fatty liver by USG abdomen based on American Gastroenterology Association (AGA) classification of NAFLD[41] were included in the study. Patients were excluded if they were consuming alcohol more than 20 g/ day in female and more than 30 g / day in male, or having HBV, HCV, HIV, Auto immune liver disease, Wilson disease, chronic liver disease , and Pregnancy.[42] Controls were the out-patients with normal USG abdomen. All participants underwent detailed interviews, clinical examination and necessary investigations as required. Patient details were collected in predefined case record forms.

1. Demographic (age, gender, and occupation), behavioral (smoking, alcohol consumption, physical activity, dietary patterns), Personal history of CAD and risk factors were noted.
2. Height was measured at the nearest 0.5 cm using Stature meter. Weight was measured in light clothing, without shoes, to the nearest 0.5 kg, using calibrated weighing machine. Body mass index (BMI) was calculated using formula weight (kg)/height (m^2). Body circumference measurements were recorded using tapes. Waist circumference was taken as circumference at a midpoint between lower rib and iliac crest at the end of expiration.
3. Hepatic steatosis, Visceral Fat Thickness (VFT), Subcutaneous Fat Thickness (SFT) was assessed in all patients by USG abdomen with convex probes (2.5- 5 MHz). Steatosis was defined as the presence of diffuse hyperechoic echotexture, bright liver, increased liver echotexture compared with kidneys, vascular blurring and deep attenuation of the ultrasonic beam. VFT and SFT were measured with the probe placed 1 cm above the umbilicus. VFT was defined as the distance between the linea alba and the anterior wall of aorta and SFT was defined as the distance between skin and linea alba. Carotid Intima-medial Thickness (c-IMT) was measured using high frequency (7.5-13 MHz) (Philips) linear probed Carotid Doppler. All measurements concerning c-IMT and plaques were performed according to the Mannheim Consensus. Longitudinal images of both the left and right side at the level of the common carotid artery, bulb and internal carotid were obtained in each patient. The arterial wall was assessed with appropriate focus, frame rate and gain setting to obtain a symmetrical brightness on the near and far wall. C-IMT was measured in the far wall of the common carotid artery, along a 15 mm section free of plaque from the bifurcation, as the distance between the leading edges of the lumen-intima and media-adventitia interfaces. Maximum rather than mean values of c-IMT were considered, and edge detection was performed manually. C-IMT measurements

from the left and right side were averaged. Values > 0.90 mm should be considered as increased in all cases. A plaque was defined as a focal structure encroaching into the arterial lumen by at least 0.5 mm or 50% of the surrounding IMT value, or having a thickness > 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface. The presence of plaques was evaluated in a 30 mm-long segment both in the left and right common carotid, internal carotid and bulb. All investigations were performed by experienced operators (for abdominal and carotid US), and they were unaware of patients' clinical data.

Definitions used in this study

1. Systolic blood pressure greater than 140 mm of Hg and diastolic blood pressure greater than 90 mm of Hg was considered as hypertension or patients taking antihypertensive medications was considered as hypertension.[39]
2. A random, or casual, blood glucose level of greater than 200 mg/dL or patients taking antidiabetic drugs were considered as having diabetes.[40]
3. Serum total cholesterol, LDL, triglyceride concentrations above ninetieth percentile or HDL below tenth percentile for general population or patients taking antihyperlidemic medications was defined as having dyslipidemia.[40]
4. Past and present smokers were termed as smokers.
5. BMI were categorized according to the international standards into four groups, < 18.5 kg/m² (Chronic energy deficiency), 18.6 kg/m^2 - 24.9 kg/m² (Normal), 25.0 kg/m^2 - 29.9 kg/m^2 (Overweight) and >30 kg/m² (obesity) (National Cholesterol Education Program, 2001).[40]
6. Metabolic syndrome was diagnosed using the guidelines of the National Cholesterol Education Program –modified ATP 3 guidelines (2001).[40]

Statistical Analysis:

All continuous data was expressed as Mean±SD. Categorical data was expressed as numbers and percentages. Unpaired 't' Test and Chi-square Test was used to detect differences between groups. A two tailed probability value less than 0.05 was considered statistically significant.

RESULTS

A total of 5276 patients were attended to medical gastroenterology OPD during the period of April 2010 to Nov 2011. USG abdomen was done in 826 patients for various reasons, among them 306 (37%) were diagnosed to have fatty liver and 111 (13.5%) out of them were having NAFLD. The Mean age of patients in our NAFLD group was 54.32 ± 9.60 yrs. There were 36 males and 14 females, 41 were having age ≥ 45 years and 9 had age < 45 years. Controls were age and gender matched. The Mean age of controls was 54.72 ± 9.73 yrs. 33 were males and 17 were female and

43 were having age ≥ 45 years and 7 had age < 45 years.(Table 1)

It can be seen from the table-1 that the risk factors for cardio vascular diseases such as BMI, DM, HTN, DL, MS, smoking, were similar between two groups. The mean BMI of patients with NAFLD was 27.84±4.02 Kg/m^2, where as in control group it was 27.31±4.05 Kg/m^2 (p=0.51). The prevalence of Diabetes, hypertension, dyslipidaemia, metabolic syndrome, and smoking were 10, 22, 17, 16, and 20 in the NAFLD group respectively. Where as in control group it was 7 (p=0.42), 14(p=0.09), 12(p=0.27), 11(p=0.26) and 18 (p=0.68) respectively. (Table 2)

Table1 Demographic, Anthropometric and Cardiovascular risk profile of study population

Variable	No NAFLD (n=50)		NAFLD (n=50)		P value
Age (Yr)	54.72±9.73		54.32± 9.60		0.83
Age ≥45 Yrs	43 (86%)		41(82%)		0.18
Gender (M/F)	33/17		36/14		0.51
BMI (Kg/m^2)	27.31± 4.05		27.84± 4.02		0.51
WC (cm)	89.90±7.77		94.77±8.63		0.01
Diabetes	7	14.00%	10	20.00%	0.42
Hypertension	14	28.00%	22	44.00%	0.09
Dyslipedemia	12	24.00%	17	34.00%	0.27
Metabolic syndrome	11	22.00%	16	32.00%	0.26
Smoking	18	36.00%	20	40.00%	0.68
VFT (mm)	40.64± 11.46		53.14 ±17.42		< 0.001
SFT (mm)	16.07 ±3.29		20.81± 4.71		< 0.001
c-IMT	0.81±0.08		0.86±0.13		< 0.05
c-IMT ≥ 0.9 mm	9	18.00%	24	48.00%	0.003
Carotid plaque	6	12.00%	18	36.00%	0.009

Table-2: Association of c-IMT with cardiovascular risk factors in NAFLD patients

Variable	c-IMT Mean ±SD (mm)	P value
Gender		0.44
Male (36)	0.84±0.14	
Female (14)	0.88±0.12	
Age		< 0.001
< 45 Yrs (9)	0.74±0.18	
≥ 45 Yrs (41)	0.88±0.10	
Diabetes		0.001
Yes (10)	0.96±0.09	
No (40)	0.83±0.13	
Hypertension		0.001
Yes (22)	0.92±0.10	
No (28)	0.81±0.13	
Dyslipidaemia		0.000
Yes (17)	0.97±0.04	
No (33)	0.80±0.12	
Smoking		0.002
Yes (20)	0.98±0.13	
No (30)	0.82±0.13	
Metabolic syndrome		0.001
Yes (16)	0.94±0.10	
No (34)	0.82±0.12	
Waist circumference		0.002
Abnormal (18)	0.93±0.07	
Normal (32)	0.82±0.14	
BMI (kg/m2)		
<25 (19)	0.76 ± 0.13	a vs. b= 0.009
25-29.9(12)	0.88 ± 0.11	b vs. c= 0.23
>30(19)	0.95 ± 0.06	a vs. c< 0.001

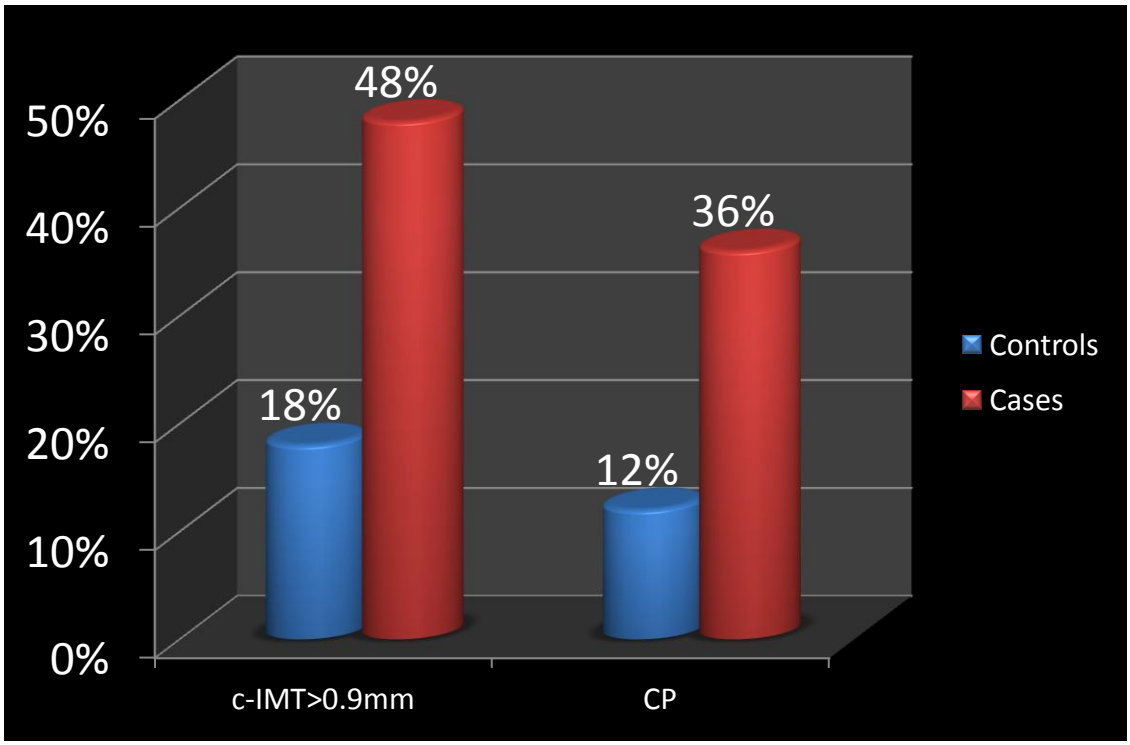

Figure 1: Comparison of C-IMT ≥ 0.9mm (p=0.003) and presence of CP (p=0.009) in Cases and Controls

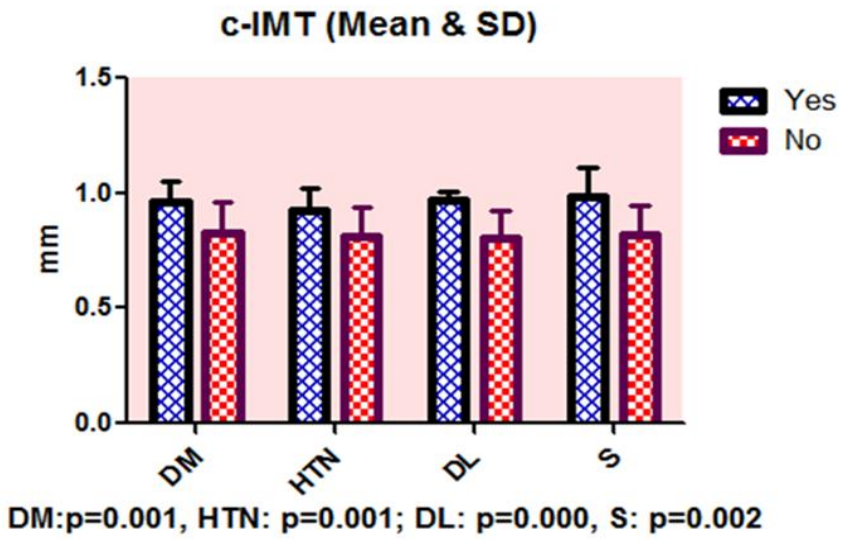

Figure 2: Comparison of C-IMT in patients with or without DM, HTN, DL, Smoking (S)

Mean WC in the NAFLD group was 94.77±8.63 cm, where as in control group it was 89.90±7.77 cm (p=0.01).The mean SFT and VFT in the NAFLD group were 20.81±4.71 mm and 53.14±17.42 mm respectively, where as it was 16.07±3.29, (p<0.001) and 40.64±11.46 mm, (p<0.001) in control group.

We measured Carotid Intima Media Thickness (c-IMT) in our study population and found that, there was a statistically significant higher mean c-IMT vales in the NAFLD group compared to controls. The mean c-IMT in NAFLD group was 0.86±0.13 mm, where as in control group it was 0.81±0.08 mm, ($p < 0.05$). On inspection we found that the proportion of NAFLD patients with c-IMT value ≥ 0.9 mm were 48%(24), whereas it was 18%(9) in control group (p=0.003). The prevalence of plaques in subjects with NAFLD was 18/50 (36%) versus 6/50 (12%) in those without (p= 0.009), which corresponds to a relative risk of 1.375.

It can be noticed from the table-2, that in NAFLD patient group, the c-IMT was significantly higher in patients with risk factors for cardiovascular disease than those who were not having; however such a difference was not noticed in relation to gender.

The mean c-IMT in male vs. females was 0.84±0.14 mm and 0.88±0.12 mm (p=0.44). The mean c-IMT in the patients < 45 years age was 0.74±0.18 mm, whereas in patients with age ≥ 45 years was 0.88±0.10 mm. ($p<0.001$). The mean c-IMT value was 0.96±0.09 mm in diabetes patients, whereas 0.83±0.13 mm in non diabetics (p=0.001). The mean c-IMT value was 0.92±0.10 mm in hypertensive patients, whereas 0.81±0.13 mm in non hypertensive patients (p=0.001). (Figure 1 &2). The mean c-IMT value was 0.97±0.04 mm in dyslipedemic patients whereas it was 0.80±0.12 mm in non dyslipedemic patients. (p=0.000). The mean c-IMT value in smokers was 0.98±0.13 mm, whereas in nonsmokers it was 0.82±0.13 mm (p=0.002). The mean c-IMT value in patients who were having metabolic syndrome was 0.94±0.10 mm, whereas those without metabolic syndrome were 0.82±0.12 mm (p-0.001). The mean c-IMT value noticed in < 25 Kg/m^2 BMI group was 0.76±0.13mm, 25-29.9 Kg/m^2 BMI group was 0.88±0.11mm, and >30 Kg/m^2 BMI group was 0.95±0.06mm. Significant difference in c-IMT values were found between <25 Kg/m^2 & 25-29.9 Kg/m^2 group (p=0.009), and <25 Kg/m^2 & >30 Kg/m^2 group (p=0.0001). The mean c-IMT value was 0.93±0.07 mm in abnormal waist circumference patients whereas 0.82±0.14 mm seen in normal waist circumference patients (p=0.002).

DISCUSSION

In the present study, we evaluated the prevalence of NAFLD, and its association with carotid lesions such as c-IMT and carotid plaques. The prevalence of NAFLD according to The National health and Nutrition Examination Survey ranged 2.8% to 5.5% of participants.[13] At least 10 % of general population in Asia is having NAFLD.[14] Epidemiological studies in India suggest prevalence of NAFLD in around 9% to 32% of general population. A study from coastal regions of India evaluated 159 healthy individuals by ultrasound and found that 24.5% individuals were having NAFLD.[15] In Mumbai railway colony study, the overall prevalence of NAFLD was 16.6%.[16] Another study involving 541 urban south Indian subjects showed prevalence of NAFLD was 32%.[17] In West Bengal community based study involving 1,911 of rural inhabitants the prevalence rate was 8.7 %.[18] The prevalence

of NAFLD in our study was 13.5%. The prevalence of NAFLD may vary with ethnicity, genetic and environmental factors.

We observed significantly higher values of c-IMT in NAFLD patients as compared to Non-NAFLD group. Association of nonalcoholic fatty liver disease and Carotid atherosclerosis was observed across various cross sectional studies.[7,19,20] Su-yeon et.al.,[21] proposed that NAFLD might be an independent risk factor for cardiovascular disease. Recently, a relationship between nonalcoholic fatty liver disease and atherosclerosis has been reported even in healthy men.[7] Additionally, we noticed that the prevalence of plaques in our NAFLD patients was higher than in patients without NAFLD (P=0.009), which corresponds to a relative risk of 1.375. Few studies reported no significant association between NAFLD and carotid intima media thickness.[6] However, Henry Volzke et.al[20] found that the hepatic steatosis was associated only with carotid atherosclerotic plaques but not with c-IMT . The lack of concordance of these results may not only relate to non standardized measurement methods but duration of disease, genetic and environmental factors could have accounted for such a variation.

The mechanism linking NAFLD and atherosclerosis is not completely understood. Potential mechanisms which may increase cardiovascular risk in patients with NAFLD includes metabolic syndrome, obesity, HTN, DM, DL, smoking, abnormal lipoprotein metabolism[3], and flat postprandial apoB responses.[22] However, Vincent wai-sun wong et.al.,[23] showed that the association between NAFLD and coronary artery disease is independent of these metabolic factors. Similarly we also observed in our study that the mean c-IMT and carotid plaque prevalence was statistically significantly different between NAFLD group and controls, even though there was no significant difference in other traditional cardiovascular risk factors such as age, sex, obesity, diabetes, hypertension and dyslipidemia.

One interesting observation noted in our study that, for a similar demographic such as age and gender, anthropometric such as BMI and cardiovascular risk profiles such as diabetes, hypertension, dyslipidemia, smoking, metabolic syndrome, between NAFLD patients and controls, WC&VFT a marker of central obesity were significantly higher in NAFLD than control group. Angulo P *et.al*[24] and Fan J G *et.al*[25] observed that that central obesity is associated with NAFLD, even in patients with normal BMI.

In our NAFLD group c-IMT values were significantly associated with all cardiovascular risk factors such as ageing, diabetes, hypertension, dyslipidemia, smoking, metabolic syndrome, and obesity except for gender.

The Valpolicella Heart Diabetes Study found the association between NAFLD and vascular diseases in 250 patients with type 1 diabetes[12] and the same group observed the risk of cardiovascular disease was twice in type-2 diabetic patients[11] Similarly, we also found higher values of c-IMT in diabetic NAFLD patients. However, Jean Michel et.al did not find association of NAFLD and increased C-IMT in diabetics.[6]

He S et.al.[26] showed significant association of NAFLD with Hypertension and the same was confirmed by Zuou et.,al.[27] In the Cardiovascular Health Study which consisted of 5201 patients done by Daniel H O'Leary et al[9] observed that hypertension and SBP significantly correlated with c-IMT. In our study we observed significantly higher values of c-IMT in Hypertensive NAFLD patients. In patients with essential hypertension, carotid wall thickening is associated with reduced endothelium-dependent vasodilation.

Yamasaki Yoshimitsu et al. (2000) [28] concluded that total cholesterol was significantly related to progress of c-IMT. Michael Davidson et.al showed the inverse relationship between HDL cholesterol and c-IMT by studying the beneficial effect of pioglitazone on HDL cholesterol which predicted less progression of c-IMT. Ananth Oren et al in the ARYA study[29] and Lloyd et al in ARIC study[10] demonstrated significant positive association between LDL and c-IMT. Similar observations were found in dyslipedemic NAFLD patients in our study. It appears that Hepatic steatosis results in increased insulin resistance and such a mechanism might be responsible for increased c-IMT.

Similarly cigarette smoking is an independent risk factor for the onset of NAFLD partly through its effect on insulin resistance.[30] Large multicenter trial (Zein et.al)[31] pointed that cigarette smoking increases the severity of fibrosis in NAFLD patients. However no difference in NAFLD prevalence were observed between current smokers and nonsmokers by Chavez-tapia et.al.[32]

The Cardiovascular study - Young Finnsh study done by Olli T Raitakeri et al(2003) [33] demonstrated that smoking significantly contributes to atherosclerosis. C-IMT values were higher among smokers in our NAFLD group. It is possible one or multiple mechanisms such as, hepatic disease, insulin resistance, reduced HDL levels, Oxidative stress and hypoxia in the arterial wall might be responsible for such higher c-IMT values in our study group.

The majority of NAFLD occurs in obese or overweight individuals. Olli T Raitakari et al (2003)[33] and the Atherosclerosis Risk in Young Adults (ARYA) study by Anath Oren et al (2003) [29] concluded that increase in BMI is associated with increase in c-IMT. Targher et al found a significant increase in c-IMT in patients with non-alcoholic hepatic steatosis and hypothesized that visceral fat accumulation might be the possible reason for such an increase in c-MIT. In concordance with the above studies the mean c-IMT values in our study were statistically significant between <25kg/m2 & 25-29.9kg/m2 BMI groups (p-0.009), and <25kg/m2 &>30kg /m2 BMI groups (p-0.0001). Mean c-IMT was significantly higher in abnormal waist patients (p-0.002). The high prevalence rate of obesity in NAFLD may be explained by its association with hepatic steatosis.107 It should be emphasized that the distribution of fat such as visceral fat may be more important than the total fat mass.[34,35]

It is now recognized that metabolic syndrome is important in the pathophysiology of NAFLD and is present even in NAFLD patients who are normal weight and have normal carbohydrate

tolerance.[36, 37] There is a strong association of NAFLD with individual features of metabolic syndrome, such as obesity, type 2 diabetes,, and dyslipidemia, or with complete syndrome. Matteoni et.al [38] observed the association of NAFLD with Metabolic syndrome and cardiovascular mortality in their study. In our study the mean c-IMT value was significantly higher in NAFLD patients with metabolic syndrome (p-0.001). Insulin resistance and enhanced oxidative stress plays a major role in the development of the metabolic syndrome and cardiovascular disease.

CONCLUSIONS

- The prevalence of NAFLD in our study was 13.5%.
- The mean c-IMT and prevalence of carotid plaques were significantly higher in NAFLD subjects when compared to controls. The relative risk of plaque prevelance in NAFLD patients was 1.37.
- Significantly higher mean c-IMT values noted in NAFLD patients in relation to cardiovascular risk factors such as Diabetes, Hypertension, Dyslipidemia, Smoking, Metabolic Syndrome, and obesity.
- Screening of c-IMT might be valuable in NAFLD patients, for detecting subclinical atherosclerosis and prevention of cardiovascular mishaps. However to prove this association large number of subjects with or without NAFLD need to be studied for carotid atherosclerosis and cardiovascular risk factors.

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