

Non-alcoholic Fatty Liver Disease (NAFLD) and its association with metabolic syndrome and cardiovascular diseases

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Abstract— *Non Alcoholic Fatty Liver Disease is also becoming public health importance nowadays. So this study was aimed to determine the association of Non Alcoholic Fatty Liver Disease with metabolic syndrome and Cardio-Vascular disease along with assessment of degree of severity of NAFLD with respect to number of components of metabolic syndrome. This study includes a total of 222 subjects were enrolled as per the inclusion/exclusion criteria, out of which 110 cases who had NAFLD with hepatic steatosis on ultrasonography and 112 subjects who did not have NAFLD were considered control. These cases and controls were interrogated and investigated further. Observations were recorded and association of Non Alcoholic Fatty Liver Disease with metabolic syndrome and Cardio-Vascular disease along with assessment of degree of severity of NAFLD with respect to number of components of metabolic syndrome. Statistical methods used were unpaired student's t-test for continuous variables, Fischer's and chi-sq test for categorical variables using bivariate analysis by Graph Pad Instat Version 3.10. Risk was assessed in terms of Odd's Ratio. The patients with MS and NAFLD had a higher proportion of CVD compared with those who did not have NAFLD (29.1 vs 18.1 %). This study concludes that NAFLD is significantly associated with MS; most significant with WC, followed by TG and FBS and thus can be considered as hepatic component of MS. This needs more research with large multi-centric prospective studies to evaluate NAFLD as an independent risk factor for CVD.*

Keywords—*CVD: Cardio Vascular Disease, MS: Metabolic Syndrome, NAFLD: Non-Alcoholic Fatty Liver Disease, NASH: Non-Alcoholic Steato Hepatitis*

I. INTRODUCTION

Non - alcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function tests among adults ¹⁻⁴. NAFLD refers to a wide spectrum of hepatic disorders in clinical practice ranging from simple fatty liver to non - alcoholic steatohepatitis (NASH) to cirrhosis. The definition of non-alcoholic fatty liver disease (NAFLD) requires that (a) there is evidence of hepatic steatosis, either by imaging or by histology and (b) there are no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders ⁵. Approximately 20 to 30% of adults in the general population in Western countries have non-alcoholic fatty liver disease, and its prevalence increases to 70 to 90% among persons who are obese or have diabetes; such patients are also at increased risk for the development of advanced fibrosis and cirrhosis ^{1, 6-8}. Obesity is a common and well documented risk factor for NAFLD. Both excessive BMI and visceral obesity are recognized risk factors for NAFLD. The metabolic syndrome, as well as each of its five components, is strong risk factors for the presence of NAFLD ⁹⁻¹². This has led investigators to suggest that NAFLD is a component of the Metabolic Syndrome ^{13, 14}. ATP III introduced the metabolic syndrome into its clinical guidelines in the effort to achieve CVD risk reduction beyond LDL-lowering therapy. People with NAFLD harbour the same cardiovascular risk factors (hypertension, dyslipidemia, obesity, physical inactivity, insulin resistance, endothelial dysfunction, and inflammation) as metabolic syndrome that places them at higher risk of cardiovascular events.

There is paucity of Indian studies on prevalence of NAFLD and its relationship to metabolic syndrome in the normal population. With the presence of metabolic syndrome in epidemic proportions in the Indian population it is fair to say that there may be an underlying silent epidemic of NAFLD but its prevalence, rate of progression, impact on quality of life as well as life expectancy needs to be explored further. Similarly, there are strong suggestions linking cardiovascular disease to NAFLD as that to the metabolic syndrome but limited conclusive data are available at present. So this present study is conducted with the primary objective of this study was to determine the association of NAFLD with metabolic syndrome and thus with CVD in the Indian population, by using clinical and laboratory data and simple cost effective, non invasive method; ultrasonogram of the liver. A secondary objective of this study is to determine an association between NAFLD and CVD independent of the metabolic syndrome.

II. METHODOLOGY

This hospital based case-control type of analytic observational study was conducted at Government Medical College Hospital, Kota (Rajasthan) over a period of two and a half years from January 2012 to January 2013.

Study population: This study was conducted in patients attending medical out-patient department and indoor patients during the study period of one year since 15th January 2012 – 14th January 2013. In this study any patient >18 years either of sex attending at medical out-patient department as well as indoor patients during the study period. Patient who taking alcohol or had H/o alcohol consumption and h/o taking drugs known to cause steatosis including Amiodarone, Corticosteroids, Tamoxifen, Methotrexate and high dose Estrogen etc were excluded from study. Even patient with chronic viral hepatitis and /or drug induced hepatitis were also excluded from study. Females who were pregnant at the time of study were also excluded from study. Out of these subjects, patients showing hepatic steatosis or fatty liver on Ultrasonography (USG) were included in study group and other who did not show hepatic steatosis or fatty liver on Ultrasonography (USG) were considered as control.

Ultimately a total of 222 eligible subjects were enrolled in this study as per the inclusion/exclusion criteria, out of which 110 case subjects had hepatic steatosis on ultrasonography and 112 control subjects with no hepatic steatosis on ultrasonography.

These 222 subjects were further divided into following four groups;

1. Group 1 (n=72) - NAFLD with Metabolic Syndrome
2. Group 2 (n=38) - NAFLD without Metabolic Syndrome
3. Group 3 (n=44) - Non NAFLD with Metabolic Syndrome
4. Group 4 (n=68) - Non NAFLD without Metabolic Syndrome

Detailed history was taken and scrutiny of previous medical record was done with thorough clinical examination of every patient included in the study. Subjects were enrolled after satisfying the inclusion/exclusion criteria. Complete Laboratory work up data were collected from patient like Fasting plasma glucose (FBS), Total Lipid Profile: Serum total cholesterol, HDL, LDL and triglycerides after overnight fasting, Liver function Tests: Serum Bilirubin, AST, ALT, ALP, HbsAg and Anti HCV antibody. Data regarding Standard 12 lead ECG, Abdominal Ultrasonography, Anthropometric measurement (Height (m), Weight (Kg), Waist circumference (cm)) were also collected along with laboratory work up to identify cases with metabolic syndrome.

All subjects underwent trans-abdominal ultrasonography performed by a single radiologist for evidence of fatty liver disease. The diagnosis of hepatic steatosis was made based on characteristic

ultrasonographic findings (diffuse increase in echogenicity as compared to that of the spleen or renal cortex). The severity of fatty liver was recorded as mild, moderate or severe fatty liver according to the findings of bright liver, hepato-renal echo contrast, the blurring of vessels and deep attenuation of ultrasound signal¹.

Metabolic syndrome was diagnosed by the NCEP ATP III (Adult treatment Panel III)¹⁴ in accordance with this definition a subject is classified as having the features of Metabolic Syndrome if one had at least three of the following five components: Waist circumference >102cm in men or >88cm in women, Serum Fasting Triglycerides >150mg/dl, Serum Fasting HDL <40mg/dl in men and <50mg/dl in women, Blood pressure >130/85 mm Hg or receiving treatment, Fasting plasma glucose \geq 100 mg/dL.

Cardio Vascular Disease (CVD) was considered on the basis of clinical assessment for recent or past coronary artery disease, any vascular event suggestive of recent or past cerebral infarction and peripheral vascular disease, with detailed review of available medical records for supportive documentary evidence. In the lack of available medical data, cardiovascular disease was considered on the basis of electrocardiogram using the Minnesota criteria. The Minnesota Code¹⁶ has become the most widely used ECG classification system in epidemiologic studies, and its application significantly improved standardization of ECG measurements. Problems are encountered with this approach if many features are used in classification criteria such as the thresholds are not optimal and the sensitivity of the criteria tends to be low, also there may be considerable degree of classification instability whereby a single error can easily result in misclassification.

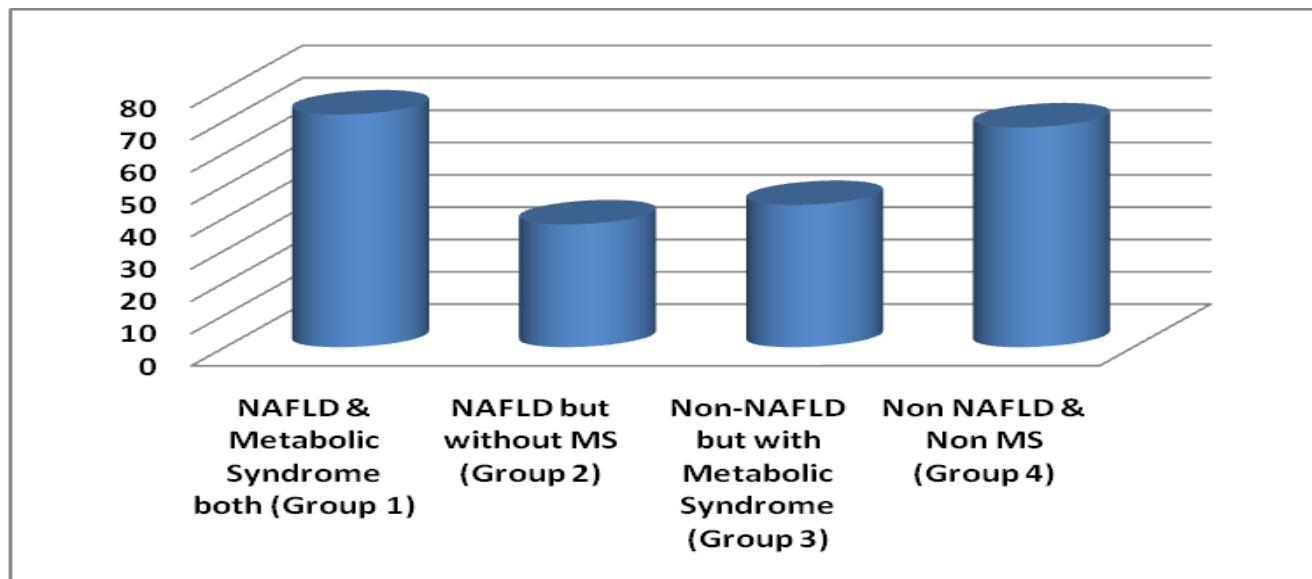
Statistical Analysis:

Statistical methods used were unpaired student's t-test for continuous variables, Fischer's and chi-sq test for categorical variables using bivariate analysis by Graph Pad InStat Version 3.1. $P < 0.05$ was considered as significant. Odds ratio with 95% confidence interval was defined as OR=1 Exposure does not affect odds of outcome, OR>1 Exposure associated with higher odds of outcome, OR<1 Exposure associated with lower odds of outcome.

III. RESULTS

This study was conducted on 222 eligible subject, out of which 110 subjects were with hepatic steatosis on ultrasonography i.e. Non alcoholic Fatty Liver Disease (NAFLD) and 112 subjects were without hepatic steatosis on ultrasonography. Out of these 110 NAFLD cases, 72 (65.45%) were with one or other Metabolic syndrome whereas in Non-NAFLD subjects only 44 (39.29%) were with one or other Metabolic syndrome. This difference in proportion of subjects with one or other Metabolic syndrome in Study group (NAFLD) and control group (Non-NAFLD) was found significant ($p < 0.001$). (Figure 1)

Likewise metabolic disorder, it was also found that 79% of the subjects in the study group (Group 1&2) were either overweight or obese as compared to only 20.4% in the corresponding control group (Group 3&4). (Figure 1)

Figure 1**Distribution of Study population as per various of Groups**

Chi-square (between NAFLD with and without MS) = 14.202 with 1 DF P < 0.001 LS=S

Figure 2 shows the mean values of FBS, TC, TLDL, TG, HDL, WC and BMI along with age of subjects in group 1 and group 3. Mean age of both the group i.e. group1 and group 3 male were 55.3 and 48.54 years respectively, while for female these values were 52.7 and 47.73 years respectively. Overall mean age \pm SD of all subjects of group1 and group 3 were 53.55 ± 11.35 and 51.39 ± 13.77 years respectively. This difference in mean age of group 1 and group 3 was not found significant ($p > 0.05$).

When it was further analyzed, it was found that FBS was higher in Group 1 subjects (123.3 ± 40.4) in comparison to Group 3 subjects (104.5 ± 12.6). This difference in mean FBS level of group 1 and group 3 was found significant ($p < 0.05$). Likewise FBS, Serum triglycerides (TG) in the group 1 was higher with mean TG 176 ± 78.6 and among controls it was 127.7 ± 40.42 . This difference in mean TG level of group 1 and group 3 was found significant ($p < 0.001$).

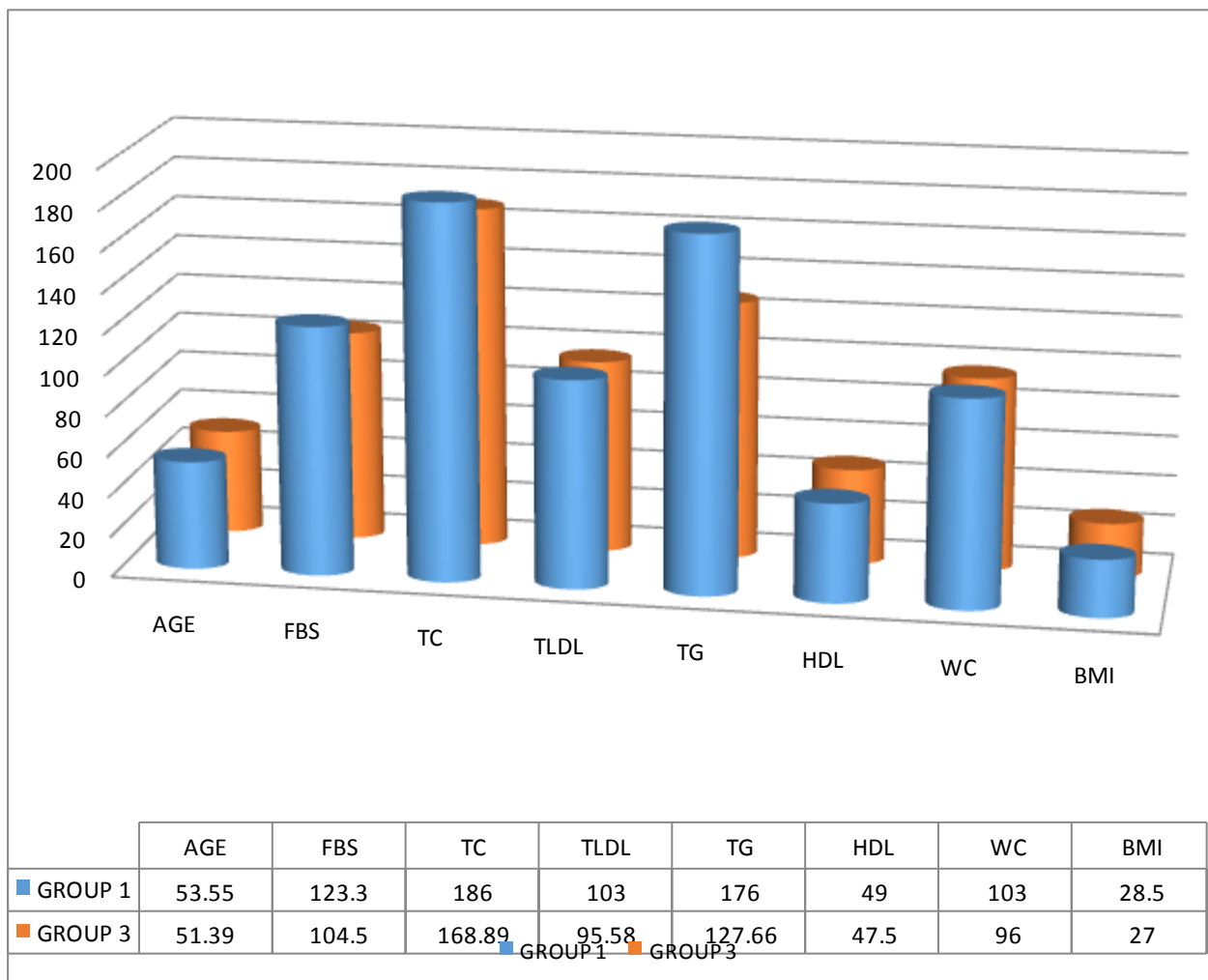
But unlikely to FBS and TG, mean TLDL, HDL and TC level in both the group i.e. group 1 and group 3 were not found significant ($p > 0.05$). Mean Fasting Serum LDL in Group 1 subjects were 103 ± 35.9 and in Group 3 subjects was 95.9 ± 39.5 . Likewise mean serum HDL in group 1 subjects and group 3 subjects were 49 ± 13.4 and 47.5 ± 10.89 respectively.

Mean fasting serum TC was observed higher in Group 1 subjects (186.00 ± 44.9) in comparison to Group 3 subjects (168.89 ± 49.3). But this difference in mean TC level of group 1 and group 3 was also not found significant ($p = 0.053$).

When mean WC in Group 1 subjects which observed was 103 ± 12 was compared with mean WC in Group 3 subjects i.e. 96 ± 13.9 . This difference in mean WC level of group 1 and group 3 was found significant ($P = 0.005$) (Figure 2)

Figure 2

Comparison of Mean values of Quantitative Variables of Group (1) and Group (3)



1. Unpaired t test for Age= 0.916 with 114 degrees of freedom; P = 0.361 LS=NS
2. Unpaired t test for FBS= 2.995 with 114 degrees of freedom; P = 0.003 LS=S
3. Unpaired t test for TC= 1.953 with 114 degrees of freedom; P = 0.053 LS=NS
4. Unpaired t test for TLDL= 1.040 with 114 degrees of freedom; P =0.301 LS=NS
5. Unpaired t test for TG= 3.781 with 114 degrees of freedom; P <0.001 LS=S
6. Unpaired t test for HDL= 0.626 with 114 degrees of freedom; P = 0.532 LS=NS
7. Unpaired t test for WC= 2.869 with 114 degrees of freedom; P = 0.005 LS=S
8. Unpaired t test for BMI= 1.389 with 114 degrees of freedom; P = 0.168 LS=NS

Overall mean age \pm SD of all subjects of group1 and group2 were 53.55 ± 11.35 and 47.57 ± 13.28 years respectively. Likewise overall mean age \pm SD of all subjects of group 3 and group 4 were 51.39 ± 13.77 and 46.87 ± 14.61 years respectively. These mean ages in different groups were found comparable with significant difference only in group 1 and group 4. (Table 1)

Table 1
Comparison of various Groups as per Age of subject

S. No.	Various Groups	No. of Subjects	Mean Age (Yrs)	SD of Age (Yrs)
1	NAFLD & Metabolic Syndrome both (Group 1)	72	53.55	11.35
2	NAFLD but without MS (Group 2)	38	47.57	13.28
3	Non-NAFLD but with Metabolic Syndrome (Group 3)	44	51.39	13.77
4	Non NAFLD & Non MS (Group 4)	68	46.87	14.61

ANOVA= 3.59 P = 0.015 LS=S

--- Multiple Comparisons - By Post-hoc Tukey Test---

Comparison	Difference of means	SE	P<.05
1 vs 4:	53.55 - 46.87 = 6.68	1.581	Yes
1 vs 2:	53.55 - 47.57 = 5.98	1.875	No
1 vs 3:	53.55 - 51.39 = 2.16		Do not test
3 vs 4:	51.39 - 46.87 = 4.52	1.809	No
3 vs 2:	51.39 - 47.57 = 3.82		Do not test
2 vs 4:	47.57 - 46.87 = 0.7	1.894	No

When association of NAFLD and metabolic syndrome was observed it was found that although metabolic syndrome was observed higher in NAFLD (group 1 & 2) than non NAFLD group (group 3 & 4) i.e. 65.45% and 39.29% respectively but this difference was not found significant. But when association of NAFLD and CVDs was observed it was found that CVDs were observed significantly (p<0.05) higher in NAFLD (group 1 & 2) than non NAFLD group (group 3 & 4) i.e. 24.55% and 10.71% respectively. (Table 2)

Table 2
Association of NAFLD with Cardiovascular Diseases and Metabolic Syndrome

S. No.	Group Variables	CVD Present		CVD Absent		Total	
		No.	(%)	No.	(%)	No.	(%)
1	NAFLD & Metabolic Syndrome both (Group 1)	21	29.17	51	70.83	72	32.43
2	NAFLD but without MS (Group 2)	6	15.79	32	84.21	38	17.12
3	Non-NAFLD but with Metabolic Syndrome (Group 3)	8	18.18	36	81.82	44	19.82
4	Non NAFLD & Non MS (Group 4)	4	5.88	64	94.12	68	30.63
5	Total	39	17.57	183	82.43	222	100.00

Chi-square = 13.195 with 3 degrees of freedom; P = 0.005

When CVDs risk analysis was done in this study it was found that although there were more chances to have CVDs in cases with either of NAFLD and Metabolic syndrome but Odd's ratio was found significant only in cases who had both NAFLD and Metabolic syndrome (group 1) than who did not have any of two (group 4). (Table 3)

Table 3**Risk Analysis of Cardiovascular Diseases with and without NAFLD and Metabolic Syndrome**

Group Combinations	Group Elaboration	CVD Present	CVD Absent	Total	OR with 95% CL.	
					P Value LS	
Group 1 v/s Group 2	NAFLD & Metabolic Syndrome both (Group 1)	21	51	72	2.196 (0.8 to 6.025)	P=0.188 NS
	NAFLD but without MS (Group 2)	6	32	38		
Group 1 v/s Group 3	NAFLD & Metabolic Syndrome both (Group 1)	21	51	72	1.853 (0.739 to 4.646)	P=0.269 NS
	Non-NAFLD but with Metabolic Syndrome (Group 3)	8	36	44		
Group 1 v/s Group 4	NAFLD & Metabolic Syndrome both (Group 1)	21	51	72	6.588 (2.127 to 20.411)	P<0.001 S
	Non NAFLD & Non MS (Group 4)	4	64	68		
Group 2 v/s Group 3	NAFLD but without MS (Group 2)	6	32	38	0.844 (0.264 to 2.693)	P=0.994 NS
	Non-NAFLD but with Metabolic Syndrome (Group 3)	8	36	44		
Group 2 v/s Group 4	NAFLD but without MS (Group 2)	6	32	38	3 (0.790 to 11.394)	P=0.185 NS
	Non NAFLD & Non MS (Group 4)	4	64	68		
Group 3 v/s Group 4	Non-NAFLD but with Metabolic Syndrome (Group 3)	8	36	44	3.556 (1.001 to 12.633)	P=0.081 NS
	Non NAFLD & Non MS (Group 4)	4	64	68		

Approximately 80% of subjects with NAFLD had ≥ 1 characteristic feature of metabolic syndrome and about 65.5% had the complete diagnosis with ≥ 3 characteristic features. But on further analysis severity of NAFLD was not found to be associated with number of components of metabolic syndrome. (Table 4)

Table 4**Association of Severity of NAFLD with number of components of Metabolic Syndrome**

S. No.	Severity of NAFLD	Number of components of Metabolic Syndrome			Total
		3	4	5	
1	Mild	22	17	3	42
2	Moderate	11	11	5	27
3	Severe	0	3	0	3
4	Total	33	31	8	72

Chi-square = 6.540 with 4 degrees of freedom; P = 0.162

LS=NS

IV. DISCUSSION

As an entity NAFLD has only recently been defined and therefore there is limited literature available on its spectrum to fully understand its behavior in the population and assess its impact on the health of an individual and thus burden on morbidity and mortality across the population. An ultrasonography based study conducted in India had showed a prevalence of 24.5%¹⁷.

In this study NAFLD was more prevalent among women (69.4%) as compared to men (30.6%). Although many authors have reported that NAFLD is 3 to 5 times more common in men than in women^{18,19} but findings similar to this study was made by Malnick SD et al.²⁰ Thus, Data on sex differences in the prevalence of NAFLD are conflicting. The underlying reasons for this could be more number of females being enrolled in our study compared to males due to the limited practice of consumption of alcohol amongst women in the local Diaspora.

Present study showed that 79% of the subjects in the study group were either overweight or obese as compared to only 20.4% in the control group, thus showing a higher preponderance of obesity in NAFLD or vice versa. A similar Indian study conducted by Uchil D et al²¹ also found only 21% of the study population with normal BMI while the remaining 79% were either overweight or obese.

People with central obesity have large amounts of Visceral Adipose Tissue (VAT), and it is major source for free fatty acids, Interleukin-6 and adipokine delivered to liver and hence involved in hepatic steatosis. Waist circumference is highly correlated with VAT in both genders and is used as a clinical marker for abdominal obesity. In our study mean waist circumference values were significantly higher in both men and women of group 1 compared with Group3 (103±12 vs 96±13.9, P < 0.05).

In this study, approximately 80% of subjects with NAFLD had ≥ 1 characteristic feature of metabolic syndrome and about 65.5% had the complete diagnosis with ≥ 3 characteristic features. In a study quoted by Marchesani G et al²², they stated that approximately 90% of patients with NAFLD have ≥ 1 characteristic feature of metabolic syndrome, however in their study only about 33% present with complete diagnosis.

Amongst the NAFLD and Non NAFLD group, subjects with NAFLD showed higher mean values for established risk factors for metabolic syndrome. Among various Cardio metabolic risk factors only FBS and TG cholesterol have shown statistically significant difference between NAFLD and non NAFLD groups.

It is to be clarified that we have chosen not to compare AST/ALT levels as evidence suggests that the AST/ALT levels measured alone are not reliable to detect NAFLD or NASH. The NAFLD Fibrosis score and ELF panel, are non-invasive methods for identifying advanced fibrosis in patients with NAFLD^{23,24}. It has been ascertained that patients with normal transaminase levels may also have advanced fibrosis²⁵. This was not the case in this study, so a spectrum of patients ranging from no hepatic steatosis to severe hepatic steatosis were chosen.

We tested the hypothesis that increasing number of components of metabolic syndrome might correlate with the severity of Non Alcoholic Fatty Liver Disease. However, on statistical analysis, there was no significant association ($P > 0.05$) found between the degrees of severity of hepatic steatosis on ultrasonography with number of components of metabolic syndrome. This result was similar to a study conducted previously where they analyzed data of subjects from National Health and Nutrition Examination Survey (NHANES) for association of metabolic syndrome and its components with severity of NAFLD and did not find any association²⁶.

On further analysis to determine if there was any association between NAFLD and cardiovascular disease, it was found that patients in these groups who had features of metabolic syndrome had a higher prevalence of cardiovascular disease. The prevalence was also found higher in subjects with NAFLD in both metabolic and non-metabolic groups, though it was not statistically significant ($P > 0.05$). This could be because of the difference in type of study, sample size and method of evaluation for CVD compared to previous studies^{27,28}. These studies evaluated CVD risk on the basis of plaque formation as seen by carotid artery intima-media thickness, hsCRP, atheroma formation, mediastinal fat pad, endothelial dysfunction and coronary calcium scores. All these methods look at both manifest and yet to manifest CVD and are thus far more sensitive than crude methods used in our study. The studies mentioned above indicate that NAFLD may herald underlying cardiovascular disease in the absence of metabolic syndrome. Indeed, this requires further research and this may be an important area of future studies to explore and to establish whether NAFLD is an independent risk factor for CVD.

This study is only limited to patients attending hospital facility, thus large multi centric population based study is required for further evaluation.

V. CONCLUSION

This study concludes that NAFLD is associated with MS and thus can be considered as hepatic component of MS. Amongst the various cardiometabolic factors association was most significant with WC, followed by TG and FBS. Degree of severity of NAFLD does not correlate with the increasing components of metabolic syndrome. Only presence of NAFLD on ultrasonogram has its own importance. Presence of NAFLD may predict higher CVD risk irrespective of presence or absence of MS. This needs more research with large multi-centric prospective studies to evaluate NAFLD as an independent risk factor for CVD. Waist circumference as an anthropometric measurement of body fat distribution is simple and non-invasive tool for prediction of NAFLD in metabolic syndrome.

CONFLICT OF INTEREST

None declared till now.

List of abbreviations:

1. NAFLD : Non alcoholic fatty liver disease
2. MS : Metabolic syndrome
3. SD : Standard deviation
4. OR : Odds ratio
5. OPD : Out patient department

6. CVD : Cardiovascular disease
7. NASH : Non alcoholic steato hepatitis
8. ATP III : Adult treatment panel
9. WC : Waist circumference
10. TG : Triglyceride
11. FBS : Fasting blood sugar
12. LDL : Low density lipoprotein
13. HDL : High density lipoprotein
14. TC : Total Cholesterol
15. AST : Aspartate amino transferase
16. ALT : Alanine amino transferase
17. ALP : Alkaline phosphatase
18. HBsAg : Hepatitis B surface antigen
19. HSCRP : high sensitivity C reactive protein
20. VAT : Visceral adipose tissue
21. HCV : Hepatitis C virus
22. ECG : Electrocardiogram
23. WHO : World health organisation
24. NCEP : National cholesterol education programme

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