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A Study of Non Alcoholic Fatty Liver Disease in Metabolic Syndrome Patients

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study aims to determine the association of nonalcoholic fatty liver disease in metabolic syndrome patients to find out the prevalence of nonalcoholic fatty liver disease in the already diagnosed metabolic syndrome patients selected from the South Indian population. Find out the correlation and general characteristics of nonalcoholic fatty liver disease in patients with metabolic syndrome. To determine the potential risk factors for developing steatohepatitis in nonalcoholic fatty liver disease cases with metabolic syndrome and establish the risk categories for developing steatohepatitis in these patients. There is an increased prevalence of all the factors of metabolic syndrome and changes are seen in biochemical markers in nonalcoholic fatty liver cases.

Keywords: Metabolic syndrome; diabetes mellitus; cirrhosis; and hypertension.

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1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases affecting a large number of people. NAFLD denotes liver with excessive fat accumulation and occurs insignificant proportion of people who do not consume alcohol. It ranges from simple steatosis to steatohepatitis, advanced fibrosis. Studies have also shown its progression to cirrhosis and even hepatocellular cancer. NAFLD is strongly related to metabolic syndrome. Diabetes, obesity, and metabolic syndrome are considered some of the risk factors for NAFLD [1-3].

Metabolic syndrome (MS) on the other hand is a group of metabolic abnormalities in which the chance of developing cardiovascular disease, diabetes mellitus, chronic kidney disease is high. Risk factors contributing are abdominal obesity, dyslipidemia, hypertension, elevated plasma glucose. NAFLD has now become a serious health issue due to increasing obesity and aging. NAFLD progresses to cirrhosis in many cases, this cirrhosis comes under the category of Cryptogenic cirrhosis which is the term used where no identifiable viral, alcoholic, autoimmune, or drug-related cause is detected due to lack of complete awareness of the burden of liver disease occurrence in these cases of metabolic syndrome. Many clinicians now believe that a considerable number of these patients have cirrhosis due to Nonalcoholic steatohepatitis (NASH) [4]. Therefore knowledge about the potential risk factors and new preventive, diagnostic, and management protocols for NAFLD should be among the priorities for the treatment of metabolic syndrome patients.

Multiple studies have been conducted all over the World on metabolic syndromes associated with liver disease but still, a complete understanding of the pathophysiology is still lacking. Also, the burden of non-communicable diseases like Diabetes mellitus, hypercholesterolemia, systemic hypertension, and metabolic syndrome are on a steep rise in our country which prompts the need for a study among our population to better understand the implications and the burden associated with these diseases [5-8]. Hence this study has been conducted to establish the prevalence and the burden of NAFLD in the South Indian population having metabolic syndrome and to determine the potential risk factors in an attempt to better understand the process of the disease and also to determine steps to provide a better clinical outcome for these patients.

2. MATERIALS AND METHODS

This study was conducted in the Department of Biochemistry, Sree Balaji Medical College and Hospital, Chromepet, Chennai between December 2016 and July 2018 among 100 patients with Metabolic syndrome attaining outpatient and inpatient services of the Department of General Medicine. The study was explained to the participants and before taking the blood sample, informed consent was taken from them. These patients were selected based on the criteria for metabolic syndrome as established by National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III), JAMA [8].

2.1 Sample Size

The total number of the sample (n) – 100 metabolic syndrome patients fulfilling the above criteria.

2.1.1 Inclusion criteria

The age group of 20-50 years who are willing to participate, 100 patients with metabolic syndrome, Both genders equally (50 - F 50- M), Non-alcoholics, and No critical illness.

2.1.2 Exclusion criteria

Alcoholics, Chronic hepatitis, Cirrhosis and steroid use.

Chart 1. Nutritional status based on "Asian criteria" values

Nutritional status	Asian criteria (BMI cut off)
Underweight	<18.5
Normal	18.5-22.9
Overweight	23-24.9
Pre-obese	25-29.9
Obese	≥30
Obese type 1(obese)	30-40
Obese type 2(morbidly obese)	40.1-50
Obese type 3(super obese)	≥50

Waist circumference is an indicator of health risk associated with excess fat around the waist. The hip bone is felt by the thumb and the index finger.

The measuring tape is aligned with the top of the hip bone and is wrapped around the waist. Measurement is done during exhaling. Blood Pressure (systolic and diastolic) of the patient was measured in the supine position after ten minutes of rest, and the average of two measurements (with a 5-minute interval) was used for analysis.

2.2 Sample Collection

Under aseptic precautions, the fasting and postprandial venous blood samples were collected from Subjects by the method of venipuncture in specific vacutainers. All the Biochemical investigations were done using BS390 fully automated analyzer. Processing of blood samples was done within half an hour. Plasma glucose was estimated immediately. Serum separated and stored under -20°C for estimation of serum insulin.

2.3 Methods

Estimation of Fasting plasma glucose and postprandial plasma glucose: Method: GOD/POD: Enzymatic Photometric Method.

Estimation of HbA1c Method:

Immunoturbidimetry method

Estimation of lipid profile:

a. Total Cholesterol (TC): Method: Cholesterol oxidase and Peroxidase (CHOD-POD)
b. Triglycerides (TGL)
c. High-Density Lipoprotein (HDL): Direct method
d. Low-Density Lipoprotein (LDL):

The concentration of LDL-cholesterol can be calculated using the Fried Wald equation i.e.

LDL =Total Cholesterol - (VLDL + HDL)

e. Very Low-Density Lipoprotein (VLDL):

Estimation of liver function test:

a. spartate aminotransferase/SGOT Method: UV kinetic- IFCC
b. Alanine aminotransferase/SGPT Method: UV kinetic- IFCC method
c. Alkaline phosphatase (ALP)
d. Gamma-glutamyl transferase (GGT) Method: Szasz method
e. Total Protein
f. Albumin Method: Bromocresol Green (BCG) method
g. Globulin is calculated by subtracting albumin from Total Protein.

Fasting plasma insulin:

Chemiluminescence Immunosorbent Assay.

2.4 Statistical Analysis

SPSS version 20 was used for the statistical analysis, Qualitative (categorical) variables were represented using frequency/percentage. Quantitative (continuous) variables were represented by mean and standard deviation. Analysis of variance (ANOVA) was used to compare quantitative variables between three groups (very high risk, high risk, and moderate risk). An independent sample t-test (Student's t-test) was performed to compare quantitative variables between steatohepatitis and non-steatohepatitis cases to find the risk factors for steatohepatitis. The ROC were again used to find the risk factors with the outcome variables namely, fasting plasma glucose, postprandial plasma glucose, HbA1c, fasting plasma insulin, HOMA-IR, and the cut-off values for steatohepatitis. The p-value <0.05, 95% CI was taken as statistically significant.

3. RESULTS

In this cross-sectional observational study, 100 individuals were selected based on the criteria for metabolic syndrome, and their history, general anthropometric measurements, biochemical parameters, and USG findings were all studied and are enumerated below.

Table 1. Frequency distribution of age

Age (Years)	Frequency	Percent
25 - 30	9	9.0%
31 - 35	10	10.0%
36 - 40	25	25.0%
41 - 45	18	18.0%
46 - 50	35	35.0%
51 - 55	3	3.0%

The average age of the patients was 41. 36 with a standard deviation of 7.17. More cases are notified between 46-50 years. The minimum and maximum age were 25 and 55 years respectively.

Table 2. Frequency distribution of sex

Sex	Frequency	Percent
Male	50	50.0%
Female	50	50.0%

Out of 100 patients taken for the study, exact 50.0% of the cases were male and 50.0% of the cases were female.

Table 3. Frequency distribution of Fatty Liver

Fatty Liver	Frequency	Percent
Grade 1	18	52.9%
Grade 2	14	41.2%
Grade 3	2	5.9%

Out of 34 fatty liver cases identified among 100 patients, almost 52.9% of the cases have grade 1 fatty liver and 41.2% of the cases have grade 2 fatty livers. Only two cases (5.9%) with grade 3 fatty liver were also noted.

Here the p-value corresponding to BMI, waist circumference, systolic blood pressure, PPBS, fasting insulin, HOMAIR, total cholesterol, TGL, HDL, LDL, VLDL, and CHO: HDL is $p<0.05$, and affecting steatohepatitis. These factors are significantly higher in steatohepatitis cases compared to non-steatohepatitis cases. HDL is significantly lower in steatohepatitis cases compared to non-steatohepatitis cases. Diastolic blood pressure, FBS, and HbA1c are not affecting steatohepatitis as the corresponding p-values are greater than the significance level. Diastolic blood pressure, FBS, and HbA1c are almost the same in both steatohepatitis and non-steatohepatitis cases.

3.1 ROC Analysis

On performing receiver operative characteristics analysis with the above-set criteria we noticed the patients getting demarked into 3 risk categories:

1. Moderate risk
2. High risk
3. Very high risk

From the above ROC analysis, we can understand that certain factors have a more significant relationship with predicting the risk for steatohepatitis in NAFLD and other factors are less significant and some no acceptable significance. The factors which are highly significant with $p<0.01$ CI 99% are BMI, WC, Systolic BP, Fasting Insulin, HOMAIR, HDL, LDL, CHO: HDL ratio. The factors which are significant with $p<0.05$ CI 95% are PPBS, TC, TGL, VLDL, for all 3 categories, this 3 ROC analysis again shows that diastolic blood pressure, FBS, and HbA1c are not affecting steatohepatitis as the corresponding p-values are greater than the significance level. Based on these findings we have deduced 3 levels of risk categories with significant sensitivity, specificity, and accuracy factors. The potential risk factors identified were those study parameters that showed 100% sensitivity and/or 100% specificity and /or 100% accuracy. Systolic BP–100% sensitivity, 100% specificity, 100% accuracy in all 3 categories. The other factors showed significant differences among the different categories.

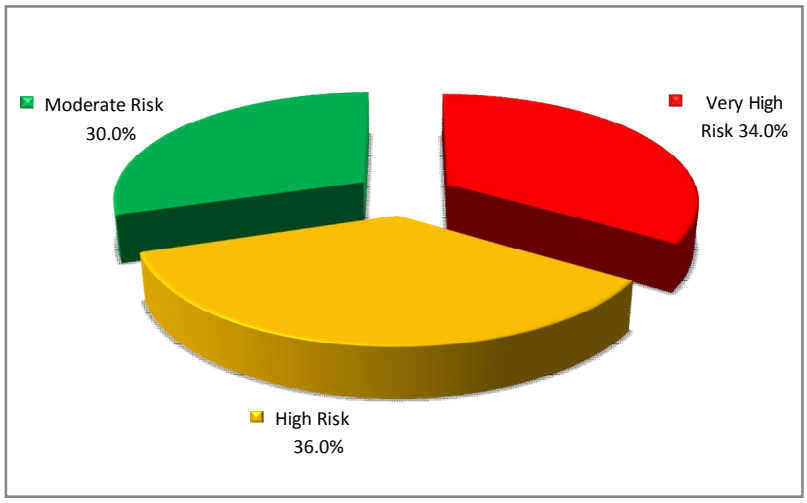

Fig. 1. Distribution of risk groups

Table 4. Risk factors cut off value, significance, sensitivity, specificity, and accuracy for very high risk

Parameters	AUC (95% CI)	p - value	Cut-off	Sensitivity	Specificity	Accuracy
BMI	1.00 (1.00 - 1.00)	0.001	40.05	5 (100.0%)	29 (100.0%)	34 (100.0%)
Waist Circumference	1.00 (1.00 - 1.00)	0.001	111. 1	5 (100.0%)	29 (100.0%)	34 (100.0%)
Systolic BP	1.00 (1.00 - 1.00)	0.001	153. 0	5 (100.0%)	29 (100.0%)	34 (100.0%)
Diastolic BP	0.69 (0.44 - 0.94)	0.181	91.00	2 (40. 0%)	26 (44.8%)	28 (82.4%)
FBS	0.77 (0.58 - 0.96)	0.058	146. 5	3 (60. 0%)	24 (82.8%)	27 (79.4%)
PPBS	0.83 (0.68 - 0.98)	0.020	234. 0	3 (60. 0%)	24 (82.8%)	27 (79.4%)
HbA1 c	0.77 (0.59 - 0.95)	0.058	7.210	3 (60. 0%)	24 (82.8%)	27 (79.4%)
Fasting Insulin	0.87 (0.72 - 1.00)	0.010	15.10	3 (60. 0%)	26 (89.7%)	29 (85.3%)
HOMAIR	1.00 (1.00 - 1.00)	0.000	5.000	5 (100.0%)	29 (100.0%)	34 (100.0%)
Total Cholesterol	0.84 (0.65 - 1.00)	0.016	337. 5	3 (60. 0%)	29 (100.0%)	32 (94.1%)
TGL	0.80 (0.57 - 1.00)	0.032	354. 5	3 (60. 0%)	29 (100.0%)	32 (94.1%)
HDL	0.88 (0.76 - 1.00)	0.007	37.00	5 (100.0%)	22 (75.9%)	27 (79.4%)
LDL	0.87 (0.73 - 1.00)	0.009	199. 0	3 (60. 0%)	26 (89.7%)	29 (85.3%)
VLDL	0.80 (0.57 - 1.00)	0.032	70.90	3 (60. 0%)	29 (100.0%)	32 (94.1%)
CHO: HDL	0.99 (0.95 - 1.00)	0.001	8.520	5 (100.0%)	28 (96.6%)	33 97.1%)

3.2 Statistical Significance

- Highly significant – BMI, WC, Systolic BP, Fasting Insulin, HOMA-IR, HDL, LDL, CHO: HDL with p<0.01, CI 99%
- PPBS, TC, TGL, VLDL with p< 0.05, CI 95%

Here the p-value is <0.05 so, the difference in BMI between groups is significantly different. The table reveals that BMI is significantly higher in very high risk (33.90 ± 0.990) and significantly lower in moderate risk (23.72 ± 0.125) compared to high risk (27.51 ± 0.695).

Here the p-value <0.05; the difference in HbA1c between groups is significant. The table reveals that HbA1c is significantly higher in very high risk (7.565 ± 0.221) and high risk (7.328 ± 0.235) compared to moderate risk (6.519± 0.090).

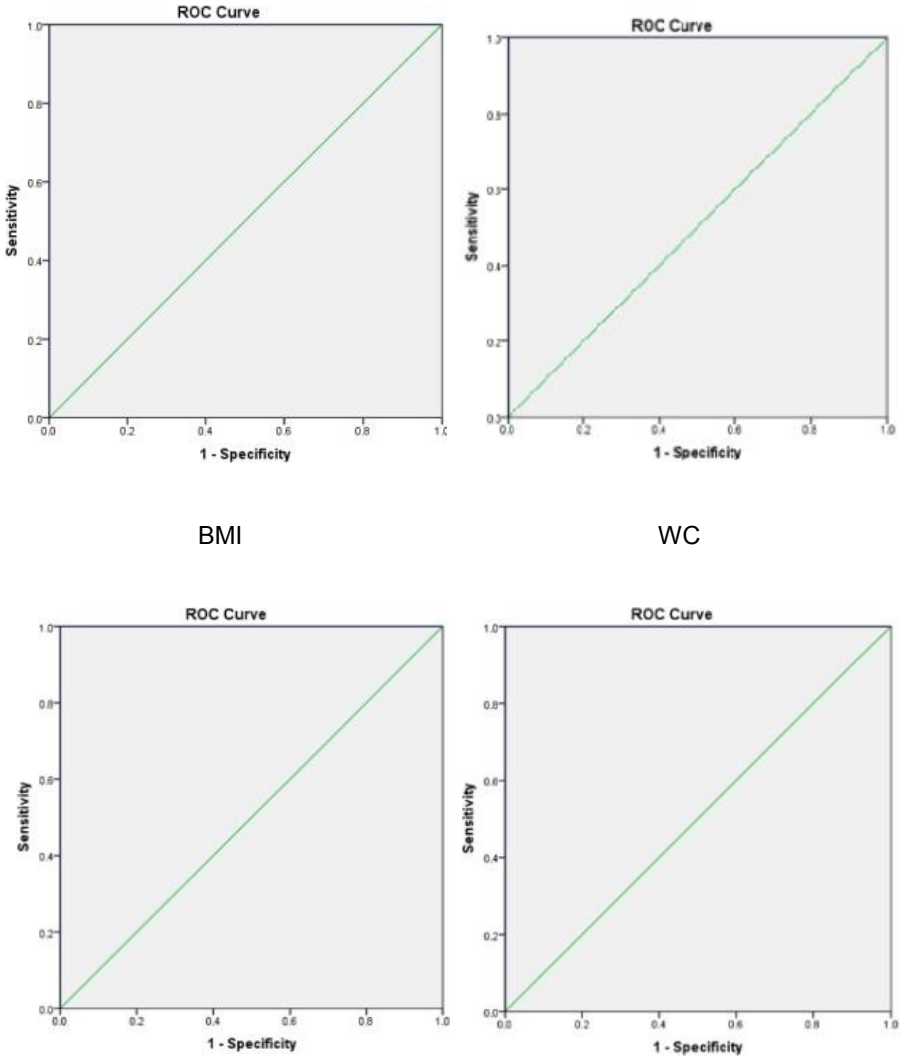

Fig. 2. ROC graph for BMI, WC, systolic BP, and HOMA-IR Comparison of BMI between groups

Table 5. Comparison of BMI

Group	Mean	SE	Range	p-value
Very high risk	33.90	0.990	23.0 - 45.2	
High risk	27.51	0.695	23.0 - 33.5	
Moderate risk	23.72	0.125	22.0 - 24.9	0.000

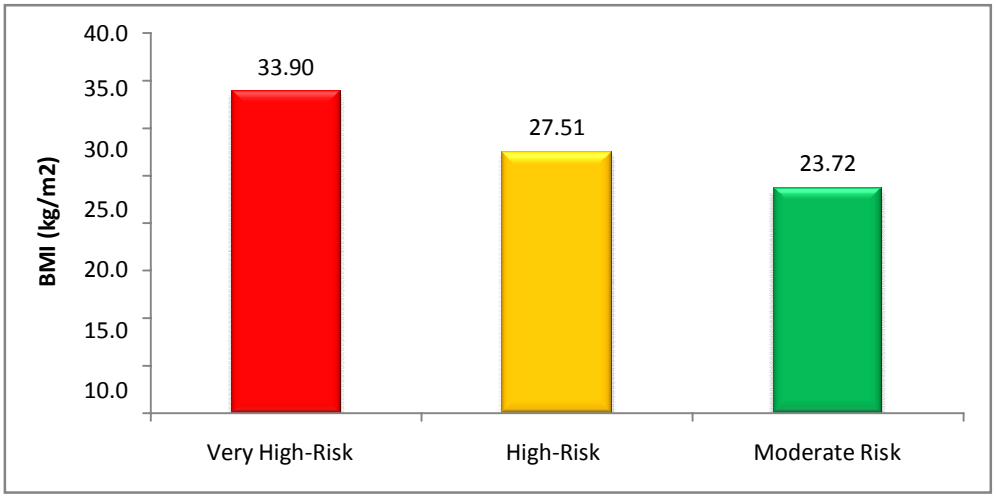

Fig. 3. Comparison of BMI between groups

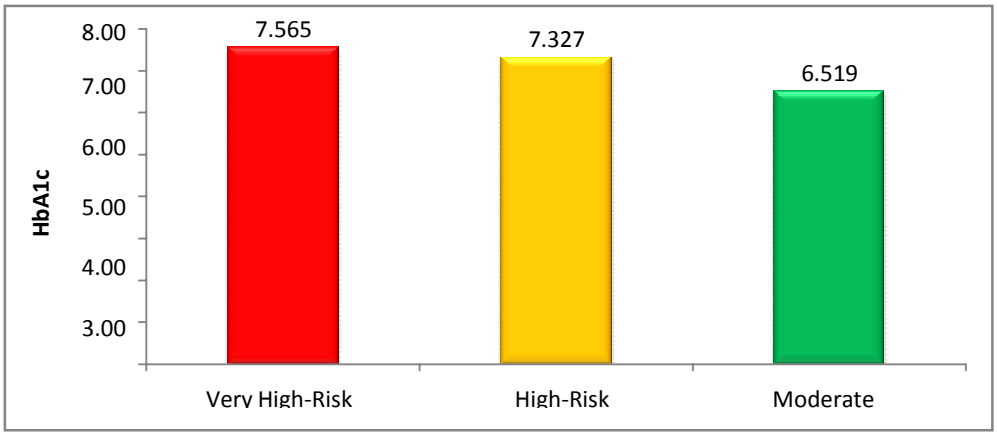

Fig. 4. Comparison of HbA1c between groups

Table 6. Comparison of Fasting Insulin between groups

Group	Mean	SE	Range	p-value
Very high risk	11.69	0.485	7.0 - 17.0	
High risk	9.656	0.246	5.8 - 12.9	0.000
Moderate risk	7.020	0.241	3.8 - 10.0	

Here the p-value <0.05; the difference in fasting insulin between groups is significant. The Table 6 reveals that fasting insulin is significantly higher in very high risk (11.69 ± 0.485) and significantly lower in moderate risk (7.020 ± 0.241) compared to high risk (9.656 ± 0.246).

Here the p-value <0.05; the difference in globulin between groups is significant. The Table 7 reveals that globulin is significantly higher in very high risk (3.488 ± 0.092) compared to high risk (3.053 ± 0.050) and moderate risk (3.180 ± 0.073).

4. DISCUSSION

Metabolic syndrome has become one of the more prevalent diseases in Asian countries and with an increased incidence of obesity and insulin resistance especially among the Indian population, we have found a rise in NAFLD due to metabolic syndrome excluding other causes. NAFLD has been recognized as the common liver disease causing morbidity and mortality. It progresses from simple steatosis to steatohepatitis to cirrhosis and hepatic failure. The main pathophysiology being insulin resistance producing steatosis and mitochondrial reactive oxygen species increasing lipid peroxidation leading to increased hepatic damage which has increased undetected liver disease in these patients [9].

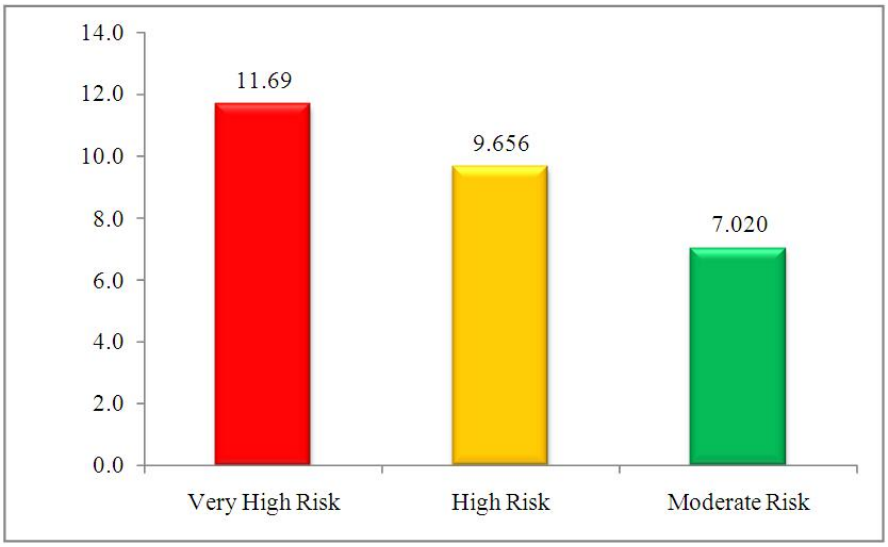

Fig. 5. Comparison of Globulin between groups

Table 7. Comparison of Globulin between groups

Group	Mean	SE	Range	p-value
Very high risk	3.488	0.092	2.4 - 5.3	
High risk	3.053	0.050	2.6 - 4.0	
Moderate risk	3.180	0.073	2.1 - 4.0	0.000

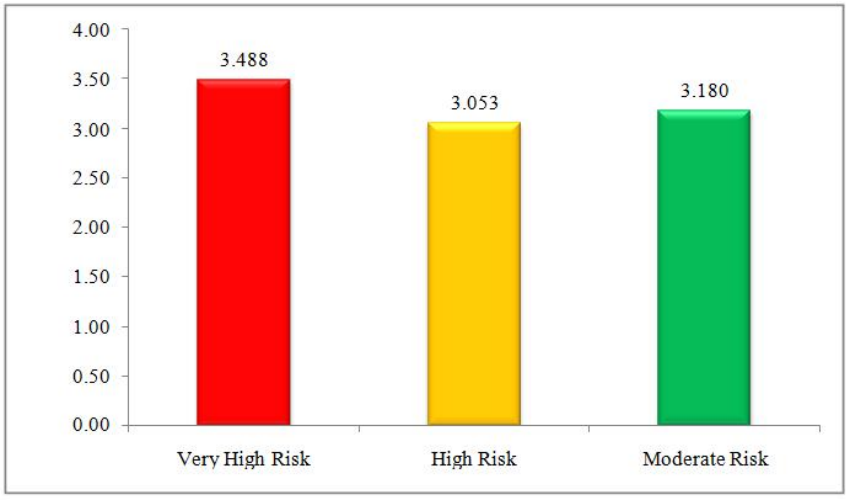

Fig. 6. Comparison of Globulin between groups

In our study with 100 patients diagnosed with metabolic syndrome, we found that 34 patients had USG evidence of fatty liver and out of the 18 had grade 1, 14 had grade 2 and 2 had grade 3 fatty liver. Obesity, one of the most important risk factors associated with fatty liver is commonly observed in developed countries. Diabetes mellitus along with obesity, hyperlipidemia, and hypertension are the main manifestations of metabolic syndrome associated with insulin resistance [10]. In our study, BMI is significantly higher in very high risk (33.90 ± 0.990) and significantly lower in moderate risk (23.72 ± 0.125) compared to high risk (27.51 ± 0.695) proving the increase of obesity among our population also which may be due to causes like westernization of diet, sedentary lifestyle, etc Visceral fat shows a better predictor of steatosis than subcutaneous fat which is also associated with severity of NAFLD [11]. Waist circumference is considered as a marker of visceral fat tightly related to triglyceride content, hepatic inflammation, and fibrosis. In our study, WC is significantly higher in very high risk (100.6 ± 1.591) and significantly lower in moderate risk (83.96 ± 0.153) compared to high risk (96.12 ± 1.841). Shou-Wu Lee et al in a study show a positive correlation between the obese parameters and NAFLD [12-15]. Higher BMI and higher waist circumference have additional risk factors for NAFLD with BMI showing an increased positive correlation. Another study had 250 NAFLD and 240 non-NAFLD shows waist circumference as the effective factor for non-alcoholic fatty liver disease [16].

Insulin resistance is the hallmark of MS and the laboratory finding is associated with the presence of non-alcoholic fatty liver disease irrespective of fat distribution, BMI, and glucose tolerance as shown by Marchesini et al. [17]. In our study, HOMA-IR was significantly higher in very high risk (4.009 ± 0.165) and significantly lower in moderate risk (2.080 ± 0.043) compared to high risk (2.897± 0.050). Found in his study mean HOMA-IR of 2.6 ± 1.3 and IR in 80% of the study population concerning liver function test profile of patients, increased SGOT (≥40 IU/L) and SGPT (≥42 IU/L) and observed in a majority of patients (98.4 with mean 76.05 ± 41.74 and 97.6% with mean 100.31 ± 43.74 respectively).

Non-alcoholic fatty liver disease is considered as the major cause of hepatic morbidity and mortality ranging from lipid accumulation to non-alcoholic steatohepatitis which is characterized by steatosis, liver cell injury, inflammation, fibrosis, and ultimately necrosis [18]. In our study, the total cholesterol is significantly higher in very high risk (272.1 ± 8.591) compared to high risk (241.2 ± 3.901) and moderate risk (231.5 ± 4.498). TGL is also significantly higher in very high risk (263.9 ± 13.70) compared to high risk (202.1 ± 6.531) and moderate risk (183.7 ± 7.6 50). HDL between groups is not significant. It is almost the same in very high risk (43.09 ± 1.533), high risk (40.44 ± 0.996), and moderate risk (42.53 ± 1.088). On the other hand, LDL is significantly higher in the very high-risk group (177.9 ± 4.255) and high risk (169.4 ± 3.190) compared to the moderate risk (155.7 ± 3.098). VLDL is significantly higher in very high risk (52.78 ± 2.739) compared to high risk (40.43 ± 1.306) and moderate risk (36.73 ± 1.530). Also, CHO: HDL is significantly higher in very high risk (6 .648 ± 0.366) compared to moderate risk (5.560 ± 0.207). High risk (6.060 ± 0.156) is not significantly different from very high risk and moderate risk. Thus, TC, TGL, LDL, VLDL, and CHO: HDL is significant while HDL did not have any significance.

The uptake of fatty acids in the liver causes accumulation of fat especially triglyceride, liver toxicity, and the inflammatory cytokines, tumor necrosis factor causes non-alcoholic fatty liver disease and also fatty liver with mild to moderate elevation of liver enzymes [19]. NAFLD can be diagnosed following abnormal liver function tests but these tests alone are not sensitive to detect NAFLD. So, ultrasonography is usually done to detect any fatty liver.

However, liver biopsy is accepted as the gold standard to distinguish NASH from other liver diseases. In our study, there is no significant difference in AST between the groups which reveals that AST is almost the same in very high risk (30.03 ± 4.143), high risk (20.56 ± 1.473) and moderate risk (25.90 ± 3.147), and moderate risk (3.180 ± 0.073). So ALT, GGT, Globulin are significant while AST, ALP, TP, Albumin are considered not significant. A similar study is seen in Oh et al where the incidence of metabolic syndrome and NAFLD have a positive correlation with ALT and γ-glutamyltransferase (GGT) levels within the reference ranges. A study that shows that the liver enzymes ALT, GGT, AST are elevated and are the signs of liver inflammation. They may be the potent markers of non-alcoholic fatty liver disease [20].

5. CONCLUSION

The results of this study are that nonalcoholic fatty liver disease is linked with metabolic

syndrome which is both the cause and also the consequences in the study population. There is an increased prevalence of all the factors of metabolic syndrome and changes are seen in biochemical markers in nonalcoholic fatty liver cases. A high range in anthropometric parameters like BMI, BP, WC, Insulin resistance is associated with a greater risk of developing NAFLD. There is a positive correlation between obesity, waist circumference, blood pressure, insulin resistance, triglyceride, fasting glucose, LDL, VLDL, and a negative correlation with HDL-C. Ultrasound fatty liver index is a cheap, simple, and accurate detector of the risk of metabolic syndrome. Most cases of NAFLD are asymptomatic. So, frequent checking, timely diagnosis, and treatment help in delaying the complications and prevents cardiac disease as its relation with metabolic syndrome are frequent.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

After getting clearance from the Institutional human ethical committee and research committee (reference number for approval: 002/SBMC/IHEC/2016/836) of Sree Balaji Medical College and Hospital, Chromepet, Chennai, the study was proceeded.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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