A STUDY OF NON ALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH METABOLIC SYNDROME

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ABSTRACT: This study was conducted to know the relation of non-alcoholic fatty liver disease (non-alcoholic steatohepatitis & cirrhosis) with metabolic syndrome. MATERIAL AND METHODS: 60 cases were selected. Out of them 30 were non-alcoholic steatohepatitis and 30 cirrhosis along with 30 healthy controls. Parameters of metabolic syndrome and liver function which are waist circumference, blood pressure, fasting plasma glucose, total triglycerides, high density lipo-protein cholesterol, total bilirubin, alanine amino transferase, alkaline phosphatase, total proteins and albumin were measured. STATISTICAL ANALYSIS: All values were expressed as mean ± SD. The results obtained were analyzed statistically using the unpaired student ‘t’test to evaluate the significance of differences between the mean values. RESULTS: The values of waist circumference, fasting plasma glucose, systolic blood pressure, total triglycerides, total bilirubin, alanine-amino-transferase and alkaline phosphatase were raised in non-alcoholic steatohepatitis and cirrhosis patients. The level of high density lipoprotein cholesterol was decreased in non-alcoholic steatohepatitis and cirrhosis patients. The level of albumin was decreased in cirrhosis patients. CONCLUSION: On the basis of our results it may be concluded that metabolic syndrome causes nonalcoholic fatty liver disease. KEYWORDS: metabolic syndrome, non-alcoholic fatty liver disease, hepatic steatosis, non-alcoholic steatohepatitis, cirrhosis

INTRODUCTION: Non-alcoholic fatty liver disease (NAFLD) is the occurrence of fatty liver in people who have no history of alcohol consumption. NAFLD includes hepatic steatosis (accumulation of fat in the hepatocytes), non-alcoholic steatohepatitis and cirrhosis. Clark et al stated that when inflammation occurs to hepatic steatosis the condition was then called nonalcoholic steatohepatitis (NASH) which is a clinical form of non-alcoholic fatty liver disease and a major cause of cirrhosis of the liver(1). Cirrhosis is a consequence of chronic liver disease.
characterized by replacement of liver tissue by fibrosis, scar tissue and regenerative nodule leading to loss of liver function. Complications of cirrhosis are ascitis, esophageal varices and hepatic encephalopathy that can cause coma and death. Different studies have revealed that cirrhosis is generally irreversible resulting in liver failure or liver related death in approximately one third of cases.\(^{(2,3)}\)

The incidence of non-alcoholic fatty liver disease has been in upward trend in the people in all parts of the world including India. According to different studies the prevalence of NAFLD ranges from 9 to 36.9% of the population in different parts of the world.\(^{(4,5,6)}\) Epidemiological studies suggest prevalence of NAFLD is around 9% to 32% of general population in India with higher prevalence in those with overweight or obesity and those with diabetes or prediabetes.\(^{(7)}\) NASH may be present in about 1/3 of NAFLD cases, while 20-25% of NASH cases could progress to cirrhosis.

There are some studies at different geographical areas which have proposed strongly that NAFLD is associated with metabolic syndrome.\(^{(8,9,10,11)}\) Metabolic syndrome is associated with insulin resistance. Insulin resistance is a physiological condition where the natural hormone insulin becomes less effective at lowering blood sugars. Insulin resistance increases free fatty acids and triglycerides in liver and serum. Current concepts suggest that the development of NASH is a two-hit process. With the initial hit macrovesicular steatosis results from insulin resistance and subsequent hyperinsulinemia which leads to alterations in the hepatic pathways of synthesis of fatty acids and ultimately to accumulation of lipids in the hepatocytes. These changes make the liver susceptible to a second hit resulting in inflammatory response and progress to liver damage. Oxidative stress mainly as a consequence of mitochondrial dysfunction and proinflammatory cytokines such as TNF-\(\infty\) are thought to play a central role in the progression from simple steatosis to steatohepatitis and cirrhosis and these may be potential causes for second hit phenomenon\(^{(12)}\).

Sedentary lifestyle, a low physical activity and excess caloric intake\(^{(13)}\) increase adipose tissue (predominantly central), reduce HDL cholesterol and is a trend towards increased triglycerides, blood pressure, and glucose causing metabolic syndrome thereby resulting in liver damage. It is observed that individuals who watch television or videos or sit before computers for more than four hours daily have a twofold increased risk of metabolic syndrome due to low physical activity.

Although much progress has been made more studies are needed to clearly define the progression of NAFLD from metabolic syndrome. Aim of the present study was to assess the parameters of metabolic syndrome and liver function in NAFLD (NASH & cirrhosis) to know the relation between metabolic syndrome and NAFLD.

**MATERIAL AND METHODS: STUDY GROUP:**

The present study was carried out during the years 2009 to 2012 in the Department of Biochemistry, Mamata Medical College and General Hospital, Khammam, Andhra Pradesh. A total of 60 cases of Non-alcoholic fatty liver disease were selected in this study group after obtaining their consent. Out of these 30 were non-alcoholic steatohepatitis and 30 were cirrhosis cases in the age group of 35 to 65 years. Diagnosis of cases was based on:

- **a.** History: Non-alcoholics
- **b.** Biochemical: abnormal liver function tests
c. Radiological: liver ultrasound showing steatosis was used to exclude gallstone problems (cholelithiasis). Ultrasound was used in the evaluation of cirrhosis. It showed a small and nodular liver in advanced cirrhosis along with increased echogenicity with irregular appearing areas.

d. Liver biopsy: liver biopsy (tissue examination) was conducted to distinguish NASH from other forms of liver disease and was used to assess the severity of the inflammation and resultant fibrosis.

e. Cases with three or more of the five markers of metabolic syndrome as per NCEP ATP III guidelines were included in this study. As per the Updated NCEP ATP III\textsuperscript{(14,15,16)} guidelines the markers of metabolic syndrome are: 1. Elevated waist circumference: men-greater than 40 inches, women-greater than 35 inches; 2. Elevated triglycerides: equal to or greater than 150 mg/dl; 3. Reduced HDL (good) cholesterol: men-less than 40 mg/dl, women-less than 50 mg/dl; 4. Elevated blood pressure: equal to or greater than 130/85 mm Hg; and 5. Elevated fasting glucose: equal to or greater than 100 mg/dl.

Exclusion criteria of cases: 1. Alcoholics, 2. Subjects on medication for HTN, 3. Subjects not having metabolic syndrome

Controls: 30, sex and age matched healthy persons, were recruited as controls.

Sample collection:

5 ml venous blood was taken from each subject and divided into two parts. 1 ml blood was transferred to an EDTA containing test tube and then centrifuged. Plasma was separated and used to determine fasting plasma glucose (FPG). Remaining 4 ml of venous blood was also centrifuged at 3000 rpm for 15 minutes. Serum was separated and used for the estimation of total triglycerides (TAG), high density lipoprotein cholesterol (HDL-C), total bilirubin, alanine-amino-transferase (ALT), alkaline phosphatase (ALP), total proteins and albumin.

Biochemical estimation:

FPG was determined by using the Glucose Oxidase- Peroxidase (GOD- POD) method.\textsuperscript{(17)} TAG was determined by using Enzymatic method (Glycerol Phosphate Oxidase). HDL-C was determined by using Enzymatic method (Poly Ethylene Glycol/Cholesterol Oxidase method). Total bilirubin was estimated by using the Modified Jendrassik & Grof's method.\textsuperscript{(18)} ALT was estimated by using the Modified International Federation of Clinical Chemistry (Mod. IFCC) method. ALP was estimated using the Para Nitro Phenyl Phosphate Kinetic method. Total protein was determined by using the Biuret method. Albumin was determined by using the Bromocresol Green (BCG) Dye Binding method.

STATISTICAL ANALYSIS: All values were expressed as mean ± SD. The results obtained were analyzed statistically using the unpaired student's t-test to evaluate the significance of differences between the mean values. P values <0.05 were considered as significant and <0.001 as highly significant.

RESULTS: Results of the parameters of metabolic syndrome and liver function in NASH and cirrhosis comparing with healthy controls are in the tables 1&2. Table 1 contains the mean, SD and p value of waist circumference, FPG, systolic blood pressure, diastolic blood pressure, TAG, and HDL-C of NASH, cirrhosis and controls. Table 2 contains the mean, SD and p value of total bilirubin, ALT, ALP, total proteins, albumin and globulins of NASH, cirrhosis and controls.

From the results of our study the following observations were made:
PARAMETERS OF METABOLIC SYNDROME:

1. The mean levels of waist circumference, FPG, systolic blood pressure and TAG were increased in NASH and cirrhosis cases when compared with controls and the mean difference was statistically significant.
2. The mean HDL-C level was decreased in NASH and cirrhosis cases when compared with controls and the mean difference was statistically significant.
3. The mean diastolic blood pressure level was increased in both NASH and cirrhosis cases when compared with controls. The mean difference was significant between NASH and controls but not significant between cirrhosis and controls.

PARAMETERS OF LIVER FUNCTION:

1. The mean levels of total bilirubin, ALT, ALP, and globulins were increased in the cases of NASH and cirrhosis. The mean difference was statistically significant when compared with controls.
2. The mean levels of total proteins and albumin were decreased in NASH and cirrhosis cases when compared with controls and the mean difference was statistically significant except total proteins between NASH group and control group.

DISCUSSION: Amarapurkar et al in their population based study in 2007 on prevalence of NAFLD stated that there was an increase in incidence of NAFLD in India.\(^{(19)}\) One study reports that approximately 23% of the United States population suffers from non-alcoholic fatty liver and continues to trend upward.\(^{(20)}\)

Abdominal obesity or clinically known as central obesity is the accumulation of abdominal fat resulting in an increase in waist size. The severity of central obesity is determined by taking waist measurement. Our results showed that the measurement of waist circumference was highly significantly raised \((p<0.001)\) in the patients with NASH and cirrhosis than control group. Central obesity is common among NAFLD patients and reflects an increase in visceral adipose tissue which appears to be particularly resistant to the effects of insulin\(^{(21)}\). Central obesity is associated with a higher risk of hypertension, insulin resistance and diabetes mellitus type 2.

Several reports indicated the relation of NAFLD to diabetes mellitus\(^{(22,11,23)}\) Our results also showed highly significantly raised \((p<0.001)\) level of FPG in the patients of NASH as well as cirrhosis than controls. Insulin resistance prevents the body from converting glucose into glycogen which in turn makes it difficult or impossible to remove excess glucose from the blood. Younossi et al. reported that patients with NAFLD and diabetes mellitus and associated with insulin resistance had more aggressive disease with a higher percentage of cirrhosis and higher odds of liver cell necrosis compared with those without diabetes.\(^{(24)}\)

The levels of both systolic blood pressure and diastolic blood pressure increased in this study in the patients when compared with controls. These are in accordance with earlier findings.\(^{(12,1)}\) High blood pressure damages the entire complex circulatory system. Therefore managing the blood pressure is an important part of prolonging the health of the organs. The
risk increases even more if we have high blood pressure along with other risk factors such as overweight or obesity, high cholesterol, diabetes and physical inactivity.

In our study we observed highly significantly raised \((p<0.001)\) level of TAG in patients when compared with controls. Assy et al in their study reported that NAFLD was found in up to 50% of patients with hypertriglyceridemia.\(^{(25)}\) Insulin resistance increases free fatty acids and triglycerides in liver and serum causing hepatic steatosis and progress to NASH and cirrhosis. Obesity, diabetes mellitus and insulin resistance increase triglyceride levels.

The level of HDL-C was highly significantly decreased \((p<0.001)\) in our study in NASH and cirrhosis than controls. Various previous studies also revealed that NAFLD was related to dislipidemia.\(^{(21,22,26,27)}\) HDL particles transport cholesterol back to the liver for excretion or re-utilization. HDL-C level decreased in metabolic syndrome due to a low physical activity.

Bilirubin is a breakdown product of heme (a part of hemoglobin in red blood cells). The results of the present study showed that the patients with NASH and cirrhosis had increased total bilirubin when compared with controls. As per our observations the level of ALT was highly significantly raised \((p<0.001)\) in patients than controls. ALT is an enzyme present in hepatocytes. When cell is damaged, it leaks this enzyme into the blood. The level of ALT rises with the damage liver cell. The level of ALP an enzyme in the cells lining the biliary ducts of the liver was also raised in patients when compared with controls. ALP levels in plasma rise with intrahepatic cholestasis or infiltrative diseases of the liver.

The levels of proteins and albumin were highly significantly decreased \((p<0.001)\) in patients with cirrhosis. This finding was in accordance with the finding of Melvin Jay Schwartz,\(^{(29)}\) who reported that hypoalbuminemia occurred in cirrhosis. Albumin which is the main constituent of total protein is produced in the liver. The levels of albumin fall as the synthetic function of the liver declines with cirrhosis.

In the present study, it was observed that the parameters of metabolic syndrome became abnormal. The abnormal parameters of metabolic syndrome cause liver damage. Free fatty acids and triglycerides in liver and serum increased in the patients of metabolic syndrome and caused fatty liver which progress to NASH and cirrhosis. It was observed that the parameters of the liver function were abnormal in the patients of NASH and cirrhosis.

Thus it may be concluded that metabolic syndrome leads to NAFLD resulting in liver failure or liver-related death. The results were consistent with other studies.\(^{(8,9,10,11)}\) There are various strategies to prevent the development of metabolic syndrome such as increased physical activity (walking 30 minutes every day),\(^{(29)}\) a healthy and reduced calorie diet.\(^{(30)}\)

REFERENCES:

28. Melvin Jay Schwartz Steatorrhea and hypoalbuminemia in cirrhosis with ascites Digestive Diseases and Sciences
31. TABLE 1: Parameters of metabolic syndrome in the patients of NASH and cirrhosis compared with healthy controls.
32. TABLE 2: Parameters of liver function in the patients of NASH and cirrhosis compared with healthy controls.

**TABLE 1: Parameters of metabolic syndrome in the patients of NASH and cirrhosis compared with healthy controls.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls mean ± S.D</th>
<th>NASH mean ± S.D</th>
<th>P value (controls vs NASH)</th>
<th>Cirrhosis mean ± S.D</th>
<th>P value (controls vs cirrhosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC (inches)</td>
<td>33.4 ± 4.3</td>
<td>41.8 ± 2.72</td>
<td>P&lt;0.001</td>
<td>42.9 ± 4.9</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>80.8 ± 8.6</td>
<td>134.1 ± 12.4</td>
<td>P&lt;0.001</td>
<td>148.2 ± 18.6</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>117 ± 4.9</td>
<td>131.1 ± 7.8</td>
<td>P&lt;0.001</td>
<td>122.4 ± 7.9</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76.3 ± 4.48</td>
<td>86.6 ± 4.8</td>
<td>P&lt;0.001</td>
<td>79.97 ± 7.7</td>
<td>P&lt;0.1</td>
</tr>
<tr>
<td>TAG (mg/dl)</td>
<td>124.3 ± 10.89</td>
<td>170.1 ± 18.3</td>
<td>P&lt;0.001</td>
<td>181.3 ± 13.9</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>47.1 ± 5.6</td>
<td>34.2 ± 5.3</td>
<td>P&lt;0.001</td>
<td>30.1 ± 3.3</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>
TABLE 2: Parameters of liver function in the patients of NASH and cirrhosis compared with healthy controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls mean± S.D</th>
<th>NASH mean± S.D</th>
<th>P value (controls vs NASH)</th>
<th>Cirrhosis mean± S.D</th>
<th>P value (controls vs cirrhosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBil(mg/dl)</td>
<td>0.5±0.2</td>
<td>4.3±1.1</td>
<td>P&lt;0.001</td>
<td>4.7±0.8</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>20.8±2.83</td>
<td>163±18.2</td>
<td>P&lt;0.001</td>
<td>59.4±7.9</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>ALP(U/L)</td>
<td>78.9±21.7</td>
<td>164.8±22.8</td>
<td>P&lt;0.001</td>
<td>155.7±19.7</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>TPro(gm/dl)</td>
<td>6.97±0.24</td>
<td>6.8±0.35</td>
<td>P&lt;0.1</td>
<td>5.7±0.1</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Alb(gm/dl)</td>
<td>4.45±0.2</td>
<td>4.09±0.4</td>
<td>P&lt;0.01</td>
<td>2.7±0.18</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Globulins (gm/dl)</td>
<td>2.5±0.13</td>
<td>2.69±0.19</td>
<td>P&lt;0.01</td>
<td>2.99±0.21</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Note: WC-waist circumference, BP- blood pressure, T Pro-total proteins, Alb-albumin, T Bil-total bilirubin