

**STUDY OF BIOCHEMICAL MARKERS IN NON ALCOHOLIC FATTY LIVER DISEASE****Babu Rao R<sup>\*</sup>, Sampath Kumar V<sup>\*\*</sup>, Rama Rao J<sup>\*</sup>, Ambica Devi K<sup>\*</sup>****<sup>\*</sup>Department of Biochemistry, Osmania Medical College, Hederabad.****<sup>\*\*</sup>Department of Biochemistry, Mallareddy Institute of Medical Sciences, Hederabad.****\*Corresponding Author Email: [sampath.surya76@gmail.com](mailto:sampath.surya76@gmail.com)****BIOLOGICAL SCIENCES****RESEARCH ARTICLE****ABSTRACT**

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease. It encompasses a spectrum of conditions associated with lipid deposition in hepatocytes. It ranges from nonalcoholic steatohepatitis to advanced fibrosis and cirrhosis. The disease is mostly silent and is often discovered through incidentally elevated liver enzyme levels. It is strongly associated with obesity and insulin resistance and is currently considered by many as the hepatic component of the metabolic syndrome. A total of 100 subjects were participated in this study 75 were diagnosed as NAFLD and 25 were normal healthy subjects. Measurements were taken for assessment of BMI and blood samples were collected for estimation of fasting blood glucose, Triglycerides, Cholesterol, Alanine transaminase, Aspartate transaminase, Total bilirubin and Uricacid. Followed by the areas under the relative operating characteristic curves, sensitivity, specificity and diagnostic efficiency was calculated for analytes by using the best cut off values. All the nonalcoholic fatty liver disease patients studied are either overweight or obese and 92% of them are glucose intolerant or diabetics. The fasting blood glucose, serum triglyceride, serum cholesterol, serum uric acid, ALT and ALT/AST ratio values are significantly increased in NAFLD patients compared to controls. Uric acid and ALT/AST ratio are showed best overall discriminatory capacity among all biochemical parameters for NAFLD. NAFLD can be seen in both male and female patients associated with Insulin resistance syndrome and oxidative stress. The ALT/AST ratio is the better biochemical marker for diagnosis of NAFLD.

**KEYWORDS:** Non alcoholic fatty liver disease, steatohepatitis, Insulin resistance.

**INTRODUCTION**

Non-alcoholic fatty liver disease (NAFLD) is one of the cause of a fatty liver, occurring when fat is deposited (steatosis) in the liver not due to excessive alcohol use and is a common disorder in the Western hemisphere. It encompasses two histological lesions, fatty liver and steatohepatitis. Steatohepatitis is characterized by the presence of varying combinations of findings that include macrovesicular steatosis, ballooning degeneration, mallory bodies, scattered predominantly lobular neutrophilic or mixed inflammatory cells and pericentral, pericellular fibrosis.<sup>1</sup> Steatohepatitis can progress to cirrhoiss of the liver. Both fatty liver and steatohepatitis, were originally described as

occurring primarily in those who consumed large amounts of alcohol. It is now well known that both lesions can occur in the absence of alcohol consumption in amounts considered to be detrimental to the liver.

It is now increasingly appreciated that NAFLD is one of the most common causes of liver function abnormalities and liver related morbidity in the western world. It is also generally believed that NAFLD is a metabolic disorder and that Insulin resistance plays a key role in its genesis. Insulin resistance along with other potential biochemical abnormalities results in fatty liver and the generation of excessive free radicals in the liver, which produce liver injury.<sup>2,3</sup>

The present study was designed to evaluate the utility of Body Mass Index (BMI), Fasting Blood sugar (FBS), Triglycerides (TGL), cholesterol (CHOL), Totalbilirubin (TB), Alanine Transaminase (ALT), Aspartate Transaminase (AST), ALT/AST ratio and uric acid (UA) in the characterization and diagnosis of Nonalcoholic fatty liver disease.

### MATERIAL AND METHODS

The present study was carried out in the Department of Biochemistry, Osmania Medical College and Hospital, Hyderabad. 100 cases of 75 Non Alcoholic fatty liver disease patients were studied. 25 subjects who were clinically healthy were taken as controls. The study was conducted with the approval of the institutional ethical committee. The clinical diagnosis of cases of NAFLD was based on the presence of insulin resistance, in the absence of alcohol abuse, viral, autoimmune, genetic and induced liver disease and was correlated by further investigation (ultrasound abdomen showing fatty liver). Most of NAFLD patients were asymptomatic while a few complained of discomfort in the right upper quadrant of the abdomen.

### EXCLUSION CRITERIA

1. Daily alcohol intake > 30gm in men or > 20gm in women.
2. Use of amiodarone, corticosteroids, tamoxifen, methotrexate or high dose estrogen.
3. Jejunioileal by pass or extensive bowel resection.
4. Other known liver diseases.
5. Malignancy.

### DATA COLLECTION

Systematic data collection was carried out from both cases and controls after obtaining the consent. Age, gender, height and weight were documented, body mass index(BMI) was calculated and is defined as weight (kg) / height (m<sup>2</sup>). The blood samples were collected for the estimation of the following parameters in the serum Total Bilirubin<sup>4</sup>, Alanine Transaminase<sup>5</sup>, Aspartate Transaminase<sup>6</sup>, Triglycerides<sup>7</sup>, Cholesterol<sup>8</sup>, Uric acid<sup>9</sup> and Fasting Blood sugar<sup>10</sup>.

### STATISTICAL ANALYSIS:

1. Calculation of Mean, Standard deviation and p values in both cases and controls for analytes.
2. Determining Best cut off values for individual markers by using ROC curves.
3. Calculating the sensitivity, specificity and diagnostic efficiency by using the best cut off values.
4. Determining the areas under the ROC curves for different analytes.
5. Comparing the discriminatory capacities of various analytes by comparing the areas under ROC curves.

### RESULTS

The parameters shown FBS, cholesterol, TGL, UA, AST, ALT, TSB, ALT/AST ratio and BMI were analyzed in 100 subjects, 75 of them are patients of NAFLD and 25 are controls. The data has been statistically analyzed. Amongst the cases, 28 (37.33%) were males and the rest 47(62.67%) were females. Amongst controls 10 (40%) were males and the remaining 15 (60%) were females. The average age of cases was  $48.28 \pm 9.26$  and  $40.40 \pm 11.67$  for controls.

**TABLE NO 1: MEAN  $\pm$  SD OF ALL PARAMETERS IN BOTH CASES AND CONTROLS**

PARAMETER	CASES	CONTROLS
BMI	32.15 $\pm$ 2.47	22.96 $\pm$ 4.64
FBS	124.19 $\pm$ 10.89	75.24 $\pm$ 8.84
Triglycerides	248.09 $\pm$ 168.37	173.60 $\pm$ 55.43
Cholesterol	210.99 $\pm$ 43.25	167.40 $\pm$ 25.88
Total Bilirubin	0.15 $\pm$ 0.28	0.52 $\pm$ 0.25
ALT	34.45 $\pm$ 10.68	25.04 $\pm$ 8.76
AST	27.77 $\pm$ 11.62	31.56 $\pm$ 7.52
ALT/AST Ratio	1.33 $\pm$ 0.41	0.78 $\pm$ 0.17
Uricacid	5.52 $\pm$ 1.43	3.69 $\pm$ 1.10

All the cases had above normal BMI values ( $> 25 \text{ kg/m}^2$ ). 22 of them are (29.33%) over weight, while 53 (70.67%) were obese. The average BMI for the cases was  $32.15 \pm 2.47$ , the same for controls being  $22.96 \pm 4.64$ . This difference was found statistically highly significant ( $P < 0.001$ ). 34 patients (45.33%) had FBS values  $\geq 126\text{mg\%}$  and an equal number have values between 110 & 125mg%. The mean FBS of NAFLD cases is  $124.19 \pm 10.89$  which is higher than the mean FBS of controls  $75.24 \pm 8.84$ , the difference being statistically significant ( $P < 0.001$ ). 62 patients (82.67%) had hypertriglyceridemia. The mean serum triglycerides in the controls  $173.6 \pm 55.43$ , while that of the cases was much higher at  $248.09 \pm 168.37$ . The difference was statistically significant with  $P < 0.001$ . 60% of cases, i.e., 45 patients had hypercholesterolemia. The mean serum cholesterol of NAFLD patients is  $210.99 \pm 43.25$  and in controls is  $167.4 \pm 25.88$ . The increase was statistically significant ( $P < 0.001$ ). The mean ALT value of controls  $24.04 \pm 8.76$  and the mean ALT of NAFLD cases  $34.45 \pm 10.63$ . P

value was  $< 0.001$ , suggesting that the elevation of ALT among patients shows statistically significant. The mean AST values of controls  $31.56 \pm 7.52$  and that of cases  $27.77 \pm 11.62$ . This difference in the values was not statistically significant. The mean  $\pm$  SD values of ALT / AST ratio in controls  $0.78 \pm 0.17$ . The value of the same ratio  $1.33 \pm 0.41$  in NAFLD patients. This shows a statistically significant increase with P values  $< 0.001$ . The mean uric acid levels in NAFLD cases  $5.52 \pm 1.43$ , whereas in controls it  $3.69 \pm 1.10$ . This difference found was statistically significant.

The mean Total serum bilirubin of controls  $0.52 \pm 0.25\text{mg\%}$  and that of cases  $0.51 \pm 0.28$ . There is no statistically significant difference in TSB of both groups.

In order to assess the significance of alterations observed in different parameters analyzed, in patients compared to those of controls, the sensitivity and specificity of these parameters are calculated. This was done with the help of best cut off values derived from ROC curves.

**TABLE NO 2: RELATIVE OPERATING CHARACTERISTIC CURVE FEATURES OF PARAMETERS**

Parameter	Best cutoff value	Sensitivity	Specificity	Diagnostic efficiency
BMI	26 kg/m <sup>2</sup>	100%	100%	100%
FBS	103 mg/dl	98%	100%	99%
Triglyceride	221 mg/dl	43%	80%	52%
Cholesterol	193 mg/dl	65%	84%	70%
Total Bilirubin	0.9 mg/dl	15%	96%	35%
ALT	55.5 U/L	9%	100%	37%
AST	49.5 U/L	11%	100%	33%
ALT / AST Ratio	1.04	85%	96%	88%
UA	3.5mg/dl	96%	64%	88%

The Best cut off values were established by selecting points of test values that provided the greatest sum of sensitivity, specificity and

diagnostic efficacy i.e., the point closest to the top left hand corner in ROC curve.

**TABLE NO 3: THE AREA UNDER CURVE OF THE ROC CURVES OF PARAMETERS**

Parameter	Area under the Curve	Standard error	Asymptomatic 95% confidence interval	
			Lower bound	Upper bound
BMI	1.000	0.000	1.000	1.000
FBS	1.000	0.000	1.000	1.000
Triglycerides	0.625	0.062	0.507	0.743
Cholesterol	0.807	0.044	0.720	0.894
Serum Bilirubin	0.478	0.063	0.354	0.602
ALT	0.743	0.062	0.622	0.864
AST	0.335	0.057	0.223	0.446
ALT / AST ratio	0.985	0.009	0.967	1.003
UA	0.842	0.046	0.752	0.932

The Area under curve table of the ROC curves of different parameters are compared to evaluate the differentiating capacities of different analyses. BMI, FBS, ALT /AST ratios discriminated controls and patients with NAFLD with higher sensitivity and specificities. AST, ALT, TSB, TGL & Cholesterol, though more specific, are not sensitive enough to identify

the NAFLD cases. Among markers of liver function, ALT/AST ratio is a better diagnostic marker, with area under curve value of 0.985.

#### DISCUSSION

Obesity is the single most consistent association described with NAFLD.<sup>11</sup> Most of the studies reported a prevalence of 40% to

100%. Some epidemiological studies reported direct correlation between BMI and the probability of having a fatty liver. The Biochemical mechanisms of progression from obesity related steatosis into NAFLD and eventually to cirrhosis are poorly defined. Recently with the help of new model of obesity associated liver disease, it has been demonstrated that hepatic macrophage dysfunction occurs in obesity and it is suggested that this might promote steatohepatitis by sensitizing hepatocytes to endotoxin.<sup>12-17</sup> All the patients (100%) in the present study have above normal BMI values, 70.67% of patients are as obese and remaining 29.33% patients are overweight.

Many authors have reported that higher plasma glucose values are observed in NAFLD patients and reported that these patients are frequently associated with type 2 Diabetes mellitus. We also observed that 62 patients presented with hypertriglyceridemia (in 82.67% of patients) and 45 patients with hypercholesterolaemia (in 60% of patients) in the present study. It is considered that NAFLD is milder than alcohol induced disease with many asymptomatic patients and a lower progression to fibrosis or cirrhosis of liver<sup>18, 19</sup>. In the present study we observed that almost all patients were asymptomatic or only presented with mild symptoms. This supports the above consideration. It has been reported that NAFLD is associated with insulin resistance which may be evident clinically with obesity, type 2 diabetes mellitus and hypertriglyceridemia. The insulin resistance and hyperinsulinaemia is reported even in NAFLD patients who are lean and with normal glucose tolerance.

It has been suggested that genetic factors that reduce insulin sensitivity and increase triacylglycerol levels may be responsible for the development of insulin resistance. Our

study supports the association between insulin resistance, and NAFLD as represented by the fact that all the patients studied are either obese or overweight; almost all the patients have either diabetes or glucose intolerance and a substantial percentage of the patients are hypertriglycerimic and / or hypercholesteremic.

Majority of the studies have shown that NAFLD patients are diagnosed during routine investigation of suspected patients or patients with mild vague symptoms. It has been reported that majority of the patients are shown to have mild abnormal liver function tests.<sup>20</sup> Many reports have shown small increases in serum liver enzyme activities (ALT and AST), mild elevation of ALT being the most common abnormality reported. Some reports have shown that when elevated the increases in ALT are higher than those of AST. In the present study we observed statistically significant increases in the ALT in NAFLD patients. However only in 11% of patients the ALT values are above the upper reference range value established in our laboratory (50 units/L). The AST values are not significantly different from those of controls and in only 11% of cases, these values are above the upper reference range value established in our laboratory (50 units / L).

Several authors have shown that there is a significant increase in the ALT / AST ratio in NAFLD patients.<sup>20</sup> However; it has been shown that the diagnostic accuracy of this ratio is lost in patients with cirrhotic NAFLD. In the present study we observed a highly significant increase in ALT / AST ratio values in patients compared to controls.

It has been reported that the complete ROC curve summarises the clinical performance of the analytical systems; Good clinical performance is characterized by a high true positive rate and low false positive rate. In the

present study we found that ALT/ AST ratio is a better diagnostic marker among the markers studied to indicate NAFLD. However the clinical diagnosis utility of this test can only be established when a study involving all other conditions which are to be considered in differential diagnosis of NAFLD is taken up.

The present study showed no significant alteration in the serum bilirubin levels. None of the studies reviewed showed any alteration in bilirubin concentrations.

The increased risk of fibrosis in NAFLD patients in the presence of slightly increased hepatic Iron stores may involve more than one mechanism. However, lipid peroxidation currently seems the most likely mechanism. It has been shown that the cyst 282 Tyr. mutation is responsible for most of the mild, iron overload found in NAFLD and thus has a significant association with hepatic damage in these patients. Hepatic Iron overload can directly cause lipid peroxidation.<sup>21</sup> In the present study we observed significantly increased Serum uric acid levels in NAFLD patients, which indicates the presence of oxidative stress in this disease which is shown to be slowly progressive. It also indicates decreased renal clearance of uric acid as a response to the day long elevations of circulating insulin.

## SUMMARY

The present study was undertaken to assess the value of various biochemical parameters in the diagnosis and progression of nonalcoholic fatty liver disease.

- Majority of the NAFLD patients were studied are asymptomatic.
- All the nonalcoholic fatty liver patients studied are either overweight or obese.
- BMI and elevated FBS are most universal abnormalities in NAFLD.

- 92% of the NAFLD patients are glucose intolerant or diabetics.
- The fasting blood sugar, serum triglyceride, serum cholesterol, serum uric acid, ALT and ALT/AST ratio values are significantly increased in NAFLD patients compared to controls.
- The serum Bilirubin and AST values in NAFLD patients are not significantly different from those of controls.
- Bilirubin, AST, ALT, TGL and cholesterol though discriminate the cases and controls; they are deficient by not having required sensitivity, specificity or diagnostic accuracy.
- Uric acid showed relatively good discriminatory capacity with relatively less sensitivity.
- The ALT/AST ratio showed best overall discriminatory capacity amongst the diagnostic tests for NAFLD.

## CONCLUSIONS

The NAFLD is a mild disease which affects both female and male

NAFLD appears to be the hepatic expression of the Insulin resistance syndrome.

The ALT/AST ratio is the best diagnostic marker for diagnosis of NAFLD.

NAFLD is associated with oxidative stress.

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