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Association of Insulin Resistance, Metabolic Syndrome And Alanine Aminotransferase Activity In Non-Alcoholic Fatty Liver Disease

Dr. S.Sailusha[1], Dr. V.Uma Lakshmi[2] , Dr. Sandhya Rani Sadula[3]
[1](Senior Resident, Department of Biochemistry, Osmania medical college, India)
[2](Professor, Department of Biochemistry, Osmania medical college, India)
[3](Senior Resident, Department of Biochemistry, Osmania medical college, India)

Abstract: *Objective: Non-alcoholic fatty liver disease (NAFLD) has been proposed as the hepatic manifestation of metabolic syndrome (MS), with insulin resistance (IR) as the common pathophysiological mechanism. The present study is to assess the association between NAFLD and insulin resistance independent of diabetes mellitus. Methods: A case control study of 90 subjects divided into three groups.1.healthy controls (n=30), 2.NAFLD without Diabetes mellitus (n=30) and 3.NAFLD with Diabetes mellitus (n=30). Fasting levels of Plasma Glucose, Serum Insulin, Lipid profile and ALT were estimated.IR was calculated by HOMA-IR. Multiple comparisons between different groups were done using ANOVA test.*
Results: *The results of the study revealed the mean±S.D of IR in controls was 0.68±0.33, in group 2 was 6.51±3.3 and in group 3 was 9.2±7.2. Fasting insulin levels, IR and Triglyceride levels were significantly increased (P<0.0001) and HDL cholesterol levels were significantly decreased (P<0.0001) in groups 2 & 3 when compared to controls. ALT levels did not show statistical significance between the cases and controls.*
Conclusions: *Nondiabetic and diabetic obese patients were simultaneously categorized by IR, those with predominant IR had a higher prevalence of NAFLD and metabolic dyslipedemia, thus confirming the pathophysiological role of hyperinsulinemia. Thus this study suggests that strategies to prevent fatty liver might focus on dietary and/or drug interventions that improve insulin sensitivity.*
Keywords: *Insulin Resistance, Metabolic syndrome, Non-alcoholic fatty liver disease, Type2 Diabetes Mellitus.*

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I. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a chronic metabolic disorder and it is characterized by lipid deposition in the hepatocytes (more than 5% of hepatocytes) of liver parenchyma in absence of excessive alcohol intake. The spectrum of disease is broad ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis[1].

NAFLD has become an important public health problem because of its high prevalence, potential progression to severe liver disease, and association with type 2 diabetes mellitus (T2DM), the metabolic syndrome and coronary heart disease (CHD). In addition, the presence of NAFLD is associated with a high risk of developing dyslipidemia (high plasma TG and/or low plasma HDL-cholesterol concentrations) and hypertension. Obesity and insulin resistance are the two major risk factors of NAFLD[2]. It is the most common liver disease in Western countries, and is becoming increasingly prevalent in Asian Pacific regions because of the increasing westernization of lifestyle, such as high-fat and high-calorie diet, less physical activity and increasing incidence of central obesity and Type2 DM[3].

The metabolic syndrome is due to group of risk factors that greatly increases an individual's probability for developing cardiovascular disease, T2DM etc. Abdominal obesity, atherogenic dyslipidemia, hypertension, elevated plasma glucose and a pro-inflammatory state are important in the list[4]. The fundamental abnormality is resistance to insulin-mediated glucose uptake in muscle and increased lipolysis, which produces elevated levels of circulating free fatty acids [5].

Insulin resistance (IR) is the inability of a known quantity of insulin to increase glucose uptake and use in an affected individual as much as it does in a normal person. IR is believed to play an important role by facilitating the transport of free fatty acid into the liver from visceral fat stores or peripheral lipolysis [6]. IR may also cause lipid peroxidation, which in turn may activate inflammatory cytokines and promote the progression of steatosis to NASH and liver fibrosis.

Obesity is an independent risk factor for NAFLD and it is strongly associated with the progression of the disease. Visceral fat is an important source of triglycerides leading to steatosis. This explains the presence of lean and centrally obese individuals with NAFLD[7]. Alanine transaminase(ALT) is a transaminase enzyme.

NAFLD causes asymptomatic elevation of the level of liver enzymes, among them ALT is most closely related to liver fat accumulation.

Thus, abnormalities in fatty acid metabolism in conjunction with adipose tissue, hepatic, and systemic inflammation are key factors involved in the development of insulin resistance, dyslipidemia and other cardio-metabolic risk factors associated with NAFLD.

Hence the present study was designed to evaluate the association between insulin resistance, metabolic syndrome and ALT levels in NAFLD cases with or without Type 2 diabetes mellitus.

II. Materials and methods

A case control study of 90 subjects was conducted in the Department of Biochemistry, Osmania General Hospital, Hyderabad. The study subjects are divided equally into 3 groups.

Group 1 - Healthy controls

Group 2 - NAFLD patients without T2DM

Group 3 - NAFLD patients with T2DM

All the subjects were in the age group 35 to 55 years and of either sex. Informed oral consent was taken from all individuals who took part in the study.

2.1 Inclusion criteria

- Group 1 included healthy controls that were matched for age and sex.
- Group 2 included NAFLD patients who were obese and not diabetic.
- Group 3 included NAFLD patients who were obese and diabetic.

Obesity was characterized by waist circumference more than 90cm in men and more than 80cm in women.

2.2 Exclusion criteria

- History of current or past alcohol intake (more than 20gm per day).
- Positive test for HBsAg and Hepatitis C antibody.
- Presence of autoimmune and congenital defects responsible for liver disease.
- Patient taking drugs known to promote fatty liver disease.

2.3 Collection of sample

Fasting venous blood samples were collected from all groups. 3ml of blood was collected into serum tubes and 2ml into sodium fluoride tubes. Grossly haemolysed and lipemic samples were excluded. Samples were analyzed for Fasting Plasma Glucose, Serum Insulin, Insulin Resistance, Serum Total Cholesterol(TC), Serum High Density Lipoprotein – Cholesterol(HDL-C), Serum Low Density Lipoprotein – Cholesterol(LDL-C), Serum Very Low Density Lipoprotein – Cholesterol(VLDL), Serum Triglyceride(TG) and Serum Alanine Transaminase. Fasting plasma glucose was estimated in plasma daily while all other parameters were estimated in serum.

2.4 Methods

All parameters except insulin, insulin resistance, LDL-C and VLDL are analyzed on semi-automated analyzers using colorimetric methods. Insulin was analyzed on Beckman Coulter Chemiluminiscence. IR was calculated on the basis of fasting values of plasma glucose and insulin, according to the homeostasis model assessment method(HOMA-IR). LDL-C was calculated by Friedewalds equation and VLDL was derived from Triglyceride.

2.5 Statistics

The data was analyzed using Graph Pad Prism Demo and software version 6 and the results were expressed as Mean and Standard deviation of various parameters in different groups. Multiple comparisons ANOVA was used to assess the significance of difference of mean values of different parameters in between the groups and represented by p values (p value < 0.05 is considered as significant). ROC curve analysis was done to assess maximum sensitivity and specificity.

III. Results

Parameter	Mean±S.D of controls	Mean±S.D of NAFLD without diabetes	Mean±S.D of NAFLD with diabetes	P-value
FPG	83.67±12.6	115.6±6.1	167.8±43.1	< 0.0001(****)
Fasting Insulin	3.33±1.71	22.6±10.9	22.8±18.4	< 0.0001(****)
IR	0.68±0.33	6.51±3.3	9.2±7.2	< 0.0001(****)
TC	126.1±10.7	199.1±27.7	214.2±44.7	< 0.0001(****)
HDL-C	43.3±4.1	34.07±4.4	30.6±6.1	<0.0001(****)

LDL-C	66.6±10.3	124.3±27.9	138.8±45.8	< 0.0001(****)
VLDL-C	16.17±3.5	40.8±12.9	44.8±15.6	< 0.0001(****)
TG	81.2±17.5	204.3±64.9	223.9±78.4	< 0.0001(****)
ALT	12.8±5.7	13.9±6.1	15.5±8.4	0.2984(n.s)

Table 1: Mean ± SD and P-value of various parameters in all groups
(**** : significant; n.s : not significant)

As reported in Table 1 Mean± S.D of fasting glucose, fasting insulin and IR were higher and statistically significant in NAFLD with and without DM cases when compared with controls. The Mean± S.D of TC, LDL-C, VLDL-C AND TG were higher and statistically significant while that of HDL-C was decreased significant in NAFLD with and without DM cases when compared with controls. Mean± S.D of ALT was within normal limits and was not statistically significant in NAFLD with and without DM cases when compared with controls.

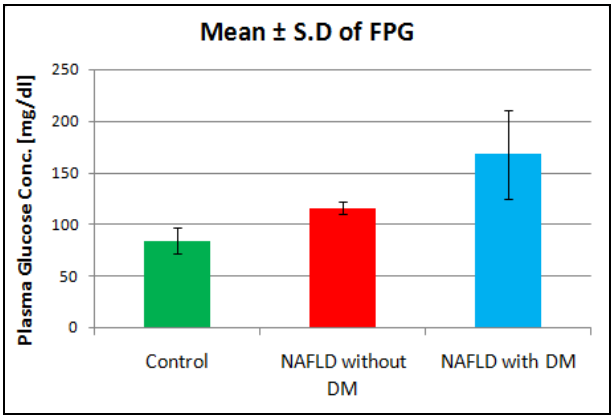

Figure 1: Graphical representation of Mean ± SD of FPG

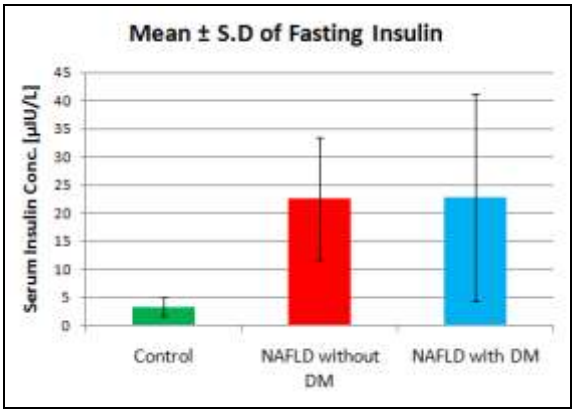

Figure 2: Graphical representation of Mean ± SD of Fasting Insulin

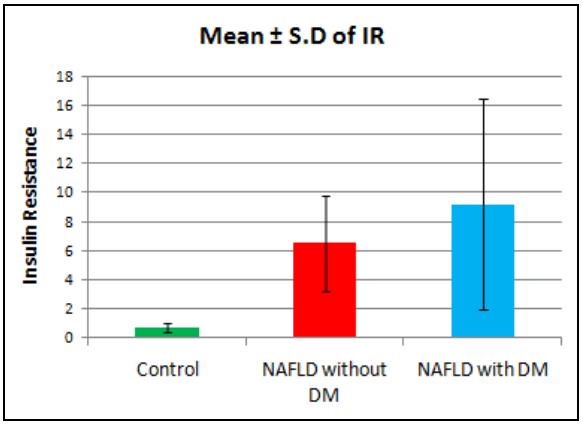

Figure 3: Graphical representation of Mean ± SD of IR

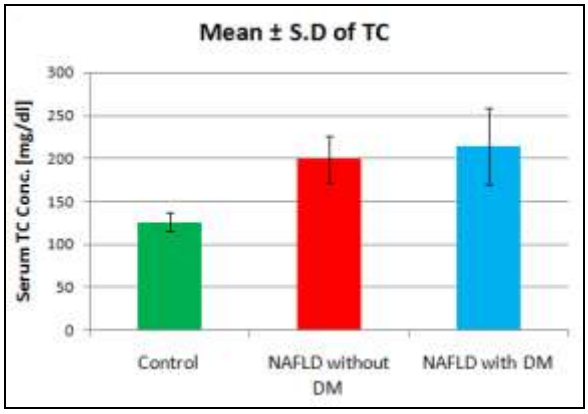

Figure 4: Graphical representation of Mean ± SD of TC

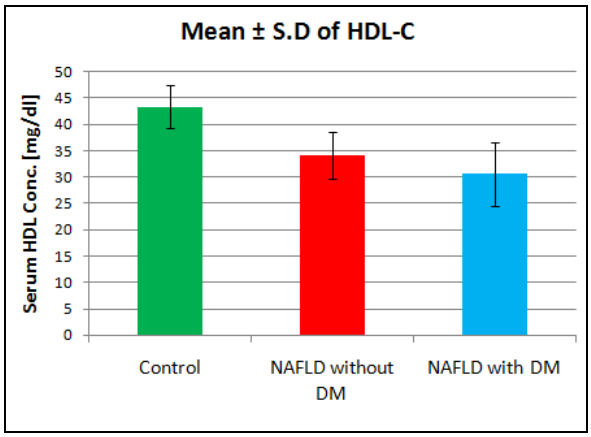

Figure 5: Graphical representation of Mean ± SD of HDL-C

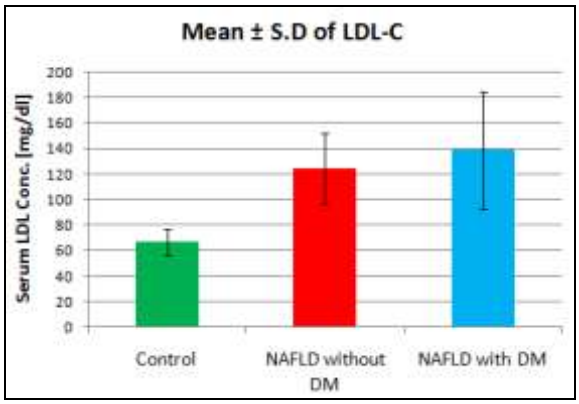

Figure 6: Graphical representation of Mean ± SD of LDL-C

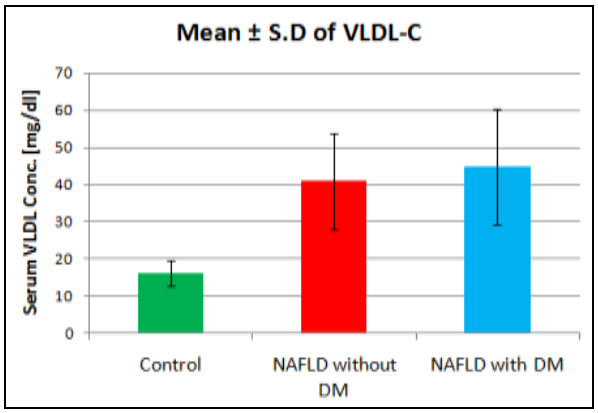

Figure 7: Graphical representation of Mean ± SD of VLDL-C

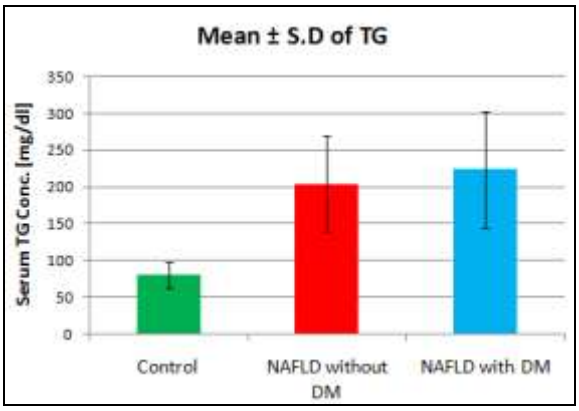

Figure 8: Graphical representation of Mean ± SD of TG

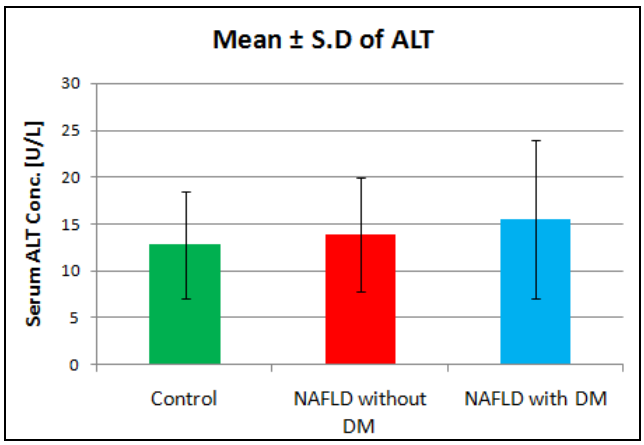

Figure 9: Graphical representation of Mean ± SD of ALT

Pearson's Correlation between different parameters in NAFLD without T2DM cases showed positively correlation of IR with ALT, VLDL-C and TG. Pearson's Correlation between different parameters in NAFLD with T2DM cases showed IR was positively correlated with FPG, VLDL-C and TG. In the above group ALT was positively correlated with IR, VLDL-C and TG which was statistically significant.

ROC analysis showed 100% sensitivity and 100% specificity of IR, TG and VLDL-C when compared to other parameters in both the case groups. IR shows a better diagnostic efficiency than other parameters in discriminating cases and controls.

IV. Discussion

Prevalence of NAFLD is high, with strong progression to severe liver disease, and association with serious cardio-metabolic abnormalities that makes it a major health problem [8,9]. Hepatic manifestation of metabolic syndrome is proposed to be NAFLD with common IR pathophysiology. Bidirectional relationship exists between NAFLD and metabolic syndrome. Liver fat content is significantly increased in subjects with metabolic syndrome as compared with those without, independent of age, gender and BMI; in turn, presence of NAFLD is a strong predictor of metabolic syndrome [10].

In the present study IR increased significantly in NAFLD with Diabetes cases when compared to other groups. Several mechanisms may be proposed to explain the role of IR in NAFLD. First, Peripheral insulin resistance in NAFLD patients causes impaired suppression of lipolysis in adipose tissue leading to increased efflux of FFA into plasma. Second, hyperinsulinemia associated with IR leads to up-regulation of the transcription factor SREBP-1c, which is a key transcriptional regulator of genes involved in DNL. Third, hyperinsulinemia may also contribute to TG accumulation in the liver by inactivating the forkhead transcription factor (Foxa2) which promotes β-oxidation of fatty acid in the liver. Fourth, hyperinsulinemia also causes degradation of apoB 100 and negatively regulates the expression of MTP, both of which are required for VLDL secretion[11-13].

Angelico et al in a similar study showed a progressive statistically significant increase in mean HOMA-IR ($P<0.0001$) in subjects with normal glucose tolerance to those with IGT and patients with T2DM. A positive linear correlation was observed between HOMA-IR and the severity of liver steatosis[14]. According to

Marchesini et al, IR was the laboratory finding most closely associated with the presence of NAFLD in a large series of patients irrespective of BMI, fat distribution or glucose tolerance[15]. Studies conducted by Quoc M N et al and Se-Yong Oh et al also showed similar results[16,17].

In the present study Fasting Insulin levels were significantly increased in NAFLD with and without DM cases when compared to controls with P-value of < 0.0001 which were in accordance with Angelico et al and Marchesini et al. Angelico et al categorized non-diabetic subjects by IR and insulin secretion status, those with a predominant IR had a higher prevalence of severe liver steatosis, thus confirming the pathophysiological role of hyperinsulinemia in the events leading to the development of fatty liver[14]. A prospective study done by Eun-Jung Rhee et al showed that presence of hyperinsulinemia at baseline predicted increased risk for NAFLD and sustained hyperinsulinemia conferred the highest risk for development of NAFLD compared to subjects with sustained low insulin levels[18].

The main features of NAFLD discussed in the above paragraphs are that IR leads to compensatory hyperinsulinemia and often presages type 2 diabetes, and Hepatic steatosis leads in a significant minority of patients to hepatic inflammation or steatohepatitis and ultimately to cirrhosis. Perhaps most pernicious of all is the associated metabolic dyslipidemia, which is atherogenic and a significant contributor to the very high mortality in T2DM due to complications of atherosclerosis, which has been estimated at up to 80%[19]. Altered lipoprotein function in NAFLD leads to hypertriglyceridemia, reduction in HDL-C, hypercholesterolemia and the appearance of small dense low density lipoproteins (sdLDL)[20]. In the present study TG levels were significantly increased in cases compared to controls which was in accordance with study by Kathleen E Corey et al and Chien-Hua Chen et al[21,22].

In the present study HDL cholesterol concentration decreased significantly in cases compared to controls and the decrease was more prominent in NAFLD with T2DM cases. A decreased concentration of HDL-C is one of the features of dyslipidemia seen in NAFLD. Increased levels of VLDL1 also alter the composition of HDL through the actions of cholesterol ester transfer protein(CETP) and hepatic lipase, leading to the formation of small dense HDL and increased catabolism of these particles[23]. These findings were in accordance with Marchesini et al and Quoc M N et al[15,16].

In the present study LDL-C levels increased significantly with P value of < 0.0001 in NAFLD with and without T2DM cases when compared with controls. These findings were in accordance with Kathleen E Corey[22]. LDL-C correlated positively with FPG, IR, fasting insulin and ALT in all cases. Quoc M N et al showed significant increase with P value <0.001 in LDL-C in diabetic and prediabetic groups when compared to controls. LDL-C levels were also more in diabetic cases than in prediabetic cases[16].

In the present study ALT levels were in the normal range in NAFLD with and without DM cases as in controls. Similar findings were found Angelico et al which are in accordance with other studies.

A prospective study done by Hamaguchi et al demonstrated that presence of metabolic syndrome is associated with increased risk of USG-defined NAFLD[24]. Another study by Tsuneto et al suggests that obesity, hypertriglyceridemia and presence of hypertension might serve as predictive variables for fatty liver[25]. A Study done by Sandhya Mishra et al showed that prevalence of NAFLD was two and half times more in metabolic syndrome and prevalence of metabolic risk factors was significantly higher in NAFLD group as compared to Non-NAFLD group[26].

NAFLD has emerged as the most common etiology of liver damage in the general population, and many metabolic disorders, such as overweight, DM, hyperlipidemia, and hyperuricemia are associated with the development of NAFLD and liver injury in NAFLD. Serum liver enzyme examinations are not sensitive enough to diagnose NAFLD and are susceptible to interference from other clinical conditions. However, we cannot rely on liver enzyme examinations alone because liver enzyme levels are often normal in most NAFLD patients. In addition, liver enzyme levels fluctuate with the development of liver disease and may even be normal at advanced stages of cirrhosis. Thus, there are obvious limitations to the use of liver enzymes as markers for the diagnosis and monitoring of the activity of NAFLD[27].

V. Conclusion

In the present study significant hyperglycemia was observed in NAFLD with DM cases and impaired glucose tolerance was observed in NAFLD without DM cases. There is increased risk of developing T2DM or poor glycemic control in NAFLD. In established T2DM cases risk of cardiovascular disease is increased. Screening for NAFLD should be done in metabolic derangement and T2DM to reduce progression and avoid complications.

To conclude non-diabetic and diabetic obese patients were simultaneously categorized by IR, those with predominant IR had a higher prevalence of NAFLD and metabolic dyslipedemia, thus confirming the pathophysiological role of hyperinsulinemia in the events leading to the development of fatty liver. Thus this study suggests that strategies to prevent fatty liver might focus on dietary and/or drug interventions that improve insulin sensitivity.

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