

Association of Fasting Lipid Profile with Insulin Resistance in Non-Alcoholic Fatty Liver Disease Patients

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Abstract

Back ground: Non-Alcoholic Fatty Liver Disease (NAFLD) is a common cause of Chronic Liver Disease (CLD) worldwide and is becoming a major public health problem. NAFLD has been recognized as a hepatic manifestation of Metabolic Syndrome (MetS) linked with Insulin Resistance (IR) and is currently considered as the hepatic component of the Metabolic Syndrome (MetS). NAFLD is strongly associated with IR and is mostly silent which is often discovered incidentally through elevated hepatic enzyme levels. The purpose of this study is to find out the association of Fasting Lipid Profile (FLP) with IR in NAFLD subjects.

Materials and methods: A prospective hospital based cross sectional study was carried out in the Department Of Biochemistry, Institute of Nuclear Medicine and Allied Sciences (INMAS) and Chittagong Medical College Hospital. One hundred & fifty (150) subjects aging between 18-60 years were included in this study by non probability consecutive sampling method. Important variables in this study were Fasting Plasma Glucose (FPG) Fasting Serum Insulin (FPI) and Fasting Lipid Profile (FLP). IR was calculated by using Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index i.e. $(FPI \mu U \times FPG \text{ mmol/L})/22.5$.

Results: In this study HOMA-IR was significantly higher in cases than that of controls (4.77 ± 0.16) and showing Insulin Resistance (IR) in 90% of the cases. There was a significant association of HOMA-IR with increased serum Triglyceride (TG) in NAFLD cases.

Conclusion: The findings of the current study suggest that independent variable such as increased Serum TG was associated with IR in NAFLD subjects.

Key words: Fasting lipid profile; Homeostasis Model Assessment of Insulin Resistance (HOMA-IR); NAFLD.

INTRODUCTION

Non-Alcoholic Fatty Liver Disease (NAFLD) includes a wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis and advanced fibrosis, with histological features of alcohol-induced liver disease in individuals who do not consume significant amounts of alcohol^{1,2}. The cutoff limit of alcohol intake that distinguishes between alcoholic fatty liver disease and NAFLD is not known, although 20g/d for women and 30g/d for men is commonly used. One standard drink typically contains 10–20 g of alcohol³. In 1980, Ludwig et al published the first systematic description of what was then an “unnamed and poorly understood” condition⁴. On liver biopsy, findings resembled those of alcoholic hepatitis, but because the patients did not have a history of heavy drinking, the condition was named “nonalcoholic steatohepatitis”. Around 90% of NAFLD patients had at least one component of MetS, and around 33% of patients met the criteria for MetS^{5,6,7}.

NAFLD is also associated with extra hepatic manifestations such as Cardiovascular Disease (CVD), Chronic Kidney Disease (CKD) etc^{8,9}. There are no evident clinical symptoms in its initial stage. Generally, absence of alcohol abuse or consumption of alcohol of < 20 g/day for prolonged periods, and negative serological tests for Hepatitis B and C should raise the suspicion of NAFLD¹⁰.

Excess intra-abdominal fat may be a key determinant in the pathogenesis of NAFLD, due to its strong association with IR¹¹. Several studies suggest that excess Free Fatty Acids (FFA) especially Non Essential Fatty Acid (NEFA) flux due to peripheral IR contributes to hepatic steatosis¹². Further more visceral adiposity is positively correlated with clinical manifestations associated with IR, such as Type 2 Diabetes Mellitus (DM), Dyslipidemia and MetS^{1,2}. Pathogenesis in NAFLD is characterized by fat deposition, inflammation, and fibrosis of liver¹⁰. Day et al proposed the 'two hits' theory¹³. The first 'hit' is peripheral insulin resistance, resulting in the steatotic liver. The presence of increased TG renders the steatotic liver more sensitive to oxidative stress (The 'second hit')¹⁴.

IR plays a fundamental role in the pathogenesis of NAFLD. IR may be defined as a condition in which higher than normal insulin concentrations are needed to achieve normal metabolic responses¹⁵. IR is evaluated according to HOMA-IR index method, as demonstrated by Marchesini et al¹⁶. The prevalence of NAFLD in the general population ranges from 5 to 20% and up to 75% in patients with Obesity and DM¹⁷. NAFLD is extremely common in USA affecting approximately 20% of the adult population¹⁸. Henceforth considering the above information gathered and based on the research works demonstrated by different relevant authors, this study was aimed to investigate the potential relation of IR in NAFLD and its association with increased serum TG in the Bangladeshi population.

MATERIALS AND METHODS

A prospective hospital based cross sectional study was carried out in the Department Of Biochemistry and Institute of Nuclear Medicine and Allied Sciences, Chittagong Medical College Hospital, which included one hundred and fifty (150) subjects aged between 18-60 years over a period of one year from June 2017- June 2018. Subjects of both the sexes were evaluated sonographically and were divided into two groups as NAFLD cases (n=80) and Non-NAFLD controls (n=70). Subjects were excluded if they tested positive for hepatitis B virus surface antigen or anti-hepatitis C virus antibody or were suffering from liver cirrhosis, Acute or chronic hepatitis, history of alcohol abuse, Type II DM and Pregnancy.

Data was collected using a pre-tested structured questionnaire containing all the variables of interest after taking informed and written consent. Using standard phlebotomy procedures 5 ml of fasting venous blood was drawn from the median-cubital vein

in between 8 and 9 am. Blood taken into clean and dry test tubes were kept for clot formation. After centrifugation serum was separated and taken into eppendorf which was then immediately transported to Biochemistry Laboratory for analysis. FPG was determined by enzymatic oxidation in the presence of Glucose Oxidase. FPI assay was a two-site sandwich immunoassay using direct chemiluminescent technology which used constant amounts of two antibodies. IR was calculated from FPG and FPI values by HOMA-IR. Fasting serum Total Cholesterol (TC) was measured using a polychromatic endpoint technique. Fasting serum Triglyceride (TG), serum High Density Lipo-protein (HDL-C) and serum Low Density Lipo-protein (LDL-C) were measured by bichromatic endpoint technique.

Statistical analyses were performed using SPSS for Windows version 20.0. Statistical inference was based on 95% confidence interval and p value ≤ 0.05 was considered statistically significant. Data were expressed as mean \pm Standard Error of Means (SEM) and comparison between two groups was done using student t-test. Correlation between HOMA-IR and FLP was tested using Spearman's correlation coefficient. The summarized data were presented in the form of tables and figure.

RESULTS

Complete clinical profile, US data and serum samples were available for 150 subjects out of which, 80 (53%) were NAFLD cases and 70 (47%) were Non-NAFLD subjects taken as controls [Fig: 1]. Mean age was significantly higher in cases (35.05 ± 1.05 years) than controls (26.53 ± 1.02 years), $p < 0.05$. Among the NAFLD subjects, 66.3% were in age group 30-49 years [Fig: 2]. FPG ($p=0.02$), FPI ($p < 0.00001$) and HOMA-IR ($p < 0.0001$) were significantly higher in NAFLD cases. Serum TG was significantly higher in NAFLD cases (203.01 ± 9.65 vs 177.94 ± 6.62 mg/dl) and HDL was significantly higher in controls ($p < 0.05$). There was no significant difference in mean LDL values between two groups [Table I]. IR status was more prevalent among the NAFLD cases ($p < 0.001$) and 90% of NAFLD cases had IR (HOMA-IR > 2.6). There was a significant Association between NAFLD and HOMA-IR ($\chi^2 46.05$, $p < 0.001$) [Table II]. There was also a significant Association between increased serum TG and IR (HOMA-IR > 2.6) among NAFLD cases ($\chi^2 6.003$, $p=0.014$) [Table: III]. Serum TG had a significant positive correlation with HOMA-IR [Fig: 3], whereas HDL had no correlation with HOMA-IR in case of NAFLD.

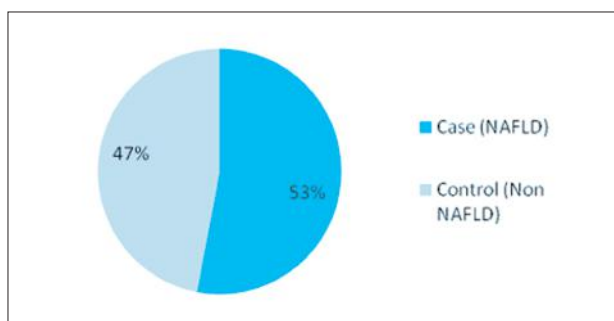


Figure 1 : Pie chart showing distribution of study population by NAFLD status (n = 150)

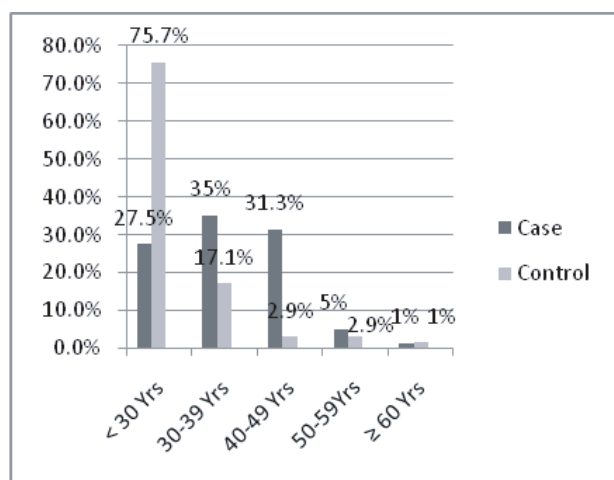


Figure 2 : Bar diagram showing the distribution of NAFLD in different age groups

Table I : Comparison of serum lipid profile amongst the study population (n = 150)

Variables (mg/dl)	Cases(n=80) (Mean±SEM)	Controls(n=70) (Mean ± SEM)	p value	Significance
Serum TC	174.64 ± 3.82	163.39 ± 4.10	p= 0.04	Significant
Serum TG	203.01 ± 9.65	177.94 ± 6.48	p= 0.032	Significant
Serum HDL	34.94 ± 0.94	36.61 ± 0.79	p = 0.035	Significant
Serum LDL	101.94 ± 2.58	99.91 ± 2.85	p = 0.61	Not significant

Table II : Association between NAFLD and HOMA-IR amongst the study population (n = 150)

Groups	Category of HOMA-IR		Total	Odds ratio (95% confidence interval)	p value & test statistic
	>2.6	2.6			
NAFLD (Cases)	72 (90%)	08 (10%)	80	15.23 (6.34-36.59)	$\chi^2 = 46.05$ p < 0.001
Non-NAFLD (Controls)	26 (37%)	44 (63%)	70		Significant
Total	98 (65%)	52 (35%)	150		

Table III : Association between increased serum TG and IR in

Serum TG (mg/dl)	Category of HOMA-IR		Total	Odds ratio (95% confidence interval)	p value & test statistics
	>2.6	2.6			
Normal (≤150)	16 (76%)	05 (24%)	21	(1.23-15.78)	$\chi^2 = 6.033$ p = 0.014 Significant
Increased (> 150)	56 (95%)	03 (05%)	59		
Total	72 (90%)	08 (10%)	80		

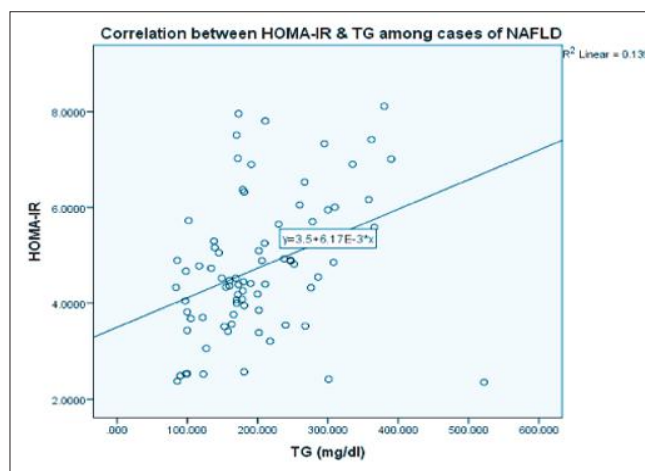


Figure 4 : Correlation between HOMA-IR and TG amongst cases (n=80)

DISCUSSION

NAFLD is the hepatic manifestation of MetS linked with lipid deposition in the hepatocytes¹⁹. Patients with NAFLD often have dyslipidemia along with other metabolic risk factors, such as obesity, DM and Hypertension (HTN)²⁰. Previous studies reported that dyslipidemia in patients with NAFLD was characterized by elevated serum TG and LDL-C, and decreased HDL-C concentrations²⁰. In view of the global epidemic of metabolic diseases in recent years, including DM, MetS or CVD, it is of great clinical and preventive significance to develop a simple and inexpensive screening tool to help clinicians quickly identify individuals who are at risk. The pathogenesis of NAFLD has been linked to IR, which was found to be associated with dyslipidemia, including low HDL-C level and high TG level²¹. The current study systematically analyzed the association between FLP and IR among NAFLD subjects. IR appears to be an intrinsic defect in NAFLD patients². Prevalence of NAFLD is rising in the Asia-Pacific region as the society becomes affluent and traditional lifestyles change i.e. increasing fat in the diet, less physical activity²². NAFLD is found to be more predominantly affecting the female patients because of their propensity to be diabetic, obesity and HTN^{22,23}.

Thus a prospective hospital based cross sectional study was carried out on One hundred and fifty (150) subjects aging between 18-60 yrs, in the Department Of Biochemistry and Institute of Nuclear Medicine and Allied Sciences, Chittagong Medical College Hospital. Complete clinical profile and US data with serum samples were recorded for one hundred and fifty (150) subjects. Among the subjects 80 were NAFLD and 70 were Non-NAFLD. In the gender distribution of the study there was female predominance. It was found that IR was more among the cases than controls. Association between NAFLD and HOMA-IR status among the study population was statistically significant ($p = < 0.001$). The result was similar to other research work done by E. Bugianesi et al². Independent variables such as FPG, FPI, HOMA-IR and Serum TG were significantly higher in cases than that of controls. TG had a significant positive correlation with HOMA-IR where as HDL had inverse correlation with IR. The results of the present study were similar to other previous research works by authors Kelley DE et al, Yoosoo, Sampath kumar.V et al, Rushad Patel et al and other relevant studies^{2,5,12,24,25,26}. Other authors failed to demonstrate an association between other components of IR with NAFLD^{27,28}.

The present study established an association between increased serum TG and IR in NAFLD patients and the observational findings were similar to other studies^{28,29,30}. There is also a well-established clinical association of NAFLD with dyslipidemia, HTN and obesity. Several studies have suggested relationship of disease with these features for these co-morbid conditions³¹.

CONCLUSION

NAFLD is a growing epidemic disease. Emerging data notes a high prevalence of NAFLD not only in the Western world but also in the South Asian population. NAFLD can progress to steatohepatitis which may further lead to HCC. The study revealed a significant association between FLP and IR in NAFLD subjects. This is likely due sedentary life style, dietary modifications from industrialization and development of metabolic risk factors.

RECOMMENDATIONS

Proper measurement of these parameters can be recommended to decrease the burden of NAFLD subjects in the community and further studies are necessary to better understand the biochemical strategy of inflammatory markers for the development of NAFLD.

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DISCLOSURE

All the authors declared no competing interest.

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