Original Article

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Association of Neutrophil to Lymphocyte Ratio and Platelet to Lymphocyte Ratio with Fatty Liver in Type II Diabetes Mellitus Patients

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Author`s Contribution

[1,2]Conception, data acquisition, revision, and approval of the final draft, Data analysis and interpretation
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ABSTRACT

Objective: To find the association between neutrophil to lymphocyte ratio, platelet to lymphocyte ratio and fatty liver in type II diabetes.

Methodology: This comparative analytical study was conducted at Shifa International Hospital on diabetic patients visiting falahee OPD clinics from June 2018 to June 2019. Nonprobability convenient sampling was used. Patients were segregated into two groups according to fatty liver status as assessed by ultrasonography. Complete blood count, lipid profile and liver profile were done. Data was analyzed by using statistical package for the social sciences (spss) version 21. Descriptive statistics were calculated for categorical variables. Kolmogorov smirnov test was used to ascertain the normality of the quantitative variables. For normal and dispersed variables, independent student t and Mann Whitney U test were applied, respectively. P Value below 0.05 was considered significant

Results: Out of total 93 patients, 33 (35.4 %) were males and 60(64.5%) were females. Female patients had increased incidence of fatty liver as compared to males. The mean duration of disease was 7.61 ± 5.8 years with 68.8% prevalence of fatty liver. BMI was elevated significantly in patients having fatty liver. There was no significant association between NLR, PLR and fatty liver. ALT, LDL and Triglycerides were increased significantly in patients having fatty liver

Conclusion: Patients having fatty liver have more deranged levels of lipid profile and hematological parameters increasing the risk of cardiovascular and inflammatory diseases.

Keywords: Fatty Liver, Neutrophil Lymphocyte Ratio (NLR), Platelet Lymphocyte Ratio (PLR),

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Introduction

Diabetes mellitus is associated with an increased incidence of Nonalcoholic fatty liver disease (NAFLD) with estimated prevalence of 70%. Non-alcoholic fatty liver disease (NAFLD) is a group of liver diseases having presence of steatosis in more than 5% percent of liver cells without having any alcohol ingestion. NAFLD consists of benign Non-alcoholic fatty liver (NAFL) and a progressive form non-alcoholic steatohepatitis (NASH). NASH is one of the leading causes of cirrhosis and is associated with an increased risk of cardiovascular morbidity and mortality. Histologically NASH manifests as steatosis and hepatocellular ballooning. Progressive forms also show lobular inflammation and fibrosis.[1,2] Noninvasive serological markers have limited role in recognition of NAFLD and usually lead to its overdiagnosis. Diagnosis of NAFLD is confirmed by histological examination of liver biopsy samples. Ultrasonography is widely used for diagnosis of fatty liver with 80% sensitivity and 99% specificity.[3,4] Increased incidences of obesity and insulin resistance has led to increased prevalence of this disease. NAFLD affects 10-24% of people in certain countries. 75% type II diabetic patients suffer from this metabolic condition. Obesity, type II DM, hyperlipidemia, and hypertension frequently accompany NAFLD.[5] Diabetes mellitus and NAFLD share same pathophysiological features which include increased oxidative stress, elevated

hepatotoxic cytokines and chronic inflammation.[6] Fatty liver results from increased fat intake, lipolysis in hepatocytes and low-density lipoproteins (LDL) secretion. Insulin resistance is associated with an increased availability of free fatty acids to the liver causing triglyceride accumulation in it. Moreover, free fatty acids can also damage hepatocytes directly. Hence there is an increased incidence of progression to cirrhosis in patients having concomitant NAFLD and type II Diabetes.[3,6] Many studies have demonstrated that diabetes mellitus, metabolic syndrome, obesity, hypertension and smoking habits are associated with chronic inflammation. This chronic inflammation is manifested by increased levels of C reactive protein and Interleukin 6 levels in conditions like insulin resistance and metabolic syndrome. This low-grade inflammation is further associated with increased incidences of thrombogenic disorders along with an increased morbidity and mortality.[7-9]

White blood cell count is one of the cheapest markers of inflammation and are available ubiquitously. Neutrophil to lymphocyte ratio (NLR) is a potential indicator of inflammation and is raised in both cardiac and non-cardiac diseases. Many cardiovascular diseases have a significantly increased neutrophil count along with lymphopenia hence disrupting NLR. Increased NLR is associated with an increased HbA1c and diabetic nephropathy in patients suffering from type II Diabetes. Similarly, NLR is also observed to be significantly elevated in patients having increased insulin resistance.[10-12] Similarly, platelet to lymphocyte ratio (PLR) is also a marker of chronic inflammation and increases in later stages of diabetes and can serve as reliable marker in complicated type II diabetes.[13]

This study aims at studying the association of fatty changes in liver with lipid profile and hematological parameters in patients suffering from type II diabetes mellitus. Study also aims at establishing a relationship between NLR, PLR, HbA1c,lipid profile and liver steatosis hence helping the healthcare professionals in understanding the patterns and progression of disease

Methodology

This comparative analytical study was conducted in Shifa International Hospital, Islamabad from June 2018 to June 2019. Patients having type II diabetes mellitus presenting to Falahee OPD of Shifa International Hospital who consented to study were consecutively included in the study. Non-probability convenient sampling technique was used for selection of patients. After approval from Institutional Research Board (IRB) and permission from authorities of Shifa International Hospital, Patients were divided into two groups according to fatty liver status as assessed by ultrasonography. All patients diagnosed with non-insulin dependent diabetes mellitus were included in the study. Pediatric diabetic patients were not included in the study. Diagnosis of diabetes was made by using WHO criteria. Patients having Fasting blood sugar above 126mg/dl and 2 hours postprandial sugar above 200mg/dl were labelled diabetic. Patients with chronic diseases, patients on any chronic medications (except oral antidiabetic agents and insulin) were excluded from the study. Patients of hypertriglyceridemia and smokers were not included in the study subjects. Patients with history of cardiovascular events were also excluded from the study. Full blood count, lipid profile, liver profile and ultrasound abdomen were done. Patients' duration of disease, body mass index (BMI), AST, ALT, cholesterol, triglycerides, HDL, LDL, NLR and PLR were assessed in relation to fatty liver. Automated analyzer was used to ascertain individual white cell counts. Absolute neutrophil and lymphocyte counts were used to calculate NLR and PLR. A structured proforma was used for data collection.

Sample size was ascertained by using WHO sample size calculator. Confidence level was taken at 95% with power of study taken at 80%. Incidence of fatty liver, NLR and PLR were used for sample size calculation.. Data was analyzed by using version 21 of Statistical Package for the Social Sciences (SPSS). Categorical variables were assessed by descriptive statistics. Kolmogorov simirnov test was used for checking the normality of quantitative variables. For quantitative normal and dispersed variables, independent student t and Mann Whitney U test were applied, respectively. Significance was decided on probability value < 0.05. An ROC curve was used for establishing the sensitivity and specificity of NLR and PLR in diagnosis of fatty liver in non-insulin dependent diabetes mellitus.

Results

Total 93 patients were included in the study. Out of which the males and females were 33(35.4%) and 60(64.5%) respectively. The prevalence of fatty liver in type II diabetes mellitus based on ultrasound was 68.8 percent.

The mean duration of diabetes was 7.61 ± 5.8 years. Females had an increased incidence of fatty liver as compared to males. BMI was significantly elevated in

patients having fatty liver. Duration of diabetes was not found to be associated with fatty liver. (Table I)

White cell counts were on lower side in patients with fatty liver. Lymphocytes and PLR were higher in people with fatty liver, but the difference was insignificant. No significant relation was ascertained between NLR, PLR and fatty liver. (Table II)

HbA1c was observed to have a more deranged value in fatty liver group. Lipid profile was more deranged in patients having fatty liver. Triglycerides and LDLs were significantly elevated in patients with fatty liver, hence describing its pathogenesis. Total cholesterol and cholesterol ratio were also increased in fatty liver group, but this difference was not significant. ALT and AST were also elevated in patients having fatty liver with ALT having a significant association with occurrence of fatty liver. (Table III)

Table I: Demographic variables (n=93)

Variable		Non fatty liver (29)	Fatty liver (64)	P value
Gender	Males	14	19	0.083
	Females	15	45	
Duration since diabetes (years)		8.69 ± 6.31	8.07 ± 5.69	0.637
BMI (kg/m^2)		27.47 ± 4.82	31.7± 5.83	0.001*

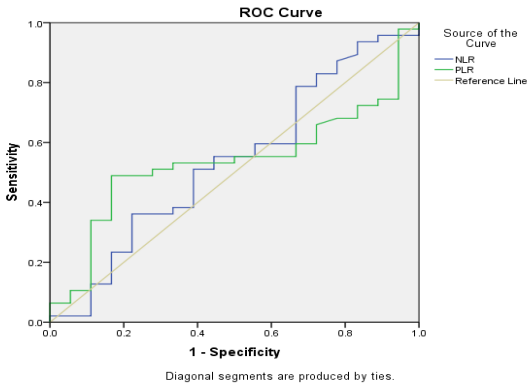
** Significant at 5% level of significance*

Table II: Comparison of hematological profile between two groups.

Measure	Non fatty liver(29)	Fatty liver(64)	P value
White blood cells (No./µL)	9274.4 ± 3018.5	8989 ± 2190	0.608
Neutrophils (No./µL)	5999 ± 2430.7	5509.16 ± 1956.10	0.303
Lymphocytes (No./µL)	2519.62 ± 790.5	2844.84 ± 1331.37	0.226
Platelets (No./µL)	256320.69 ± 110211.56	292706.25 ± 92393.93	0.101
NLR	2.58 ± 1.22	2.33 ± 1.80	0.06
PLR	109.51 ± 49.32	116.75 ± 64.46	0.593

** Significant at 5% level of significance*

ROC curve shows low sensitivity and specificity of NLR and PLR for diagnosing fatty liver although NLR has higher predictive value than PLR.



Discussion

This hospital based comparative analytical study of 93 diabetic patients showed that lipid profile and hematological parameters are more deranged in patients with liver steatosis in contrast to patients with normal liver texture. In our study, WBCs, lymphocytes and PLR is higher in patients with liver steatosis but this association was not significant. Moreover, our study showed that triglyceride level, ALT and LDL levels are significantly raised in patients with fatty liver. A Chinese study showed a significant (p value <0.001) increase in WBCs count in patients of metabolic syndrome (6.20 ± 1.62) versus patients having diabetes only (5.72 ± 1.54).[14] This is also similar to results of a study conducted by Nilufer KK and associates[15] which showed that a progressive pattern of NAFLD in diabetes is associated with increased white cell counts and an elevated NLR and PLR(p value less than 0.001). Similarly, levels of HbA1c, triglycerides**,** cholesterol and LDL were found to be deranged in patients manifesting fatty liver on ultrasonography (p value less than 0.001). Triglycerides were significantly (p value <0.001) elevated in patients of NAFLD (2.16 ± 1.53) in contrast to patients with diabetes only (1.61 ± 0.93).[14] This deranged pattern of lipid profile describes the pathogenesis

Table III: Comparison of fatty liver between two groups

Variable	Normal liver (29)	Fatty liver (64)	P value
Random blood glucose (mg/dl)	230 ± 104.7	232.63 ± 75.13	0.318
HbA1c (%)	8.55 ± 2.36	8.89 ± 2.23	0.507
ALT (IU)	24.7 ± 13.1	53.3 ± 96.57	0.02*
AST (IU)	29.6 ± 24.7	45.28 ± 72.45	0.054
AST/ALT	1.36 ± 1.48	0.95 ± 0.29	0.05*
Cholesterol (mg/dl)	182.34 ± 45.5	198.04 ± 41.35	0.104
Triglycerides (mg/dl)	211.48 ± 276.28	226.26 ± 129.23	0.009*
HDL (mg/dl)	42.10 ± 11.1	41.5 ± 13.64	0.85
LDL (mg/dl)	104.31 ± 37.07	119.93 ± 33.62	0.04*
Cholesterol ratio	4.56 ± 1.69	5.25 ± 2.57	0.084

* Significant at 5% level of significance

of liver steatosis in NAFLD in diabetics. In accordance, another study conducted by Lee Y J *et al* [16] showed that WBC count had a positive correlation with BMI, blood pressure, fasting plasma glucose, total cholesterol, triglyceride and ALT in both men and women. HDL cholesterol was negatively correlated in patients of NAFLD ($P < 0.05$ for each of them)

Our present study showed that HbA1c levels are comparatively elevated in patients of NAFLD in contrast to non-fatty liver group. According to a study, the cause of increased incidence of NAFLD is due to an increased insulin resistance with progression of diabetes. This insulin resistance is one of the main reasons of deranged lipid metabolism and chronic inflammatory state justifying the metabolic complications of late diabetes.[17,18] In contrast, another established fact is that patients who are previously suffering from NAFLD are at increased risk of developing diabetes in the future. This can also progress to metabolic syndrome in late stages of the disease .[19] The findings of these two studies are in agreement with results of our study.

In our study, prevalence of fatty liver suffering from non-insulin dependent diabetes mellitus comes out to be 68.8% using ultrasonography as a diagnostic modality. A recent study estimated the prevalence of NAFLD in type II diabetes to be about 70%. Diagnosis of liver steatosis is widely dependent on the modality used for its recognition.[20, 21] In a study using ultrasonography as a diagnostic investigation for fatty liver, about 50% of diabetic patients had NAFLD.[22]

The present study shows that patients suffering from NAFLD are more prone to have deranged lipid profile and increased inflammatory markers in the body putting them at a greater risk of developing cardiovascular complications and metabolic derangements. This study also suggests that patients suffering from diabetes mellitus type 2 are more likely to develop fatty liver thus emphasizing the regular assessment of liver histology along with other body functions tests. The study shows a positive association of deranged lipid profile and liver appearance on ultrasound showing a greater risk of development of coronary artery disease.

This study can form the basis to generate future research in this novel area to determine the effects of increased levels of blood glucose levels and lipid profile on liver. It will equally form the basis for proper education of the patients regarding keeping a strict glycemic control along with controlled use of fatty meal to prevent them from developing serious complications.

Conclusion

Patients having fatty liver have more deranged levels of lipid profile and hematological parameters increasing the risk of cardiovascular and inflammatory diseases.

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