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Research Article

Study of prevalence of non alcoholic fatty liver disease in type 2 diabetes mellitus patients and variations in liver function tests, lipid profile and mean platelet volume in patients with fatty liver in comparison with patients without fatty liver

Nagaraj S.[1], Sunitha S. Kiran[2]*, Rajeev Gandham[3], Wilma Delphine Silvia C. R.[3], Nagaraja M. R.[3], Abdul Nasar S.[3], Biswajit Das[3]

[1]Department of Radiology, Akash Institute of Medical Sciences & Research Center, Bangalore, Karnataka, India
[2]Department of Pathology, Akash Institute of Medical Sciences & Research Center, Bangalore, Karnataka, India
[3]Department of Biochemistry, Akash Institute of Medical Sciences & Research Center, Bangalore, India

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***Correspondence:**
Dr. Sunitha S. Kiran,
E-mail: sunithakiran84@gmail.com

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) patients potentially are at risk of developing non-alcoholic fatty liver disease. The aim of the study is to determine the prevalence of NAFLD among T2DM patients, diagnosed by ultrasonography of liver, to study the age & sex incidence of NAFLD and to compare the liver function tests, lipid profile and mean platelet volume (MPV) between individuals with NAFLD and without NAFLD.
Methods: Total of 97 type 2 diabetes mellitus ambulatory patients were selected for the study. Among them 62 were males (63.9%) and 35 were females (36%). 78 healthy subjects were selected as controls. Their age ranged between 27 to 75 years. Serum was used for the estimation of FBS, PPBS, total bilirubin, direct bilirubin, AST, ALT, ALP, GGT, total protein, albumin, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). EDTA blood was used for the estimation of MPV. Fatty liver was diagnosed on ultrasound based on the echogenicity and size of the liver.
Results: In this study, liver size & echotexture, liver enzymes such as AST, ALT, ALP and GGT, serum cholesterol, TGL and LDLC were found to be statistically significantly increased in T2DM patients when compared to controls. T2DM patients were divided in to two groups; group 1 included patients with NAFLD and group 2 included patients without NAFLD. Liver size, AST, ALT, ALP, GGT, Cholesterol and TGL were significantly increased in group 1 patients when compared to group 2 patients (P value<0.05). Elevation of MPV was found to be more in group 1 patients than group 2 patients ranging between 12.1±3.0 and 10.2±0.9 respectively.
Conclusions: Early detection and optimum control of diabetes mellitus is important to minimize the effect of diabetes on liver. Hence, assay of serum levels of hepatic enzymes and USG abdomen to detect NAFLD should be done in all patients with T2DM as preliminary diagnostic tests.

Keywords: ALP, ALT, AST, GGT, Non alcoholic fatty liver disease

INTRODUCTION

Diabetes mellitus is one of the major non-communicable diseases, whose prevalence is increasing expotentially. Globally, type 2 diabetes mellitus is the most common form accounting for about 90% of all the cases and is more prevalent in men than women.[1] The prevalence of diabetes mellitus worldwide was 2.8% in 2000 and is estimated to rise to 4.4% in 2030. The total number of diabetes mellitus is projected to increase from 382 million in 2013 end to 592 million by 2035.

India presently has more than 50 million diabetic patients, making India "the diabetes capital of the world".[2,3] The prevalence of diabetes mellitus in India is estimated to be 58%. Diabetic population is estimated to rise from 51 million people in 2010 to 87 million in 2030.[3]

Liver plays an important role in regulation of carbohydrate homeostasis and regulation of blood sugar.[4,5] Liver dysfunction is a known association with diabetes mellitus. Type 2 diabetes mellitus patients potentially are at risk of developing non-alcoholic fatty liver disease. Fatty liver or hepatic steatosis is characterized by diffuse accumulation of fat in liver cells. Fatty liver occurring in individuals without a history of significant alcohol intake is termed as non-alcoholic fatty liver disease (NAFLD).[6] NAFLD includes a spectrum of liver disorders from lipid accumulation without inflammation to non-alcoholic steatohepatitis (NASH) which leads to advanced parenchymal destruction such as fibrosis, cirrhosis and in some patients to hepatocellular carcinoma. NAFLD is associated with obesity, type 2 diabetes mellitus, dyslipidemia and hypertension and insulin resistance.[7]

NAFLD is a type of chronic liver disorder which is gaining significance worldwide.[6] Most of the patients with NAFLD are asymptomatic, although some may experience fatigue, malaise or pain in the right hypochondriac region of abdomen. Hepatomegaly is the common finding in majority of patients. The definitive diagnosis of NAFLD is based on the histological examination of liver biopsy, but it is an invasive and costly procedure and is associated with many complications. Hence, NAFLD is generally diagnosed by most widely available ultrasonographic examination that has a sensitivity of 90% and specificity of 95% in detection of moderate and severe hepatic steatosis.[7]

NAFLD in association with Type 2 diabetes mellitus can increase its risk and severity. Peripheral insulin resistance is a central mechanism in the pathogenesis of both conditions.[8] It has been estimated that the prevalence of NAFLD in type 2 diabetes mellitus is 70-75%. Patients with both NAFLD and type 2 diabetes mellitus have poor prognosis. The mortality rate of diabetes mellitus patients due to cirrhosis is more than the general population. With recognition and control of risk factors, the progression to fatty liver and irreversible cirrhosis can be prevented.

The presence of type 2 diabetes mellitus induced hyperglycemia results in significant increase in lipid profile, oxidative stress, elevated liver enzymes and inflammatory mediators in patients with NAFLD, in fact it has been identified as independent risk factor for development of coronary artery disease.[9,10]

Diabetes Mellitus patients are at increased risk of developing thrombosis and atherogenesis. Changes in hemostatic balance and increased platelet activity play a role in the development of vascular complications of diabetes mellitus.[11] Platelet volumes, a marker of the platelet function and activation is measured as mean platelet volume (MPV).[12] MPV is an indicator showing thrombotic function and activation. Large platelets that contain denser granules are metabolically and enzymatically more active than smaller ones and have higher thrombotic potential; hence, increased MPV might be linked with increased thrombotic potential and development of vascular complications of diabetes mellitus.[11]

The aim of the study is to determine the prevalence of NAFLD among type 2 diabetes mellitus patients, diagnosed by liver ultrasound, to study the age & sex incidence of NAFLD and to see the differences in liver function tests, lipid profile and hematological parameters between individuals with NAFLD and individuals without NAFLD.

METHODS

This study was conducted at Akash Institute of Medical Sciences & Research Centre, Devanahalli, Bangalore rural. Total of 97 diagnosed type 2 diabetes mellitus ambulatory patients of both sexes were taken as cases. Their age ranged between 27 to 75 years. 78 healthy subjects, with their age ranging between 25 to 75 years were selected as control group. Patients with type 1 diabetes mellitus, chronic liver disease, patients on hepatotoxic drugs, alcoholism, type 2 diabetes mellitus patients on insulin treatment, autoimmune diseases, congestive cardiac failure and renal diseases were excluded from the study. The clinical history and other necessary details were obtained from the patients records. The patients were divided into two groups on the basis of liver size and echo texture. Group 1 included type 2 diabetes mellitus patients with NAFLD and group 2 included type 2 diabetes mellitus patients without NAFLD.

Venous blood samples were collected after taking aseptic precautions from the study subjects. 5 ml of blood was collected in plain vaccum tubes and 2 ml of blood was collected in EDTA vaccum tubes. Samples were left for 20 minutes at room temperature, and centrifuged at 3000 rpm for 4 to 5 minutes. Serum was used for the

estimation of fasting blood sugar (FBS), PPBS, total bilirubin, direct bilirubin, Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Dyslipidemia, γ-Glutamyl transferase (GGT), Non alcoholic fatty liver disease, total protein, albumin, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) by automated chemistry analyser cobas c111. EDTA blood was used for the estimation of mean platelet volume (MPV), Hb, mean corpuscular volume (MCV) by using Sysmex xs-800i. Fatty liver was diagnosed on ultrasound based on the echogenicity and size of the liver. The examination of liver was carried by using Samsung Medison UGEO and GE voluson P8 with curvilinear array transducers starting with 3-7 MHz. The size of the liver was measured in midclaviclar line longitudinally and <14 cm was taken as controls and >14 was considered as hepatomegaly. Data were expressed as mean ±SD. P value <0.05 is considered as statistically significant. Statistical analysis was performed using SPSS 20.0.

RESULTS

Total of 97 type 2 diabetes mellitus ambulatory patients were selected for the study. Among them 62 were males (63.9%) and 35 were females (36%) with male to female sex ratio of 1.8:1. Their ages ranged between 27 to 75 years. 78 healthy subjects, with their ages ranging between 25 to 75 years were selected as control group.

In this study, liver size, liver enzymes such as AST, ALT, ALP and GGT, serum cholesterol, TGL and LDLC were found to be statistically significantly increased in type 2 diabetes mellitus patients when compared to controls. Type 2 diabetes mellitus patients were divided in to two groups; group 1 included type 2 diabetes mellitus patients with NAFLD and group 2 included type 2 diabetes mellitus patients without NAFLD.

In group 1 patients, liver size, AST, ALT, ALP, GGT, Cholesterol and TGL were significantly increased when compared to group 2 patients, as illustrated in Table 1. The hematological parameters such as Hb, MCV and MPV were not found to be statistically significant among the controls, group 1 and group 2, but the mean values of MPV were found to be increased in group 1 when compared to group 2 and controls.

Table 1: Comparison of liver size, liver function tests and hematological parameters between healthy controls, T2DM patients with NAFLD (group 1) and T2DM patients without NAFLD (group 2).

Parameters	Control (n=78) Mean ± SD	Group 1 T2 DM with NAFLD (n=50) Mean ± SD	Group 2 T2 DM without NAFLD (n= 47) Mean ± SD
Age	44.1±14.0	49.5±12.2 [a*]	51.5±10.1 [a*]
Liver size	12.9±1.0	21.3±3.5 [a*, b*]	16.75±0.9 [a*]
FBS (mg/dl)	93.9±9.2	187.2±38.3 [a*]	159.0±56.3 [a*]
PPBS (mg/dl)	114.6±24.4	284.3±45.5 [a*]	234.8±96.4 [a*]
Serum cholesterol (mg/dl)	159.4±26.4	202.4±53.4 [a*, b*]	175.6±41.8 [a*]
Serum Triglycerides (mg/dl)	141.6±65.9	231.4±33.6 [a*, b*]	167.8±91.3
HDL cholesterol (mg/dl)	37.1±10.2	36.4±9.1	38.7±11.4
LDL cholesterol (mg/dl)	93.2±26.3	125.3±53.3 [a*]	104.4±33.9 [a*]
Total Bilirubin(mg/dl)	0.6±0.3	0.72±0.55	0.76±0.45
Direct Bilirubin(mg/dl)	0.25±0.1	0.31±0.3	0.29±0.13
AST (IU/L)	21.6±6.7	36.5±12.2 [a*, b*]	21.3±7.0
ALT (IU/L)	19.8±10.3	46.9±15.3 [a*, b*]	20.2±9.1
ALP (IU/L)	76.7±21.4	99.2±34.5 [a*, b*]	80.8±17.8
GGT (IU/L)	31.8±7.9	44.1±7.4 [b*]	29.3±16.3
Total protein (gm/dl)	7.3±0.6	7.3±0.5	7.5±0.6
Albumin(gm/dl)	4.5±0.4	4.4±0.48	4.49±0.48
Hb	13.5±2.1	13.9±1.9	13.5±2.4
MCV	80.4±6.8	81.1±5.5	80.7±5.9
MPV	9.9±0.9	12.1±3.0	10.2±0.9

Data expressed as mean±SD, p value ≤0.05 considered as statistically significant.
a*= Control vs T2DM without NAFLD and T2DM with NAFLD
b*= T2DM without NAFLD vs T2DM with NAFLD.

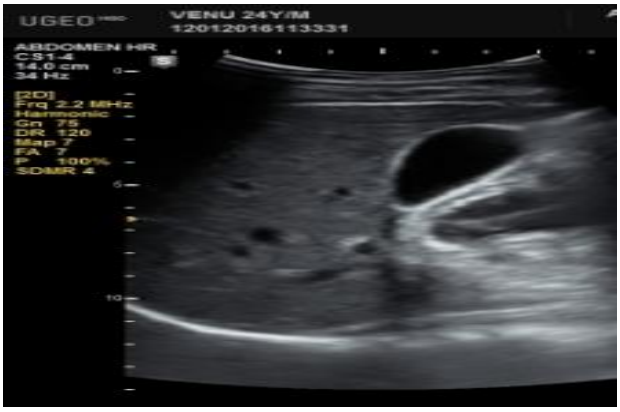

Figure1: Normal liver.

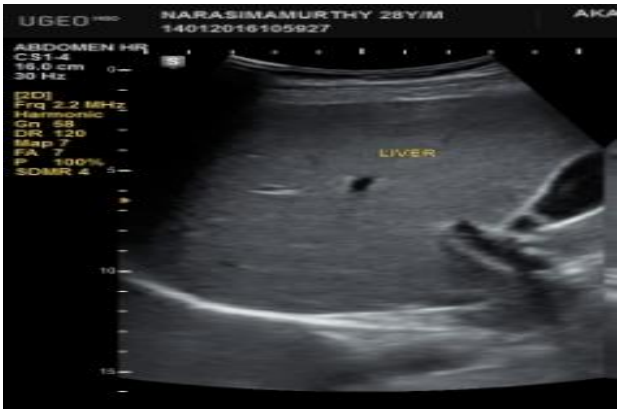

Figure 2: Fatty liver.

DISCUSSION

Patients with type 2 diabetes mellitus (T2DM) appear to have an increased risk of developing non alcoholic fatty liver disease (NAFLD) and have higher risk to develop hepatic fibrosis and cirrhosis.[7] Non alcoholic fatty liver disease is a common hepatic disorder characterized by accumulation of fat in the liver parenchyma of the patients who do not drink excessive amount of alcohol.[6] The spectrum of liver damage in NAFLD ranges from steatosis to nonalcoholic steatohepatitis (NASH), which can progress to end-stage liver disease. The etiology is unknown, but the disease is often associated with type 2 diabetes mellitus, insulin resistance, dyslipidemia, obesity and hypertension, all of them are components of the metabolic syndrome, strongly supporting the notation that NAFLD is the hepatic manifestation of the syndrome.[7] NAFLD is very common in type 2 diabetes mellitus patients, with 50-75% subjects demonstrating fat in the liver by ultrasound.[6] In general population, the prevalence of NAFLD has been reported to be 15-30%. In the present study, the prevalence of fatty liver was found to be 51.5% in type 2 diabetes mellitus patients.

NAFLD encompasses a spectrum of clinicopathologic entities, all of which include an accumulation of fat in the hepatocytes. Non alcoholic steatohepatitis (NASH) is a subtype of NAFLD accompanied by hepatocyte ballooning and necrosis with or without Mallory's hyaline and fibrosis; it carries a risk for progressive liver disease and cirrhosis.[13] Most of the patients with NAFLD are asymptomatic, although some may experience fatigue, malaise or pain in right hypochondriac region of abdomen. Hepatomegaly is the common finding in the majority of patients. NAFLD is generally diagnosed by ultrasonographic examination that has a sensitivity of 90% and specificity of 95% in detection moderate to severe hepatic steatosis.[7]

The echotexture of normal liver (Figure 1) is equal to/ or minimally exceeding that of renal cortex or of the spleen. The intra-hepatic vessels are sharply demonstrated and the posterior aspects of liver are well depicted. In fatty liver (Figure 2), the liver echogenicity exceeds that of renal cortex and spleen and there is attenuation of Ultrasound waves and loss of definition of the diaphragm and poor delineation of the intra-hepatic architecture.[14]

Type 2 diabetes mellitus has been linked with dyslipidemia and elevation of some liver enzymes. The liver fat content in type 2 diabetes mellitus patients could contribute to diabetic dyslipidemia. In fact diabetic dyslipidemia is an independent risk factor for development of coronary artery disease.[10,7] Obesity is a known association with NAFLD, body fat distribution appears to play an important role in the pathogenesis of NAFLD.[6] Increased transport of fatty acids to the liver, enhanced hepatic fat synthesis as well as decreased oxidation or removal of fat from the liver lead to fat accumulation in the liver.[15] Abnormalities of triglyceride storage and lipolysis in insulin-sensitive tissues such as liver are early manifestations of insulin resistance. The genetic, environmental, and metabolic factors lead to insulin resistance. Hyperinsulinemia might also directly lead to hepatic insulin resistance with associated fatty changes. The excess fatty acid found in insulin resistance state is known to be directly toxic to liver cells.[5] In addition, insulin resistance induces lipid peroxidation which activates inflammatory cytokines and facilitates the progression of simple steatosis to steatohepatitis and hepatic fibrosis. Serum triglycerides and/or LDL cholesterol levels might be increased in patients with NAFLD. Considering that hepatic steatosis is common in type 2 diabetes mellitus patients, it has been shown to influence both severity and composition of dyslipidemia.[7,16,17] In our study, serum total cholesterol and serum triglyceride levels were significantly increased in patients with NAFLD, indicating that they are independent risk factors for development of NAFLD.

Type 2 diabetes mellitus patients have been reported to be associated with higher incidence of abnormal liver enzymes, raised ALT levels are the most common abnormality.[5,18] In the present study, liver enzymes such as AST, ALT, ALP, GGT were significantly increased in type 2 diabetes mellitus patients with NAFLD. This is also reported in other studies as well.[19,4] The mechanism behind increased liver enzymes in NAFLD patients is

that, dyslipidemia and insulin resistance leading to lipid deposition in hepatocytes causes induction of mitochondrial swelling, increased lysosomal fragility and impaired membrane integrity, resulting ultimately in release of hepatic enzymes from the injured heaptocytes.[6] Serum amino transferases such as ALT and AST indicate the concentration of hepatic intracellular enzymes that have leaked into the circulation. These are the markers for hepatocellular injury and are used for primary screening of NASH.[5]

The increased platelet activity is emphasized to play a role in the development of micro-and macro-vascular complications of diabetes mellitus. Platelets may be involved as a causative agent with respect to altered platelet morphology and function.[13] It has been shown that diabetic patients have increased platelet adhesion and aggregation, thrombaxane synthesis and platelet factor 4 plasma levels.[11] Mean platelet volume (MPV) is an indicator of platelet function and activation. MPV has been shown to be associated with a variety of inflammatory conditions.[20] Expansion in size and an increase in the amount of cytoplasmic granules noticed in activated platelets is seen in response to inflammation. Inflammatory products that increase in serum in inflammatory diseases may interact with megakaryopoesis in bone marrow resulting in production of larger platelets. Increased insulin levels in type 2 diabetes mellitus also causes generation of bigger platelets from megakaryocytes. Swelling of platelets due to hyperglycemia causes higher levels of MPV.[11,20] In the present study, the mean value of MPV was significantly increased in type 2 diabetes mellitus patients with NAFLD.

Limitations of the study include small study group, non availability of data regarding HbA1c to assess diabetic control and diagnosis of fatty liver solely on the basis of ultrasonographic examination. A large trail of a longer follow up period is necessary to establish the prognosis of liver disease caused by diabetes mellitus. Although liver biopsy is the gold standard method to diagnose fatty liver, studies suggest that liver biopsy is seldom necessary to diagnose NAFLD as the sensitivity and specificity of ultrasound for detecting hepatic steatosis varies from 60 to 94% and 88 to 95% respectively.

CONCLUSION

In the present study, the prevalence of NAFLD is 51.5% among type 2 diabetes mellitus patients. Dyslipidemia and elevated liver enzymes such as AST, ALT, ALP and GGT are seen more frequently in group 1 than in group 2. Early detection and optimum control of diabetes mellitus is important to minimize the effect of diabetes on liver. Hence, assay for serum levels of hepatic enzymes and USG abdomen to detect NAFLD plays an important role and should be done in all patients with T2DM as preliminary diagnostic tests. Although rise in MPV was found in both the groups of T2DM patients, the rise was found to be more in group 1 than in group 2 T2DM patients in our study. MPV acts as an inflammatory marker for cardiovascular disease in T2DM patients. But it cannot be used to detect and/or to assess the severity of NAFLD in T2DM patients. Further large prospective studies are required to study the clinical significance of MPV in these patients.

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