Original Article
Associated liver enzymes with hyperlipidemic profile in type 2 diabetes patients

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Abstract: Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia and is associated with dyslipidemia and disturbed liver function. Aim of the present work is to assess the liver enzymes and to find its association with hyperlipidemic profile in T2DM. Total of 157 subjects were studied and divided into two groups; diabetes (n=81) and non-diabetes (n=76). Various biochemical parameters like fasting glucose, post prandial glucose, HbA1c, total cholesterol (TC), triglycerides (Tg), high density lipoprotein cholesterol (HDL-C), alanine amino transferase (ALT), aspartate amino transferase (AST) and gamma-glutamyl transferase (GGT) were analyzed by ROCHE module Cobas 6000 (C501 & C601) analyzer, kits were procured by ROCHE diagnostics. Low density lipoprotein cholesterol (LDL-C) was estimated by Freidwald's formula. Statistical analysis was performed by applying student t test and Pearson's correlation coefficient, at 0.0001 and 0.05 level of significance, respectively. All the glycemic control parameters, lipid profile parameters except HDL-C and liver enzymes were found increased in diabetes group and significantly differ from non-diabetes group (p<0.0001). ALT showed significant positive correlation with fasting glucose, post prandial glucose, HbA1c, TC, Tg, LDL-C and GGT at p<0.05. AST showed very weak relation with all parameters while GGT was positively associated with fasting glucose, post prandial glucose, HbA1c, TC, Tg, LDL-C and ALT at p<0.05. In conclusion, T2DM incline to elevate liver enzymes, especially ALT and GGT were of significance. Routine screening of ALT and GGT in T2DM patients may assists early detection of liver abnormalities and to arrest the progress of disease.

Keywords: Diabetes, ALT, AST, GGT, dyslipidemia

Introduction

Diabetes mellitus is a metabolic disease known by chronic hyperglycemia which results from defective insulin action and secretion. World Health Organization projects that number of diabetes will exceed 350 million by 2030 [1, 2]. Various studies have documented liver disease as a major cause of mortality in patients with type 2 diabetes [3, 4]. It is well known that liver plays an important role in maintenance of normal glucose levels during fasting as well as in the post prandial period. ALT, AST and GGT are the common liver enzymes which together comprises liver function tests [5]. ALT and AST are the well-known markers of hepatocellular health while GGT also shows biliary tract function. ALT is most specific marker of liver function, but AST and GGT are the less specific markers because they are present in other tissues [6, 7]. The scope of liver disease in type 2 diabetes includes abnormal liver enzymes and non-alcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma (HCC), acute liver failure [8]. NAFLD is a pathological condition characterized by histological findings from hepatic steatosis or fat accumulation in hepatocytes. No virtual symptoms can be seen even if the liver enzymes get increased and disease progress up to hepatic failure, thus asymptomatic individuals with mild elevation of ALT, AST reveals the chances of liver disease, mainly NAFLD and hepatitis [9]. Although the pathogenesis is unclear but insulin resistance is thought to play an important role in triglyceride accumulation. Altered lipoprotein pattern and liver enzymes have been identified as independent risk factors for the development of cardiovascular disease (CVD) [10, 11]. The present work is designed to estimate the liver enzymes and to find the association with lipid profile in type 2 diabetes patients.

Table 1. Various biochemical parameters of diabetes and non-diabetes group

	Diabetes n=81	Non-diabetes n=76	*P* value
Males	52	40	
Females	29	36	
Age (years)	51.3±18.1	39.0±6.7	<0.0001
Fasting glucose (mmol/l)	13.6±4.2	3.7±0.9	<0.0001
Post parandial glucose (mmol/l)	15.7±2.2	4.0±1.0	<0.0001
HbA1C (%)	10.3±2.6	4.1±0.8	<0.0001
TC (mmol/l)	13.7±6.1	4.2±1	<0.0001
Tg (mmol/l)	8.9±3.1	1.1±0.3	<0.0001
HDL-C (mmol/l)	1.3±0.4	1.2±0.3	0.39
LDL-C (mmol/l)	8.0±5.6	2.4±1.0	<0.0001
ALT (u/l)	57.1±15.8	20.3±6.3	<0.0001
AST (u/l)	51.5±13.1	16.9±4.1	<0.0001
GGT (u/l)	52±14.7	18.2±4.0	<0.0001

Mean ± SD values of each group.

Materials and methods

Patients and sample collection

The study was carried out at College of Applied Medical Sciences, and the subjects were selected from the outpatient department of King Abdul Aziz University Hospital, Riyadh. A total of 157 subjects were studied; 92 males and 65 females. An informed consent was taken from all patients and institutional ethical committee approved the study. Age of all subjects ranged between 38-85 years, and the patients were diagnosed with type 2 DM not less than 3 years. Patients suffering from hepatitis B or C, jaundice, liver cirrhosis, liver cancer, under treatment of drugs affecting normal liver metabolism, other chronic illness and alcohol intake were excluded from the study.Studied subjects were categorized as diabetes patients according to American diabetes association of fasting glucose ≥7 mmol/l, post prandial glucose ≥11 mmol/l, along with physician diagnosis. After overnight fasting, 8 ml of venous blood sample was collected in clean glass tubes, of which 1 ml of sample was taken in an EDTA coated tube for the estimation of HbA1c. For further biochemical investigations serum was separated by centrifugation at 3000 rpm for 10 minutes and kept at -20°C until analysis.

Chemical and techniques

Serum analysis for fasting glucose, post prandial glucose, TC, Tg, HDL-C, ALT, AST and GGT was performed by the fully automatic analyzer, ROCHE module Cobas 6000 (C-501 and C-601), and kits were procured by ROCHE. LDL-C was estimated by Fried Wald's formula [12]. HbA1c was estimated by direct enzymatic method [13]. Reference ranges of various parameters according to the kits manufacturer are as follows; HbA1c (5.5%), TC (3.2-6.2 mmol/l), Tg (0.4-2 mmol/l), HDL-C (0.9-2.3 mmol/l), LDL-C (2.2-5.2 mmol/l), ALT (6-37 u/l), AST (10-31 u/l), GGT (7-32 u/l).

Statistical analysis

Data were represented as mean ± SD values. Statistical analysis was done by using student *t* test to find out the difference between the two unpaired groups at 0.0001 level of significance. Pearson's correlation was performed to know the correlation, and the correlation coefficient (r) values were represented at 0.05 level of significance.

Result

In the present study two groups were comprised i.e. diabetes (n=81) and non-diabetes (n=76) out of 157 total subjects. There were 52 male & 29 female patients in diabetes group and 40 males & 36 females in non-diabetes group. All patients under diabetes group were of higher mean age (51.3±18.1), compared to non-diabetes.

Data of various biochemical parameters is presented as Mean ± SD values in **Table 1**. Fasting

Table 2. Correlation coefficient (r) between liver enzymes and other studied parameters

	ALT	AST	GGT
Fasting glucose	0.34*	0.18	0.21*
Post prandial glucose	0.42*	0.19*	0.28*
HbA1C	0.32*	0.18	0.20*
TC	0.42*	0.15	0.30*
Tg	0.37*	0.15	0.37*
HDL-C	-0.08	0.01	0.02
LDL-C	0.42*	0.12	0.30*
ALT	1	0.19*	0.19*
AST	0.17	1	0.18
GGT	0.19*	0.18	1

*P value<0.05.

glucose, post prandial glucose, HbA1C, total cholesterol, triglycerides, LDL-C, AST, ALT, and GGT were significantly increased from the reference range in diabetes patients. Upon applying t test it was observed that all the biochemical parameters of diabetes group differ significantly from non-diabetes group (p<0.0001) at 155 degree of freedom (df) and 95% confidence interval (CI), except HDL-C.

Table 2 reveals the correlation coefficient, established between the liver enzymes (ALT, AST, GGT) and parameters of glycemic control, lipid profile. Level of significance was set at 0.05. ALT was observed in significant positive correlation with fasting glucose (0.34), post prandial glucose (0.42), HbA1C (0.32), TC (0.42), Tg (0.37), LDL-C (0.42) and GGT (0.19) while negatively correlated with HDL-C (-0.08). AST showed weak association with post prandial glucose (0.19) and ALT (0.19). GGT was found positively correlated to all estimated parameters except HDL-C (0.02) and AST (0.18) respectively.

Discussion

The incidence of diabetes is increasing day by day, and an increase in prevalence rate occurs in developing countries. Studies showed that most of the people aged 45-64 years in developing countries and people of age ≥65 years in developed countries suffer from diabetes [11, 14]. Present studies showed diabetes patients were of significantly higher mean age when compared to non-diabetes subjects.

Conditions like insulin resistance, hypertriglyceridemia, and hypercholesterolemia are descri-

bed as a cause of NAFLD [15] and are reported around 40-70% in all diabetic patients of both sexes [16, 17]. We observed significant hyperglycemia, hyperlipidemia with elevated ALT, AST, GGT in diabetes patients, **Table 1**. Our study is consistent with the study of Han Ni, who reported elevated determinants of liver function tests with hyperlipidemia in T2DM [18]. Adeniran et al. investigated that increased ALT and AST with dyslipidemia in patients from Nigeria were diagnosed with T2DM [19].

We observed ALT and GGT show significant positive association with FBS, PPBS, HbA1C, TC, Tg, LDL-C and negative correlation with HDL-C in diabetes, **Table 2**. Our findings agree with Idris et al. [20]. Nannipieri et al. [21] with the promising role of insulin resistance in pathogenesis of NAFLD. Studies have also noted the positive correlation of elevated liver enzymes with fasting and postprandial glucose with the duration of DM [22]. Salmella et al. reported poor glycemic control, oral hypoglycemic agents, statins as a cause of increased ALT with histological changes in liver [23]. On contrary Saligram et al. discussed the association of increased ALT levels with elevated triglycerides and low HDL-C but not with glycemic control [15]. Liver as a central organ involved in carbohydrate and lipid metabolism and due to insulin resistance in diabetes, its function get disturbed. Insulin contributes proinflammatory effect to liver abrasion [10]. Hyperlipidemic profile is observed due to increased transportation of fat to liver with respect to decreased oxidation. The impairment of normal process of synthesis and elimination of triglycerides may progress to fibrosis, cirrhosis and hepatocellular carcinoma [24, 25]. Marchesinia reported association between ALT activity and hyperlipidemia, insulin resistance in T2DM [26]. One study has also correlated ALT activity with increased hepatic fat [27].

GGT is known as a marker of hepatobiliary disorders and is associated with other pathological conditions like DM. Free radicals generated by DM consume glutathione which induces the expression of GGT in liver. Various studies have suggested the association of GGT concentrations with the incidence of type 2 diabetes [6, 7, 28, 29] obesity, hypercholesterolemia, hypertriglyceridemia, cholelithiasis and oxidative stress [17, 30]. Our study is in agreement with these findings as GGT was observed in significant positive correlation with hyperglycemic and

hyperlipidemic profile. However in a study no association of GGT concentration was reported with development of type 2 diabetes [31]. We observed ALT and GGT together were positively correlated. Few studies had also reported elevated GGT levels with ALT in T2DM cases with dyslipidemia [28, 29, 32]. Reason behind the pathogenesis of NAFLD appears to be insulin resistance which results lipolysis and excess deposition of fat on liver and together create inflammatory affect, oxidative stress and lead to elevate liver enzymes [33]. The current study is consistent with the hypothesis. Although we did not confirm the presence of fatty liver due to lack of imaging techniques but we showed the relationship of ALT, AST, GGT with the predictors of diabetes and lipid profile parameters, presenting hepatocellular injury.

We observed significantly increased AST showed weak positive relation with the parameters of glycemic control and lipid profile. Being a marker of hepatocellular health AST is less specific than ALT and GGT [7]. Hultcrantz et al. showed that in asymptomatic individuals with mild elevations of ALT and AST 98% has liver disease commonly fatty liver disease [34]. In a study of male Korean workers AST was found independently associated with diabetes [35] while in a study of male Japanese office workers AST was not associated with diabetes risk [28]. Vozarova et al. reported ALT as a significant predictor of diabetes while AST is not [31]. Our study is in agreement with Vozarova et al. as AST does not show considerable relationship with the studied parameters. Present work is limited to the standard method of liver biopsy for the prediction of NAFLD but it goes with the analysis of Third National Health and Nutritional Examination Survey where individuals with NAFLD are known to have elevated transaminase concentrations, Clark et al. have also suggested that mild or chronic elevations of these markers may be due to NAFLD [36, 37]. We conclude that ALT and GGT are the salient markers for NAFLD in T2DM patients with hyperlipidemia, and should be considered in routine analysis to forbid the progress of disease to chronic conditions. However further investigations are required to sustain these associations.

Disclosure of conflict of interest

There is no conflict of interest.

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