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Research Article

Liver Enzymes and its Correlates in Treated and Newly Diagnosed Type 2 Diabetes Mellitus Patients in Osogbo, South West, Nigeria

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Abstract: Previous studies suggested that alteration in liver enzymes may be a predisposing and probable risk factor of Type 2 Diabetes Mellitus (T2DM) and complication of long standing T2DM. The aim of this study was to evaluate the prevalence of elevated liver enzymes in T2DM on treatment and the newly diagnosed in a Nigerian population and the relationship between the duration of the T2DM and the levels of the enzymes. A total of 117 subjects comprising 52 and 25 T2DM on treatment and newly diagnosed respectively; and 40 controls were recruited. Ten milliliter of blood were collected from antecubital vein of the subjects for analysis. There was no significant difference in the level of gamma-glutamyl transferase (GGT) in both T2DM patients, whereas there were significant elevations ($p<0.05$) of alanine amino transferase (ALT) and alkaline phosphatase (ALP) in newly diagnosed and ALT ($p<0.001$) and ALP ($p<0.01$) in patients on treatment when compared with the control. There were significant ($p<0.001$) differences in the levels of fasting plasma glucose (FPG) in both T2DM patients compared with the control. Prevalence of elevated ALT was 30.77%, ALP was 55.76% and GGT was 0% in patients on treatment; ALT was 32.0%, ALP was 56.0% and GGT was 4.0% in newly diagnosed patients compared with ALT of 0%, ALP of 30% and GGT of 0% in the control subjects. A positive correlation between ALT ($r = 0.265$, $p<0.05$), FPG ($r = 0.474$, $p<0.01$) and T2DM duration and between FPG and ALT ($r = 0.311$, $p<0.01$) and ALT and ALP ($r = 0.234$, $p<0.05$) were observed in diabetic patients. Overall T2DM in both categories of patients predisposes to elevated levels of ALT and ALP but only ALT may be a useful biomarker for monitoring hepatic complication in T2DM without underlying hepatitis.

Keywords: Diabetes, duration, liver enzymes, prevalence

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disease of multiple aetiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, action or both (World Health Organization, 1999; Ikekpeazu *et al*., 2010). Diabetes mellitus occurs throughout the world, but is more common in the developed countries. The prevalence of diabetes in the Western countries is estimated to be between 6.0 and 7.6% (King *et al*., 1998). There is a global trend towards the increase of the incidence and prevalence of diabetes mellitus in African populations and it is predicted that there would be a 35% increase in the worldwide prevalence of diabetes. The rising number of people with diabetes will occur mainly in populations of developing countries, leading to more than 300 million people with diabetes globally by 2025 (King *et al*., 1998). Moreover, in 1999, the World Health Organization projects that the number of diabetes will exceed 350 million by 2030. The increase in the incidence of diabetes in the developing countries follows the trend of urbanization and life style changes (American Diabetes Association, 2005). Recently, a study on Nigerian population shows a high prevalence of 23.4% among the high socio-economic group and 16% among the low socio-economic group with about 18.9% of study population unaware of their diabetes status (Nwarfor and Owhoji, 2001).

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Emerging evidence suggests that a strong link exists between certain liver enzymes such as gamma-glutamyl transferase (GGT) (Vozarova *et al*., 2002; Marchesini *et al*., 2001; Dixon *et al*., 2001) and alanine amino transferase (ALT) (Robinson and Whitehead, 1989) and diabetes. GGT and the ratio between aspartate amino transferase (AST) and ALT were used as markers of alcohol abuse (Dixon *et al*., 2001). These liver enzymes may be involved in several critical processes that affect the risk of developing conditions such as diabetes and cardiovascular disease (Bogoch *et al*., 1955) since circulating liver enzymes such as alkaline phosphatase (ALP), alanine amino transferase (ALT) and gamma glutamyl transferase (GGT) are commonly elevated in asymptomatic patients with Non-Alcoholic Fatty Liver Disease (NAFLD) (Mulhall *et al*., 2002; Angulo and Lindor, 2002), the prospective association between the hepatic markers and type 2 diabetes is expected, as has been reported in many longitudinal studies (Andre *et al*., 2005). However, the results of these observations are variable. For example while most of the studies demonstrated that serum GGT predicted type 2 diabetes independent of common diabetes risk factors, a study in PimaIndians did not (Nakanishi *et al*., 2004). Moreover, some (Vozarova *et al*., 2002; Sattar *et al*., 2004), but not all (Lee *et al*., 2003) studies have demonstrated independent and significant associations of ALT with incidenttype 2 diabetes mellitus. This study investigated the relationship between liver enzymes and duration of type 2 diabetes mellitus in a Nigerian population.

MATERIALS AND METHODS

This cross-sectional study was carried out at the Diabetic Clinic and Department of Chemical Pathology of the State Hospital, Osogbo, South West, Nigeria. The centre is a state-owned secondary health institution located in the state capital to serve as a referral centre to local governments' health clinics and General hospitals of neighboring towns. Participants were selected by random sampling and comprised of 117 subjects (66 females and 51 males) that met inclusion criteria out of about 350 subjects enlisted. Fifty two subjects (29 females and 23 males) were type 2 diabetics on treatment, 25 subjects (14 females and 11 males) were newly diagnosed type 2 diabetics and the remaining 40 subjects (23 females and 17 males) served as control. Patients, aged 30-70 years were diagnosed using the (American Diabetes Association, 2008) diagnostic criteria of fasting plasma glucose (FPG) of ≥7.0 mmol/L and/or 2-h post-prandial plasma glucose (2HPPG) of ≥11.1 mmol/L. In order to ensure that individuals with underlying conditions that may influence results of the present study were not recruited, exclusion criteria were set, among others, to include systemic or topical treatment with drugs which might affect normal liver metabolism (such as Thiazolidinediones Rosiglitazone and Pioglitazone), use of local native medicinal preparations or concoctions. Patients who were seropositive for hepatitis B and C or had visible jaundice of any cause as well as those who use alcoholic beverages of any type were excluded. The Medical Ethics Committee (MEC) of the Osun State Hospital Management Board, Osogbo approved the study and participants gave informed written consent in accordance with the Helsinki Declaration of 1964 as amended in 1983 (World Medical Organization, 1996). Anthropometric, pulse and blood pressure measurements of subjects were also carried out.

Determination of plasma glucose: Fasting Plasma Glucose (FPG) was determined according to the spectrophoto metric method described by Barham and Trinder (1972) using commercial kits obtained from Randox Laboratories Ltd (Crumlin, UK).

Assay of liver enzymes: Plasma alanine aminotransferase (ALT) activities were determined according to the principle described by Retiman and Frankel (1957), while the alkaline phosphatase (ALP) activity was carried out according to the method described by Roy (1970) and gamma glutamyl transeferse (γGT) was estimated according to the principle described by Teschke *et al*. (1977).

Reference ranges: The median normal concentration of ALT is 3-12U/L, ALP 65-105 U/L, GGT is 6-15U/L.

Statistical analysis: Results are presented as mean±standard deviation. Data are analyzed using Statistical Package for the Social Sciences (SPSS) version 16.0. Comparison between patients and control was performed using Student's t test for unpaired data and Pearson's correlation coefficient. The statistical significance was set at $p<0.05$.

RESULTS

Biophysical characteristics: The biophysical data of diabetics and control subjects are presented in Table 1. Significant difference ($p<0.05$) was observed in age of the newly diagnosed patients (D_2) relative to controls with no significant difference ($p>0.05$) in patients on treatment (D_1) when compared to control. WHR increased significantly ($p<0.01$) in the diabetic patients when compared with control. There were no significant ($p>0.05$) changes between BMI of diabetic patients group and those of the control group.

Liver enzymes' activities: Table 2 depicts the plasma activities of ALT, ALP and GGT. ALT and ALP were significantly increased in Diabetics ($p<0.05$ and $p<0.001$, respectively) compared with control, but no significant difference was observed in GGT level ($p>0.05$). ALT increased by 72.32%, ALP by 38.64%

Table 1: Biophysical data of diabetics and control subjects

Parameters	D_1 (n = 52)	D_2 (n = 25)	Control (n = 40)	F-value	p-value
Age (years)	45.60±10.00	51.84±8.98	48.83±2.06	3.269	0.042
BMI (kg/m^2)	26.05±5.34	26.65±4.95	25.87±5.32	0.133	0.875
WHR	0.91±0.06[a]	0.90±0.05[a]	0.86±0.06	4.798	0.010

Values are expressed as mean±standard deviation (S.D.); n: Number of subjects; BMI: Body mass index; WHR: Waist to hip ratio; D_1: Type II diabetes mellitus patients newly diagnosed; D_2: Type II diabetes mellitus patients on treatment; [a]: Significantly different from control

Table 2: Changes in liver enzyme activity status in diabetic patients

	Subjects		
		Diabetics	
Parameters	Control N = 40	D_1 = 25	D_2 = 52
FPG (mmo1/L)	4.11±0.71	11.89±4.40[b] (189.30%)[a]	11.59±4.55[b] (182.00%)[a]
ALT (U/L)	5.78±2.06	9.96±6.36[c] (72.32%)[a]	9.83±5.47[c] (70.07%)[a]
ALP (U/L)	86.50±56.46	119.92±61.29[c] (38.64%)[a]	121.19±58.59[c] (41.00%)[a]
GGT (U/L)	9.03±3.84	10.40±6.78 (15.18%)[a]	10.42±5.95 (15.73%)[a]

Values are expressed as mean±standard deviation (S.D.); n = Number of subjects; [a]: Percentage change relative to control group; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transferase; FPG: Fasting plasma glucose; [b]: $p<0.001$ when compared with control group; [c]: $p<0.05$ when compared with control group; D_1: Type II diabetes mellitus patients newly diagnosed; D_2: Type II diabetes mellitus patients on treatment

Table 3: Prevalence of ALT, ALP and GGT in untreated type 2 DM, treated type 2 DM and controls

	ALT (%)	ALP (%)	GGT (%)
Type 2 DM on treatment			
Elevated values	28.84	55.76	0
Upper limit of normal	5.78	7.60	0
Within normal range	65.38	36.53	100
Untreated type 2 DM			
Elevated values	32	56	0
Upper limit of normal	4	8	0
Within normal range	64	36	100
Controls			
Elevated values	0	30	0
Upper limit of normal	0	2.50	0
Within normal range	100	67.50	100

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transferase

Table 4: Coefficient of correlation between parameters and duration of DM (n = 77)

	Correlation coefficient (r)				
Parameter/duration	FBG	ALT	ALP	GGT	Duration
Duration	0.474[b]	0.265[a]	0.155	0.008	1.000
FBG	1.000	0.311[b]	0.178	0.134	0.473[b]
ALT	0.311[b]	1.000	0.234[a]	-0.072	0.265[b]
ALP	0.178	0.234[a]	1.000	0.219[a]	0.155
GGT	0.134	-0.072	0.219[a]	1.000	0.008

FPG: Fasting plasma glucose; ALT: Alanine aminotransferase; ALP: Alkaline phosphate; GGT: Gamma glutamyl transferase; Duration: Duration of DM; DM: Diabetes mellitus; [a]: $p<0.05$; [b]: $p<0.01$

and GGT by 15.18% in diabetics on treatment while ALT increased by 70.07%, ALP by 41.00% and GGT by 15.73% in newly diagnosed diabetics relative to control. Table 3 shows the prevalence of elevated ALT, ALP and GGT in the subjects. Prevalence of elevated ALT was 30.77%, ALP was 55.76% and GGT was 0% in patients on treatment; ALT was 32.0%, ALP was 56.0% and GGT was 4.0% in newly diagnosed patients compared with ALT of 0%, ALP of 30% and GGT of 0% in control subjects.

Correlation of disease duration and some of the analyzed parameters: Table 4 shows the degree of association between FPG, ALT, ALP and GGT and disease duration. ALT activity ($r = 0.265$; $p<0.05$) and FPG ($r = 0.474$; $p<0.01$) exhibited significant positive correlation, respectively, with disease duration. ALP and GGT showed no significant correlation with disease duration. Furthermore, significant correlation between FPG and ALT activity ($r = 0.311$, $p<0.01$) and ALT and ALP ($r = 0.234$, $p<0.05$) were observed in diabetic patients but such correlation was not seen in control group.

DISCUSSION

In the present study, it was observed that the mean values of ALT and ALP of the patients with type 2 DM on treatment and the newly diagnosed are significantly elevated relative to control subjects. These findings are in agreement with that of Vogarova *et al*., (2002), Lebovit *et al*. (2002) and Andre *et al*. (2005). Perry *et al*. (1998) suggested Non-Alcoholic Fatty Liver Disease (NAFLD) or Nonalcoholic Steatohepatitis (NASH) as the probable reason for elevated liver enzymes in his report. NAFLD is a well recognized complication of diabetes with a reported frequency of 40-70% and affects both male and female. Fat is stored in the form of triglyceride and may be a manifestation of increased fat transport to the liver, enhanced hepatic fat synthesis and decreased oxidation or removal of fat from the liver. The steatosis may be micro- or macro-vesicular and may progress to fibrosis, cirrhosis and hepatocellular carcinoma (Chatila and West, 1996; Tolman *et al*., 2004). The GGT levels in patients on treatment show no significant difference with that of the control subjects. This finding is similar to the observation of Nakanishi *et al*. (2004). GGT apart from being a marker of hepatic steatosis is strongly associated with visceral fat, obesity, cholelithiasis and oxidative stress. Though considered to be a sensitive

indicator of liver damage, GGT is not specific (Marchesini *et al*., 2001; Teschke *et al*., 1977). GGT has been reported to be elevated in alcoholics and the values obtained in the present study were expected as studied subjects were non-alcoholics. The prevalence of elevated enzymes revealed that for the patients with type 2 DM on treatment, prevalence of elevated ALT was 30.77%, ALP was 55.77% and GGT was 1.92% while for the patients with newly diagnosed type 2 DM prevalence of elevated enzymes were ALT 32%, ALP 56% and GGT 4%. In the controls, prevalence of elevated ALT, ALP and GGT were 0, 36 and 0%, respectively. Both ALT and ALP followed the same pattern in the two groups with DM, but there was a wide disparity in the value obtained for GGT. γ-glutamyl transferase (GGT) is used as a sensitive indicator of hepatobiliary disorders including alcohol-related liver disease and fatty liver. Serum GGT activity is also associated with other pathological conditions, such as diabetes (Oli *et al*., 1983; Sakuta *et al*., 2005). Elevated GGT in DM on treatment had been previously reported in Nigerian population but this was linked to possible clinical complications (Oli *et al*., 1983) whereas subjects for the present study were screened for any possible complications which might explain reason for the low prevalence of elevated values of GTT observed. The observed difference may be attributed to GGT role in oxidative stress. Oxidative stress is suggested to be a potential contributor to the development of DM and the associated complications during which free radicals are generated. Free radical production leads to depletion of glutathione, induces the expression of GGT in liver (Moriya *et al*., 1994) and subsequently elevates serum activity of GGT (Whitfield, 2001). It has been shown that GGT counteracts oxidative stress by breaking down extracellular glutathione and making component amino acids of glutathione available to the cells (Whitfield, 2001). It has also been shown that GGT shows a prooxidant property at the extracellular level, while it exerts a protective effect at intracellular levels (Whitfield, 2001). Oxidative stress has been implicated in a number of pathological conditions, including aging, atherosclerosis and diabetes (West, 2000), it has also been speculated that chronic mild elevations in GGT may predispose to diabetes by mediating/inducing oxidative stress (Nannipieri *et al*., 2005). This might explained why the percentage of GGT in newly diagnosed was 4% and patients on treatment was 1.92% where oxidative stress is slightly ameliorated as a result of treatment.

The present study provides evidence of higher prevalence of elevated ALT and ALP in both the newly diagnosed Type 2 diabetics and those on treatment than in the control group. There was no significant difference in the liver enzymes status of both the treated and untreated Type 2 diabetics. We further observed that ALT and FPG exhibited significant positive correlation with disease duration. Significant correlation between FPG and ALT level and between ALT and ALP was also observed in diabetic subjects but such correlation was not seen in the control subjects.

CONCLUSION

In conclusion, the plasma levels of liver enzymes (ALT and ALP) assessed in this study were significantly elevated in both diabetics on treatment and the newly diagnosed. This revealed that hepatic processes mechanisms are important biochemical and pathophysiological changes that contribute to the predisposition to development of type 2 DM. Our study also revealed that both ALT and ALP are rational liver biomonitors, but the former (ALT) shows better reliance for monitoring hepatic complication in type 2 DM on treatment and in those who may be predisposed to DM without underlying hepatitis in a Nigerian population. It is, therefore, recommended that estimation of ALT be included in the management of diabetic patients for the prediction of insulin resistance while diabetic patient with a mild chronic elevation of ALT should be screened for treatable causes of liver disease.

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