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Liver function tests in diabetic and non-diabetic patients in dhaka city of bangladeshi population

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ABSTRACT

These studies evaluated the liver function test in diabetic and non diabetic patients. The measuring parameters were fasting blood sugar, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin and globulin. The study was carried out in BIRDEM hospital (Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders). This study investigated a total of 459 diabetic patients and 193 non diabetic individuals to compare the level of liver function tests between diabetic and non-diabetic patients. Their ages ranged between 30 and 60 years. The biochemical parameters did not differ significantly between non-diabetic male and female patients. In this study, mean values of ALT ($p<0.041$), ALP ($p<0.001$) and total bilirubin ($p<0.001$) were significantly higher in diabetic patients than in the non diabetic patients. Total protein ($p<0.001$) and albumin ($p<0.007$) concentrations in patients were lower compared to non diabetic patients. The mean of serum fasting blood sugar in patients revealed significant difference ($P<0.001$) in comparison to the non diabetic patients. Overall BMI of diabetic patients were high. Although the differences were statistically significant, the means of ALT, AST, and ALP were falling within the abnormal range. Moreover, 27.66% (127) patients had increased one or more liver enzymes in diabetic patients.

KEYWORDS

Diabetic;
Hyperglycemia;
Glycogen;
Liver function;
Metabolism;
Metabolic disorders.

INTRODUCTION

The liver plays a major role in the regulation of carbohydrate metabolism, as it uses glucose as a fuel, it has the capability to store glucose as glycogen and also synthesize glucose from non-carbohydrate sources. This

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type of role makes the liver more susceptible to diseases in subjects having metabolic disorder, especially for diabetes[17]. Elevated serum enzymes activity of the aminotransferases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is the most frequently measured indicator of liver disease and occurs in diabetics more frequently than in the healthy population[11]. The endogenous substances such as drugs are excreted through kidney or billiary channel after metabolic processes in liver[13]. In type-2 diabetes, excessive hepatic glucose output contributes to the fasting hyperglycemia. Maintaining glucose homeostasis is one of the crucial roles of the liver and this may provide a clue to pathogenesis in glucose intolerance but it is not known that liver disease is directly associated with diabetes mellitus. Recent studies indicate that increased gluconeogenesis plays a predominant mechanism for the increased glucose output but glycogenolysis is not to be involved in the type-2 diabetes patients[8]. Excessive deposition of glycogen in liver may cause hepatomegaly and liver enzyme abnormalities; sometimes it may also cause abdominal pain, even nausea and vomiting and rarely ascities for the patients having this feature. Proper control of glucose level may assure to overcome all these abnormalities[9].

Usually in clinical practice, liver function tests (LFTs) are done to diagnose liver diseases but the tests are also done to follow up the progression of known disease, monitor the function of potentially hepatotoxic drugs etc. Serum billirubin, aminotransferases, alkaline phosphatase, total protein and albumin are the most common tests in LFTs. Aminotransferase such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), total protein (TP), albumin (Alb) and globulin (Glo) levels are sensitive indicators of liver-cell injury and are helpful in recognizing hepatocellular diseases[4]. Chronic mild elevation of liver enzymes is frequently found in type 2 diabetic patients. However, though all these reports suggests that the liver function is involved in the development of diabetes but no study so far have been known to show which of these enzymes is the best markers for the development of diabetes. Moreover, it is also not known whether these liver enzymes parameters are indicators or predictors of future diabetes than the well-known risk factors for diabetes, such as adiposity, inflammation, insulin resistance etc. The purpose of this study is to examine the effects of serum liver enzymes, i.e. AST, ALT and ALP, on the development of diabetes in a prospective study of a defined Bangladeshi population, taking into account comprehensive risk factors, including fasting blood sugar, total bilirubin, total protein, albumin, globulin and BMI. The present study was aimed to evaluate the liver function variables in diabetic patients compared to non-diabetic patients.

MATERIALS AND METHODS

Study design and subjects

This study was done including diabetic and non diabetic patients in Dhaka city of Bangladesh. This study was carried out in BIRDEM hospital from January 2010 to December 2010. Patients those who had diabetes registration number at BIRDEM were considered diabetic and the overnight fasting normal individuals were taken as non-diabetic subjects. Patients who had been following interview, clinical examinations were done and blood sample were collected for the assay of fasting blood sugar, total bilirubin, AST, ALT, ALP, total protein, albumin, and globulin.

Determination of liver function test (LFT)

Liver function test (LFT) was done by enzyme kinetic and end point assay through the determination of the activity of serum enzyme from blood samples. Blood samples were collected by venepuncture after an aseptic measure. The samples were allowed to clot and the serum was separated by centrifugation at 10,000 rpm for 15 minutes at room temperature. Serum samples were stored at 2-4^{0}C until tested. For enzyme kinetic assay, commercially available kits (Bio-Rad Laboratories, Richmond, USA; Randox laboratories Ltd., Antrim, UK; Merck, Germany; Sigma Chemicals Co, USA; Roche international Inc. USA; Jhonson & Jhonson Inc. USA.) were used. Absorbance of reaction mixture was measured after performing the assay according to the supplied instruction. Then absorbance was converted by plotting a standard curve to determine sample values. All tests were done at 37^{0}C.

The glucose test method is an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method, presented as a general clinical laboratory method by[15]. The absorbance due to NADH (and thus the glucose concentration) is determined using a bichrimatic (340

and 383 nm) endpoint technique. The instrument automatically calculates and prints the activity of glucose in mmol/L.

The total bilirubin test was determined by the modification of the Doumas reference method, which was modification of the diazo method described by Jendrassik and Grof[10]. The reaction forms a red chromophore representing the total bilirubin, which absorbs at 540 nm and was measured using a bichromatic (540,700 nm) end point technique. The instrument automatically calculates and prints the activity of total bilirubin in mg/dl.

Serum AST test method is an adaptation of the methodology recommended by the International Federation of Clinical Chemistry (IFCC)[10]. The change in absorbance with time due to the conversion of NADH to NAD is directly proportional to the AST activity and is measured using a bichromatic (340,700 nm) rate technique. The instrument automatically calculates and prints the activity of AST in U/L.

Serum ALT test method is an adaptation of the recommended procedure of the IFCC as described by Bergmeyer[7]. The change in absorbance is directly proportional to the ALT activity and is measured using a bichromatic (340,700 nm) rate technique. The instrument automatically calculates and prints the activity of ALT in U/L.

The alkaline phosphatase test method is based on a procedure published by Bowers and McComb[2,5,6,12,] and more recently reviewed by Rej[7]. This method responds to all ALP isoenzyme in human serum. The change in absorbance at 405 nm due to the formation of p-NP is directly proportional to the ALP activity, since other reagents are present in non-rate limiting quantities and is measured using a bichromatic (405, 510 nm) rate technique. The instrument automatically calculates and prints the activity of alkaline phosphatase in U/L.

Serum total protein method is a modification of the bi-uret reaction first introduced by Kingsley[16]. The blue (II) protein complex thus formed is proportional to the protein concentration in the sample and is measured using bichromatic (540, 700nm) endpoint technique. The instrument automatically calculates and prints the activity of TP in gm/L.

Serum albumin test method is an adaptation of the bromocresol purple (BCP) dye-binding method reported by[10,18]. The complex absorbs at 600 nm and is measured using a polychromatic (600, 540, 700 nm) endpoint technique. The instrument automatically calculates and prints the activity of Alb in gm/L.

Diabetes was diagnosed based on drug treatment for diabetes (insulin or oral hypoglycemic agents) and/ or criteria laid by the WHO Consultation Group report i.e. fasting plasma glucose (FPG) 126 mg/dl[1].

The diagnosis is based on BIRDEM laboratory reference values as ALP >120 and 119 U/L for men and women, respectively; ALT >41 and 31 U/L for men and women, respectively; AST >37 and 31 U/L for men and women, respectively; Serum total bilirubin >1.2 mg/ dl, total protein (63-82) mg/L, and albumin (35-57) mg/L for both male and female.

Statistical analysis

SPSS 17 (Chicago, IL, USA) software package was used for the analysis and p values less than o.05 were considered as statically significant. In the study of analyses, the relationship between liver function test in non-diabetic and diabetic variables was examined by Pearson correlation coefficients. Multiple linear regression and partial correlations were used to examine the relationships after adjusting for covariates. The results were presented as mean ± SE.

RESULTS

A total of 652 subjects with 459 (male, 256 and female, 203) diabetic patients and 197 (male 96, female 97) non-diabetic patients were included in this study. Three hundred and fifty two (53.98%) were males and three hundred (46.02%) were females. The mean ages of non-diabetic and diabetic patients were 45.06 ± 0.62 and 47.27 ± 0.42 years, ranging between 30 and 60 years in non-diabetic and diabetic male and female. In the study of BMI, it was observed that non-diabetic and diabetic patients had 22.97 ± 0.11 and 23.53 ± 0.09 kg/m^2 respectively.

In this study, ALT, AST, ALP, bilirubin, total protein, albumin and serum fasting blood sugar were estimated in diabetic patients and non-diabetic patients. As presented in (TABLE-1), mean (±SE) values of fasting blood sugar, total bilirubin, ALT, AST, and ALP were significantly higher in diabetic patients than in non-diabetic patients. Total protein ($p<0.001$), albumin

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(p<0.007) and globulin concentrations were significantly lower in comparison to the non -diabetic patients. The mean value of serum fasting sugar (p<0.001) was clearly higher in diabetic patients (7.01 ± 2.70 mmol/L) than in non-diabetic patients (5.55 ± 2.63 mmol/L).

In univariate analysis, AST and globulin were not significantly associated with fasting blood sugar whereas bilirubin, ALT, ALP, total protein and albumin had significant ORs of 0.032 (0.007 to 0.056), 2.177 (-0.222 to 4.576), 2.795(-0.321 to 5.912), 6.298 (4.243 to 8.349), - 0.391 (-0.579 to -0.203), - 0.332 (- 0.484 to - 0.180) respectively (TABLE 2). After adjustment for age, sex and BMI, birubin, ALT, ALP, total protein and albumin ORs were 0.031 (0.006 to 0.056), 2.793 (0.335 to 5.921), 6.244 (4.191 to 8.296), - 0.389 (-0.577 to -0.202), - 0.332 (- 0.483 to - 0.180) independently associated with fasting blood sugar. When the model was adjusted age, sex and BMI, the associati on of birubin, ALT, ALP, total protein and albumin with fasting blood sugar remained significant (P<0.014, P=0.040, p<0.001, p<0.001 and P <0.001 respectively).

TABLE 1: Mean values of descriptive characteristics and biochemical parameters of the non-diabetic and diabetic patients.

No. of subjects (male/female)	Non-diabetic Mean ± SE	Diabetic Mean ± SE	p-value
	193 (96/97)	459 (256/203)	
Age (years)	45.06 ± 0.62	47.27 0.42	0.004
BMI (kg/m^2)	22.97 ± 0.11	23.53 ± 0.09	0.002
Fasting blood sugar (mmol/L)	5.55 ± 0.18	7.01 ± 012	<0.001
Bilirubin (mg/dl)	0.66 ± 0.12	0.78 ± 0.02	0.001
D. Bil (mg/dl)	0.18 ± 0.003	0.20 ± 0.007	0.111
GOT (U/L)	32.89 ± 2.28	45.01 ± 4.79	0.109
GPT (U/L)	38.36 ± 2.76	66.04 ± 6.46	0.041
ALP (U/L)	86.64 ± 1.27	140.0 ± 3.38	<0.001
Total protein (gm/L)	75.65 ± 0.32	73.78 ± 0.30	<0.001
Albumin (gm/L)	45.32 ± 0.24	44.18 ± 0.25	0.007
Globulin (gm/L	30.27 ± 0.31	29.57 ± 0.21	0.073

Data presented are mean ± standard error. *P*-value obtained from Independent-Samples "t" test.

The Pearson's correlations (r) between biochemical parameters were shown in table-3. Non diabetic patients, fasting blood sugar showed no significant correlations with ALT, AST, ALP total protein, albumin and BMI; whereas, fasting blood sugar showed very high correlations with ALP (p<0.001) and total protein (p<0.008): albumin (p<0.003) were observed more frequent and significant in diabetic than non-diabetic subjects (TABLE 3).

TABLE 2 : An association between fasting blood sugar with biochemical parameters of liver function test on non-diabetic and diabetic patients.

Dependent variable	Independent variable	
	FBS	
	Unadjusted	Adjusted
Birubin (total)		
β-Coefficient (95% CI)	0.032 (0.007 to 0.056)	0.031 (0.006 to 0.056)
p-value	0.012	0.014
GOT		
β-Coefficient (95% CI)	2.177 (-0.222 to 4.576)	2.169 (-0.239 to 4.577)
p-value	0.075	0.077
GPT		
β-Coefficient (95% CI)	2.795 (0.321 to 5.912)	2.793 (0.335 to 5.921)
p-value	0.045	0.040
ALP		
β-Coefficient (95% CI)	6.298 (4.243 to 8.349)	6.244 (4.191 to 8.296)
p-value	< 0.001	< 0.001
Total Protein		
β-Coefficient (95% CI)	- 0.391 (-0.579 to -0.203)	- 0.389 (-0.577 to -0.202)
p-value	< 0.001	< 0.001
Albumin		
β-Coefficient (95% CI)	- 0.332 (- 0.484 to - 0.180)	- 0.332 (- 0.483 to - 0.180)
p-value	< 0.001	< 0.001
Globulin		
β-Coefficient (95% CI)	- 0.042 (- 0.184 to 0.100)	- 0.042 (- 0.185 to 0.100)
p-value	0.560	0.560

***P-values* were from Multivariate linear regression, adjusted for age, sex and BMI. β- Standard regression coefficient.**

DISCUSSION

This study compared the biochemical parameters, commonly used for liver function tests (LFT), between diabetic and non-diabetic patients. This study has been comparatively conducted on Bangladeshi population with some limitations. Socio-demographic and clinical variables have not been taken properly and could not be analyzed. Nutritional status and lipid profile level could have been important biophysical variables for determination of association with liver enzymes.

Measurements of albumin are used in the diagnosis

and treatment of numerous diseases involving primarily the liver or kidneys. Albumin is the highest concentration in plasma. Albumin is formed exclusively in the liver and serves as a transport and binding protein for calcium, fatty acids, bilirubin, hormones, vitamins, trace elements and drugs. It is also prime importance in maintaining the colloidal osmotic pressure in both the vascular and extra vascular spaces. Decreased serum albumin concentration can result from liver disease.

Table 3 : Pearson's Correlation between Fasting blood sugar and liver function test biomarkers among non-diabetic and diabetic subjects.

liver function test biomarkers	Non diabetic		Diabetic	
	r	*p*-value	*r*	*p*-value
Bilirubin (mg/dl)	0.005	0.95	0.002	0.967
GOT (U/L)	-0.007	0.925	0.005	0.912
GPT (U/L)	-0.011	0.874	0.161	0.003
ALP (U/L)	-0.011	0.874	0.171	<0.001
Total protein (gm/L)	-0.36	0.616	-0.123	0.008
Albumin (gm/L)	0.026	0.721	-0.139	0.003
Globulin (gm/L	0.000	0.999	-0.004	0.932

***r*- Pearson's correlation coefficient**

Overall, the prevalence of increased aspartate aminotransferase (AST) was 27.3% (n=131) with the different key wise prevalence of 15.4% (n=74) and 11.9% (n=57) in male and female respectively. The prevalence of increased alanine aminotransferase (ALT) was 21.2% (n=102) with the different key wise prevalence of 8.5 %(n=41) and 12.7 % (n=61) in male and female respectively. Also, alkaline phosphatase, total protein, globulin and BMI were the prevalence of 30.6% (n=147),5.4% (n=26), 9.6% (n=46) and 18.3% (n=88); with the different key wise prevalence of 20.2% (n=97), 10.4% (n=50); 3.1% (n=15), 2.3% (n=11); 6.5% (n=31), 3.1% (n=15) and 10.2% (n=49), 8.1% (n=39) respectively for both male and female diabetes patients. These findings are in agreement with Salmela et al.,[20]. Our results also agree with Ayman S. Idris et al[3].

We found that the prevalence of increased ALT, AST and ALP were higher in diabetic patients (459 patients, male 256, female 203) than in non diabetic patients. The prevalence of increased ALT and AST in type 2 diabetic patients was higher than general population[19] but lower than studies done in diabetic patients[21]. High waist circumferences were associated with an increased risk of elevated ALT levels. Nutritional status and lipid profile were not included in the study for what these could not be compared.

CONCLUSIONS

The values of the liver function tests in diabetic patients were significantly higher than non diabetic patients. Diabetic patients had lower albumins in comparison to the non diabetic patients. A significant number of patients had increased one or more liver enzymes. Total protein and albumin concentration had normal levels in overall all patients. Thus performance of liver function tests is highly recommended for diabetic patients. Nonetheless, more studies need to be performed on diabetic patients on the liver function tests.

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