LIVER ENZYMES IN DIABETIC AND NON DIABETIC SUBJECTS WITH CLINICALLY DIAGNOSED HEPATITIS

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Abstract

The occurrence of liver disease and raised liver enzymes is common in diabetic patients and the increasing level of enzymes indicates the severity of hepatic injury. Very few studies have addressed this issue in Bangladesh though Bangladeshi population is very much susceptible to diabetes.

This study investigated a total of 1400 diabetic patients and 100 non diabetic individuals to compare the level of liver enzymes between diabetic and non-diabetic subjects. The comparisons were made among subjects who were referred to the department of Gastro-hepato-pancreatic diseases (GHPD) of BIRDEM with the clinical diagnosis of chronic hepatitis and other gastro-intestinal disorders. The investigations included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin levels. The subjects were categorized with and without hepatitis based on these investigations.

The biochemical markers (ALT, AST, ALP, bilirubin) did not differ significantly between non-diabetic male and female subjects. Neither the differences were significant between diabetic males and females though the diabetic patients had higher level of markers. In contrast, when compared between diabetic and non-diabetic subjects there were striking differences in either sex. Compared with the non-diabetic the diabetic subjects had significantly higher level of ALT (48.3 vs. 277.0), AST (42.0 vs. 213.0) and ALP (148 vs. 302) in males ($p < 0.005$ for all). Similarly, these values were found significantly higher in diabetic females than their non-diabetic counterparts ($p < 0.01$). For bilirubin, it was also found significant in males ($p < 0.001$).

The study revealed that the liver enzymes were found elevated in both diabetic and non-diabetic subjects who were referred with clinically diagnosed hepatitis. The enzymes were found markedly elevated among the diabetic than non diabetic patients, which indicate hepatic injury was more marked among the diabetic patients. Further study may confirm these findings. It is suggested that other socio-demographic and biophysical risk factors are important to be investigated in order to prevent increased hepatic damage among the diabetic subjects.

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Key words: Liver function tests (LFTs), bilirubin, ALT, AST, ALP, hepatitis.

Introduction

Disturbance of liver function in some patients with diabetes mellitus is well recognized.[1] Liver is the largest and complex gland of the body. It plays a central role in the metabolism of nutrients. The liver metabolizes endogenous substances such as drugs, which are then excreted via kidney or billiary system

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(Guyton, 1991).[2] The liver plays a central and crucial role in the regulation of carbohydrate metabolism. Its normal functioning is essential for the maintenance of blood glucose levels and of a continued supply to organs that require a glucose energy source. This central role for the liver in glucose homeostasis offers a clue to the pathogenesis of glucose intolerance in liver diseases but little insight into the mechanisms of liver disease in diabetes mellitus. In type-2 diabetes, excessive hepatic glucose output contributes to the fasting hyperglycemia. Increased gluconeogenesis is the predominant mechanism responsible for this increased glucose output, while glycogenolysis has not been shown to be increased in patients with type 2 diabetes.[3] Patients showing solely excessive glycogen deposition may exhibit hepatomegaly and liver enzyme abnormalities and may have abdominal pain and even nausea and vomiting and rarely ascites. All these abnormalities may improve with sustained glucose control.[4]

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, Bilirubin. Aminotransferase such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), levels are sensitive indicators of liver-cell injury and are helpful in recognizing hepatocellular diseases such as hepatitis. The elevated level of ALT is more specific indicator of liver injury. Both enzymes are released into the blood in increasing amounts when the liver cell membrane is damaged.[5] Alkaline phosphatase (ALP) and bilirubin act as markers of biliary function and cholestasis. Chronic mild elevation of transaminases is frequently found in type 2 diabetic patients.

Materials and Methods

Study population: This study included all patients with or without diabetes, clinically diagnosed as chronic hepatitis, referred to the Department of Gastro-Hepato-Pancreatic Diseases (GHPD) of BIRDEM from January 2008 to December 2009. All the referred patients were interviewed. The patients who had diabetes registration (reference) number of BIRDEN were considered diabetes and the rest were taken as non-diabetic subjects. Following interview, clinical examination was done and blood samples were collected for the assay of ALP, ALT, AST and total bilirubin.

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 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 alkaline phosphatase method is based on a procedure published by Bowers and McComb[6,7,8,9] and more recently reviewed by Rej.[10] 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 ALT method is adaptation of the recommended procedure of the IFCC as described by Bergmeyer.[10] 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.

Serum AST method is an adaptation of the methodology recommended by the International Federation of Clinical Chemistry (IFCC).[11] 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.

Table-1: *Proportion of Diabetic and non-diabetic subjects referred to BIRDEM with the clinical diagnosis of chronic hepatitis*

Liver Function tests (Values for CLD†)	Non Diabetic (n=100)		Diabetic (n=1400)	
	Normal %	Hepatitis† %	Normal %	Hepatitis %
ALT (m>41/f>31 U/L†)	64.0	36.0	49.7	50.3
AST (m>37/f>31 U/L†)	82.0	18.0	33.6	66.4
ALP (m>129/f>104 U/L†)	70.0	30.0	43.1	56.9
Bilirubin (m & f>1.2 mg/dl†)	99.0	1.0	62.2	37.8

m – male, f – female.

The total bilirubin was determined by the modification of the Doumas reference method, which was modification of the diazo method described by Jendrassik and Grof.[11] 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 cut-offs for the diagnosis of hepatitis are 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 for both male and female.

Statistical Analysis: Data analysis was performed using SPSS software version 10 (Chicago, IL, USA). The results were presented as mean ± SE.

Results

Overall, 1500 referred subjects were investigated at BIRDEM. A total of 1400 (male 808, female 592) diabetic patients and 100 ((male 50, female 50) non diabetic individuals aged 25-77 years participated. Eight hundred and eight (54.5%) were males and 592 (45.5%) were females.

As mentioned in "diagnostic cut-off values for hepatitis", the values for ALT, AST, ALP and bilirubin were found normal, or proved not having hepatitis, in 64%, 82%, 70% and 99% of non-diabetic patients as against 49.7%, 33.6%, 43.1% and 62.2% of diabetic patients, respectively (table1). All these values were found markedly elevated in diabetics than their non-diabetic counterparts. However, these values did not differ significantly.

These biochemical markers when tested only for the abnormally elevated groups with the exclusion of normal values, as shown in table 2, the mean (±se) values were found markedly elevated in diabetic than their non diabetic counterparts in either sex. For example, male and female of non-diabetic groups showed mean (U/L) of ALT (48.3 vs. 51.9), AST (42.0 vs. 41.7) and ALP (148 vs. 154) almost within similar range. Similar range, though at much higher level, were also observed between male and female in diabetic groups.

In contrast, when compared between diabetic and non-diabetic referred subjects there were striking differences. The mean values showed markedly elevated in diabetic than non-diabetic groups. Thus, these observations were for ALT (48.3 vs. 277.0),

Table-2: *Comparison of biochemical variables between diabetic and non-diabetic subjects with hepatitis (m/f = 858/642)*

Liver Function tests	**Male**		**Female**	
	Non diabetic n=50 % (Mean ± SE)	**Diabetic** n=808 % (Mean ± SE)	**Non diabetic** n=50 % (Mean ± SE)	**Diabetic** n=592 % (Mean ± SE)
ALT (m>41 / f>31 U/L)	14.0 (48.3 ± 1.6)	50.7 (277 ± 25)	58.0 (51.9 ± 3.2)	49.8 (228 ± 22)
AST (m>37 / f>31 U/L)	4.0 (42.0 ± 5.1)	67.7 (213 ± 18)	64.5 (41.7 ± 2.8)	32.0 (205 ± 21)
ALP (m>129 / f>104 U/L)	10.0 (148 ± 9)	58.5 (302 ± 11)	50.0 (154 ± 8)	54.6 (238 ± 9)
Bilirubin (both>1.2mg/dl)	2.0 (1.4 ± 0.1)	44.1 (7.6 ± 0.4)	0 (0.0)	29.2 (7.3 ± 0.6)

SE – standard error of mean. m – male, f – female.
Comparison between non-diabetic and diabetic among male and female subjects: t –tests used for comparison between diabetic and non-diabetic groups in either sex: ALT, AST and ALP $p < 0.005$. For bilirubin, $p < 0.001$ in male; not applicable for female.

Table-3: *Pearson's correlations (r) between biochemical markers for hepatitis in non-diabetic (n=100) and diabetic (802) subjects.*

Biochemical variables		Non-diabetic (n = 100)			Diabetic (n = 1400)		
		ALT	AST	ALP	ALT	AST	ALP
Bilirubin (bili)	*r*	.114	.190	.155	.320**	.341**	.207**
	p (2-tailed)	.259	.058	.124	.000	.000	.000
ALT	*r*	-	.886**	.225*	-	.869**	.056
	p (2-tailed)		.000	.024		.000	.115
AST	*r*		-	.269*		-	.103*
	p (2-tailed)			.007			.004

AST (42.0 vs. 213.0) and ALP (148 vs. 302) in males. Likewise, in females these were ALT (51.9 vs. 228.0), AST (41.7 vs. 205.0) and ALP (154 vs. 238). In either sex, the differences of ALT, AST and ALP between diabetic and non-diabetic subjects were found significant ($p < 0.005$). For bilirubin, it was also found significant in male ($p < 0.001$); whereas, in females, comparison could not be made due to lack of non-diabetic patients (table 2).

The correlations (*r*) between biochemical markers were shown in table 3. For the non diabetic patients, serum bilirubin showed no significant correlations with ALT, AST and ALP; whereas, bilirubin showed very high correlations ($p < 0.001$) with all these enzymes. Again, the correlations among ALT, AST and ALP were more frequent and significant in diabetic than non-diabetic subjects (table 3).

Discussion

This study compared the biochemical markers, commonly used for liver function tests (LFT), between diabetic and non-diabetic subjects. The study is unique in the sense that there has been no such comparative study conducted on Bangladeshi population. But, the study has some limitations. Socio-demographic and clinical variables have not been taken properly and could not be analyzed. The study could have taken the final or confirmed diagnosis of the patients. Age, nutritional status, fasting blood glucose and lipids could have been the important biophysical variables for determination of association between liver enzymes.

The study findings are consistent with other studies. Elevated activities of serum aminotransferases are a common sign of liver disease and are observed more frequently among diabetics than in the general population.[12] In a previous study by Erbey *et al*, type-2 diabetes has been reported to be associated with mild (asymptomatic) elevations in the serum levels of certain enzymes including serum ALT.[13] Elevated ALT levels have been reported as more frequently observed for diabetics than for the general population studies by Everhart JE.[14]

We found that the prevalence of elevated ALT and AST was higher in diabetic patients (1400 patients, male 808, female 592) than in non diabetic patients. The prevalence of elevated ALT and AST in type 2 diabetic patients was higher than general population,[15] but lower than studies done in diabetic patients.[16] M. A. Meybodi *et al*, of 348 patients that entered the study, mean age was 58.8 ± 11.5. Elevated ALT and AST were found in 10.4 and 3.3% of type 2 diabetic patients, respectively. The prevalence of elevated ALT increased with increasing age.

Overall, the prevalence of elevated alanine transaminase (ALT) was 10.4% (n=105) with the gender-wise prevalence of 12.8% (n=71) in men, and 7.4% (n=34) in women. The prevalence of elevated AST was 5.4% (n=56) with the gender-wise prevalence of 5.6 %(n=31) in men and 5.4 % (n=25) in women. Only 4.5% (n=44) showed elevated levels of both ALT and AST. Of patients with high ALT levels, 88 patients (83.8%) had mild, 13 (12.4%) had moderate, and only four patients (3.8%) had marked elevation of the enzyme activity. Male gender and high waist circumference were associated with an increased risk of elevated ALT levels. Younger patients had a higher tendency to have elevated ALT compared to those over 65 years. As age and nutritional variables were not included in the study these could not be compared.

Conclusion

It may be concluded that the liver enzymes were found elevated in both diabetic and non-diabetic subjects who were referred with a clinical diagnosis of hepatitis. Very markedly elevated enzymes were found among the diabetic than non diabetic patients indicating hepatic injury was more likely among the diabetic patients. Further study may confirm these findings. It is suggested that other socio-demographic and biophysical risk factors are important to be investigated in order to prevent hepatic damage among the diabetic subjects.

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