



Association of Insulin Resistance with Liver Biomarkers in Type 2 Diabetes Mellitus

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ABSTRACT

Objective: Objectives of our study were to compare liver markers in type 2 diabetic patients with that in non-diabetic healthy volunteers, and also to find the correlation between insulin resistance and liver markers. **Methods:** The cross-sectional study was conducted in the Clinical Biochemistry laboratory. 114 type 2 DM patients in the age group 18-65 years, diagnosed as per ADA guidelines were included in the study. 100 age and gender matched non-diabetics, healthy volunteers or those having health packages were chosen as controls. Blood sample was collected and fasting blood glucose, AST, ALT and Alkaline phosphatase, total bilirubin, direct bilirubin, total protein, albumin and insulin were estimated. HOMA-IR was calculated. Statistical analysis was carried out using SPSS 16. **Results:** A significant elevation was seen in AST, ALT, ALP, GGT, TB, DB, TP, A:G ratio and insulin levels in diabetics. A lowered albumin and A:G ratio were observed in diabetics compared to controls. A positive relationship was seen between insulin resistance (HOMA-IR) and ALP, total and direct bilirubin. A very significant negative correlation was revealed between albumin and HOMA- IR. **Conclusion:** We conclude that diabetic patients had high liver enzymes as compared with non-diabetics. A correlation was found between type 2 diabetes mellitus, liver markers and insulin resistance.

Key Words: Insulin Resistance, Diabetes Mellitus, Liver Markers

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INTRODUCTION

Liver disease is reported to be one of the important causes of death in diabetes mellitus (DM). A population-based diabetes research by De Marco et al suggested that cirrhosis accounted for 4.4% of diabetes related deaths [1]. In a study Balkau B et al suggested that cirrhosis accounted for 12.5% of deaths in DM [2]. Various reports suggest that diabetes is becoming one of the most common causes of liver disease. A spectrum of liver disorders can occur in DM. Trombetta et al suggests that prevalence of diabetes in cirrhotics is 12.3 -57% [3]. These suggest higher prevalence of DM in liver diseases. The cause and effect relationship between diabetes mellitus and liver disease are yet to be established in our settings. Since this is a less explored area, we would like to find an association between liver markers and insulin resistance, which are associated with type 2 DM.

Insulin resistance is a condition where cells are non-responsive to insulin. IR is associated with type 2 diabetes mellitus [4]. IR is independently associated with NAFLD and a close association was found between NAFLD and metabolic syndrome [5]. NAFLD is in turn consistently associated with DM. Since IR is associated with both DM and liver disorders, liver markers could be elevated in DM. Clinical trials report suggests that serum ALT, AST or alkaline phosphatase were 1-2.5 times higher in type 2 DM [6]. In a retrospective study, we found that ALT and AST were 1.3 and 1.4 times respectively higher in diabetes patients. It has been suggested that diabetics have an inclined tendency towards alterations of liver enzymes [7]. Increased activity of the liver enzymes is associated with Insulin resistance.

From literature review, association between IR & DM, DM & liver disorders, liver disorders and IR is evident. However the cause and effect relationship among these is

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not well established.

As insulin resistance (IR) is associated with DM as well as liver disorders, it is justifiable to measure liver markers in diabetics. It is very much essential to find out the relationship between insulin resistance, liver markers and diabetes mellitus.

Research question/hypothesis: We hypothesize that

- i. liver biomarkers are positively associated with insulin resistance in diabetics
- ii. possibility of elevation of liver markers is high in diabetics

Objectives:

Aims of our study were to

1. compare liver markers in type 2 diabetic patients with that in non-diabetic healthy volunteers
2. find the correlation between insulin resistance and liver markers

METHODOLOGY

Study design

The Cross-sectional study was conducted in the department of Biochemistry. Institutional ethics committee approval was sought before starting the study. Informed consent was obtained from the subjects.

Inclusion criteria:

Cases: 114 type 2 DM patients (18-65 years), diagnosed as per ADA 2017 guidelines

Controls: 100 age and gender-matched non-diabetics, healthy volunteers or those having health packages undergoing surgery

Exclusion criteria

Alcoholics, acute and chronic hepatitis, other liver disorders

Sample collection and analysis

5 ml Blood sample was collected, centrifuged for 10 min at 3000 rpm. Parameters like fasting blood sugar, AST, ALT and Alkaline phosphatase, total bilirubin, direct bilirubin, total protein, albumin and insulin were estimated. Fully automated chemistry analyzer, Cobas C-311 was used for liver markers assay and hormone analyzer, e411 which works on the principle of electrochemiluminescence, was used for insulin assay.

Insulin resistance is calculated by homeostasis model assessment (HOMA-IR) using the following formula;
 $HOMA-IR = \frac{\text{fasting glucose} \times \text{fasting insulin}}{22.5}$
 insulin expressed in $\mu U/L$, glucose in $mmol/l$.

Statistical analysis

Statistical analysis was done using the software SPSS version 16. Mann Whitney U test was used for the comparison of liver markers between cases and controls. Spearman's correlation was used to find the correlation between liver markers and HOMA- IR

RESULTS

Insulin levels were raised 1.76 times in diabetics compared to controls. In liver profile, total bilirubin, direct bilirubin, liver enzymes like AST, ALT, ALP, GGT were 1.2, 1.12, 1.63, 1.43, 1.09, 1.59 times higher respectively in cases compared to controls. Albumin levels were decreased and total protein and globulins were increased significantly in cases compared to control. Homeostatic model for assessment of insulin resistance was 2.7 times higher in T2DM.

A significant positive correlation was found between insulin levels and total and direct bilirubin, ($r=0.279$, $P=0.003$ and $r=0.233$, $P=0.014$ respectively). ALP, total and direct bilirubin had a significant positive correlation with HOMA-IR ($r=0.228$, $P=0.033$, $r=0.231$, $P=0.030$; $r=0.242$, $P=0.023$ respectively). A very significant negative correlation was found between albumin and HOMA- IR ($r= -0.306$, $P=0.004$).

Table 1: Comparison of Liver markers and insulin resistance in diabetics and non-diabetics

	CASES (T2DM)	CONTROLS (Non diabetics)	P VALUE
INSULIN ($\mu U/L$)	20.51 \pm 3.37	11.66 \pm 1.34	0.001
TP (G/dl)	7.47 \pm 0.06	7.22 \pm 0.06	0.01
ALB(G/dl)	4.1 \pm 0.04	4.2 \pm 0.057	0.026
Globulin (G/dl)	3.2 \pm 0.78	1.4 \pm 0.49	0.000
A:G ratio	1.29 \pm 0.03	1.48 \pm 0.03	<0.0001
TB (mg/dl)	0.94 \pm 0.066	0.78 \pm 0.96	0.026
DB (mg/dl)	0.37 \pm 0.03	0.33 \pm 0.05	0.016
AST (U/L)	52.28 \pm 5.75	32.1 \pm 3.6	0.0179
ALT (U/L)	40.17 \pm 3.74	28.1 \pm 3.845	0.0001
ALP (IU/L)	94.54 \pm 2.96	86.5 \pm 3.91	0.04
GGTP	68.09 \pm 13.44	42.89 \pm 5.2	0.011
GLU (mg/dl)	192.7 \pm 9.12	105.97 \pm 2.29	0.000
GLU (MMOL/L)	10.69 \pm 0.50	5.88 \pm 0.12	0.000
HOMA IR	8.17 \pm 1.25	3.01 \pm 0.36	0.000

P<0.05 is significant

P< 0.01 highly significant

P<0.001 very highly significant

DISCUSSION

A significant increase in Bilirubin, liver enzymes and total proteins were observed in diabetics compared to non-diabetics (Table 1). A hyperinsulinemia and high insulin resistance were also noted in diabetics.

Elevation of serum alanine aminotransferase (ALT), while uncommon in apparently normal individuals is usual in subjects with type 2 diabetes [5]. A clinical trial



report revealed that 2- 24% of screened type 2 DM patients had liver enzyme tests above the upper limit of normal [8]. Another research on various clinical trials with type 2 diabetes showed that diabetics had higher levels of serum alanine amino transferase (ALT), aspartate amino transferase (AST), or alkaline phosphatase than the normal limits [6]. Liver has a key role in the carbohydrate metabolism and adjusting blood glucose. It is the main site for glycogenesis and gluconeogenesis. This role of liver makes it susceptible in diabetes mellitus [9].

A significant positive correlation was recorded between ALP and bilirubin with HOMA-IR. This finding is supported by increased activity of the liver enzymes related to Insulin resistance [10]. The cause and effect relationship between diabetes mellitus and liver diseases are well-documented, but less explored area in the field of study in our settings.

In our previous study, ALT and AST levels were in the normal range, but AST levels were 1.3 times higher in diabetes patients as compared to normal controls. ALT levels were 1.4 times higher in diabetes individuals. This suggests that diabetes patients have an inclined tendency towards changes of liver enzymes [7]. But the limitation of the research was that insulin resistance was not examined.

Apart from this, there are several studies which report that there is an elevation in liver enzymes in diabetics. In a research involving clinical trials with type 2 diabetes subjects, serum ALT, AST or alkaline phosphatase were 1-2.5 times higher than the upper normal. 5.6% had serum ALT values between 1 and 2.5 times upper normal limit [6]. Asymptomatic subjects with mild increase of ALT and AST showed that 98% had liver illness, fatty liver disease and chronic hepatitis [11]. The main reason of a mild increase of serum ALT is non-alcoholic fatty liver disease [12], which is the most common liver disease in type 2 diabetes.

Comparatively high liver enzymes reveal a possible risk of chronic liver disease in future. Since we have not investigated the histopathology of liver biopsy specimens, we cannot specify whether there is a fatty alteration or to which liver disorder they are prone. Our study is supported by a recent review report by Paola et al., who revealed that subjects with type 2 DM are at the highest risk of non-alcoholic steatohepatitis (NASH), even in the setting of normal plasma aminotransferases [13].

An increase in total proteins and decrease in albumin levels in diabetics observed in our study could be due to reduced fractional synthetic rate of albumin in insulin deficiency. Lowered albumin levels are widely reported by the study by Rehman et al [14]. Total proteins are reported to be elevated in diabetics [15].

Our own study suggests that, a significantly higher ($p=0.0013$) total protein level was found in diabetics as compared to the control group. Globulin was extremely significantly elevated ($p=0.0001$) in type 2 DM. However, an insignificantly lowered albumin levels were noted in patients. A/G ratio was lowered in extremely significant ($p=0.001$) manner in patient group in comparison to the control group [16].

Comparatively high total proteins found in our study is supported by findings of various studies [14, 17]. This increase could be attributed to the elevation of different acute phase proteins, fibrinogen and globulins in DM which contribute to the increase in plasma proteins. In diabetics, reports are available which suggest an elevation in acute phase proteins CRP, α 1-acid glycoprotein, plasminogen, complement C3, ceruloplasmin, etc. [18-21]. Fibrinogen levels are reported to be increased in type 2 DM due to increased synthesis [15, 22]. Study by Ardavi and colleagues suggests that diabetics might exhibit hypergamma globulinemia [23].

Limitations of our study are various confounding factors like obesity, metabolic syndrome and infection with hepatitis C, being not taken in to account.

CONCLUSION

We conclude that diabetic patients had high liver enzymes as compared to non-diabetics. An association was found between type 2 diabetes mellitus, liver markers and insulin resistance.

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