Association of Insulin Based Insulin Resistance with Liver Biomarkers in Type 2 Diabetes mellitus

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Abstract

Aims of the study were to compare liver markers in T2DM patients with that in non-diabetic healthy volunteers and also to find the correlation between insulin resistance(IR) and liver markers. The objective of the study was also to find out whether PON1 can be an alternative liver marker. 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 recruited in the study. 100 age and gender-matched non-diabetics, healthy volunteers or those having health packages were taken as controls. The blood sample was collected and fasting blood glucose, transaminases and Alkaline phosphatase, bilirubin (total and direct), total protein, albumin, and insulin were assayed. HOMA-IR was calculated. Statistical analysis was done by using SPSS 16. A significant elevation was seen in AST, ALT, ALP, GGT, TB, DB, TP, A: G ratio in diabetics. A lowered albumin and A: G ratio were observed in diabetics as compared to controls. Fasting insulin levels were 1.7 times higher in diabetics compared controls, suggesting hyperinsulinemia in cases. Homeostatic model for assessment of insulin resistance (insulin based) was 2.7 times greater in T2DM compared to controls. 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). A significant positive correlation was observed between PON1 and HOMA-IR (P=0.000), PON and insulin (P=0.015). It can be concluded from that diabetics had high liver enzymes as compared to non-diabetics. An association was found between T2DM, liver markers, and IR. It was observed that PON1 was not a good liver marker in T2DM.

Keywords: Insulin resistance, diabetes mellitus, liver markers, Paraoxanase 1.
INTRODUCTION

Liver disease is reported to be one of the important causes of death in diabetes mellitus (DM). A report by De Marco et al suggests that cirrhosis accounted for 4.4% of diabetes-related deaths, in a population based study. A study by Balkau B et al reported that cirrhosis was the cause of deaths in DM in 12.5% of population. Various reports suggest that diabetes has merged as one of the commonest causes of liver disease. A spectrum of liver disorders can occur in DM. Trombetta et al suggest that prevalence of diabetes in cirrhotics is 12.3 - 57%. These suggest a higher prevalence of DM in liver diseases. The relationship between diabetes mellitus and liver disorders are yet to be established in our settings. Since this is a less explored area, we focused to establish an association between liver markers, insulin resistance (IR) and T2DM.

The rationale of the study: As IR is associated with DM as well as liver disorders, it is justifiable to measure liver markers in diabetics. As traditional liver markers proved to be nonspecific in identifying liver disorders, there is a need to explore, a better non-invasive marker. Hence this is an attempt to find out whether paraoxonase 1 (PON1) can be a better marker compared to traditional liver markers. It is very much essential to establish a relationship between insulin resistance, liver markers, and diabetes mellitus.

IR and DM

IR is a condition where cells are non-responsive to insulin. Insulin resistance is associated with T2DM. IR and liver disorders

IR is independently associated with NAFLD and a close association was found between NAFLD and metabolic syndrome. 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.

DM & Liver markers

A clinical trials report suggests that serum transaminases or alkaline phosphatase were 1-2.5 times elevated in type 2 DM. In a retrospective study, we found ALT and AST were 1.3 and 1.4 times respectively higher in diabetics. It has been suggested that diabetics may be more prone for alterations of liver enzymes. Enhanced activity of the liver enzymes is associated with IR.

From the literature review, an association between IR & DM, DM & liver disorders, liver disorders, and IR is evident. However, the cause and effect relationship between these is not well established. This necessitates a study which explores an association between insulin resistance and liver markers. It has been widely accepted that standard biochemical tests which assess liver functions have low sensitivities. Histopathological study of liver biopsy specimen is the gold standard. Invasive procedure and complications are its limitations. Hence, an accurate, reliable, and noninvasive hepatic marker is needed. Under such circumstances parameter of choice appears to be paraoxonase 1 (PON1), which originates from the liver and its gene expression is confined to the liver.

PON1 is an antioxidant enzyme, associated with DM, IR as well as liver disorders.

PON1, IR, and DM

PON1 is HDL bound antioxidant, found to be significantly reduced in diabetics with insulin resistance. It has also been suggested that PON1 activity is positively correlated to IR, as assessed by HOMA index. PON1 & liver disorders

PON1 has been reported to be reduced significantly in acute viral hepatitis, chronic hepatitis, cirrhosis, and sepsis. These findings suggest that PON1 may serve as a useful additional marker in the evaluation of liver conditions.

All the published data available are international, there are only a few Indian studies which focus on IR, liver markers and PON1 to the best of our knowledge.

Objectives

Aims of the study were to compare liver markers in T2DM patients with that in non-diabetic healthy volunteers find the correlation between insulin resistance (insulin based) and liver markers find out the effectiveness of PON1 activity as a liver biomarker as compared to traditional liver parameters.

MATERIALS AND METHODS

Study design

The cross-sectional study was carried out in the Department of Biochemistry, KS...
Hegde Medical Academy, Mangalore, Karnataka. Institutional ethics committee approval was obtained to conduct the study. Informed consent was taken from the study subjects.

**Inclusion criteria**

114 type 2 diabetics in the age group of 18-65 years, diagnosed as per ADA 2016 guidelines were included as cases. Hundred age and gender-matched nondiabetics, healthy volunteers were considered as controls.

**Exclusion criteria**

Alcoholics, diagnosed cases of acute and chronic hepatitis, other liver disorders.

**Sample collection and analysis**

Five ml of fasting venous blood sample was collected using aseptic precaution. The blood sample was centrifuged at 3000rpm for 20 min and serum was separated. Fasting blood glucose, AST, ALT, ALP, GGT, bilirubin, total protein and albumin were estimated using fully automated chemistry analyzer, Cobas C-311.

Insulin levels were assayed using hormone analyzer, Cobas e411 which works on the principle of electrochemiluminescence.

Insulin resistance was calculated by the homeostasis model assessment (HOMA).

$$\text{HOMA-IR} = \frac{\text{fasting glucose} \times \text{fasting insulin}}{22.5}; \text{insulin expressed in µU/L, glucose in mmol/l.}$$

PON1 activity was assayed using the spectrophotometric method 10.

**Statistical analysis**

Statistical analysis was done using the software, SPSS version 16.

Mann Whitney U test was applied to compare liver markers in diabetics and non-diabetics. Spearman’s correlation coefficient was used to find the correlation between liver markers and insulin resistance. Receiver Operative characteristic curve (ROC) was constructed to find out whether PON1 can be used as an alternative liver marker.

**RESULTS**

Insulin levels were raised 1.76 times in diabetics compared to controls. In liver profile, total and direct bilirubin, liver enzymes like AST, ALT, ALP, GGT, bilirubin, total protein and albumin were estimated using fully automated chemistry analyzer, Cobas C-311.

<table>
<thead>
<tr>
<th>Test Variable</th>
<th>The area under the curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>0.908</td>
</tr>
<tr>
<td>ALT</td>
<td>1</td>
</tr>
<tr>
<td>ALP</td>
<td>0.720</td>
</tr>
<tr>
<td>GGT</td>
<td>0.848</td>
</tr>
<tr>
<td>TB</td>
<td>0.617</td>
</tr>
<tr>
<td>DB</td>
<td>0.634</td>
</tr>
<tr>
<td>TP</td>
<td>0.484</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.450</td>
</tr>
<tr>
<td>PON1</td>
<td>0.472</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Cases (T2DM)</th>
<th>Controls (Non diabetics)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INSULIN</td>
<td>20.51±3.37</td>
<td>11.66±1.34</td>
<td>0.001**</td>
</tr>
<tr>
<td>TP (G/dl)</td>
<td>7.47±0.06</td>
<td>7.22±0.06</td>
<td>0.01*</td>
</tr>
<tr>
<td>ALB (G/dl)</td>
<td>4.1±0.04</td>
<td>4.2±0.057</td>
<td>0.026*</td>
</tr>
<tr>
<td>Globulin</td>
<td>3.2 ± 0.78</td>
<td>1.4± 0.49</td>
<td>0.000**</td>
</tr>
<tr>
<td>A:G ratio</td>
<td>1.29±0.03</td>
<td>1.48±0.03</td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>TB (mg/dl)</td>
<td>0.94±0.066</td>
<td>0.78±0.96</td>
<td>0.026*</td>
</tr>
<tr>
<td>DB (mg/dl)</td>
<td>0.37±0.03</td>
<td>0.33±0.05</td>
<td>0.016*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>52.28±5.75</td>
<td>32.1±3.6</td>
<td>0.0179*</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>40.17±3.74</td>
<td>28.1±3.845</td>
<td>0.0001***</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>94.54±2.96</td>
<td>86.5±3.91</td>
<td>0.04*</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>68.09±13.44</td>
<td>42.89±5.2</td>
<td>0.011*</td>
</tr>
<tr>
<td>GLU (mg/dl)</td>
<td>192.7±9.12</td>
<td>105.9±2.29</td>
<td>0.000***</td>
</tr>
<tr>
<td>GLU (mmol/l)</td>
<td>10.69±0.50</td>
<td>5.88±0.12</td>
<td>0.000***</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>8.17±1.25</td>
<td>3.01±0.36</td>
<td>0.000***</td>
</tr>
<tr>
<td>PON1</td>
<td>0.84±0.03</td>
<td>0.69±0.04</td>
<td>0.003**</td>
</tr>
</tbody>
</table>

*P<0.05 is significant
**P< 0.01 highly significant
***P<0.001 very highly significant

When the utility of PON1 as a biomarker of liver disease, it was observed that, PON1 was not a good liver marker in T2DM. Liver transaminases, especially ALT and AST were good markers (AUC =1 and 0.908 respectively whereas that for PON1 was 0.472). GGT was a better marker compared to ALP (AUC 0.848 VS 0.720).

PON1 was found to bear a significant positive correlation with HOMA-IR and insulin (P=0.000 and P=0.015 respectively).
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.

Insulin level showed a significant positive correlation with 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). Albumin and HOMA-IR showed a very significant negative correlation \( (r=-0.306, P=0.004) \).

**DISCUSSION**

A significant increase in Bilirubin, liver transaminases and total proteins were observed in diabetics compared to non-diabetics (Table 1).

Elevation of ALT is commonly reported in patients with type 2 diabetes, while uncommon in apparently normal subjects\(^5\). A clinical trial by Belcher et al suggests that 2- 24% of screened type 2 DM patients had transaminase levels higher than normal limit\(^13\). Another report by Lebovitz et al involving multiple clinical trials with DM suggests that diabetics had higher levels of serum transaminases and ALP\(^6\). The liver plays a key role in the carbohydrate metabolism and plasma glucose maintainance. It is the key organ for glycogenesis and gluconeogenesis. This role of the liver makes it susceptible in DM\(^12\).

ALP and bilirubin showed a significant positive correlation with HOMA-IR. This finding is supported by the increased activity of the liver enzymes associated with Insulin resistance\(^13\). The relationship between diabetes mellitus and liver diseases, the cause and effect aspect are yet to be established. This is the less explored area in the field of research in our settings.

In our previous study, serum transaminases levels were in the normal range, but AST levels were 1.3 times high in diabetes patients as compared to non-diabetics. ALT levels were 1.4 times high in diabetics. These findings suggest that diabetics may be more prone for an altered hepatic transaminases\(^9\). However our previous study had a few limitations that insulin resistance was not studied.

There are several studies which suggest an elevation in serum transaminases in diabetics. In a clinical trial report by Yamada et al, hepatic enzymes were 1-2.5 times in DM. Serum ALT values were 1 - 2.5 times higher than normal range in 5.6% patients\(^8\). A mild elevations of transaminases in asymptomatic individuals could be due to fatty liver disease or chronic hepatitis\(^13\). Non-alcoholic fatty liver disease is the most common cause of a mild elevation of serum ALT and it is the most prevalent hepatic disorder in T2DM\(^14,15\). Findings of our study are supported by a review report by Paola et al, which opined that type 2 diabetics are more prone for non-alcoholic steatohepatitis (NASH), even though they have a normal liver enzyme levels\(^16\). Comparatively elevated hepatic enzymes suggest a probable risk of hepatic disorder in the future. As the histopathology of liver biopsy specimens were not analyzed in the study, it is not possible to specify whether a fatty change is involved or which type of liver disease is likely.

An elevation in total proteins and a decline in albumin levels in diabetics found in the study could be attributed to a low rate of synthesis of albumin due to insulin deficiency. A study by Rehman et al also reported a lowered albumin levels in diabetes mellitus patients\(^17\). A study by Mohammed et al observed an elevated total proteins in diabetics\(^18\).

Our previous study report suggests an elevation of total protein in diabetics as compared to non-diabetics. Globulin was extremely significantly high in diabetics. Lowered albumin levels found in diabetics was insignificant. A/G ratio was lowered in an extremely significant manner in diabetics\(^19\).
Comparatively elevated total proteins observed in our present study is supported by various reports. This could be due to the elevation of various acute phase proteins, fibrinogen, and globulins in T2DM which in turn rise plasma proteins. Studies suggest an elevation in acute phase proteins CRP, α1-acid glycoprotein, plasminogen, complement C3, ceruloplasmin in type 2 diabetes mellitus. Elevated fibrinogen levels in diabetics could be attributed to increased hepatic synthesis. A study by Ardavi and colleagues reported a hypergamma-globulinemia in diabetes mellitus.

Albumin had a significant negative correlation with C-peptide based insulin resistance in the present study. Studies reported that an elevated albumin level was associated with IR. However, we couldnot establish an independent association of albumin on the development of diabetes. Although the causal relationship between IR and serum albumin levels is not clear, our results suggest that IR may affect serum albumin levels. IR is by definition associated to hyperinsulinemia which was observed in our study.

ROC curve is the graphical plot which is used to compare the diagnostic ability of two diagnostic tests. If a ROC curve follows the left-hand border and top border of ROC space, it suggests that the test could be accurate. If an area under the curve (AUC) equal to 1, it suggests that the test is perfect. If AUC lies between 0.9 -1, the test is said to be excellent. The AUC value of 0.80-0.90 suggests a good accuracy.

Based on our results, transaminases have an excellent area under the curve implying that they are the better markers of liver disease compared to PON1 which has the AUC of 0.472. AUC for GGT is in the acceptable range (AUC = 0.848).

Our finding is in contradictory to the reports by Pyati et al, which compared the diagnostic accuracy of PON1 versus routine liver markers. The study showed that PON1 had an area under the curve 0.990 which was in agreement with the other parameters like, ALT (AUC = 0.999), TB (AUC = 0.977) and ALP (AUC = 0.904).

However, a significant increase (1.25 times) in PON1 levels was observed in diabetics compared to non-diabetics. Our results are in agreement with the study by Suvarna et al, which reported an elevated PON1 in uncomplicated diabetics compared to non-diabetics. PON1 is an antioxidant enzyme, its elevation could be a compensatory increase so as to fight the enhanced oxidative stress in diabetics.

A positive correlation was established between the Homeostasis Model Assessment (HOMA) index and PON1 activity in non-diabetic Japanese subjects by Yamada et al. Tabur et al reported in Turkish population that PON1 activities were not different between non-diabetic subjects with and without metabolic syndrome.

Beer et al reported that PON1 concentration and activity were same in diabetic patients, impaired glucose tolerant patients and non-diabetics. However postprandial hyperlipidemia was associated with alterations in PON1 activity in diabetic subjects. In the same study, it was observed that there was no difference in the postprandial PON1 response between diabetics and non-diabetics. Kopprasch et al suggested that PON1 activity was not significantly different in normoglycemic subjects, glucose-intolerant subjects and newly ascertained diabetics. It may be concluded from these studies that PON1 activity may be lost in the course of diabetes mellitus and hyperglycemia, rather than in the initial stage of IR. Studies suggest a significantly lowered serum PON1 activity in diabetics compared to the healthy subjects.

A study by Pyati et al suggests that PON1 activity has a better diagnostic accuracy compared to other liver markers. It also reports that assay of PON1 activity may improve the efficiency significantly, of a laboratory’s evaluation system.

However, we could not establish the role of PON1 as an effective liver marker in predicting liver diseases associated with T2DM.

CONCLUSION

It could be concluded from the study that diabetics had elevated serum transaminases, bilirubin and total protein as compared to non-diabetic controls. An association was found between type 2 diabetes mellitus, liver markers and insulin resistance. Paraoxonase 1 activity may not be a good marker to predict liver disease in diabetes mellitus.
ACKNOWLEDGEMENTS
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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION
UA designed the study, wrote the protocol and did statistical analysis. KP helped in sample collection. NP helped in standardization of procedure. UA wrote the manuscript.

FUNDING
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DATA AVAILABILITY
Data was collected from Department of Biochemistry, KS Hegde Medical Academy, NITTE-Deemed to be University, Mangaluru, Karnataka, India. All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT
The study was approved by Institutional Ethics committee of KS Hegde Medical Academy, NITTE-Deemed to be University, Mangaluru, Karnataka, India. Approval Number: INST.EC/EC/021/2017-18.

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