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ABSTRACT

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INSULIN RESISTANCE AND HEPATIC MARKERS IN TYPE 2 DIABETES MELLITUS: A CROSS-SECTIONAL STUDY

Aim: The objective of this study was to compare liver markers and insulin resistance between diabetic and non-diabetic individuals and determine the correlation between diabetic and non-diabetic parameters.

Methods: A cross-sectional study was conducted at the Department of Biochemistry after being approved by the Institutional Ethics Committee. Fasting venous blood was collected for serum analysis of various biochemical parameters, including liver enzymes AST and ALT. Total and direct bilirubin and plasma proteins like total protein, albumin, globulin, A:G ratio were assessed using automated chemistry analyzers. Insulin levels were measured using a hormone analyzer based on electrochemiluminescence. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) formula, and PON1 activity was determined using a spectrophotometric method.

Results: The study showed a significant increase in the levels of insulin, liver enzymes, bilirubin, and insulin resistance in T2DM patients compared to controls. Notably, AST and ALT exhibited excellent discriminatory ability as liver markers, with perfect AUC values, while PON1 showed lower AUC values, indicating its limited utility as a liver marker in T2DM. Positive correlations were observed between PON1 and HOMA-IR, as well as between PON1 and insulin levels. These findings suggest that while liver enzymes like AST and ALT are robust markers of liver disease in T2DM, PON1 may not be as effective in predicting liver disease in this population.

Conclusion: The study provides valuable insights into the association between liver markers and insulin resistance in type 2 diabetes mellitus (DM), highlighting the importance of comprehensive liver function assessment in diabetic individuals.

Keywords: insulin, C peptide, diabetes mellitus, insulin resistance, liver markers, PON 1.

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INTRODUCTION

T2DM is a progressive metabolic disorder with micro and macrovascular complications. The prevalence of T2DM is correlated with obesity and unhealthy sedentary lifestyles. Several environmental, metabolic, and genetic risk factors are associated with T2DM, and obesity is considered an important risk factor for T2DM. According to the World obesity atlas 2022, by 2030, approximately 1 billion people globally will be living with obesity. This means that 1 in 5 women and 1 in 7 men will be obese. Obesity is more prevalent among women than men. International Classification of Disease (ICD) defines obesity as a chronic, relapsing, multifactorial disease. This seems to affect low and middle-income countries (LMIC) where malnutrition is a public health concern [1].

Liver disease is recognized as a significant cause of mortality in individuals with diabetes mellitus (DM), with cirrhosis contributing notably to diabetes-related deaths [2-4]. There is a growing understanding of diabetes as a prevalent factor in liver disorders, yet the precise relationship remains largely unexplored. This study seeks to investigate the correlation between liver markers and insulin resistance in type 2 DM.

Given the link between insulin resistance (IR) and both DM and liver disorders, it is logical to examine liver markers in diabetic patients. Conventional liver markers have limitations, prompting exploration into alternative non-invasive markers, such as paraoxonase 1 (PON1). Understanding the relationship between insulin resistance, liver markers, and diabetes mellitus is of paramount importance.

Insulin resistance is closely associated with type 2 DM [5], although its measurement using HOMA-IR has limitations in specific patient populations [6]. Alternative indices, such as the C-peptide-based index, may offer enhanced accuracy [7]. Insulin resistance is independently linked to non-alcoholic fatty liver disease (NAFLD) and is closely associated with metabolic syndrome [8], which in turn correlates with DM.

Clinical trials and retrospective studies have indicated elevated serum ALT, AST, and alkaline phosphatase levels in individuals with type 2 DM [9, 10], indicating a tendency towards alterations in liver enzymes among diabetics.

The literature review suggests intricate connections among IR, DM, liver disorders, and PON1, highlighting the need for further exploration. Paraoxonase 1 (PON1), primarily expressed in the liver, exhibits reduced activity in diabetics with insulin resistance and various liver conditions [11, 12].

While international literature provides valuable insights, there is a scarcity of national studies focusing on the interplay of insulin resistance, liver markers, and PON1. This study aims to address this gap and contribute to the understanding of these complex associations.

The objective of the study was to compare the liver markers and insulin resistance between diabetics and non-diabetics and to find the correlation between them.

METHODS

The cross-sectional study, conducted in the Department of Biochemistry, received approval from the institutional ethics committee, and informed consent was obtained from all participants. Inclusion criteria comprised 114 type 2 DM patients aged 18-65 years, diagnosed according to ADA 2016 guidelines, while 100 age and gender-matched non-diabetic individuals, including healthy volunteers and those undergoing health packages or surgery, served as controls. Exclusion criteria encompassed alcoholics and diagnosed cases of acute and chronic hepatitis, among other liver disorders. Five milliliters of fasting venous blood were collected aseptically and centrifuged to obtain serum for analysis. Various parameters, including fasting blood glucose, liver enzymes (AST, ALT, alkaline phosphatase, GGT), total bilirubin, direct bilirubin, total protein, and albumin, were assessed using automated chemistry analyzers. Insulin levels were measured using a hormone analyzer based on electrochemiluminescence. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) formula. PON1 activity was determined using a spectrophotometric method. Statistical analysis was performed using SPSS version 16, employing the Mann-Whitney U test to compare liver markers between diabetics and non-diabetics, Spearman's correlation coefficient to assess the correlation between liver markers and insulin resistance, and receiver operating characteristic (ROC) curve analysis to evaluate PON1 as a potential liver marker.

RESULTS

In diabetic individuals compared to controls, insulin levels showed a 1.76-fold increase. Liver profile analysis revealed elevated levels of total bilirubin, direct bilirubin, and liver enzymes such as AST, ALT, ALP, and GGT, with increases of 1.2, 1.12, 1.63, 1.43, 1.09, and 1.59 times, respectively. Conversely, albumin levels decreased while total protein and globulins increased significantly in cases versus controls. The Homeostatic Model

Assessment of Insulin Resistance (HOMA-IR) was 2.7 times higher in individuals with type 2 diabetes mellitus (T2DM).

A notable positive correlation was observed 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 also exhibited significant positive correlations with HOMA-IR (r=0.228, P=0.033; r=0.231, P=0.030; r=0.242, P=0.023, respectively). Conversely, a significant negative correlation was found between albumin and HOMA-IR (r=-0.306, P=0.004).

In controls compared to diabetics, fasting C-peptide levels were 1.26 times higher. The C-peptide-based HOMA-IR was 3.5 times higher in T2DM compared to controls. The index 20/(fasting C-peptide x fasting plasma glucose) was 1.45 times higher in controls. No significant correlation was observed between C-peptide levels and liver function tests. However, C-peptide-based HOMA-IR showed a significant negative correlation with albumin (r=-0.230, P=0.035), while the index 20/(fasting C-peptide x fasting plasma glucose) exhibited a significant positive correlation with albumin (r=0.261, P=0.016).

When assessing PON1's effectiveness as a liver disease biomarker, it was noted that PON1 did not perform well as a liver marker in T2DM. Liver transaminases, particularly ALT and AST, proved to be strong markers (with AUC values of 1 and 0.908, respectively), whereas the AUC for PON1 was 0.472. GGT emerged as a superior marker compared to ALP (with AUC values of 0.848 and 0.720, respectively).

Furthermore, a significant positive correlation was identified between PON1 and HOMA-IR (P=0.000), as well as between PON1 and insulin (P=0.015).

Among the tested variables, AST exhibited the highest area under the curve (AUC) at 0.908, followed by ALT with a perfect AUC of 1, indicating their excellent performance as markers of liver disease. ALP and GGT also demonstrated good discriminatory ability with AUC values of 0.720 and 0.848, respectively. In contrast, total bilirubin (TB), direct bilirubin (DB), total protein (TP), albumin, and PON1 showed lower AUC values, suggesting less accurate performance in predicting liver disease.

DISCUSSION

A notable increase in bilirubin, liver enzymes, and total proteins was observed in diabetic individuals compared to non-diabetics (Table 1). Hyperinsulinemia

Table 1 – Comparison of liver markers and insulin resistance in diabetics and non-diabetics

Parameters	Cases (t2dm)	Controls (non diabetics)	P-value
INSULIN	123.06 ± 20.22 pmol/L	69.96 ± 8.04 pmol/L	0.001**
C -peptide	0.94 ± 0.07 nmol/L	1.1862 ± 0.079 nmol/L	0.046*
TP	74.7 ± 0.6 g/L	72.2 ± 0.6 g/L	0.01*
ALB	41 ± 0.4 g/L	42 ± 0.57 g/L	0.026*
Globulin	2 ± 7.8 g/L	14 ± 4.9 g/L	0.000**
A:G ratio	1.29 ± 0.03	1.48 ± 0.03	<0.0001**
TB	16.074 ± 1.1286 µmol/L	13.338 ± 16.416 µmol/L	0.026*
DB	6.327 ± 0.513 µmol/L	5.643 ± 0.855 µmol/L	0.016*
AST	52.28 ± 5.75 U/L	32.1 ± 3.6 U/L	0.0179*
ALT	40.17 ± 3.74 U/L	28.1 ± 3.845 U/L	0.0001***
ALP	94.54 ± 2.96 IU/L	86.5 ± 3.91 IU/L	0.04*
GGTP	68.09 ± 13.44 U/L	42.89 ± 5.2 U/L	0.011*
GLU (mmol/l)	10.69±0.50	5.88±0.12	0.000***
HOMA IR	8.17±1.25	3.01±0.36	0.000***
HOMA-IR(C-peptide)	1.27±0.14	0.36±0.038	0.000***
20/ (Fasting C-peptide x FPG)	4.99±0.65	7.25±1.39	0.041*
PON 1 (nmol/ml/min)	0.84±0.03	0.69±0.04	0.003**

*Note: *P<0.05 is significant; **P< 0.01 highly significant; ***P<0.001 very highly significant*

and high insulin resistance were also evident in diabetics. Elevated ALT levels, though uncommon in apparently healthy individuals, are frequently observed in type 2 diabetes [5]. Clinical trial data suggests that 2–24% of screened type 2 DM patients exhibit liver enzyme test abnormalities [12]. Additionally, multiple clinical trials have demonstrated higher levels of ALT, AST, or alkaline phosphatase in diabetics compared to normal ranges [7]. The liver's pivotal role in carbohydrate metabolism and blood glucose regulation makes it vulnerable to the effects of diabetes mellitus [13].

A significant positive correlation was noted between ALP and bilirubin with HOMA-IR, supported by increased liver enzyme activity associated with insulin resistance [14]. However, the cause-and-effect relationship between diabetes mellitus and liver diseases remains insufficiently documented, representing a less explored area in our research context.

Figure 1 – ROC for PON 1 and liver markers

In our previous study, while ALT and AST levels were within the normal range, AST levels were 1.3 times higher, and ALT levels were 1.4 times higher in diabetic patients compared to controls. This indicates a propensity for liver enzyme alterations in diabetes patients [8]. However, the previous study's limitation was the lack of investigation into insulin resistance.

Moreover, several studies have reported elevations in liver enzymes in diabetics. Involving clinical trials with type 2 diabetes patients, some studies found serum ALT, AST, or alkaline phosphatase levels to be 1-2.5 times higher than the upper normal limit, with 5.6% exhibiting serum ALT values between 1 and 2.5 times the upper normal limit [7]. Asymptomatic individuals with mild ALT and AST elevations often have liver diseases such as fatty liver disease and chronic hepatitis [15]. Non-alcoholic fatty liver disease is the most common liver disease in type 2 diabetes, with a mild elevation in serum ALT being indicative [16]. Our study is corroborated by a recent review suggesting that type 2 DM patients are at increased risk of non-alcoholic steatohepatitis (NASH), even with normal plasma aminotransferases [17]. Elevated liver enzymes suggest a potential risk of chronic liver disease in the future. However, since we did not assess liver biopsy histopathology, we cannot specify the presence of fatty changes or the specific liver disorder to which patients are prone.

The observed increase in total proteins and decrease in albumin levels in diabetics in our study could be attributed to reduced albumin fractional synthetic rate due to insulin deficiency. Lowered albumin levels have been widely reported [18]. Additionally, elevated total protein levels in diabetics are supported by various studies [18, 19]. This elevation may be attributed to increased acute phase proteins, fibrinogen, and globulins in diabetes, contributing to plasma protein elevation. Diabetic patients often exhibit increased levels of acute-phase proteins such as CRP, α1-acid glycoprotein, plasminogen, and complement C3, as well as increased fibrinogen synthesis [20-24]. Studies suggest that diabetics may also present with hypergammaglobulinemia [25]. Elevated total and direct bilirubin levels, along with significant positive correlations with insulin levels and HOMA-IR, suggest that increased bilirubin may act as a compensatory

mechanism to improve insulin sensitivity. This hypothesis is supported by studies showing that bilirubin contributes to increased insulin sensitivity and glucose tolerance in mouse models by regulating cholesterol metabolism, adipokines, and PPAR γ [26, 27].

ROC curve analysis evaluates the diagnostic accuracy of a binary classifier system, with an AUC value of 1 indicating perfect accuracy. Our results indicate that ALT and AST exhibit excellent AUC values, making them superior markers of liver disease compared to PON1, which had an AUC of 0.472. GGT's AUC falls within an acceptable range (AUC = 0.848). However, our findings contradict those of Pyati et al., who reported high diagnostic accuracy of PON1 compared to routine liver markers [28]. Nonetheless, we observed a significant increase (1.25 times) in PON1 levels in diabetics compared to non-diabetics, consistent with the findings of Suvarna et al. [29]. This elevation in PON1 activity could be a compensatory response to heightened oxidative stress in diabetics. Studies have shown varying results regarding the association between PON1 activity and diabetes mellitus. Some report a positive correlation between the HOMA index and HDL-corrected PON1 activity in non-diabetic subjects [7], while others found no difference in PON1 activities between diabetic and non-diabetic subjects [30]. Serum PON1 activity loss may occur later in the course of diabetes mellitus and hyperglycemia rather than in the insulin resistance stage [31, 32]. However, serum PON1 activity is significantly decreased in both type 1 and type 2 diabetics compared to healthy controls [33–35].

CONCLUSION

In conclusion, diabetic patients exhibit elevated liver enzymes, bilirubin, and total proteins compared to non-diabetics. An association was observed between type 2 diabetes mellitus, liver markers, and insulin resistance. However, PON1 activity may not be an effective marker for predicting liver disease in diabetes mellitus.

AUTHOR CONTRIBUTIONS

All authors substantively contributed to the drafting of the initial and revised versions of this paper. They take full responsibility for the integrity of all aspects of the work.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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