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ORIGINAL ARTICLE

LIVER ENZYMES AND BIOCHEMICAL FUNCTION TESTS IN T2DM: IMPACT OF DURATION, GLYCEMIC CONTROL AND SOME OTHER CO-VARIABLES

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Summary

Background and Purpose: T2DM is the most common cause of end- stage liver diseases, and different mechanisms contribute to diabetic hepatopathy's wide spectrum presentation. In this study, we aimed to identify abnormalities in liver function tests (LFTs) for a group of Iraqi T2DM patients, determine their prevalence, and investigate the influence of some independent co-variables (duration of DM, HbA1c, BMI, age, and gender).
Methods: This case-control study enrolled 43 T2DM patients alongside 40 healthy, age- and sex- matched non-diabetic subjects. After overnight fasting, blood was collected, and fasting plasma glucose (FPG), HbA1c, and serum LFTs (AST, ALT, ALP, total proteins, albumin, and bilirubin) were measured in addition to serum lipids.
Results: T2DM patients exhibited significantly higher FPG, HbA1c, AST, and ALT mean values than the controls. Serum aminotransferases were increased in 30% of patients. Serum albumin and total bilirubin (TSB) decreased in 18.6 and 37.2% respectively. Diabetics with HbA1c>7.0% had significantly higher AST, ALT, ALP, and STP values and lower serum albumin and TSB. The logistic regression analysis revealed that duration, BMI, HbA1c, and age are independent co-variables significantly linked to increased ALT activity.
Conclusions: LFTs (mainly aminotransferases) are altered in DM. The duration of diabetes, the age of the patient, BMI, and glycemic control influence this change. We recommend monitoring LFTs in DM and maintaining good glycemic control.

Key words: ALT; liver function tests; HbA1c; T2DM

Diabetes mellitus (DM) is a global health problem that affected about 534 million people in 2021 and is expected to affect 780 million in 2045 worldwide (1). Type 2 diabetes (T2DM) accounts for almost 90% of all diabetic cases and is characterized by chronically elevated blood glucose levels due to combined insulin deficiency and poor responsiveness (2). Chronic hyperglycemia, and metabolic complications produce damage to many body organs with time and yield life-threatening complications (3). T2DM adversely affects the liver, and diabetes is currently the most common cause of end- stage liver diseases. In the USA, liver cirrhosis due to DM is the third most common indication for liver transplantation (4, 5).

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Different studies revealed a wide range of liver problems in T2DM patients. This large scale includes raised blood levels of liver enzymes, non-alcoholic fatty liver disease (NAFLD), liver cirrhosis, and acute liver failure. In addition, diabetes has been associated with hepatocellular carcinoma and hepatitis C (6, 7). The exact mechanism by which DM produces liver dysfunction is not fully understood. However, one possible reason is fat deposition in the liver, the characteristic feature of NAFLD. Another explanation may be the higher susceptibility to inflammation in the hepatic tissue of diabetes (8).

Liver function tests (LFTs) are widely used for screening liver diseases, monitoring their progress, and observing the impact and success of their treatment. Among the most common LFTs are serum transaminases (as biomarkers of hepatocyte integrity), albumin, total protein, and prothrombin time (as indicators of liver synthetic functions), as well as bilirubin, alkaline phosphatase (ALP), and gamma-glutamyltranspeptidase (GGT), which are markers of cholestasis (9). Awareness that DM is a significant cause of liver diseases may help to prevent future liver damage. In this study, we aimed to determine the prevalence of abnormal LFT among adult Iraqi T2DM patients. We also investigated the relationship between the LFTs abnormalities and some independent co-variables such as the duration of DM, and the extent of glycemic control in addition to BMI, age, and gender differences.

Materials and Methods

Study Design

This is a case- control study conducted over a five- month duration starting from 11- May to 11- October- 2022 in Mosul City, Northern Iraq.

Adherence to Ethical Standards

This study was approved by the Medical Research Ethics Committee of the College of Medicine, University of Mosul [Approval ID: UOM/COM/MREC/21-22(72)]. After being informed of the study's aims, procedures, and possible benefits, all participants provided their written informed consent. The study adhered to all universal standards of medical research ethics.

Subjects

Fifty known cases of T2DM were randomly selected from a private clinic in Mosul City for this study. These patients have been diabetic for variable periods and are on different oral hypoglycemic regimens. The exclusion criteria included T1DM, previous hepatitis history, alcohol consumption, smoking, pregnancy, treatment with hepatotoxic drugs and history of malignancy. Finally, 43 patients (21 males and 22 females) aged 38- 73 years gave their written consent to participate. Group 1constituted the patients group.

Forty- five healthy age- and sex- matched non- diabetic subjects with a negative history of previous glucose homeostasis abnormalities were recruited to participate. They were randomly selected from among the auditors of a few private clinics for complaints other than diabetes or liver diseases and consented to participate. Subjects with a history of hepatitis, renal diseases or malignancies were excluded, as well as those with fasting plasma glucose (FPG) levels > 5.56 mmol/L as recommended by the American Diabetes Association (ADA) (10). People with acute infections and inflammations were also excluded. Group 2, the control group, comprised 40 (21 males and 19 females) subjects aged (34- 69).

Data Collection and Anthropometric Measurements

Subjects in both groups were interviewed and asked to provide a brief medical history including name, age, sex, duration of diabetes (patients group), current medical history, and any co-morbidities. Blood pressure was measured in a sitting position after enough rest, and height was recorded while the subjects had their shoes off. His/her weight was measured while wearing light clothes. Body mass index (BMI) was calculated as weight (kg)/ height $(m)^2$ (11). They were asked to fast for 12-14 hours prior to blood collection as appointed.

Blood samples of 8 ml were obtained from every participant under aseptic conditions. A total of 1 ml was transferred into a sodium fluoride- containing tube (for FPG measurement), while 6 ml was allowed to clot in a plain tube prior to centrifugation. Plasma and serum samples were respectively separated by centrifugation at 3000 rpm for 15 minutes. Serum samples were aliquoted and frozen at -20 °C for serial measurements of lipids (total cholesterol, HDL-cholesterol, and triglycerides) and LFTs (AST, ALT, ALP, total proteins, albumin, and bilirubin). At the same time, plasma glucose was measured without delay. We mixed 1 ml of whole blood in an EDTA- containing tube and refrigerated at 2-8 ˚C for subsequent measurement of glycated hemoglobin (HbA1c) within 48 hours. All biochemical analyses were conducted at the Clinical Biochemistry Lab, Department of Biochemistry, College of Medicine, University of Mosul.

Biochemical Analyses

All investigations were conducted with kits purchased from international suppliers and used according to the manufacturer's instructions. FPG was measured using the glucose oxidase- peroxidase method (12), while the GPO- PAP method measured serum triglycerides depending on hydrolysis by lipase enzyme. Serum total cholesterol was measured using the enzymatic endpoint method (CHOD-PAP), while HDL-cholesterol levels were determined by the precipitation method (13). Low- density lipoprotein cholesterol (LDL-c) was calculated mathematically using Friedewald's equation (14).

Serum activities of aminotransferases (AST and ALT) were determined using 2,4-dinitrophenylhydrazine (DNPH) in an alkaline solution (15). Using the kit from Biolabo, France, ALP serum activity was measured by the reaction of phenol produced from phenylphosphate hydrolysis with 4-amino-antipyrine (16). Serum albumin and total protein (STP) were determined by the bromocresol green (BCG) (17), and Biuret methods (18), respectively. Total serum bilirubin (TSB) was measured according to the Malloy-Evelyn principle modified by Walters and Gerarde (19- 20). However, HbA1c was measured by the ion exchange resin separation method (21).

Statistical Analysis

Data analysis was conducted using SPSS version 20 for Windows 10. Continuous (quantitative) variables were expressed as mean ± SD and their mean values were compared using unpaired Student's t- test. However, categorical (qualitative) variables were displayed as N (%) and compared using the Chi- square test. Analysis of variance (ANOVA) was used to analyze mean values of LFTs among subgroups of diabetics (based on DM duration). The cut-off values of increased and decreased LFT variables were determined as mean+2SD and mean-2SD respectively. Linear Regression Analysis was conducted to study the relationship between HbA1c levels and serum ALT Activity. Logistic regression analysis was employed to determine the impact of co-variables such as age, sex, BMI, duration, and glycemic control on LFTs. A p- value of <0.05 was considered statistically significant.

Results

The results are displayed according to the participants' grouping, as follows:

Group 1 (Diabetics): Consists of 43 known T2DM cases (21 males and 22 females) aged 38-73 years (mean 49.2±1.3) who have had diabetes for 2-21 years (mean 8.58±0.91). The mean BMI of subjects in this group was 27.22±0.81 kg/m^2.

Group 2 (Control Group): Comprising 40 healthy age- and sex- matched non- diabetic subjects (21 males and 19 females) aged 34-69 years (mean 47.5±3.2). The mean BMI of control subjects was 25.5±3.2 kg/ m^2. The basic clinical, anthropometric and biochemical characteristics of subjects in both groups are shown in Table 1.

Comparing Biochemical Parameters and LFTs between Diabetics and Non- diabetics

In Table (1), using unpaired Student's t- test, diabetic patients exhibited significantly higher mean values of FPG and HbA1c than controls ($p<0.001$) with non-significant differences in mean values of the serum lipid profile.

In addition, BMI, SBP, and DBP mean values did not significantly differ. However, comparing liver enzymes and other LFTs, the mean values of serum AST and ALT activities were significantly higher among T2DM patients ($p<0.001$ and 0.004 respectively).

Table 1. Basic characteristics of the study subjects. Data are expressed as mean± SD or N(%) as appropriate.

Variable		Group (1) Or Diabetics Group (N=43)	Group (2) Or Control Group (N=40)	p- value
Age (years)		49.2 ± 1.3	47.5 ± 3.2	0.065
Sex	Male	21 (48.8%)	21 (52.5%)	0.74
	Female	22 (51.2%)	19 (47.5%)	
Duration of Diabetes (years)		8.58 ± 0.91	0.000	<0.001
SBP (mmHg)		135 ± 2.75	128.1 ± 17.3	0.19
DBP (mmHg)		81.42 ± 1.5	80.9 ± 10.3	0.66
BMI (kg/m^2)		27.22 ± 0.81	25.5 ± 3.2	0.06
FPG (mmol/L)		8.61 ± 0.50	4.91 ± 0.39	<0.001
Total Cholesterol (mmol/L)		5.51 ± 0.88	4.82 ± 0.16	0.29
Triglycerides (mmol/L)		1.68 ± 0.18	1.15 ± 0.64	0.11
HDL-c (mmol/L)		1.24 ± 0.05	1.33 ± 0.3	0.53
LDL-c (mmol/L)		3.61 ± 0.77	2.76 ± 0.12	0.55
AST (U/L)		29 ± 2.17	24 ± 4.1	<0.001
ALT (U/L)		39.23 ± 3.11	27.48 ± 9.14	0.004
ALP (U/L)		91.5 ± 4.37	69.2 ± 19.1	0.10
STP (g/L)		73.85 ± 4.35	73.5 ± 0.58	0.41
Albumin (g/L)		43.67 ± 0.36	44.86 ± 2.3	0.66
TSB (µmol/L)		10.85 ± 1.18	11.35 ± 0.65	0.71
HbA1c (%)		8.32 ± 1.33	4.12 ± 0.97	<0.001

SBP_ systolic blood pressure; DBP_ diastolic blood pressure; BMI_ body mass index; FPG_ fasting plasma glucose; HDL-c_ high density lipoprotein cholesterol; LDL-c_ low density lipoprotein cholesterol; AST_ aspartate transaminase; ALT_ alanine transaminase; ALP_ alkaline phosphatase; STP_ serum total proteins; TSB_ total serum bilirubin.

Frequency of Abnormal Liver Function Tests in Patients with T2DM

The cut-off values for abnormally increased LFTs (AST, ALT, ALP, and STP) were determined in the control group as mean+2SD. Patients with values above the cut-off limits for these variables were considered to have elevated LFTs. However, the cut-off values for other LFTs (albumin and TSB) were determined as mean- 2SD. Patients with values less than the mean- 2SD for these variables were also considered to have abnormal LFTs. Serum ALT and AST levels- significantly different from controls- were elevated in about one third of T2DM patients, Table 2.

Table 2. Frequency of abnormal LFTs among diabetic patients.

LFT	Cut- off	Number (%) of Patients with Abnormal LFT
*AST (U/L)	32.20	13 (30.2)
*ALT (U/L)	45.76	13 (30.2)
*ALP (U/L)	107.4	11 (25.6)
*STP (g/L)	74.66	7 (16.28)
**Albumin (g/L)	40.26	8 (18.6)
**TSB (µmol/L)	10.05	16 (37.2)

*cut- off is mean+2SD; ** cut- off is mean- 2SD

Impact of Diabetes Duration on LFTs

The diabetic patients in Group (1) were sub-divided into three sub-groups based on disease duration; < 5, 5-10, and ≥10 years. Liver function tests were evaluated and compared according to the duration sub-classes. An ANOVA test determined significant differences in the mean values of AST, ALT, ALP, and TSB among diabetic patients according to diabetes duration, Table 3. The mean values of serum transaminases increased within (5-10 years) of having diabetes and declined afterward (three patients had DM for ≥20 years).

Table 3. Impact of diabetes duration on changes in LFTs. Data are expressed as mean ± SD.

	DM < 5 yrs (N=11)	DM 5-10 yrs (N=18)	DM ≥ 10 yrs (N=14)	*p-value
AST (U/L)	24.51 ± 9.9	34.16 ± 11.1	26.5 ± 7.7	< 0.001
ALT (U/L)	37.0 ± 12.2	45.67 ± 13.4	31.45 ± 10.1	< 0.001
ALP (U/L)	86.2 ± 19	84.5 ± 15.7	100.5 ± 34	< 0.001
STP (g/L)	72.8 ± 5.3	73.1 ± 2.5	73 ± 2.7	0.414
Albumin (g/L)	43.7 ± 2.6	43.5 ± 2.11	43.5 ± 2.11	0.594
TSB (µmol/L)	11.7 ± 9.4	10.9 ± 2.7	9.63 ± 1.35	< 0.001

STP: Serum total proteins; TSB: Total serum bilirubin. * Using one-way ANOVA test.

Influence of Glycemic Control on LFTs

The influence of glycemic control (as reflected by HbA1c values) on LFTs was tested. Patients were classified into two sub-groups: well- controlled (HbA1c< 7%) and poorly- controlled (HbA1c≥ 7%) (22). Serum transaminases and almost all other LFTs were significantly influenced by glycemic control, Table 4. However, the correlation between serum ALT levels and HbA1c values was not significant (R2=0.00, p= 0.891).

Table 4. Influence of glycemic control on liver function tests in diabetics. Data are expressed as mean ± SD.

	HbA1c< 7.0%	HbA1c≥ 7.0%	*p-value
AST (U/L)	28.62 ± 11.3	29.8 ± 9.13	0.013
ALT (U/L)	31.5 ± 10.9	39.2 ± 12.3	< 0.001
ALP (U/L)	90.1 ± 23.8	96.3 ± 28.5	0.027
STP (g/L)	72 ± 3.9	74 ± 4.26	0.003
Albumin (g/L)	44.9 ± 1.77	43 ± 2.1	< 0.001
TSB (µmol/L)	12.1 ± 4.1	10 ± 1.98	< 0.001

*Unpaired student's t- test was used for comparisons.

Logistic regression analysis determined which co-variables affect liver functions in Type 2 diabetic patients. Serum ALT activity was chosen as the dependent variable (since it is highly specific for liver cells and liver enzymes differ significantly between diabetics and controls, Table 1. The co-variables assigned to this test were age, sex, BMI, and HbA1c along with the duration of the disease. This test has shown that advanced age, longer duration of DM, poor glycemic control (higher HbA1c), and obesity (higher BMI) adversely affect LFTs, Table 5.

Table 5. Logistic regression analysis of co-variables' effects on serum ALT activity among T2DM patients.

	F	*p-value
Age	10.36	0.001
Sex	3.46	0.064
Duration	40.36	<0.001
BMI	142.85	<0.001
HbA1c	67.16	<0.001

Discussion

Diabetes mellitus is a systemic disease resulting in severe irreversible complications including hepatopathy. The spectrum of liver involvement in diabetes ranges from elevated liver enzymes to cirrhosis and even hepatocellular carcinoma. It may also manifest as NAFLD or acute hepatitis (23).

Uncontrolled chronic hyperglycemia and insulin resistance associated with hyperinsulinemia can precipitate hepatocellular damage by different mechanisms- mainly oxidative stress and inflammatory reactions (24). In addition, excessive fat deposition in the liver can exacerbate insulin resistance and aggravate metabolic derangements. Some patients with NAFLD develop steatohepatitis where liver inflammation and fibrosis can result in hepatic failure (25, 26).

The current study found significantly elevated serum aminotransferases among diabetic patients compared to non- diabetic controls. AST and ALT activities were elevated in about 30% of diabetic patients. In 2021, Ahamed *et al*. found a significant elevation in serum ALT activity in 30% of type 2 diabetic subjects while AST and ALP were elevated in 7 and 6% of patients respectively (27). In 2022, Noorozi-Karimabad *et al*. found that serum AST and ALT levels were higher in T2DM patients compared to non-diabetic subjects (4.38 vs 2.55% and 11.40 vs 8.17% respectively) and that increased serum aminotransferases activities, ɤ- glutamyl transferase (GGT) and ALP were associated with a significantly higher odds ratio of DM (28).

A cross- sectional study in China demonstrated that about 10 and 6.1% of diabetics had significantly elevated serum ALT and AST respectively. However, the prevalence of increased serum transaminases varied between the sexes (29). Similarly, researchers found that serum ALT was elevated in 15.9% of women and 10.9% of men respectively. This finding confirms differences between the sexes in raised liver enzymes (30). Based on logistic regression analysis, the current study found that sex did not significantly influence ALT activity, as shown in Table 5. On the contrary, some researchers found that while ALT, ALP, and GGT were significantly elevated in diabetic patients, AST was not (31). Differences in the prevalence rates of abnormally elevated serum liver enzymes in different studies worldwide may be attributed to different cut-off values, BMI and age intervals.

The current study found no significant difference between serum albumin levels in diabetic patients and healthy controls ($p=0.66$). About 19% of diabetic patients had serum albumin values below the selected cut-off. Serum albumin is one of the hepatic synthetic functions influenced by kidney function and nutritional status. A limitation of the present study is the lack of data about the socio-economic status and nutrition of the study subjects.

Patients with poor glycemic control (HbA1c>7.0%) had a significantly lower mean serum albumin value than those with good control (Table 4). In 2017, Nazki *et al*. found a negative correlation between serum albumin and HbA1c levels among 204 T2DM patients (32). The negative association between glycated hemoglobin and serum albumin levels has also been reported by other researchers (33, 34). The glycation process in diabetes promotes albumin degradation and reduces its half- life. Many long-term DM complications can be attributed to the glycation of proteins. It is also worth investigating renal functions and excluding proteinuria (including microalbuminuria) in future studies.

Despite about 16% of T2DM patients having increased serum proteins, there were no statistically significant differences in mean STPs between Groups 1 and 2. Researchers have inconsistent findings about serum proteins in DM. In 2006, Gul and Rahman found increased STPs in diabetic patients with and without retinopathy (35). Other researchers have also reported this finding (32, 36). STP's increase in DM may be due to the increase in acute phase proteins (such as alpha-1 acid glycoprotein, ceruloplasmin, C-reactive protein and alpha-1 antitrypsin) (37), fibrinogen (38, 39), and globulins (40). In other studies, however, advanced protein glycosylation and renal hyperperfusion caused STP to decrease early in the course of diabetic nephropathy (41, 42).

The difference in the mean TSB level between the diabetic and control groups was not statistically significant. However, TSB was significantly reduced with increased disease duration ($p< 0.001$). Meanwhile, diabetics with good glycemic control showed higher mean total bilirubin values than those with poor control (as reflected in HbA1c values). Erkus *et al*. have also revealed a significantly lower mean bilirubin value among poorly controlled diabetics with a significant inverse correlation with BMI, fasting blood sugar and HbA1c (43). T2DM is a disease where

a known interaction between chronic inflammation and oxidative stress is present (44). Bilirubin is a compound with natural anti-oxidant power that may protect against T2DM macrovascular and microvascular complications (45). Glycation of serum proteins which plays an important role in diabetic sequelae is; opposed by serum bilirubin to some extent (46). This finding may explain the association between reduced TSB levels and poor glycemic control; thus, it may be used as a follow- up measure.

The current study showed that glycemic status, DM duration, age, and MBI are independent determinants of increased serum ALT activity in DM (Table 5). Other researchers have also revealed the association between serum transaminases and glycemic status. Kashinakunti *et al*. have revealed that liver enzymes are raised in uncontrolled T2DM and are significantly and positively correlated with markers of glycemic control. They also concluded that serum AST of>34 U/L and ALT of >28U/L could predict NAFLD in diabetic patients (47). In this aspect, Asada *et al*. reported that good glycemic control in T1DM improved LFTs (48). The longer duration and poor control of hyperglycemia in DM have significantly impacted liver functions deterioration. These findings may be partly due to excessive formation of irreversible advanced glycation end- products (AGEs) (49). It is likely that diabetic patients will also experience increased LFTs as they age. In 2020, Islam *et al*. showed that serum AST and GGT activities were significantly associated with age in T2DM patients (50).

In the current study, gender variance did not independently influence altered liver function tests in T2DM patients. However, the logistic regression study showed that BMI independently affects serum ALT activity in T2DM. This finding may be due to increased total body fat content, altered lipid metabolism, and visceral fat accumulation. These changes can worsen insulin resistance and precipitate NFLD in T2DM (51).

In conclusion, LFTs (mainly aminotransferases) are altered in T2DM. Both ALT and AST are elevated in around one- third of the patients studied. Based on practical results, the elevation is influenced by the duration of diabetes, the patient's age, obesity, and poor glycemic control. In addition, serum albumin was significantly reduced when HbA1c was increased. Monitoring LFTs in DM regularly and always having a good glycemic status is recommended.

Limitations of the Study

Among the limitations of the current study was the small number of patients included. In addition, there was insufficient information about the nutritional status, physical activity, and socio- economic levels of T2DM patients. Exclusion was based on self- reporting and lab investigations of viral hepatitis were not performed. These are considered as our study's weaknesses. Despite these limitations, this study's major strength is that it is one of few similar local studies that have determined the frequency of disturbed liver functions in diabetes. Knowing that about one third of T2DM patients have abnormal liver functions is an alarm for those ignoring risk factors such as obesity and uncontrolled hyperglycemia.

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Conflict of Interest

This article and its publication contain no conflicts of interest.

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