**Alqalam Journal of Medical and Applied Sciences**. 2026;9(8):2448-2462
https://doi.org/10.54361/ajmas.269818

*Research Article*

# Paradoxical Hepatic-lipid Associations in Libyan Type 2 Diabetes: A Case-control Study

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**Abstract**
Prevalence of type 2 diabetes mellitus (T2DM) in Libya is high and often accompanied by the presence of metabolic dysfunction-associated steatotic liver disease (MASLD) and atherogenic dyslipidemia. This case-control study explored associations of liver biomarkers with lipid profile parameters in Libyan adults with T2DM versus non-diabetic controls. The study included 176 individuals (89 patients with T2DM and 87 age- and sex-matched controls). Levels of biochemical parameters, including ALP, ALT, AST, TB, DB, TP, Alb, TG, TC, HDL-C, and LDL-C, were measured using automated assays. Statistical methods used in this study include the Mann-Whitney U test, Spearman's correlation, MANOVA, and linear regression. The T2DM group demonstrated statistically significant decreases in ALP (p=0.030), TB (p=0.010), and DB (p=0.001) levels compared to the control group. No statistically significant difference was found for ALT, AST, TP, albumin, TG, TC, HDL C and LDL C levels. Inverse correlation was found between the following parameters: ALT with HDL C (r=−0.187, p=0.013); TB with HDL C (r=−0.153, p=0.043) and LDL C (r=−0.217, p=0.004); DB with TC (r=−0.215, p=0.004) and LDL C (r=−0.279, p<0.001); TP with HDL C (r=−0.226, p=0.003). FBS was found to be an independent predictor of ALP (B=−0.262, p=0.010), AST (B=−0.038, p=0.047) and TG (B=0.774, p=0.002). Gender had a significant impact on several parameters: males had higher ALT (p=0.032), TB (p=0.032), DB (p=0.002), and TG (p=0.015), whereas females had higher TC (p=0.039), HDL C (p=0.001) and LDL C (p=0.008). Neither age nor BMI category had a significant effect on any studied parameter (all p>0.05). Decreased levels of ALP and bilirubin in Libyan patients with T2DM and inverse correlation between liver biomarkers (ALT, bilirubin fractions, TP) and lipid parameters (HDL C, LDL C, TC) demonstrate an association of hepatic function with lipid metabolism independent of elevation of traditional liver biomarkers. Gender-specific changes underline the necessity of developing a sex-tailored monitoring strategy. Regular measurement of ALP, bilirubin, and FBS along with lipid profiles can help detect early stages of metabolic impairment in this population.
***Keywords***. Type 2 Diabetes Mellitus, Liver Biomarkers, Lipid Profile, Gender Differences.

## Introduction

Type 2 Diabetes Mellitus (T2DM) represents a serious global public health problem due to the rapid increase in its prevalence, particularly in developing countries [1]. Besides its well-known microvascular and macrovascular complications, T2DM is closely associated with two related metabolic conditions: metabolic dysfunction-associated steatotic liver disease (MASLD) and dyslipidemia, causing atherosclerosis. The coexistence of these entities synergistically amplifies the risk of atherosclerotic cardiovascular disease and accelerates hepatic deterioration [4, 5]. *Understanding the mechanistic basis of this deleterious triad requires examining the central pathophysiological driver—insulin resistance—which fundamentally mediates hepatic dysfunction and systemic lipid derangements by promoting hepatic de novo lipogenesis and disrupting free fatty acid metabolism [5,10].* Accordingly, routine liver biomarkers and lipid parameters have emerged as accessible biomarkers that may reflect underlying cardiometabolic risk, even in the absence of overt liver disease [6]. The clinical benefit of this pathophysiological framework lies in the ease of detecting these hepatic and metabolic disorders through routine blood biomarkers, which may constitute early warning signals for systemic diseases. These metabolic disorders are typically associated with elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as increased triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL-C), and decreased high-density lipoprotein cholesterol (HDL-C) [6,8].

Collectively, these biochemical changes may serve as early indicators of systemic cardiovascular and metabolic risk rather than being isolated signs of hepatocellular injury [9]. Previous studies viewed liver enzymes as systemic risk markers, which is strongly supported by a growing body of epidemiological evidence across different population groups, and have shown a strong association between liver enzyme abnormalities and changes in blood lipid levels across different populations. It has been shown that a high concentration of ALT is positively associated with triglycerides and negatively associated with high-density lipoprotein cholesterol (HDL-C) [6, 8, 11]. In addition, epidemiological evidence indicates that elevated concentrations of liver biomarkers are independently associated with an increased risk of type 2 diabetes [12]. A recent meta-analysis conducted in Africa showed the prevalence of MASLD is 48.1% among patients with type 2 diabetes [13]. The prevalence of type 2 diabetes in Libya is among the highest in North Africa, affecting approximately 16% of Libyan adults [2,3]. Previous studies conducted in Libya have shown abnormal lipid levels in

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***Received:*** *04-06-2026 -* ***Accepted:*** *02-08-2026 -* ***Published:*** *09-08-2026*

patients with type 2 diabetes, with elevated levels of LDL cholesterol, decreased levels of HDL cholesterol, and elevated triglyceride levels [14, 15]. However, published studies on the relationship between liver biomarkers and lipid profiles in Libyans are insufficient. Additionally, there is no study conducted in Libya that investigates the relationship between abnormal liver biomarkers and lipid profile disturbances while adjusting the main influencing variables such as obesity, blood sugar levels, high blood pressure, and duration of diabetes. To address this gap, we designed the present case-control study, in which we hypothesized that Libyan adults with T2DM exhibit a distinct profile of liver biomarkers—specifically lower levels of ALP and bilirubin that are inversely correlated with atherogenic lipid parameters, regardless of age, sex, and body mass index, and that these correlations are influenced by sex-specific factors. Accordingly, the primary objectives of this study were to compare liver biomarkers and lipid profiles between T2DM patients and controls, to investigate the correlation between these parameters, and to evaluate the impact of demographic and metabolic factors on these relationships.

## Methods

This comparative case-control study was conducted between September 2025 and March 2026 at two specialized diabetes centers: the Diabetes and Endocrinology Center in Al-Khums and the Diabetes and Endocrinology Center in Misrata, Libya. The study protocol was approved by the ethics committees of the Diabetes and Endocrinology Centers in Al-Khums and Al-Musallata, Libya. All procedures were followed in accordance with the ethical guidelines of the Declaration of Helsinki (2013), and informed consent was obtained from all participants before their enrollment. A total of 200 participants aged between 25 and 85 were selected for this study. Based on the exclusion criteria, 176 eligible participants remained, and they were divided into two groups: 89 patients with type 2 diabetes (43 females and 46 males) as the case group, and 87 healthy individuals as the control group (53 females and 34 males). Type 2 diabetes was diagnosed according to the American Diabetes Association (ADA) diagnostic criteria [16]. All participants with diabetes were undergoing regular clinical follow-up at the participating centers. Participants were excluded from the study if they had chronic liver disease, renal insufficiency (eGFR <60 ml/min/1.73 $m^2$), cardiovascular disease, active malignancies, thyroid disorders, familial hyperlipidemia, pregnancy or gestational diabetes, a history of alcohol abuse, or current use of hepatotoxic drugs [17]. The sample size estimate was based on previously published studies that assessed liver enzymes and lipid profile abnormalities among patients with type 2 diabetes [18,19]. Although increasing the number of participants is generally preferred for population-based studies and may lead to increased confidence in the study, 176 participants were selected, including 89 in the case group and 87 in the control group. This sample size was sufficient to detect clinically significant differences between study groups with acceptable statistical power. Subsequent statistical power analysis showed that the statistical power achieved at a significance level of 5% is approximately 80%, with the sample size calculated based on the main biochemical parameters studied. A structured questionnaire and personal interviews were employed to collect demographic and clinical data, including age, sex, medications, duration of diabetes, and family history of diabetes or hyperlipidemia. The questionnaire was pilot tested prior to the commencement of the study to ensure clarity and consistency. Body weight and height were measured using standard anthropometric measuring devices, and participants wore light clothing and were barefoot. Body mass index (BMI) was calculated according to the following equation:

BMI = Weight (kg) / Height squared (m)

According to the World Health Organization's standards, the Body Mass Index (BMI) is divided into five categories: normal weight (18.5-24.9), overweight (25.0-29.9), Class I obesity (30.0-34.9), Class II obesity (35.0-39.9), and Class III obesity (≥40.0) [20]. Individuals who were underweight, with a BMI less than 18.5, were enrolled but subsequently processed, as described in the Statistical Analysis section. After an overnight fast of 10-12 hours, a venous blood sample of approximately 10 ml was collected from each participant under aseptic conditions using sterile, single-use instruments. Blood samples were distributed into two vacuum tubes: one tube containing fluoride-oxalate for measuring fasting blood glucose (FBG), and a plain tube for serum separation and measuring of other biochemical study variables. The samples were allowed to clot at room temperature, then centrifuged at 3000 rpm for 10-15 minutes. Serum and plasma samples were processed and stored according to standard laboratory procedures prior to biochemical analysis [21,22]. All biochemical parameters were analyzed using an automated chemical analyzer (Cobas 6000, Roche Diagnostics) in the central laboratory of Al-Khums Diabetes Hospital. Commercial enzyme test kits (Roche Diagnostics) were used according to the manufacturer's instructions, and internal quality control procedures were performed daily. The biochemical study variables were assessed, specifically fasting blood sugar, as well as liver biomarkers including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (T.B), direct bilirubin (D.B), total protein (T.P), and serum albumin (S.Alb). In addition, the lipid profiles were evaluated, which included total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).

### *Statistical analysis*

Statistical analyses were performed using IBM SPSS version 27.0. The normality of the data was assessed using the Shapiro-Wilk test, and the homogeneity of the variances was evaluated using the Levene test.

Continuous variables were presented as median (IQR) or mean±SD, as appropriate. Categorical variables were summarized as frequencies and percentages. Comparisons between T2DM patients and control groups were performed

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***Received:*** *04-06-2026 -* ***Accepted:*** *02-08-2026 -* ***Published:*** *09-08-2026*

using the Mann–Whitney U test for non-normally distributed variables and the independent t-test for normally distributed variables. The chi-square test was used to assess the correlations between categorical variables, while Spearman's rank correlation coefficient was used to assess correlations between liver enzymes and lipid parameters. Regarding the categories of BMI, the participants have been classified according to the World Health Organization standards into five groups: normal weight (18.5-24.9), overweight (25.0-29.9), Class I obesity (30.0-34.9), Class II obesity (35.0-39.9), and Class III obesity (≥40.0). One case of underweight, with a BMI less than 18.5 was excluded (n=1). To assess the effect of the remaining five BMI categories on biochemical parameters, a one-way multivariate analysis of variance (MANOVA) was performed using Pillai's Trace test. Despite Box's M violation ($p<0.001$), the MANOVA was considered robust given the non-significant results and group sizes (13-58). This was followed by a one-way analysis of variance (ANOVA) with Tukey's post-hoc HSD test. To examine the association between FBS levels and biochemical parameters, multiple linear regression analysis was performed with FBS levels considered as a continuous variable. To assess the combined effects of age and sex separately on liver and lipid parameters, one way MANOVA (Pillai's Trace) was used, followed by univariate analyses of variance for each parameter.

## Results

### *Distribution Characteristics and Normality Testing of Study Variables*

An assessment of the data distribution showed that most of the continuous variables, including BMI, FBS level, liver biomarkers, and lipid profile parameters, were non-normally distributed in at least one study group. In contrast, LDL-C levels showed a normal distribution across both groups. Q-Q plots and box plots (Figure 1) showed skewness and outliers in most variables, supporting the results of formal normality testing.

### *Basic characteristics of study participants*

### *Demographic and anthropometric characteristics of study participants*

The gender distribution was not statistically different between the control and patient groups ($\chi^2$= 2.819, df= 1, p=0.093). Similarly, the BMI categories did not differ significantly between the two groups ($\chi^2$ = 6.865, df = 4, p = 0.231), with comparable distributions across the normal weight, overweight, and Class I-III obesity categories. Regarding the continuous variables, age was normally distributed in both groups and was significantly higher in the patient group (58.18 ± 12.05 years) compared to the control group (50.22 ± 13.34 years; t = -4.11, p < 0.001), while BMI as a continuous variable was not normally distributed and did not show a significant difference between the groups (U = 3742, p = 0.678). Overall, the study groups were comparable in terms of sex and BMI categories, but differed significantly in age (Table 1).

***Table 1. Baseline Demographic and Clinical Characteristics of Study Participants***

| Variable | Control Group (n = 87) | Patient Group (n = 89) | Test statistic | p-value |
|---|---|---|---|---|
| Age (years), mean ± SD | 50.22 ± 13.34 | 58.18 ± 12.05 | t = -4.11 | <0.001 |
| Gender, n (%) | | | $\chi^2$ = 2.819 (df=1) | 0.093 |
| Male | 34 (39.1%) | 46 (51.7%) | | |
| Female | 53 (60.9%) | 43 (48.3%) | | |
| BMI (kg/m$^2$), median (IQR) | 27.5 (24.1–31.2) | 27.2 (23.8–30.9) | U = 3742 | 0.678 |
| BMI categories, n (%) | | | $\chi^2$ = 6.865 (df=4) | 0.231 |
| - Normal weight (18.5–24.9) | 15 (17.2%) | 23 (25.8%) | | |
| - Overweight (25.0–29.9) | 30 (34.5%) | 28 (31.5%) | | |
| - Obesity class I (30.0–34.9) | 21 (24.1%) | 24 (27.0%) | | |
| - Obesity class II (35.0–39.9) | 15 (17.2%) | 6 (6.7%) | | |
| - Obesity class III (≥40.0) | 6 (6.9%) | 7 (7.9%) | | |

*Data are presented as mean ± SD for normally distributed variables (Age) and median (IQR) for non-normally distributed variables (BMI). Independent t-test was used for Age; Mann-Whitney U test for BMI continuous; $\chi^2$ = Chi-square test for categorical variables. percentages represent column percentages within each group. BMI, body mass index.*

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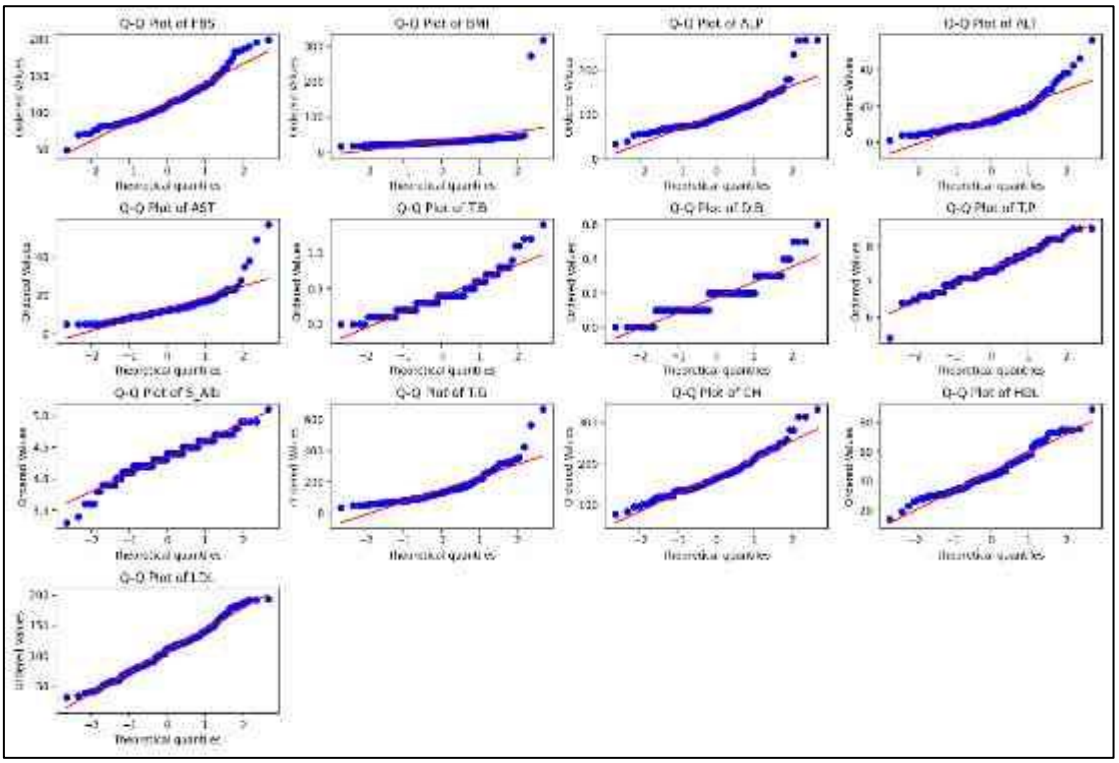


***Figure 1. Q-Q plots and box plots of study variables in control and patient groups.***

***Comparison of Biochemical Parameters Between Study Groups***

Appropriate statistical tests were selected based on the data distribution. Most variables were non-normally distributed and were analyzed using the Mann-Whitney U test, while the LDL-C was normally distributed, it was assessed using the independent samples t-test.

Most of the variables were abnormally distributed and were analyzed using the Mann-Whitney U test, while LDL-C was normally distributed and was assessed using the independent samples t-test. The Mann-Whitney U test showed statistically significant differences between the two study groups (patients and controls) for three of the ten non-parametric variables: alkaline phosphatase (ALP), total bilirubin (TB), and direct bilirubin (DB). For all three of these non-parametric variables, the median values were lower in the patient group, with p-values of 0.030, 0.010, and 0.001, respectively (Table 2). The distribution of these three liver biomarkers across the study groups is illustrated in (Figure 2).

The remaining non-parametric variables (ALT, AST, TP, Alb, TG, TC, and HDL-C) did not differ significantly between the two study groups (all p-values > 0.05; Table 2). Similarly, the LDL-C level as the parametric variable was evaluated using the independent samples t-test. The results showed that the LDL-C level was comparable between the control group (109.92 ± 36.62 mg/dL) and the patient group (106.47 ± 33.28 mg/dL), with no statistically significant difference between these groups (p = 0.514; Table 3).

***Table 2. Non-parametric Comparison of Biochemical Parameters Between Control and Patient Groups (Mann–Whitney U test)***

| Variable | Control (Median) | Patient (Median) | p-value |
|---|---|---|---|
| ALP (U/L) | 105.37 | 94.52 | 0.030 |
| ALT (U/L) | 12.93 | 14.79 | 0.200 |
| AST (U/L) | 13.85 | 12.67 | 0.120 |
| TB (mg/dL) | 0.43 | 0.35 | 0.010 |
| DB (mg/dL) | 0.20 | 0.15 | 0.001 |
| TP (g/dL) | 7.30 | 7.41 | 0.110 |
| Alb (g/dL) | 4.34 | 4.34 | 0.790 |
| TG (mg/dL) | 142.66 | 160.65 | 0.060 |
| TC (mg/dL) | 172.55 | 167.40 | 0.700 |
| HDL-C (mg/dL) | 47.17 | 45.04 | 0.210 |
| ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; DB, direct bilirubin; | | | |

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TP, total protein; Alb, albumin; TG, triglycerides; TC, cholesterol; HDL-C, high-density lipoprotein

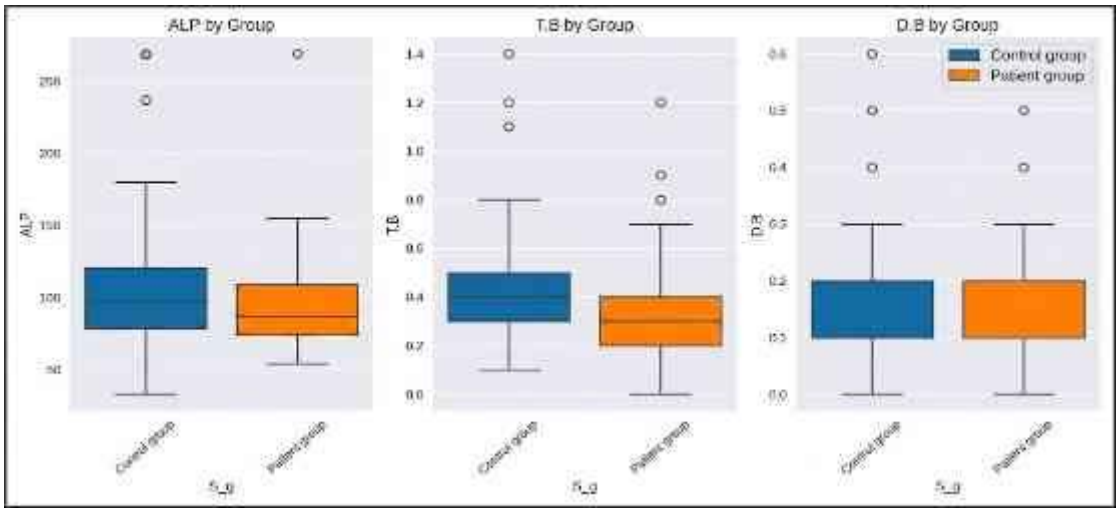


***Figure 2. Comparison of ALP, TB, and DB between study groups***

***Table 3. Parametric Comparison of LDL-C Between Control and Patient Groups (Independent Samples t-test)***

| Variable | Control (Mean ± SD) | Patient (Mean ± SD) | p-value |
|---|---|---|---|
| LDL-C (mg/dL) | 109.92 ± 36.62 | 106.47 ± 33.28 | 0.514 |
| Abbreviations: SD, standard deviation; LDL-C, low-density lipoprotein cholesterol. | | | |

***Correlation between Liver Biomarkers and Lipid Profile Parameters***

Spearman's correlation analysis was performed to assess the association between liver biomarkers and lipid profile parameters in the study population (n = 176). Statistically significant inverse correlations were observed between ALT enzyme and HDL-C (r = −0.187, p = 0.013). TB showed negative correlations with both HDL-C (r = −0.153, p = 0.043) and LDL-C (r = −0.217, p = 0.004), while DB was inversely correlated with both TC (r = −0.215, p = 0.004) and LDL-C (r = −0.279, p < 0.001). TP also showed a negative correlation with HDL-C (r = −0.226, p = 0.003). No statistically significant correlations were identified between ALP, AST, Alb and any of the lipid profile parameters (Table 4).

***Table 4. Correlations Between Hepatic Biomarkers and Lipid Profile Parameters***

| Liver Enzyme | Lipid Parameter | r | p-value |
|---|---|---|---|
| ALP (U/L) | TG (mg/dL) | 0.057 | 0.452 |
| ALP (U/L) | TC (mg/dL) | 0.006 | 0.938 |
| ALP (U/L) | HDL-C (mg/dL) | 0.007 | 0.924 |
| ALP (U/L) | LDL-C (mg/dl) | -0.024 | 0.751 |
| ALT (U/L) | TG (mg/dL) | 0.098 | 0.194 |
| ALT (U/L) | TC (mg/dL) | 0.027 | 0.721 |
| ALT (U/L) | HDL-C (mg/dL) | -0.187 | 0.013 |
| ALT (U/L) | LDL-C (mg/dl) | 0.106 | 0.162 |
| AST (U/L) | TG (mg/dL) | 0.003 | 0.970 |
| AST (U/L) | TC (mg/dL) | 0.073 | 0.336 |
| AST (U/L) | HDL-C (mg/dL) | -0.077 | 0.313 |
| AST (U/L) | LDL-C (MG/DL) | 0.102 | 0.180 |
| TB (mg/dL) | TG (mg/dL) | 0.045 | 0.552 |
| TB (mg/dL) | TC (mg/dL) | -0.136 | 0.072 |
| TB (mg/dL) | HDL-C (mg/dL) | -0.153 | 0.043 |
| TB (mg/dL) | LDL-C (mg/dl) | -0.217 | 0.004 |
| DB (mg/dL) | TG (mg/dL) | -0.044 | 0.558 |
| DB (mg/dL) | TC (mg/dL) | -0.215 | 0.004 |
| DB (mg/dL) | HDL-C (mg/dL) | -0.036 | 0.638 |
| DB (mg/dL) | LDL-C (mg/dl) | -0.279 | <0.001 |
| TP (g/dL) | TG (mg/dL) | 0.035 | 0.641 |

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| | | | |
|---|---|---|---|
| TP (g/dL) | TC (mg/dL) | 0.106 | 0.163 |
| TP (g/dL) | HDL-C (mg/dL) | -0.226 | 0.003 |
| TP (g/dL) | LDL-C (mg/dl) | 0.094 | 0.213 |
| Alb (g/dL) | TG (mg/dL) | 0.016 | 0.832 |
| Alb (g/dL) | TC (mg/dL) | 0.060 | 0.426 |
| Alb (g/dL) | HDL-C (mg/dL) | -0.132 | 0.081 |
| Alb (g/dL) | LDL-C (mg/dl) | 0.055 | 0.471 |

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; DB, direct bilirubin; TP, total protein; Alb, albumin; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
r, Pearson correlation coefficient. Significant correlations ($p < 0.05$) are indicated in bold.

***Associations of Clinical Factors (FBS and BMI Categories) with Biochemical Parameters***

To investigate the association between two clinical factors- FBS as a continuous variable and BMI categories (normal weight, overweight, Class I obesity, Class II obesity, and Class III obesity)—with biochemical parameters (liver biomarkers and lipid profile), two separate analytical approaches were used. Univariate linear regression analyses were performed to study the association between FBS and each biochemical parameter individually. Separately, MANOVA followed by univariate ANOVA was performed to assess the overall effect of BMI categories on the combined biochemical parameters.

***Associations of FBS and BMI with Liver Biomarkers***

Univariate linear regression analyses were conducted to examine the correlations of FBS with liver biomarkers (ALP, ALT, AST, TB, DB, Alb). FBS level was statistically significantly associated with ALP and AST levels (B = -0.262, p = 0.010 and B = -0.037, p = 0.047, respectively). The effect sizes ($R^2$) for these correlations were 0.038 for ALP and 0.022 for AST, indicating small effects. No statistically significant associations were observed with the remaining liver biomarkers (all p values > 0.05) (Table 5).

***Table 5. Univariate Linear Regression Analysis of the Associations of FBS with Liver Biomarkers***

| Independent Variable | Dependent Variable (Liver Biomarkers) | B | t | p-value | $R^2$ |
|---|---|---|---|---|---|
| FBS | ALP (U/L) | -0.262 | -2.603 | 0.010 | 0.037 |
| | ALT (U/L) | 0.040 | 1.716 | 0.088 | 0.017 |
| | AST (U/L) | -0.038 | -1.998 | 0.047 | 0.022 |
| | TB (mg/dL) | -0.001 | -1.213 | 0.227 | 0.008 |
| | DB (mg/dL) | 0.000 | -1.108 | 0.269 | 0.007 |
| | TP (g/dL) | 0.001 | 0.861 | 0.391 | 0.004 |
| | Alb (g/dL) | -0.00005 | -0.061 | 0.951 | 0.000 |

Abbreviations: FBS, fasting blood sugar; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; DB, direct bilirubin; TP, total protein; Alb, albumin.
B, unstandardized regression coefficient; t, t-statistic. Significant associations ($p < 0.05$) are indicated in bold.
*Note: B is unstandardized regression coefficient. $R^2$ represents the proportion of variance explained by FBS.*

To examine the effect of BMI categories on liver biomarkers (ALP, ALT, AST, TB, DB, TP, Alb), a one-way MANOVA was conducted after excluding one case of underweight (n = 1) due to insufficient sample size. The result of the one-way MANOVA test showed no statistically significant overall effect of the BMI categories (Pillai's Trace = 0.179, p = 0.935). Subsequent univariate ANOVA also showed no statistically significant differences between BMI categories and any of the liver biomarkers (all p values > 0.05). The effect sizes ($\eta^2$ partial) were small for all parameters (ranging from 0.006 to 0.028). Descriptive statistics and results of ANOVA are presented in (Table 6) with standardized units.

***Table 6. Univariate ANOVA Results for the Effect of BMI Categories on Liver Biomarkers***

| Biomarker / | BMI Categories | F (df=4,170) | p-value | Partial $\eta^2$ |
|---|---|---|---|---|

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**Received**: 04-06-2026 - **Accepted**: 02-08-2026 - **Published**: 09-08-2026

| Parameter | Normal weight (n=38) mean ± SD | Overweight (n=58) mean ± SD | Obesity I (n=45) mean ± SD | Obesity II (n=21) mean ± SD | Obesity III (n=13) mean ± SD | | | |
|---|---|---|---|---|---|---|---|---|
| ALP (U/L)[2] | 94.84 ± 29.26 | 105.52 ± 41.22 | 96.67 ± 36.11 | 108.33 ± 38.77 | 88.69 ± 20.09 | 1.231 | 0.299 | 0.028 |
| ALT (U/L)[2] | 13.97 ± 10.96 | 14.21 ± 7.27 | 14.27 ± 7.94 | 12.05 ± 7.53 | 14.08 ± 5.95 | 0.304 | 0.875 | 0.007 |
| AST (U/L)[2] | 12.71 ± 7.16 | 14.22 ± 7.29 | 12.80 ± 4.28 | 13.52 ± 9.29 | 11.92 ± 4.48 | 0.549 | 0.700 | 0.013 |
| TB (mg/dL)[3] | 0.405 ± 0.244 | 0.371 ± 0.193 | 0.424 ± 0.240 | 0.376 ± 0.221 | 0.392 ± 0.350 | 0.387 | 0.818 | 0.009 |
| DB (mg/dL)[3] | 0.184 ± 0.092 | 0.164 ± 0.087 | 0.184 ± 0.102 | 0.162 ± 0.087 | 0.192 ± 0.150 | 0.576 | 0.680 | 0.013 |
| TP (g/dL)[4] | 7.374 ± 0.395 | 7.364 ± 0.494 | 7.402 ± 0.443 | 7.310 ± 0.385 | 7.269 ± 0.795 | 0.271 | 0.896 | 0.006 |
| Alb (g/dL)[4] | 4.347 ± 0.253 | 4.362 ± 0.255 | 4.342 ± 0.289 | 4.252 ± 0.284 | 4.331 ± 0.386 | 0.621 | 0.648 | 0.014 |

Abbreviations: BMI, body mass index; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; DB, direct bilirubin; TP, total protein; Alb, albumin.
Note: Partial $\eta^2$ (effect size) was calculated from the Type III sum of squares (provided by SPSS). All effect sizes are small ($\eta^2 < 0.06$). No significant associations were observed (all $p > 0.05$).

***Associations of FBS and BMI Categories with Lipid Parameters***

To assess the relationship between FBS and each of the lipid parameters individually, univariate linear regression analyses were performed. The result showed that FBS was a significant predictor of TG levels ($p = 0.002$) ; however, no other significant associations were observed (Table 7; Figure 3).

***Table 7. Table 7. Univariate Linear Regression Analysis of the Associations of FBS with Lipid Parameters***

| Independent Variable | Dependent Variable | B | t-value | p-value |
|---|---|---|---|---|
| FBS | TG (mg/dL) | 0.774 | 3.152 | **0.002** |
| | TC (mg/dL) | 0.022 | 0.179 | 0.858 |
| | HDL-C (mg/dL) | -0.063 | -1.695 | 0.092 |
| | LDL-C (mg/dL) | -0.002 | -0.017 | 0.986 |

*Abbreviations: TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.*

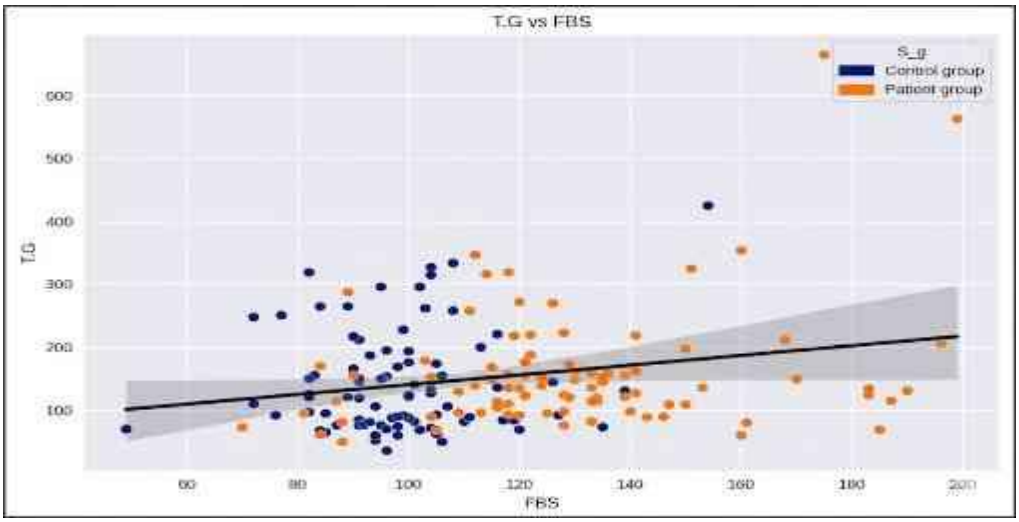


***Figure 3. Linear regression plots illustrating the relationship between FBS and TG levels in control and patient groups.***

To examine the overall effect of BMI categories separately on combined lipid profiles (TG, TC, HDL-C, and LDL-C), a one-way MANOVA was performed, and the Pillai's Trace effect test result showed no statistically significant multivariate effect

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***Received:*** *04-06-2026 -* ***Accepted:*** *02-08-2026 -* ***Published:*** *09-08-2026*

(Pillai's Trace = 0.179, F = 0.693, p = 0.935). Although the result of the Pillai's Trace test was not significant, univariate ANOVAs were performed for each lipid parameter as follow-up tests, which also showed no statistically significant differences between BMI categories (all p-values > 0.05), as presented in (Table 8). The effect sizes (partial $\eta^2$) were small, ranging from 0.016 to 0.050.

***The association between demographic factors (gender and age) and biochemical parameters***

The effect of demographic factors (gender and age) on the combined biochemical parameters (liver biomarkers and lipid profiles) was assessed using MANOVA and follow-up univariate ANOVA analysis.

***Multivariate analysis of age and gender***

The result of MANOVA (using Pillai's Trace) showed a statistically significant overall effect of gender on biochemical parameters ($p < 0.001$) with a large effect size (partial $\eta^2 = 0.247$), indicating that gender explains approximately 24.7% of the variance in the combined dependent variables. In contrast, age did not show any statistically significant multivariate effect ($p = 0.646$; partial $\eta^2 = 0.051$), as shown in (Table 9).

***Table 8. One-way ANOVA for the Effect of BMI Categories on Lipid Parameters***

| Parameter | BMI Categories | | | | | F (df=4,170) | p-value | Partial $\eta^2$ |
|---|---|---|---|---|---|---|---|---|
| | Normal weight (n=38) mean ± SD | Overweight (n=58) mean ± SD | Obesity I (n=45) mean ± SD | Obesity II (n=21) mean ± SD | Obesity III (n=13) mean ± SD | | | |
| TG (mg/dL) | 156.79 ± 110.78 | 145.41 ± 76.38 | 161.44 ± 99.94 | 161.90 ± 73.82 | 120.15 ± 37.03 | 0.712 | 0.585 | 0.016 |
| TC (mg/dL) | 158.95 ± 32.64 | 168.00 ± 43.07 | 168.60 ± 44.09 | 192.90 ± 54.93 | 174.46 ± 40.28 | 2.220 | 0.069 | 0.050 |
| HDL-C (mg/dL) | 47.42 ± 14.01 | 45.26 ± 13.20 | 43.64 ± 11.51 | 49.86 ± 15.92 | 47.46 ± 10.68 | 1.008 | 0.405 | 0.023 |
| LDL-C (mg/dL) | 99.58 ± 31.45 | 107.71 ± 34.80 | 109.36 ± 33.73 | 120.33 ± 40.70 | 107.38 ± 35.75 | 1.244 | 0.294 | 0.028 |

Abbreviations: TG, triglycerides; TC, total cholesterol; HDL□C, high□density lipoprotein cholesterol; LDL□C, low□density lipoprotein cholesterol; BMI, body mass index categories (WHO, kg/m$^2$): normal (18.5–24.9), overweight (25.0–29.9), obesity class I–III (30.0–34.9, 35.0–39.9, ≥40.0). One underweight case excluded. One□way ANOVA: no significant differences (all p > 0.05).

Note: Partial $\eta^2$ (effect size) derived from SPSS R-squared values. All effect sizes are small to medium ($\eta^2 < 0.06$). No significant differences were observed (all p > 0.05).

***Table 9. MANOVA of the effects of age and gender on the biochemical study variables***

| Effect | Test | Value | F | df (H) | df (E) | p-value | Partial $\eta^2$ |
|---|---|---|---|---|---|---|---|
| Age | Pillai's Trace | 0.051(5.1%) | 0.794 | 11 | 163 | 0.646 | 0.051 |
| Gender | Pillai's Trace | 0.247(24.7%) | 4.874 | 11 | 163 | <0.001 | 0.247 |

MANOVA (Pillai's Trace). p < 0.05 in bold. df(H), hypothesis degrees of freedom; df(E), error degrees of freedom; $\eta^2$, partial eta squared.

***Association between age, gender, and liver biomarkers.***

Univariate ANOVA was conducted to examine the independent effects of age and gender on liver biomarkers (ALT, AST, TB, DB, and TP), and the analysis result showed significant effects of gender on ALT (p=0.032, partial $\eta^2$=0.026), TB (p=0.032, $\eta^2$=0.026), and DB (p=0.002, $\eta^2$=0.056), with levels being significantly higher in males than females. For AST and TP, no statistically significant effects were observed, although there was a slight tendency towards this at a statistical significance level of α=0.05 (p=0.056 for AST and p=0.053 for total protein). Age had no significant effect on any of the measured liver biomarkers (p> 0.05 for all). Effect sizes (partial $\eta^2$) were small for age (ranging from 0.000 to 0.010), while gender showed small to medium effect sizes (partial $\eta^2$ from 0.021 to 0.056) as presented in (Table 10).

***Association between age, gender, and lipid profiles.***

Univariate ANOVA was used to examine the effects of age and gender on lipid profile parameters. The result of Univariate ANOVA showed that gender had a significant main effect on all lipid parameters. Males had significantly higher TG levels ($p = 0.015$, partial $\eta^2 = 0.034$), whereas females had higher TC ($p = 0.039$, $\eta^2 = 0.024$), HDL-C ($p = 0.001$, $\eta^2 = 0.064$), and LDL-C ($p = 0.008$, $\eta^2 = 0.040$), as shown in (Table 11). Age showed no significant effect on any parameter ($p > 0.05$ for all), with small effect sizes (partial $\eta^2$ ranging from 0.000 to 0.006).

**Table 10. Univariate ANOVA Results for the Effects of Age and Gender on Liver Biomarkers**

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***Received:*** *04-06-2026 -* ***Accepted:*** *02-08-2026 -* ***Published:*** *09-08-2026*

| Dependent Variable | Source | Type III SS | df | MS | F | p-value | Partial $\eta^2$ | Direction (Gender Effect) |
|---|---|---|---|---|---|---|---|---|
| ALT (U/L) | Age | 85.404 | 1 | 85.404 | 1.279 | 0.260 | 0.007 | — |
| | Gender | 310.283 | 1 | 310.283 | 4.648 | 0.032 | 0.026 | Male > Female |
| AST (U/L) | Age | 3.409 | 1 | 3.409 | 0.077 | 0.782 | 0.000 | — |
| | Gender | 163.011 | 1 | 163.011 | 3.687 | 0.056 | 0.021 | Male > Female (n.s.) |
| TB (mg/dL) | Age | 0.089 | 1 | 0.089 | 1.703 | 0.194 | 0.010 | — |
| | Gender | 0.244 | 1 | 0.244 | 4.688 | 0.032 | 0.026 | Male > Female |
| DB (mg/dL) | Age | 0.006 | 1 | 0.006 | 0.721 | 0.397 | 0.004 | — |
| | Gender | 0.091 | 1 | 0.091 | 10.253 | 0.002 | 0.056 | Male > Female |
| TP (g/dL) | Age | 3.108e-6 | 1 | <0.001 | 0.000 | 0.997 | 0.000 | — |
| | Gender | 0.844 | 1 | 0.844 | 3.809 | 0.053 | 0.022 | Male > Female (p=0.053) |
| *Notes: Significant p-values (<0.05) are shown in bold. n.s., non-significant.* | | | | | | | | |

**Table 11. Univariate ANOVA Results for the Effects of Age and Gender on Lipid Profile**

| Dependent Variable | Source | Type III SS | df | MS | F | p-value | Partial $\eta^2$ | Direction (Gender Effect) |
|---|---|---|---|---|---|---|---|---|
| TG (mg/dL) | Age | 4937.29 | 1 | 4937.29 | 0.647 | 0.422 | 0.004 | — |
| | Gender | 46419.29 | 1 | 46419.29 | 6.080 | 0.015 | 0.034 | Male > Female |
| TC (mg/dL) | Age | 2086.75 | 1 | 2086.75 | 1.127 | 0.290 | 0.006 | — |
| | Gender | 8025.96 | 1 | 8025.96 | 4.335 | 0.039 | 0.024 | Female > Male |
| HDL-C (mg/dL) | Age | 0.663 | 1 | 0.663 | 0.004 | 0.949 | 0.000 | — |
| | Gender | 1930.53 | 1 | 1930.53 | 11.788 | 0.001 | 0.064 | Female > Male |
| LDL-C (mg/dL) | Age | 452.09 | 1 | 452.09 | 0.382 | 0.537 | 0.002 | — |
| | Gender | 8593.53 | 1 | 8593.53 | 7.265 | 0.008 | 0.040 | Female > Male |
| *Notes:* Significant p-values (<0.05) are shown in **bold**. Partial $\eta^2$ indicates effect size (small = 0.01, medium = 0.06, large = 0.14). Direction of gender effect was derived from estimated marginal means. | | | | | | | | |

## Discussion

In this Libyan case-control study of adults with type 2 diabetes mellitus (T2DM) and non-diabetic controls, significantly lower levels of ALP, TB, and DB were observed among individuals with T2DM compared to controls (p = 0.030, p = 0.010, and p = 0.001,respectively). Several significant negative correlations between liver-related biomarkers and lipids were observed: ALT with HDL-C (r = -0.187, p = 0.013); TB with HDL-C (r = -0.153, p = 0.043) and LDL-C (r = -0.217, p = 0.004); DB with TC (r = -0.215, p = 0.004) and LDL-C (r = -0.279, p < 0.001); and TP with HDL-C (r = -0.226, p = 0.003). Gender significantly influenced multiple biochemical parameters, with males showing higher ALT, TB, DB, and TG, while females exhibited higher HDL-C, TC, and LDL-C. Age was not significantly associated with any measured parameter. FBS showed a significant association with ALP (B = -0.262, p = 0.010), AST (B = -0.038, p = 0.047), and TG (B = 0.774, p = 0.002). BMI categories did not significantly affect any liver biomarkers or lipid parameters (all p > 0.05).

### *Interpretation of observed statistical relationships*

It is noteworthy that the levels of ALP, TB, and DB are significantly lower in patients with type 2 diabetes compared to control groups. Although previous studies often describe the rise of ALPamong diabetic subjects as an indicator of steatohepatitis or cholestasis [23, 24], the low ALPlevels in our study may have several possible explanations. Firstly, statin or another lipid-lowering therapy, not systematically measured in this study, could play a role. Secondly, Libya's specific dietary habits, particularly the presence of Mediterranean dietary patterns, might influence these parameters differently. The negative correlation between liver-related biomarkers (ALT, TB , DB, TP) and lipids (HDL-C, LDL-C, TC) observed among the studied population suggests a link between liver function and lipid metabolism in this Libyan cohort. Our data revealed a negative correlation between DB and LDL-C (r = -0.279, p < 0.001), representing the strongest association observed in this study. This finding may reflect the antioxidant capacity of bilirubin, which can inhibit lipid peroxidation and reduce the formation of atherogenic oxidized LDL particles that cause atherosclerosis, thus acting as an inhibitor of atherosclerosis [25, 26]. Moreover, the negative correlation between ALT and HDL-C (r = -0.187, p = 0.013) indicates that even mild hepatocyte damage may be followed by an unfavorable lipid profile, which may be caused by impaired apolipoprotein A-I synthesis or altered HDL maturation in the state of hepatic insulin resistance. [27]. In addition, TP has a negative correlation with HDL-C (r = -0.226, p = 0.003), which may indicate inflammatory processes

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***Received:*** *04-06-2026 -* ***Accepted:*** *02-08-2026 -* ***Published:*** *09-08-2026*

associated with diabetes, as acute phase proteins can alter the protein profile while HDL metabolism is also affected by inflammation [25].

***Comparison with Libyan studies***

The present finding of no significant differences in LDL-C and TC levels among the studied groups is consistent with some but not all Libyan studies. Ahmida et al. [28] described a high prevalence of dyslipidemia in 90% of T2DM patients from Benghazi, with extremely high TC, TG, and LDL-C levels. The discrepancy between our findings and those of Ahmida et al. may be explained by differences in geographic location, dietary habits, or the potential use of lipid‐lowering medications, such as statins, in our study groups, whose data were not systematically collected. The study by Rhayem and Ramah reported a dyslipidemia prevalence of 47.7% in T2DM patients, with elevated LDL-C being the most common abnormality [14]. In our study, although there were no significant differences in LDL-C levels between the study groups, a negative correlation was found between DB and LDL-C, which aligns with the concept that altered lipid metabolism is closely linked to liver function [25]. Ashawesh et al. [15] found higher TG levels in diabetics and lower HDL-C in females, which coincides with our gender-specific findings: elevated TG in males and elevated HDL-C in females.

***Comparison with regional studies***

The absence of significant ALT or AST elevation in T2DM patients in the present study differs from reports from Yemen [29, 30], Iraq [23, 31], and Bangladesh [32] that described significantly higher ALT levels in diabetic patients. However, a study from Mosul, Iraq [30] noted that only 30% of T2DM patients had elevated ALT/AST and that glycaemic control (HbA1c) was a critical factor, a finding that partially agrees with our observation that FBS was significantly associated with AST levels across the study population ($p = 0.047$). A Basrah study using FibroScan [31] showed that most T2DM patients had advanced steatosis (grade III) but low-grade fibrosis, thus suggesting that liver enzyme elevations are not sensitive markers of early non-alcoholic fatty liver disease; this concept supports our finding of normal ALT/AST despite metabolic disturbance. Regarding ALP, our finding of significantly lower ALP in T2DM patients contrasts with studies from Iraq [23] and India [24] that reported elevated ALP in diabetic patients. Consistent with these reports, a recent Iraqi study by Alwan [33] also found significantly higher ALP levels in T2DM patients compared to non-diabetic controls (434.99 ± 33.10 vs. 295.07 ± 35.87 U/L in males, $p < 0.05$), with ALP being notably higher in diabetic males than females. This discrepancy may be attributed to differences in medication use (particularly statins), dietary patterns, or the stage of liver disease in the study populations [34].

***Comparison with international studies***

An Italian study in obese non-diabetic subjects followed for 14 years [35] found that elevated ALT was an independent predictor of incident T2DM; the negative correlation between ALT and HDL-C in our cross-sectional study may reflect the same axis of insulin resistance and fat accumulation in the liver. An Indian study [27] indicated strong positive correlations between ALT, AST and lipid parameters (TC, TG, LDL-C) in T2DM patients, with an area under the ROC curve for ALT predicting NAFLD of 0.86. Our findings differ (weak to moderate negative correlations), possibly due to population differences, medication use, or sample size. An African meta-analysis [13] reported a pooled prevalence of metabolic dysfunction associated fatty liver disease (MAFLD) in T2DM of 48.1% and a weak positive correlation between MAFLD and HbA1c. Our observations of altered ALP and bilirubin in T2DM patients are suggestive of potential hepatic involvement, consistent with the high burden of MAFLD reported in African diabetic populations [13]. A US study in NAFLD patients [7] showed that non-traditional lipid parameters (plasma atherosclerosis index, residual cholesterol, and non-HDL-C) were superior to traditional lipids in predicting T2DM and prediabetes. Although our study did not account for these composite indices, the observed negative correlations between bilirubin and LDL-C suggest that bilirubin, an endogenous antioxidant with anti-atherosclerotic properties, could be used as a simple and unconventional biomarker for lipid-related risks in type 2 diabetes [25, 36]. However, prospective studies are needed to confirm its predictive value. A Saudi study [37] found that ALP levels were higher in patients with uncontrolled type 2 diabetes, and paradoxically, LDL-C levels were lower in the subgroup of uncontrolled diabetes. This latter finding differs from our observation that there was no significant difference in LDL-C levels between T2DM patients and controls. The discrepancy may be partly explained by differences in medication use (such as statins) among the study groups, although medication data were not systematically collected in our study. Notably, previous Libyan studies have reported a high prevalence of dyslipidaemia in T2DM patients [28, 14], which contrasts with our findings. A Bangladeshi study [8] reported independent associations between GGT and all lipid components, with 61% of dyslipidaemic participants having elevated liver enzymes. Although GGT was not measured in our study, the observed correlations between ALT, bilirubin, and lipids suggest a similar link between liver function and lipid metabolism. A large prospective Taiwanese study by Chen et al. [38] demonstrated that elevated ALP is an independent risk factor for incident type 2 diabetes (hazard ratios: 1.37 in men, 1.44 in women), contrasting with our cross-sectional observation of lower ALP in prevalent T2DM. This discrepancy may reflect differences in study design (incidence vs. prevalence) and the potential effects of unrecorded antidiabetic or lipid-lowering medications on ALP levels. A large European cohort study (the MARK-AGE study) by Schoissengeier et al. [39] demonstrated that higher serum unconjugated bilirubin concentrations were significantly associated with lower BMI, lower HbA1c, lower insulin, lower TG, lower TC, lower LDL-C, and lower C-reactive protein, as well as higher HDL-C. These findings from 2,489 participants

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***Received:*** *04-06-2026 -* ***Accepted:*** *02-08-2026 -* ***Published:*** *09-08-2026*

strongly support the inverse relationship between bilirubin levels and atherogenic lipid profiles, consistent with our observed negative correlations between bilirubin and LDL-C. Furthermore, a Czech case-control study by Jiraskova et al. [40] confirmed that patients with T2DM have significantly lower serum bilirubin concentrations compared to healthy controls (9.2 vs. 12.1 μmol/L, $p < 0.001$), with each μmol/L increase in bilirubin associated with an 11% reduction in T2DM risk. These international findings corroborate our observation of lower bilirubin levels in Libyan T2DM patients and support the potential protective role of bilirubin against metabolic dysfunction.

***Explanation of discrepancies***

The present study revealed no significant differences in LDL-C or TC between T2DM patients and controls, a result that deviated from the established literature. This divergence drew particular attention to undocumented lipid-lowering therapy; specifically, the potential for unreported statin use within the diabetic group offered a plausible context for the blunted lipid elevations otherwise expected in type 2 diabetes. However, data on medication use were not systematically collected in the current study; therefore, the extent to which such therapies influenced the lipid profile remains unknown. It is plausible that some patients were receiving statins, which could have normalised lipid levels and masked the typical diabetic dyslipidaemia observed in other Libyan populations [14, 28]. Nevertheless, this interpretation is speculative, and future studies with detailed medication histories are needed to clarify the contribution of statin therapy to lipid normalisation in T2DM patients.

***Dietary Patterns***

Dietary patterns may also contribute to the observed lipid profile. A large body of evidence supports the cardioprotective effects of the Mediterranean diet, which is rich in monounsaturated fatty acids (primarily from olive oil) and omega-3 fatty acids (from fish), showing beneficial effects on lipid profiles, blood pressure, and inflammatory markers [41,42,43]. Although specific data on Libyan dietary habits are limited, North African populations may share similar traditional dietary characteristics [44]. This dietary background could partly explain the relatively favourable lipid profile observed in our Libyan T2DM patients compared to other populations with different dietary patterns.

***Geographical and Clinical Variation***

Variations in glycaemic control, disease duration and obesity prevalence across different Libyan regions may contribute to heterogeneous metabolic profiles, as demonstrated by district-specific differences in haematological and lipid parameters [15]. Diagnostic Sensitivity of Liver Biomarkers: The lack of significant ALT/AST elevation could reflect a lower burden of non-alcoholic fatty liver disease in this specific cohort, or it may indicate that aminotransferases are less sensitive markers of early hepatic steatosis in this genetic and environmental context [23,29], as suggested by the Basrah study where most patients had advanced steatosis but low-grade fibrosis [23].

***Antioxidant and Anti-inflammatory Properties of Bilirubin***

In our study, bilirubin showed a negative association with LDL-C ($r = -0.217$ to $-0.279$). This finding is consistent with the well-established antioxidant properties of bilirubin, which can inhibit lipid peroxidation and reduce the formation of atherogenic oxidised LDL particles [25,26]. Mechanistically, bilirubin has been shown to function as a metabolic hormone by binding to and activating the peroxisome proliferator-activated receptor alpha (PPARα), thereby enhancing fatty acid oxidation and improving insulin sensitivity [26].

***Biological and mechanistic plausibility***

The observed associations are biologically plausible. Insulin resistance, the core defect in T2DM, promotes hepatic de novo lipogenesis and impairs fatty acid oxidation, leading to intrahepatic lipid accumulation, which can in turn exacerbate insulin resistance and dyslipidaemia [45]. Bilirubin possesses well-documented antioxidant and anti-inflammatory properties [25]. Its negative association with LDL-C ($r = -0.217$ to $-0.279$) may reflect direct inhibition of lipid peroxidation, reducing the formation of atherogenic oxidised LDL particles. This finding is further supported by recent studies which demonstrate that lower serum bilirubin levels are significantly associated with atherogenic lipid abnormalities, including increased concentrations of small, dense LDL particles---a highly atherogenic lipoprotein phenotype common in type 2 diabetes [46,47]. Bilirubin is a potent antioxidant that inhibits lipid peroxidation; higher levels may protect LDL from oxidative modification, thereby reducing its atherogenicity and mitigating cardiovascular risk [25]. The negative correlation between ALT and HDL-C ($r = -0.187$, $p = 0.013$) may be explained by the role of the liver in HDL metabolism; hepatocellular injury or dysfunction could impair the synthesis of apolipoprotein A-I and HDL maturation [27]. The significant associations of FBS with ALP ($B = -0.262$, $p = 0.010$), AST ($B = -0.038$, $p = 0.047$), and TG ($B = 0.774$, $p = 0.002$) underscore the central role of glycaemic control in modulating both liver function and lipid metabolism. These findings align with the Italian study, where the triglyceride-glucose index was a strong predictor of incident diabetes [35]. Gender differences are likely mediated by oestrogen, which enhances HDL synthesis and may modulate hepatic enzyme expression. This is supported by experimental evidence showing that ERα is the most abundant estrogen receptor in hepatocytes and regulates lipogenesis, cholesterol homeostasis, and glucose metabolism in both sexes. Furthermore, lower circulating

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***Received:*** *04-06-2026 -* ***Accepted:*** *02-08-2026 -* ***Published:*** *09-08-2026*

estrogen levels in postmenopausal women and men are associated with increased plasma cholesterol and LDL-C, promoting hepatic fat accumulation [34]. The finding that females had higher TC and LDL-C than males in this study is interesting and may reflect age-related hormonal changes, as the majority of female participants were postmenopausal. A recent comprehensive review by Lee et al. [26] further elucidates that bilirubin acts as a metabolic hormone by binding to peroxisome proliferator-activated receptor alpha (PPARα), thereby enhancing fatty acid oxidation and improving insulin sensitivity. The heme oxygenase-biliverdin reductase-bilirubin axis plays a critical role in regulating oxidative stress and inflammation, and its dysfunction contributes to insulin resistance and metabolic disease. These mechanistic insights provide strong support for our observation that lower bilirubin levels in T2DM patients are associated with adverse lipid profiles and poorer metabolic health. Regarding alkaline phosphatase, Raizada et al. [48] reported that unexplained elevations in total ALP in T2DM patients are attributable to increased bone-specific ALP(BAP), with high ALP levels being associated with lower vitamin K2 and higher fetuin-A levels, suggesting that total ALP elevation may reflect vascular calcification risk rather than primary liver disease. In our study, ALP was significantly lower in T2DM patients. This could be consistent with lower bone turnover, possibly influenced by statin therapy or other factors not captured in our dataset. The bone-specific ALP fraction (BAP) was not measured, and future studies should explore whether the observed lower ALP in Libyan T2DM patients is driven by reduced bone-specific isoform activity.

***Clinical and translational implications***

The findings of the study have several important clinical implications for diabetes care in Libya. Firstly, routine measurements of ALP and bilirubin besides typical liver enzymes, may provide useful information about the state of the liver and metabolism in T2DM patients, particularly since these liver biomarkers were significantly different between study groups despite normal ALT/AST levels. Secondly, the gender differences indicate that the prevention and management strategies for dyslipidemia and liver dysfunction in diabetics may require a gender-specific approach, which was also recommended by Ashawish et al. [15], who noted sex-specific haematological differences. This was consistent with the observation that sex hormones regulate not only the biochemistry of the liver but also the immune system, with the description of the sexual duality of both innate and adaptive immune responses [34]. Thirdly, the absence of a clear abnormality in dyslipidaemia and elevated liver enzymes in this cohort should not lead to complacency but may reflect the effectiveness of current dietary and pharmacological interventions. In the context of public health and in line with previous recommendations [14, 28], these findings support continued emphasis on Mediterranean dietary patterns and the judicious use of statins in Libyan diabetic patients. Fourthly, the significant association between FBS and TG reinforces the importance of glycaemic control in managing lipid abnormalities. This is of particular importance given the high prevalence of poor glycaemic control in Libyan diabetic populations. Given the high prevalence of MAFLD in African T2DM population [13], screening for hepatic dysfunction using simple biomarkers such as ALP and bilirubin could be a cost-effective method in resource-limited conditions. Furthermore, the recognition that gender-affirming hormone therapy can impact nutrition-relevant biochemical measures [46] underscores the need for individualized reference ranges when interpreting laboratory data in diverse patient populations. Finally, as suggested by Raizada et al. [48], the relationship between ALP and vascular calcification in T2DM merits further investigation in Libyan patients, particularly using bone-specific ALP assays and imaging for arterial calcification.

***Interpretation of demographic and metabolic factors***

Gender was a significant determinant of multiple biochemical parameters in this study: Males showed higher levels of ALT, TB, DB, and TG, while females had higher levels of HDL-C, TC, and LDL-C. These differences are consistent with the effects of known hormones on lipid metabolism, where oestrogen upregulates HDL synthesis and may reduce hepatic fat accumulation, whereas testosterone is associated with increased visceral adiposity and hepatic lipogenesis [34]. However, the finding of elevated TC and LDL-C levels in females requires further research and investigation, as it may be influenced by menopausal status, which was not mentioned in this study. The comprehensive review by Lee et al. [26] highlights that bilirubin acts through PPARα to regulate lipid metabolism, and the sexual dimorphism in PPARα expression and activity may contribute to the gender differences observed in our study. FBS significantly affected TG levels in the patient group (B = 0.774, p = 0.002), reinforcing the central role of glycaemic control in modulating lipid metabolism, a finding consistent with the Italian study that indicated that the triglyceride-glucose index was a strong predictor of diabetes incidence [35]. In addition, FBS was significantly associated with ALP (B = -0.262, p = 0.010) and AST (B = -0.038, p = 0.047), suggesting that glycaemic status may affect liver enzyme levels even within the normal range. BMI did not significantly differ between the groups and was not a determinant of liver enzymes or lipids in regression analysis, which suggests that other factors (genetic predisposition, dietary fat intake, and physical activity) may have a greater effect in this population study. This finding contrasts with some Asian studies that have shown strong associations between BMI and liver enzymes, possibly due to differences in fat distribution between Libyan and Asian populations. It is noteworthy that sex hormones cause changes in lipoprotein metabolism, with estradiol typically increasing the level of high-density lipoprotein (HDL)/apolipoprotein A1 (ApoA1), which is protective against atherosclerosis in women, while testosterone is associated with increased VLDL and more serious markers of atherosclerosis [47].

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***Received:*** *04-06-2026 -* ***Accepted:*** *02-08-2026 -* ***Published:*** *09-08-2026*

### *Methodological strengths and limitations of this study*

Strengths of this study include its case-control design, comprehensive panel of liver and lipid parameters, appropriate use of nonparametric statistics, combination of clinical and demographic variables in the multivariate models, and use of the validated methods for laboratory measurements. The following methodological limitations should be considered when interpreting the results of this study: (1) The cross-sectional design precludes any inference of causality; (2) limited sample size (n = 176), reducing generalizability and statistical power for detecting smaller effect sizes; (3) no systematic data on medication use (particularly statins, metformin and insulin), dietary intake, and physical activity, causing possible confounding; (4) The lack of liver imaging (ultrasound, transient flexography) means that it is not possible to confirm or rule out subclinical non-alcoholic fatty liver disease, although studies from Basra [23] and the African meta-analysis [13] indicate that a significant proportion of type 2 diabetic patients suffer from hepatic steatosis even with normal levels of liver enzymes; (5) the single timepoint measurement of biochemical parameters does not account for within-individual variability over time; (6) selection bias, as study subjects were recruited from two northwestern cities of Libya (Al-Khums and Msallata) and may not reflect the whole population of Libya, as regional differences in metabolic parameters have been documented [15]; and (7) absence of GGT measurement, which is useful in the detection of liver dysfunction and dyslipidemia in other populations. Also, bone-specific ALP was not measured, which prevented differentiation between hepatic and bone-specific origins of ALP in T2DM patients.

## Conclusion

This study concluded that ALP, TB, and DB levels in Libyan type 2 diabetic patients were significantly lower compared to non-diabetic controls and that there were inverse correlations between specific liver-related biomarkers (ALT, TB, DB, and TP) and lipid parameters (HDL-C, LDL-C, and TC). Gender significantly influences these relationships, whereas age and BMI do not significantly influence them. FBS levels were associated with ALP, AST, and TG levels, underscoring the importance of blood glucose control. Future research should focus on: (1) longitudinal studies to determine the timing and causality of the relationships between changes in liver enzymes and the development of dyslipidemia; (2) prospective interventional studies to evaluate the effect of lifestyle modification (Mediterranean diet) and pharmacological interventions on the liver enzymes and lipid profiles; (3) larger multi-center studies in different Libyan regions with the standardization of data collection on medication use and diet; (4) mechanistic studies using assessments of genetic and inflammatory markers to study and elucidate the pathways linking bilirubin metabolism to lipid peroxidation in diabetes; (5) Studies based on imaging assessment using ultrasound, FibroScan and transient elastic imaging to directly assess hepatic steatosis and fibrosis in relation to biochemical parameters. (6) Other studies to determine non-traditional lipid indices (plasma atherosclerosis index, residual cholesterol, and non-HDL-C) and gamma-glutamyl transferase (GGT) enzyme, as well as bone-specific alkaline phosphatase measurement to determine the skeletal contribution to total alkaline phosphatase in type 2 diabetic patients.

### *Acknowledgments*

The authors acknowledge the Diabetes and Endocrinology Centers in Al-Khums and Msallata for providing the clinical and laboratory facilities used in this study. They also extend their sincere thanks to the medical laboratory staff for their support in conducting the biochemical measurements.

### *Conflicts of Interest*

The authors declare that there are no financial, personal, or professional conflicts of interest that could affect the design, conduct, or interpretation of this study. The study was conducted without any external commercial support, and all costs were covered by the institutional resources of the participating diabetes centers, where the biochemical analyses were carried out in the non-profit public sector.

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***Received****: 04-06-2026 -* ***Accepted****: 02-08-2026 -* ***Published****: 09-08-2026*

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***Received****: 04-06-2026 -* ***Accepted****: 02-08-2026 -* ***Published****: 09-08-2026*

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***Received****: 04-06-2026 -* ***Accepted****: 02-08-2026 -* ***Published****: 09-08-2026*