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Original Article

# Circulating betatrophin in relation to metabolic, inflammatory parameters, and oxidative stress in patients with type 2 diabetes mellitus

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ABSTRACT

*Aims:* Recently, it was suggested that betatrophin has a role in controlling pancreatic β cell proliferation and lipid metabolism, however, its role in human subjects has not been established yet. The predicting role of betatrophin and MDA along with other biochemical indicators in type 2 diabetes mellitus (T2DM) in a sample of the Iraqi population was examined in the present investigation.
*Methods:* A total of 31 patients diagnosed with T2DM and 30 adult subjects without diabetes were matched in age and gender in a case-control study. Logistic and linear regression models were performed to examine the role of MDA and betatrophin in T2DM and triglyceride, respectively.
*Results:* The study confirmed a higher concentration of LDL (124.45 vs. 102.70 mg/dL; P = .001) and TG (191.13 vs. 103.83 mg/dL; P < .0001), insulin (18.40 vs. 10.97 μU/mL; P < .0001), and Hs. CRP (5.39 vs. 2.80 mg/L; P = .033) in diabetic patients compared to the controls. No significant difference in betatrophin and MDA was found between diabetic patients and non-diabetic healthy subjects. The study showed triglyceride as the only predictor of T2DM (P = .028). Similarly, total cholesterol (P < .0001), HDL (P = .001), LDL (P < .0003), and MDA (P = .010) were shown as predictors of triglyceride in diabetic patients.
*Conclusion:* The present study that triglyceride is a direct and MDA is an indirect predictor for T2DM.


## 1. Introduction

Type 2 diabetes mellitus (T2DM), which is responsible for close to 90 percent of all diabetes is one of the four mortality risks in the Pacific and the Middle Eastern regions. In addition, it is a major cause of premature illness and death and an increased risk of up to 80 percent of cardiovascular diseases (CVD). Importantly, cardiovascular complications associated with diabetes are the main leading cause of blindness, kidney failure, amputation, and account for much of the social and financial burden [1].

The progressive loss in mass and function of beta cells in the pancreas is considered to be a significant pathophysiological feature of diabetes. The proliferation of pancreatic beta cells has a potential clinical benefit for insulin-dependent diabetic patients. The known factors have an influence on beta-cell proliferation are placental lactogen, serotonin, glucagon-like peptide-1, and pregnancy physiological effects, but inducing a proliferative effect in

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beta cells is difficult [2,3]. In the context of this background, Yi and Park et al. [4] found that betatrophin (a fat and liver-derived hormone) has a strong stimulatory impact on the beta cell proliferation in mice. It is also a regulator of lipid metabolism and is known as angiopoietin-like protein 8 (ANGPTL8), TD26, RIFL, lipasin [5,6]. Espes and Lau et al. [7] found higher concentrations of betatrophin in plasma of patients with type 1 diabetes. The higher serum concentrations of betatrophin were reported by Hu and Sun et al. [8] in patients with T2DM compared to healthy controls.

There are contradictory of findings with respect of serum level of betatrophin and its role in disease onset in T2DM. For example, Gokulakrishnan and Manokaran et al. [9] reported a higher significance level of betatrophin in type 2 diabetic patients compared to healthy controls (803 vs 1104 pg/ml, $p < .001$) in contrast with a lower concentration of betatrophin reported by Gómez-Ambrosi and Pascual et al. [10].

High sensitivity C-reactive protein (Hs. CRP) is a protein produced in the liver to respond to cytokine production in acute inflammation. It is considered to be an indicator of low-grade inflammation. An increased level of Hs-CRP has been shown to associate with cardiovascular diseases or T2DM [11].

Currently, the role of betatrophin and oxidative stress in predicting T2DM has not been clarified yet. This study would have a particular interest in the betatrophin study as the literature has not sufficient information. Thus, further research is needed for evaluating the role of betatrophin in type 2 diabetic patients in relation to some metabolic and inflammatory parameters, and oxidative stress.

The predicting role of betatrophin and malondialdehyde (MDA) along with other biochemical indicators in T2DM in a sample of Iraqi population was examined in the present investigation. The authors did not anticipate the significant difference in circulating betatrophin and MDA between non-diabetic healthy subjects and patients with T2DM.

## 2. Patients and methods

### 2.1. Study design and sampling

In the present case-control study, the patients diagnosed with non-complicated T2DM (range: 29–50 years; $n = 31$) were entered in the case group and non-diabetic healthy subjects (range: 28–50 years; $n = 30$) in a control group. The groups of the study were matched in age ($P = .076$), gender ($P = .252$), and smoking ($P = .848$) prior to study analysis. The subjects were non-alcoholic Kurdish and recruited from Duhok Diabetes Center of the Azadi Teaching Hospital over three months from October 2017 to January 2018. The subjects were consecutively screened for the eligibility criteria by an internist.

The subjected aged 18 years and older and regardless of gender and socio-demographic perspectives were eligible to participate in the study. The subjects diagnosed with the autoimmune disorder and renal diseases, thyroid dysfunction, pregnancy, women on lactation, and those were consuming triglyceride or cholesterol-lowering drugs since the last months until data collection were not included in the study. Moreover, those diabetic patients recognized with the following complications were not included in the study: cardiovascular diseases, retinopathy, nephropathy, feet ulcer, kidney failure, heart failure, hypothyroidism, and neoplasia, acute generalized inflammation, end-stage malignant disease, acute infectious disease, viral hepatitis, and a history of myocardial infarction.

### 2.2. Diagnostic and measurement tools

The baseline and demographic characteristics of the patients, inducing age, gender, physical activity (yes/no), and smoking (yes/no) were collected through the self-reported technique. The patients visited the diabetes center were screened through clinical and physical examinations for the eligibility criteria and patients with A1C equals to or greater than 6.5% were considered to have T2DM in line with American Diabetes Association. The patients' companions were screened for the control group following taking ethical approval from the corresponded department and consent form and were included in this group if they A1C was less than 6.5% [12].

The blood pressure of the participants was measured from the right arm upon the patients sitting using a mercury sphygmomanometer in accordance with standards.

**Biochemical measurements:** 10 ml venous blood samples were taken from all subjects after an overnight fast (=>12 h). The samples were centrifuged at 3000 rpm at 4 °C for 15 min and their supernatants were decanted and frozen at −30 °C and assayed. The other biochemical measurements and Hs-CRP were determined using Roche autoanalyzer 6000 cobas (Roche diagnostics, Mnnheim, Germeny) in the medical lab of the hospital. In the current study, Serum Betatrophin was performed using Human angiopoietin-like protein 8 (ANGPTL8) ELISA Kit, Catalog Number: MBS2021947 - 96 tests from MyBioSource, Inc. USA. ELx800 Universal Microplate Reader with ELx50 Auto Strip Washer was used for betatrophin measurement. The biochemical measurements were calculated form the serum samples.

**Malondialdehyde measurement**: 0.5 ml of plasma was shaken with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge tube for MDA measurement. Accordingly, 1 ml of 0.6% TBA was added to the mixture, shaken, and warmed in a boiling water bath for 30 min. It was cooled rapidly and was shaken into a 4 ml of nbutyl-alcohol layer in a separation tube and the mixture was centrifuged for 10 min. Subsequently, MDA contents in the plasma were determined from the absorbance at 535 and 520 nm by spectrophotometer against butanol. 1,1,3,3 tetraethoxypropane (TEP) (TEP, Sigma Chemical) as an external standard curve with different concentrations (10.45, 5.22, 4.86, 2.43, 1.22 and 0.61 nmol/ml) and the results were read as nmol/ml in plasma [13].

**Insulin resistance** was measured by the homeostatic model (HOMA-IR) by the following formula: HOMA-IR = fasting insulin (mIU/ml) × fasting glucose (mmol/L) ÷ 22.5. The cut-off HOMA≥2 was considered as insulin resistance [14].

**Anthropometric Measurements:** The patients' weight was measured in Kg scaled by Bathroom Scale, a digital scale nearest to 0.1 cm. Waist circumference (WC) was measured in the horizontal plane by non-stretch tape in the midway place between the lateral lower ribs and the iliac crests by Double-Scale Soft Tape with the nearest 0.1 cm.

### 2.3. Statistical analysis

The descriptive purposes of the study were expressed as mean (standard deviation) for continuous and frequency (percentage) for nominal variables. The difference in baseline characteristics and biochemical indicators between cases and controls were examined through the Pearson chi-square tests, independent *t*-test, or Mann-Whitney *U* test as appropriate. The predictors of study groups and triglyceride were examined in logistic and linear regressions, respectively. The *P*-value of less than 0.05 was used to reject the null hypothesis. The statistical calculations were performed in SPSS version 25:00 (IBM).

### 2.4. Ethical considerations

The ethical approval of the present investigation was obtained from the local corresponded department in Duhok registered as reference number: 04012017–1 on 4th January 2017. In addition, their written and informed consents were taken prior to clinical assessment. The entire procedures performed in this study were in line with the ethical standards of Helsinki declaration. The subjects were given guarantee for confidentiality of their personal information. The stern aseptic techniques were applied during blood sampling.

## 3. Results

Of the total 61 adult subjects (30 healthy and 31 type 2 diabetic patients) were included in the study. The controls and patients were comparable in age (38.33 vs. 41.19 years, P = .076), smoking (10.0% vs. 14.3%, P = .848), and gender (53.3% and 38.7% males, P = .252), respectively. While, the patients had higher SBP (13.26 vs. 12.07 mm-Hg, P < .0001), DBP (8.37 vs. 7.82 mm-Hg, P = .003), waist circumference (104.74 vs. 98.30 cm, P = .007), and BMI (29.28 vs. 26.55; P = .035 compared to non-diabetic healthy diabetic subjects; see Table 1.

The difference in biochemical indicators between controls and patients were shown in Table 2. The study showed that subjects in the case group had a greater level of LDL (124.45 vs. 102.70 mg/dL, P = .001), TG (191.13 vs. 103.83 mg/dL, P < .0001), and A1C (8.76 vs. 5.18%, P < .0001), and glucose (6.79 vs. 5.29 mg/dL; P = .001). Similarly, the substantial higher concentrations of insulin (18.50 vs. 10.97 μU/mL, P < .0001) and Hs. CRP (5.39 vs. 2.80 mg/L, P = .033) were found in patients in comparison to controls. No substantial difference of betatrophin (P = .210) and MDA (P = .258) and other biochemical markers (P > .05) between the study groups.

Being healthy or type 2 diabetic was considered as a dichotomous dependent variable in logistic regression and the biochemical indicators as explanatory factors. The study found the having a higher concentration of triglyceride was only predictor of being diabetic in our sample size (P = .028; OR: 1.053, 95% CI: 1.006–1.103); see Table 3. The range of TG in controls and cases were 32.00–215.00 and 43.00–429.00 mg/dL, respectively.

For the further analysis, triglyceride was considered as the dependent variable and biochemical markers as explanatory factors in linear regression. The study recognized TC (P < .0001), HDL (P = .001), LDL (P < .0001), and MDA (P = .010) as predictors of triglyceride in diabetic patients (see Table 4). Triglyceride was positively correlated with total cholesterol and LDL and negatively with HDL and MDA in diabetic patients (data not shown). The ranges of the predictors in **controls** were: TC: 115.00–889.00 mg/dL; HDL: 23.00–62.0 mg/dL; LDL: 49.00–134.00 mg/dL; and MDA: 0.11–4.70 nmol/ml and in **cases** were: TC: 143.00–276.00 mg/dL; HDL: 26.00–79.00 mg/dL; LDL: 78.00–176.00 mg/dL; and MDA: 0.02–5.84 nmol/mL.

## 4. Discussion

The present study did not show a substantial difference in betatrophin and MDA between healthy subjects and patients with T2DM. However, in logistic regression, higher serum concentrations of triglycerides was shown to be the only predictor of T2DM. Accordingly, the higher levels of total cholesterol and LDL and lower levels of HDL, and MDA were found as predictors of higher levels of triglycerides in diabetic patients.

The literature has reported contradictory findings on the level of betatrophin between healthy individuals and the patients with T2DM. For example, Al-Daghri and Rahman et al. [15] reported higher betatrophin levels in T2DM patients compared to healthy individuals (882.19 ± 329.06 vs 657.14 ± 261.04 pg/ml, p < .001). In addition, it was positively associated with blood pressure and serum fasting glucose (p < .05) in patients. Some other studies reported a higher level of betatrophin [8,16], while Gómez-Ambrosi and Pascual et al. [10] reported a reduction in betatrophin in patients with T2DM. Gokulakrishnan and Manokaran et al. [9] reported a lower level of betatrophin in Asian patients with T2DM compared to healthy controls (803 vs 1104 pg/ml, p < .001). In regression analysis, they found that betatrophin is independently related to T2DM following age, gender, and waist circumference adjustment (OR per standard deviation: 0.562, 95% CI: 0.342–0.899, p = .019), While, the association was lost when HOMA was considered in model (OR: 1.141, 95% CI: 0.574–2.249; p = .646).

We did not find that the levels of serum triglyceride in T2DM is regulated through an increase or decrease in serum betatrophin levels. Although, this finding does not reflect the role of betatrophin in controlling the growth of a beta cell, nor it can induce such expansion in pancreatic beta cells. The findings that plasma triglyceride levels are decreased by an increase in betatrophin and vice versa represents an important therapeutic strategy for hypertriglyceridemia.

In pharmacological and genetic mouse models of insulin resistance, hepatic over-expression has been contributed to an increase in the rate of proliferation of beta cells, islet size, and insulin contents [4]. Hence, betatrophin has received the emergence for a compensatory growth of beta cells in response to insulin resistance in diabetic patients [17].

Angiopoietin-like protein 8 (ANGPTL8) is a circulating protein found primarily in the liver and adipose tissue. Its hepatic overexpression in mice has been shown to associate with hypertriglyceridemia, while its inactivation causes a decrease in plasma

**Table 1**
Difference of baseline characteristics between healthy individuals and type 2 diabetic patients.

| Characteristics | Healthy (n = 30) Mean (SD) | Diabetes Type 2 (n = 31) Mean (SD) | Test of Significance |
|---|---|---|---|
| **Age** (Year); Mean/SD | 38.33 (6.97) | 41.19 (5.24) | .076* |
| **Systolic Blood Pressure** –mmHg; Mean/SD | 12.07 (1.05) | 13.26 (1.44) | <.0001* |
| **Diastolic Blood Pressure** –mmHg; Mean/SD | 7.82 (.68) | 8.37 (.71) | .003* |
| **Body Mass Index** | 26.55 (4.09) | 29.28 (5.11) | .035* |
| **Waist Circumference** –cm; Mean/SD | 98.30 (7.04) | 104.74 (9.51) | .007* |
| **Smoking**; F (%) | 3 (10) | 4 (14.3) | .848*** |
| **Physical activity**; F (%) | 10 (33.3) | 7 (38.9) | .697** |
| **Gender**; f (%) | | | .252** |
| Male | 16 53.3) | 12 (38.7) | |
| Female | 14 (46.7) | 19 (61.3) | |

*Independent *t*-test, ** Pearson Chi-square, and *** Fishers' exact tests were performed for statistical analyses.

**Table 2**
Difference of biochemical indicators between healthy individuals and type 2 diabetic patients.

| Biochemical Indicators | Healthy (n = 30) | Type 2 Diabetes (n = 31) | Test of Significance |
|---|---|---|---|
| Total Cholesterol, mg/dL | 189.70 (133.94) | 203.58 (35.51) | .587 |
| Glucose, mmol/L | 5.29 (.37) | 6.79 (2.23) | **.001** |
| High Density Lipoprotein, mg/dL | 43.17 (8.76) | 42.35 (11.79) | .761 |
| Low Density Lipoprotein, mg/dL | 102.70 (20.89) | 124.45 (28.51) | **.001** |
| Triglyceride, mg/dL | 103.83 (44.79) | 191.13 (101.11) | **<.0001** |
| Albumin, g/dL | 4.44 (.37) | 4.57 (.28) | .110 |
| Urea, mg/dL | 28.33 (8.23) | 28.26 (9.15) | .973 |
| Uric Acid, mg/dL | 4.79 (1.44) | 4.45 (1.34) | .347 |
| GOT, IU/L | 18.90 (6.27) | 19.03 (8.92) | .947 |
| GPT, IU/L | 20.27 (8.89) | 25.10 (13.10) | .097 |
| Alkaline Phosphatase, IU/L | 90.50 (23.47) | 101.87 (36.44) | .152 |
| HbA1c (%) | 5.18 (.43) | 8.76 (2.07) | **<.0001** |
| Betatrophin, ng/mL | .279 (.10) | .243 (.12) | .210 |
| MDA, nmol/mL | 1.88 (1.13) | 2.23 (1.26) | .258 |
| Creatinine, mg/dL | .66 (.4) | .65 (.31) | .773* |
| Insulin, μU/mL | 10.97 (8.31) | 18.40 (23.68) | **<.0001** |
| Hs.CRP, mg/L | 2.80 (3.71) | 5.39 (8.09) | **.033** |
| HOMA-IR | 2.62 (1.92) | 5.96 (6.68) | **<.0001** |

Mann-Whitney *U* test (median (interquartile range) was performed for statistical analyses and independent *t*-test (mean (Standard deviation) for others. The bold numbers show the significant differences.

**Table 3**
Logistic regression of biochemical indicators of healthy subjects and type 2 diabetic patients.

| Dependent variable: Healthy subjects and type 2 diabetes mellitus | | | | | |
|---|---|---|---|---|---|
| Predictors | B | Sig. | OR | 95% C.I. for OR (B) | |
| | | | | Lower | Upper |
| Systolic Blood Pressure-mmHg | .808 | .447 | 2.243 | .280 | 17.939 |
| Diastolic Blood Pressure-mmHg | −1.352 | .451 | .259 | .008 | 8.702 |
| Waist Circumference-Cm | .017 | .863 | 1.018 | .834 | 1.241 |
| Total Cholesterol; mg/dL | -.004 | .862 | .996 | .955 | 1.040 |
| High Density Lipoprotein (HDL); mg/dL | .156 | .203 | 1.169 | .919 | 1.488 |
| Low Density Lipoprotein (LDL); mg/dL | .016 | .742 | 1.016 | .925 | 1.115 |
| Triglyceride; mg/dL | .052 | **.028** | 1.053 | 1.006 | 1.103 |
| Albumin; g/dL | 2.450 | .291 | 11.583 | .123 | 1087.559 |
| Urea; mg/dL | -.069 | .556 | .934 | .743 | 1.173 |
| Creatinine; mg/dL | −1.437 | .785 | .238 | .000 | 7415.442 |
| Uric Acid; mg/dL | -.652 | .302 | .521 | .151 | 1.795 |
| GOT; IU/L | -.584 | .127 | .558 | .264 | 1.180 |
| GPT; IU/L | .439 | .085 | 1.552 | .941 | 2.559 |
| Alkaline Phosphatase; IU/L | -.006 | .892 | .994 | .907 | 1.089 |
| Hs. CRP; mg/L | .298 | .134 | 1.348 | .913 | 1.991 |
| Betatrophin; ng/mL | 6.823 | .475 | 918.348 | .000 | 121367215747.223 |
| MDA, nmol/mL | 1.235 | .206 | 3.437 | .508 | 23.267 |
| Age-Year | .040 | .775 | 1.041 | .790 | 1.372 |
| Gender | .827 | .693 | 2.286 | .038 | 138.986 |

triglyceride levels [18,19]. But, we did not found that overexpression of betatrophin is correlated with a reduction in serum triglyceride in patients with T2DM. Recently, betatrophin has been reported to mediate an increase in pancreatic beta cells proliferation and mass in mice in the case of insulin resistance induced by the insulin receptor antagonist S961. It is claimed that overexpression of betatrophin induces beta cell proliferation, beta cells expansion resulting in improved glycemic control [4].

Accordingly, we may say that overexpression of ANGPTL8 does not make a change in glucose tolerance or beta cell mass, it just can regulate the level of triglyceride. Gusarova and Alexa et al. [20] showed that ANGPTL8 overexpression for 8 days substantially increased plasma triglyceride levels and not impact on plasma glucose and insulin concentrations and comparable beta cells area expansion and mass between control and ANGPTL8 overexpressing mice. In agreement with these data, overexpression does not support the role of betatrophin in beta cell function and mass regulation, but its role is in triglyceride metabolism involvement in agreement with the subsequent investigation [21]. The finding is completely in disagreement with Yi and Park et al. [4] who reported betatrophin controls beta cell proliferation in mice treated with the insulin receptor antagonist S691. The authors in their correspondence confirmed that their own null mice model were similarly unaffected in beta cell mass expansion in the case of insulin resistance. Moreover, they accented that discrepancies with their previous paper have been occurred because of the high variability of the procedure employed to enhance betatrophin expression in the liver resulted in the strong response in a small number, but not among many of the cases [22].

The present investigation did not confirm the finding of previous studies son the role of betatrophin in atherogenic lipid profile in patients with obesity comorbidity or T2DM [16,23]. In addition, it is claimed that it has a role in dysfunctional lipid metabolism through its involvement to regulate hepatic very low-density lipoprotein secretion and in changed lipoprotein lipase activity [5,19]. The involvement of betatrophin in triglyceride metabolism is performed through the interaction with ANGPTL3 and regulation in the lipoprotein lipase activity. Triglyceride in chylomicrons and

**Table 4**
Linear regression of biochemical indicators of triglyceride in type 2 diabetic patients.

| Dependent Variable: Triglycerides | | | | | |
|---|---|---|---|---|---|
| Predictors | Standardized Coefficients | t | Sig. | 95.0% CI for B | |
| | Beta | | | Lower Bound | Upper Bound |
| Systolic Blood Pressure-mmHg | -.161 | −1.013 | .341 | −36.450 | 14.195 |
| Diastolic Blood Pressure-mmHg | .058 | .523 | .615 | −30.297 | 48.074 |
| Waist Circumference-Cm | .171 | 1.261 | .243 | −1.566 | 5.341 |
| Total Cholesterol; mg/dL | 1.882 | 7.664 | **.000** | 3.802 | 7.074 |
| High Density Lipoprotein (HDL); mg/dL | -.662 | −5.291 | **.001** | −8.692 | −3.415 |
| Low Density Lipoprotein (LDL); mg/dL | −1.461 | −7.187 | **.000** | −6.985 | −3.591 |
| Albumin; g/dL | -.032 | -.239 | .817 | −128.558 | 104.458 |
| Urea; mg/dL | -.105 | −1.068 | .317 | −4.921 | 1.806 |
| Creatinine; mg/dL | .339 | 1.797 | .110 | −56.860 | 458.524 |
| Uric Acid; mg/dL | -.299 | −2.217 | .057 | −46.275 | .913 |
| GOT; IU/L | -.115 | -.621 | .552 | −6.215 | 3.578 |
| GPT; IU/L | -.016 | -.084 | .935 | −3.539 | 3.291 |
| Alkaline Phosphatase; IU/L | .068 | .775 | .461 | -.367 | .739 |
| Hs. CRP; mg/L | .003 | .034 | .974 | −3.041 | 3.131 |
| Betatrophin; ng/mL | -.126 | −1.181 | .272 | −330.919 | 106.775 |
| MDA, nmol/mL | .290 | 3.377 | **.010** | 7.594 | 40.308 |
| Age-Year | -.012 | -.140 | .892 | −4.269 | 3.781 |
| Gender | .048 | .262 | .800 | −77.865 | 97.857 |

VLDL are hydrolyzed through the binding lipoprotein lipase to the surface of capillary microvascular endothelial cells resulting in free fatty acids. These free fatty acids are taken by peripheral tissues of fat, muscle, and heart [24].

Serum ANGPTL8 levels are lower in patients with low high-density lipoprotein cholesterol (HDLC < 40 mg/dL in men and <50 mg/dL in women) compared to those with high HDL-C [25]. Persons with low-density lipoprotein cholesterol and total cholesterol have higher concentrations of betatrophin [23].

Active investigations are required to identify the factors contributing to beta cell expansion. But, it must be taken into account that those factors may differ between humans and mice. For instance, Jiao and Le Lay et al. [21] showed that administration of S691 increases beta cell proliferation but not in humans.

The role of betatrophin in T2DM and obesity is still unclear. Fu and Berhane et al. [16] found an association between type 2 diabetes and body mass index with ANGPTL8 levels. We found a negative association of the betatrophin with triglyceride in this study reflecting the triglyceride as the main factor of type 2 diabetes is regulated by ANGPTL8 levels. Interestingly, it is noteworthy to mention that total cholesterol, HDL, and LDL has a stronger association with triglyceride than with betatrophin and MDA.

Betatrophin has suggested to act as a regulator for lipid metabolism and it has referred to as refeeding-induced fat and liver (RIFL), lipasin and angiopoietin-like protein 8 (ANGPTL8), and hepatocellular carcinoma-associated protein TD26 [16,18,19].

Gokulakrishnan and Manokaran et al. [9] reported that levels of betatrophin were decreased with increasing HOMA-IR in the overall groups, in type 2 diabetic patients, and in control ($P < .05$). We could not find such of this correlation in patients, even in linear regression in this study (data not shown). It is anticipated that the discrepancies in the studies about betatrophin in diabetic patients are reflected in sample sizes and use of different tools for measurement, methodological differences between the immunoassays or owing to ethnic discrepancies [16]. However, whether betatrophin is induced by insulin resistance or is linked to glucose homeostasis is unclear.

In the present study, we did not find a significant difference in MDA between patients with T2DM and healthy controls, however, it was seen to be one of the predictors for triglyceride. It was negatively associated with triglyceride in this study. Kamal and Salem et al. [26] reported a significantly higher concentration of MDA in type 2 diabetic patients compared to healthy control. It was shown that MDA has a positive correlation with triglycerides ($r = 0.315$) and LDL-c ($r = 0.354$) and negative correlation with HDL-c ($r = 0.315$). It seems that MDA is a cofactor in the pathogenesis of the T2DM. Our study showed that MDA is not as a stronger factor as HDL, TC, and LDL for triglyceride.

### 4.1. Strengths and limitations of the study

The strong point of the study must be traced in its design as the majority of the previous investigations have taken advantage of cross-sectional rather than case-control. However, the study is not exempt from the weaknesses. The entire subjects were Kurdish and taken from one setting may face us to generalize to other settings in the rest of the country or across the world.

## 5. Conclusions

We did not find the significant difference of betatrophin and MDA between healthy subjects and patients with T2DM in this study. The triglyceride was found to be the independent and MDA, HDL, LDL, and TC as indirect predictors for T2DM.

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The authors were only financial supporters of the study.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

## Authorship

I declare that all authors have made substantial contributions to all of the authors to the conception and design of the study, acquisition of data, analysis, and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be submitted.

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