



## Research Article

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# An Analysis of Circulating Betatrophin Levels in Relation with Type1 and Type2 Diabetes Mellitu Running Title: Betatrophin and Diabetes

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## Abstract

**Background:** Betatrophin is a newly identified liver-derived hormone that is associated with glucose homeostasis and lipid metabolism. Previous researches of betatrophin on glucose and lipid metabolism were mainly done under insulin resistant conditions. Only three studies from two centers investigated the association between betatrophin and type1 diabetes mellitus (T1DM). There is no consensus about the association of diabetes and betatrophin levels. Main purpose of this study was to investigate the relationship between betatrophin and various markers affecting glucose and lipid metabolisms in T1DM, type 2 diabetes mellitus (T2DM) and control subjects.

**Methods:** T1DM (n: 64), T2DM (n: 67) and control subject (n: 31) enrolled in this study. All subjects' physical examination, measurements of antropometric parameters and blood pressures were documented. Insulin, C-peptide, HbA1c, triglyceride, LDL-C, HDL-C, CRP, microalbuminuria, and betatrophin levels were measured in all groups.

**Results:** Serum betatrophin levels were significantly increased in patients with T1DM (p: 0.0001). Betatrophin was correlated with LDL-C in T1DM (r: 0.265, p: 0.034). No relationship between betatrophin and glucose metabolism (FBG, HbA1c) in T1DM was observed. Serum betatrophin levels were not increased in T2DM patients. Further more, we observed a significant positive correlation between betatrophin and FBG (r: 0.289, p: 0.019) and HbA1c (r: 0.372, p: 0.002) in T2DM. As distinct from previous studies we did not find any relationships between betatrophin and insulin resistance in any groups.

**Conclusions:** Betatrophin is significantly higher in T1DM. Betatrophin levels correlated positively with markers of glycemic control in T2DM. Circulating betatrophin levels appeared to be the metabolic parameters of the T2DM rather than T1DM.

## Keywords

Betatrophin, Type 1 diabetes mellitus, Type 2 diabetes mellitus, Glycemic control

## Introduction

Progressive loss of mass and function of beta cells are important pathophysiological features of diabetes. There by the best therapy and potential cure for both T1DM and T2DM is to replace or regenerate the insulin-producing pancreatic beta cell. Restoration of the functional beta cell mass is therefore expected to be a key aim of diabetes therapy. In this context, researchers from Harvard Stem Cell Institute (HSBI) recently found a new hormone and named it betatrophin, which could increase the quantity of insulin producing betacells in mice [1]. Before discovery of betatrophin by HSBI, different research teams have named this hormone differently according to its different functions: Including angiotensin amyloid 8(ANGPTL8), lipoprotein lipase

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inhibition (lipasin), refeeding induced in fat and liver (RIFL), hepatocellular carcinoma-association gene (TD26) [2-5].

In humans, betatrophin is encoded by the C19 or F80 gene, secreted protein of 198 aminoacides [6]. Betatrophin is recently identified as a circulatory adipokine, mainly secreted from liver and adipose tissues. Liver-derived proteins known as hepatokines have ambivalent roles; they either decrease insulin resistance or improve metabolic variable of T2DM (eg: Fetuin-A, irisin, fibroblast growth factor 21). Betatrophin was initially proposed for its action on beta cell proliferation, although this has recently been questioned [7]. Even a previously reported positive effect of betatrophin on regeneration of pancreatic beta cells is now currently considered to be negative [1,8]. As fibrinogen-like domain (FNDC 5) is absent, betatrophin is recognized to be an atypical member of the ANGPTL protein family [9]. Betatrophin has a similar gene structure to ANGPTL 3. In animal models, in the lipid tissue irisin, which is a member of classical ANGPTL protein family, secretes betatrophin via a coupling protein 1 (UCP1) [10].

Although it is now important to understand how betatrophin acts at systemic, cellular and molecular levels. So, most studies reported blood betatrophin levels to be high in patients with diabetes mellitus while some studies found no difference between DM and non-DM patients [11-13]. Furthermore, betatrophin was reported to be increased in serum of T1DM, suggesting that hepatic insulin resistance is not necessary for betatrophin release [14]. Also, betatrophin a nutritionally regulated factor and involved in the pathophysiology of lipid metabolism is an important regulator of plasma lipids. Some studies reported blood betatrophin levels to correlate with triglyceride levels, but others showed no correlation [15-18]. Hence, the impact of betatrophin in humans has not been clarified. Moreover, previous studies did not focus if betatrophin was related with both C-peptide and insulin levels. Increased betatrophin levels may be an early and better predictor of beta cell reserves and metabolic parameters in diabetic patients. We hypothesized that betatrophin concentrations which are positively correlated with the markers of glycemic control and serum lipid levels, are different among T1DM and T2DM. The aim of this study is to investigate the relationship of betatrophin levels with C-peptide and insulin in three separate groups: T1DM, T2DM and control subjects. Explorations of potential correlations with glucose and lipid metabolism are also within our goals.

## Methods

### Subjects

In the present study, the leakage difference between control and diabetic groups were found to be between 44%-72% via the power analysis conducted by G power 3.1 programs. Parametric distribution assumptions were considered, and minimum patient number was assumed to be 30. Our sample groups consisted of 67 previously diagnosed T2DM, 64 T1DM and 31 control individuals with comparable age, sex and body mass index (BMI) satisfying the requirements of a power rating of 0.8. The study protocol was approved

by the Ethics Committee of Istanbul Education and Research Hospital (no: 796, date: 11.03.2016) and it conformed to the principles outlined in the Declaration of Helsinki in 1995 (as revised in Tokyo 2004). The written informed consents were obtained from all participants before the initiation of study.

Inclusion criteria: 1. Diabetic patients with durations of diabetes at least >3 years and their medical records based on the ADA criteria (2016). 2. Control subjects, who were selected from the hospital staff, did not have a family history of diabetes and their oral glucose tolerance test (OGTT) showed 2-hour BG < 180 mg/dl. Following exclusion criteria were applied: 1. Patients with secondary diabetes or specific type of diabetes; 2. Ketoacidosis, lactic acidosis, hyperglycemic hyperosmolar state during enrolment; 3. Subjects with diabetic foot or inflammatory or infectious diseases; 4. Acute myocardial or cerebral infarction during enrolment; 5. Familial hypercholesterolemia and samples with visible lipemic and hemolysis; 6. Heart failure, severe impaired liver function or alcoholism; 7. Renal disease (diabetic nephropathy) (GFR < 60 ml/min/1.73m<sup>2</sup>), proliferative diabetic retinopathy; 8. Pregnancy or lactation; 9. Impaired hematopoietic function and malignancy.

### Physical and anthropometric measurements

Each subject underwent a complete physical examination by the same physician at the Internal Medicine Outpatient Clinic of our Hospital. Blood pressure (BP) was measured with using an Omron HEM-907XL digital sphygmomanometer. An average of 2 BP readings with 5-10 minutes rest between each was obtained. Weight and height were measured with participants wearing light clothing and bare footed, using calibrated portable electronic weight scales and portable inflexible height measuring bars. BMI was calculated using the standard BMI formula: Body weight (in kilograms) divided by height (in meters squared). Waist circumference was measured twice of the nearest 0.1 cm with flexible tape measure at the level of minimum circumference which was usually at the level of the navel.

### Laboratory analysis

All subjects had at least 12 hours of fasting before blood sampling for biochemical analysis. Fasting blood samples were collected in EDTA-containing, anticoagulant free tubes in the morning and centrifuged immediately (3000 g) for 10 min at +4°C, plasma and serum were separated in Eppendorf tubes and frozen immediately at -80°C until analysis of betatrophin. Fasting blood glucose (FBG), urea, creatinine, triglyceride, total cholesterol (TC), high-density cholesterol (HDL-C), low-density cholesterol (LDL-C) and albumin were measured spectrophotometrically using the Abbott Aeroset 2.0 (Abbott Diagnostic, USA). The analysis of C-reactive protein (CRP) was performed by nephelometric (IMAC-Beckman coulter, Germany). HbA1c % was measured by the Premier Hb9210 (Trinity Biotech, Ireland) which uses the glycation specific binding of boronated affinity to detect all the glycosylated Hb species present. GFR was calculated using the MDRD formula [19]. Fasting insulin was measured by enzymatic immunoassay using direct chemiluminescent (Bergman Coulter, Inc.). The

analysis of C-peptide was performed by two-side sandwich immunoassay using chemiluminescent (Siemens Healthcare Diagnostic, Germany). Insulin resistance was calculated using the HOMA-R formula: Fasting glucose (mg/dl) x fasting insulin (mIU/ml)/405.

## Measurement of plasma betatrophin (ANGPTL8) levels

Plasma betatrophin levels were measured by a commercially available enzyme-linked immunosorbent assay kit (Human (ANGPTL8) ELISA Kit, Sunred, Cat No: 201-12-5327). The coefficients of intra and inter-assay variation were 4.8% (n = 20) and 6.1% (n = 20) respectively.

## Statistical analysis

In this study, statistical tests were conducted with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) program. While evaluating the results descriptive statistical methods (average, standard deviation) were utilized. In multiple-group comparisons unilateral variance analysis was used. Qualitative data was evaluated with chi-square test, while variants were compared with Pearson correlation test. To demonstrate the relationship between betatrophin and C-peptide the area under the ROC curve was calculated; sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Likelihood Ratio (LR) were calculated. Results were evaluated with significance  $p < 0.05$  and confidence bounds within 95%. Mann Whitney U test was used for pos hoc test of nonparametric variables, and Tukey Test was used for pos hoc test of parametric variables. Multinomial logistic regression analysis was used for significantly relevant parameters.

## Result

The clinical characteristics of the three groups including T1DM, T2DM and control subjects are presented in (Table 1). There are no differences among three group sinage, gender, smoking / alcohol consumption. Waist circumference (WC), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were significantly higher in T2DM than T1DM or control subjects ( $p: 0.004$ ,  $p: 0.001$ ,  $p: 0.008$  respectively). Yet, duration of diabetes with T1DM was significantly longer than T2DM ( $p: 0.0001$ ).

The metabolic parameters of study groups are shown on (Table 2). Betatrophin values (cutoff: 116 ng/l according to ROC curve) were significantly higher in T1DM group than T2DM or control subjects ( $322,73 \pm 266,05$  ng/l vs.  $144,84 \pm 124,5$  ng/l vs.  $106,19 \pm 59,07$  ng/l) respectively ( $p: 0.0001$ ). As expected, C-peptide and insulin values were significantly lower in T1DM than T2DM or control groups ( $p: 0.0001$ ,  $p: 0.0001$  respectively). Glomerular filtration rate (GFR) was significantly less in T2DM than T1DM and control groups ( $p: 0.003$ ,  $p: 0.006$  respectively). T1DM and T2DM were both micro albuminuria positive, but there was no meaningful difference between T1DM and T2DM groups with regard to micro albuminuria. There was no significant difference among three groups' triglyceride, HDL-C, albumin and CRP levels. Total cholesterol (TC) and LDL-C values were

significantly higher in T2DM than T1DM1 and control groups ( $p: 0.036$ ,  $p: 0.037$  respectively).

As expected, fasting blood glucose and HbA1c values were significantly lower in control groups than diabetic groups ( $p: 0.0001$ ,  $p: 0.0001$  respectively). (Table 3) demonstrates correlation between betatrophin and C-peptide with metabolic parameters among the study groups. Betatrophin levels are significantly correlated with FBG ( $p: 0.019$ ) and HbA1c ( $p: 0.002$ ) in T2DM not in T1DM. On the other hand, in T1DM betatrophin levels are correlated with LDL-C ( $r: 0.265$ ,  $p: 0.034$ ), there are no correlations with either HDL-C or TG. According to binary logistic analysis results duration of diabetes, betatrophin, C-peptide, total cholesterol and insulin levels of patients had significant contribution to type of DM. Effects of duration of DM and betatrophin were positive, whereas effects of C-peptide, total cholesterol and insulin levels were negative.

The most effective factor was C-peptide, followed by insulin and duration of DM. There was no correlation between insulin resistance and betatrophin levels among the groups. Parameter estimates are shown on (Table 4). According to ROC curves, AUC, cut off levels of betatrophin, sensitivity, specificity, PPV, NPV and LP in control, T1DM, T2DM groups are shown on the (Table 5). The likelihood of having T1DM for patients who had betatrophin value  $\geq 116$  ng/l was 6.46 times more than patients who had betatrophin level  $< 116$  ng/l. The likelihood of having T2DM was 3.39 times more in patients who had betatrophin level  $> 116$  ng/l compared with patients having betatrophin level  $< 116$  ng/l. Among diabetic patients, prediction capacity of betatrophin for T2DM was 2,51 times more than patients who had betatrophin level  $> 94$  ng/l. These results state that betatrophin cannot be used as a diagnostic marker for diabetes.

## Discussion

We found that circulating betatrophin is elevated in Turkish T1DM patients, but not in T2DM and control groups. We were not able to find any relationships between betatrophin and glycemic control such as FBG and HbA1c in T1DM. Betatrophin had a significant positive correlation with the duration of diabetes in T1DM. There was no correlation of insulin or C-peptide and insulin resistance between betatrophin in any of the groups. No correlation was found with microalbuminuria and GFR. Furthermore, we observed a relationship of betatrophin with FBG and HbA1c in T2DM patients. We are the third center that has found elevated betatrophin levels in patients with T1DM and betatrophin is correlated with the LDL-C levels. In accordance with our results, Espeset, et al. [14] showed that increased plasma betatrophin of the Sweden's T1DM patients wastwiceof the patients with normal glucose tolerance and the plasma contents did not correlate with C-peptide or any other metabolic parameters. They also have shown that betatrophin levels were higher in older non-diabetic control subjects while T1DM patients did not show a similar trend. In our study there is no age difference among three subject groups. So, our population does not show age dependent betatrophin levels. In the early phases of the disease patients with T1DM

**Table 1:** General characteristics of the study groups.

		Control Group n: 31		Type 1 DM n: 64		Type 2 DM n: 67		p	Significant within group differences
<b>Age (years)</b>		56.84 ± 7.66		56.25 ± 9.33		59.78 ± 8.42		0.055 <sup>a</sup>	N/A
<b>Gender</b>	<b>Male</b>	10	32.3%	26	40.6%	24	35.8%	0.705 <sup>c</sup>	N/A
	<b>Female</b>	21	67.7%	38	59.4%	43	64.2%		
<b>Height (kg)</b>		162.45 ± 10.47		163.69 ± 8.78		162.03 ± 9.3		0.585 <sup>a</sup>	N/A
<b>Weight (cm)</b>		78.97 ± 13.56		77.3 ± 14.87		81.63 ± 11.81		0.182 <sup>a</sup>	N/A
<b>BMI</b>		30.07 ± 5.38		29.00 ± 6.07		31.18 ± 4.49		0.067 <sup>a</sup>	N/A
<b>Smoking (+)</b>		6	20.7%	14	21.9%	20	29.9%	0.482 <sup>b</sup>	N/A
<b>Alcohol (+)</b>		1	3.4%	5	7.8%	2	3.0%	0.418 <sup>c</sup>	N/A
<b>WC(cm)</b>		94.23 ± 11.11		91.67 ± 9.74		97.76 ± 10.66		<b>0.004<sup>a</sup></b>	Type 2 DM >Type 1 DM <sup>e</sup>
<b>SBP (mmHg)</b>		126.77 ± 10.45		126.25 ± 13.03		134.93 ± 14.81		<b>0.000<sup>d</sup></b>	Type 2 DM >Type 1 DM <sup>f</sup> Type 2 DM >Type 1 DM <sup>f</sup>
<b>DBP (mmHg)</b>		79.68 ± 3.4		78.44 ± 7.34		81.72 ± 5.47		<b>0.001<sup>d</sup></b>	Type 2 DM >Type 1 DM <sup>f</sup> Type 2 DM >Type 1 DM <sup>f</sup>
<b>Diabetes duration (years)</b>				21.34 ± 8.87		12.73 ± 5.91		<b>0.000<sup>a</sup></b>	N/A

a) One Way ANOVA Test; b) Chi-Square Test; c) Chi-Square Likelihood Ratio; d) Kruskal Wallis H test; e) Tukey Test; f) Mann Whitney U Test, N/A: Not Applicable, \*p<0.05.

**Table 2:** Metabolic parameters of the study groups.

	Control n: 31	Type 1 DM n: 64	Type 2 DM n: 67	p	Significant within group differences
<b>Betatrophin(ng/l)</b>	106.19 ± 59.07	322.73 ± 366.05	144.84 ± 124.50	<b>0.000<sup>a</sup></b>	Type 1 DM >Control <sup>e</sup> Type 1 DM >Type 2 DM <sup>e</sup>
<b>C-peptide(ng/ml)</b>	1.16 ± 0.69	0.56 ± 0.34	1.34 ± 0.78	<b>0.000<sup>a</sup></b>	Type 1 DM <Control <sup>e</sup> Type 2 DM >Type 1 DM <sup>e</sup>
<b>GFR(ml/dak -1.73m2)</b>	115.30 ± 25.31	111.07 ± 27.96	91.33 ± 41.66	<b>0.000<sup>a</sup></b>	Type 2 DM <Control <sup>e</sup> Type 2 DM <Type 1 DM <sup>e</sup>
<b>Microalbuminuria (mg/g)</b>	38.69 ± 115.05	129.50 ± 318.09	139.55 ± 298.86	<b>0.010<sup>a</sup></b>	Type 1 DM >Control <sup>e</sup> Type 2 DM >Control <sup>e</sup>
<b>Total C(mg/dl)</b>	202.55 ± 43.74	190.56 ± 51.46	211.27 ± 44.90	<b>0.046<sup>b</sup></b>	Type 2 DM >Type 1 DM <sup>d</sup>
<b>Triglyceride (mg/dl)</b>	135.77 ± 76.98	179.97 ± 194.20	196.45 ± 133.29	<b>0.022<sup>a</sup></b>	Type 2 DM >Control <sup>e</sup>
<b>HDL-C(mg/dl)</b>	45.65 ± 10.02	45.95 ± 15.17	46.75 ± 16.08	0.895 <sup>a</sup>	N/A
<b>LDL-C(mg/dl)</b>	122.81 ± 33.57	114.56 ± 35.38	131.46 ± 39.80	<b>0.037<sup>b</sup></b>	Type 2 DM >Type 1 DM <sup>d</sup>
<b>FBG(mg/dl)</b>	84.58 ± 12.91	151.86 ± 57.33	162.17 ± 43.76	<b>0.000<sup>a</sup></b>	Type 1 DM >Control <sup>e</sup> Type 2 DM >Control <sup>e</sup> Type 2 DM >Type 1 DM <sup>e</sup>
<b>Insulin(qU/ml)</b>	7.02 ± 1.19	4.48 ± 2.21	7.22 ± 3.04	<b>0.000<sup>a</sup></b>	Type 1 DM <Control <sup>e</sup> Type 2 DM >Type 1 DM <sup>e</sup>
<b>Albumin(g/dl)</b>	4.28 ± 0.26	4.23 ± 0.39	4.24 ± 0.33	0.961 <sup>a</sup>	N/A
<b>CRP(mg/dl)</b>	0.67 ± 0.50	0.58 ± 0.43	0.81 ± 1.49	0.714 <sup>a</sup>	N/A
<b>HbA1c(%)</b>	6.38 ± 1.09	8.71 ± 2.11	8.49 ± 2.02	<b>0.000<sup>b</sup></b>	Type 2 DM > Control <sup>d</sup> Type 1 DM > Control <sup>d</sup>

Kruskal Wallis H Test, b. One Way ANOVA Test, d. Tukey Test, e. Mann Whitney U Test, N/A: Not Applicable

C-peptide: 0.8-3.85 ng/ml, GFR>110 ml/dk/1.73m<sup>2</sup>, microalbuminuri: 30-300 mg/dl, T-cholesterol <200mg/dl, triglyceride <150 mg/dl, LDL-C <130 mg/dl, HDL-C: 40-60 mg/dl, FBG: 70-95 mg/dl, insulin: 1.923 qU/ml, albumin: 3.5 g/dl, CRP: 0-5 mg/dl, HbA1c: 6.5 %

are lean, but as the years pass patients increase in BMI. As our study subjects had comparable BMI, we did not observe any relationship between betatrophin and BMI. Also, Yamada, et al. [20] focused on increased betatrophin levels in Japan's T1DM patients similar to our study. Like our results, Espes, et al. [21] found a positive correlation between betatrophin and HbA1c in T2DM patients.

As a result, three studies from two centers investigated the association between betatrophin and T1DM and all of them found a significant increased level of betatrophin in T1DM, like our study. Yi, et al. and a lot of other researchers

showed increased levels of serum betatrophin in Chinese T2DM patients [22,23]). Gomes-Ambrosi, et al. [13] showed that circulating betatrophin level was reduced in T2DM patients. There is heterogeneity observed among the studies. Different race, age and samples may be the reasons of this heterogeneity. In addition, diabetic patients assessed in the studies were taking hypoglycemic medications.

Since the effects of hypoglycemic agents on serum betatrophin levels are unclear, they might cause the potential confounding effects. However, several reports have indicated that circulating betatrophin levels were generally higher

**Table 3:** Correlations between betatrophin and C-peptide among the study groups\*.

	B	Std. Error	Wald	p	Exp(B)	95% Confidence Interval for Exp(B)	
						Lower Bound	Upper Bound
Intercept	8.271	2.910	8.079	0.004			
BMI	0.123	0.069	3.171	0.075	0.884	0.772	1.012
Duration of diabetes (year)	0.111	0.041	7.447	<b>0.006</b>	1.118	1.032	1.210
Betatrophin	0.005	0.002	6.458	<b>0.011</b>	1.005	1.001	1.009
C-peptide	3.510	0.882	15.850	<b>0.000</b>	0.030	0.005	0.168
Total cholesterol (mg/dl)	0.021	0.008	6.987	<b>0.008</b>	0.980	0.965	0.995
Triglyceride (mg/dl)	0.002	0.002	1.167	0.280	1.002	0.998	1.006
Insulin (µU/ml)	0.400	0.149	7.212	<b>0.007</b>	0.670	0.500	0.898
HbA1c (%)	0.170	0.163	1.081	0.299	1.185	0.860	1.632

\*Reference category is Type 2 DM.

**Table 4:** Parameter Estimates.

dm <sup>a</sup>		B	Std. Error	Wald	df	Sig.	Exp(B)	95% Confidence Interval for Exp(B)	
								Lower Bound	Upper Bound
1	Intercept	8.271	2.910	8.079	1	.004			
	BMI	-.123	.069	3.171	1	.075	.884	.772	1.012
	Duration of DM	.111	.041	7.447	1	.006	1.118	1.032	1.210
	Betatrophin	.005	.002	6.458	1	.011	1.005	1.001	1.009
	C-peptide	-3.510	.882	15.850	1	.000	.030	.005	.168
	T-Cholesterol	-.021	.008	6.987	1	.008	.980	.965	.995
	TG	.002	.002	1.167	1	.280	1.002	.998	1.006
	Insülin	-.400	.149	7.212	1	.007	.670	.500	.898
	HBA1C	.170	.163	1.081	1	.299	1.185	.860	1.632

a. The reference category is: 2.

**Table 5:** AUC, Cut off, Sensitivity, Specificity, PPV, NPV and LR of Betatrophin levels.

Betatrophin(ng/l)	AUC	Cut off	Sensitivity	Specificity	PPV	NPV
Control/T1DM	0.740	116.00	62.50	90.32	93.0	53.8
Control/T2DM	0.468	116.00	32.84	90.32	88.0	38.4
T1DM/T2DM	0.710	94.00	62.69	75.00	72.4	65.8

AUC: Area Under Curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value

in metabolically disturbed state such as T1DM, T2DM, metabolic syndrome, and fatty liver disease Lee, et al.'s [24] study "a nested case-control study from a population-based prospective study", showed that patients with baseline betatrophin levels with significantly higher levels converted more to diabetes compared to non-converter group. So, betatrophin may be a possible biomarker for individuals at high risk for developing diabetes. Yi, et al.'s [25] study using ROC curve showed that circulating betatrophin concentration could be a diagnostic biomarker for T2DM, with optimal cut-off 501. 23 pg/ml. These two studies show that we observe increased levels of betatrophin in the early phases of the T2DM. Where as, in the present study ROC curve showed betatrophin cut-off as 116 ng/L. for diagnostic level in T2DM. Thus, due to our low PPV value we could not consider betatrophin as a diagnostic marker for T2DM.

It has been well established that betatrophin plays an important role of generalization of triglycerides. Studies on animals showed that over expression of ANGPTL8 for 8 days significantly increased plasma triglyceride levels but had no

effect on glucose or insulin concentrations [26]. A lot of studies on humans showed that serum betatrophin levels have a positive correlation with triglyceride [15,17,22,27]. ANGPTL8/betatrophin apparently induces triglycerides elevation through reducing triglycerides clearance by LPL inhibition [27-30]. As a result, betatrophin plays a significant role in the triglyceride metabolism. Fenzl, et al. [31] showed that betatrophin was associated with plasma atherogenic lipids in obesity and T2DM; but they did not observe any relationships with beta cell function and glucose homeostasis. Betatrophin also showed significant positive correlation with HDL-C in previous studies, [25,28] yet it should be noted that these associations were not found in other reports. Also, Hassan, et al. [32] showed that betatrophin was not associated with triglyceride in T2DM. In the present study, we have not found any relationship between betatrophin and triglyceride in any of the three groups. But we have found a relationship with betatrophin and LDL-C in T1DM. So, atherogenic lipid profile was observed in our T1DM patient group, mainly because they were patients with longer duration of diabetes.

Hyperglycemia and altered lipid profile are in association with diabetic nephropathy. Chen, et al. [33] found that serum betatrophin levels were positively correlated with microalbuminuria in T2DM patients. Also, they found serum betatrophin levels to be higher in patients with macroalbuminuria compared to normal albuminuria. One recent Japanese study in diabetic patients found a significant negative correlation between betatrophin and creatinine clearance as well as GFR [12,30]. In contrast, a recent German study investigating betatrophin levels in diabetic patients undergoing hemodialysis compared to diabetic individuals with sustained renal function, found significantly positive correlation between betatrophin and GFR [34].

They also found that patients on hemodialysis had significantly lower betatrophin levels compared with subjects having GFR >50 ml/min/1.73m<sup>2</sup>. Espes, et al. [20] showed a positive correlation between plasma betatrophin levels and plasma creatinine in T2DM patients, which would suggest that betatrophin, may be excreted in the urine, though there was no correlation between betatrophin and calculated GFR. In the present study, we did not find any correlation of betatrophin with microalbuminuria or calculated GFR in any of the three study groups. As we only included subjects with a calculated GFR >60 ml/min/1.73m<sup>2</sup>, this may be the reason for lack of correlation between betatrophin with creatinine in diabetic patients. Another reason may be that most of patients in our diabetic study groups have microvascular and macrovascular complications and they used ACE and AT2 drugs.

Our study has same limitations. First, we included previously diagnosed diabetic patients. They used antidiabetic medications such as metformin, thiazolidinediones that altered insulin resistance state in T2DM and exogenous insulin may also affect results. Second, postprandial levels of betatrophin could not be analyzed because we measured only fasting betatrophin levels. So fasting betatrophin levels cannot reflect betatrophin levels over time. Third, serum betatrophin levels were determined by ELISA without verification by western blotting. Lastly, liver tests like AST, ALT, ALP, GGT and upper abdominal ultrasound were not measured.

## Conclusion

In this study, we found circulating betatrophin levels were higher in Turkish T1DM patients, though they were not correlated with metabolic parameters. Given the positive correlation of betatrophin levels with FBG and HbA1c in T2DM, we suggest that betatrophin measurement may be useful in the monitorization of diabetes regulation in T2DM. Contrary to common belief, we not found any relationship between betatrophin with insulin resistance. We not known that high betatrophin levels are observed in early phases of diabetes. If yes, close monitorization of patients with high betatrophin levels, hence strict regulation of their blood glucose can prevent and/or postpone microvascular complications. This is a significant finding in improving patient life expectancy. We recommend for researchers to perform prospective studies in larger groups evaluating betatrophin first marker or not of diabetic monitorization.

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