Turk J Endocrinol Metab 2019;23:1-7

Evaluation of the Circulating Betatrophin Concentration and its Possible Correlations Among Diabetic Patients with Dyslipidemia

Dislipidemili Diyabetik Hastalarda Dolaşımdaki Betatrofin Konsantrasyonunun ve Muhtemel Korelasyonlarının Değerlendirilmesi

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Abstract

Objective: The circulating betatrophin levels in diabetic patients, treated with insulin or with oral hypoglycemic agents, were measured. The correlation between betatrophin levels and the glucose/lipid variables was also analyzed.

Material and Methods: Thirty-six diabetic patients (18 insulin-treated and 18 oral hypoglycemics-treated) and 16 age-and sex-matched non-diabetic controls were enrolled. The serum levels of betatrophin, glucose, and lipids were measured.

Results: The serum betatrophin levels were significantly lower in both the insulin-treated and oral hypoglycemics-treated diabetics compared to the controls (32.8±3.6 and 48.4±5.2 vs. 54.4±3.7, respectively, p<0.001). Pearson's bivariate correlation analysis showed that betatrophin was positively correlated with total cholesterol (r=0.303, p=0.029), LDL-cholesterol (r=0.339, p=0.014) and triglyceride (r=0.562, p=0.015) levels.

Conclusion: The circulating betatrophin levels were reduced in diabetic patients compared to the non-diabetic controls, and positively correlated with lipid parameters but not with glucose levels.

Keywords: ANGPTL8; betatrophin; Diabetes mellitus; glucose; lipids

Özet

Amaç: İnsülin veya oral hipoglisemik ajanlarla tedavi edilen diyabetik hastalarda dolaşımdaki betatrofin seviyeleri ölçüldü. Ayrıca, betatrofin seviyeleri ile glukoz/lipit değişkenleri arasındaki korelasyon analiz edildi.

Gereç ve Yöntemler: Otuz altı diyabetik hasta (insülinle tedavi edilen 18 ve oral hipoglisemik ile tedavi edilen 18), yaş ve cinsiyeti eşleştirilmiş 16 diyabetik olmayan kontrol çalışmaya dâhil edildi. Serum betatrofin, glukoz ve lipit seviyeleri ölçüldü.

Bulgular: Serum betatrofin düzeyleri hem insülinle hem de oral hipoglisemikle tedavi edilen diyabetiklerde kontrollere kıyasla anlamlı derecede düşük bulundu (sırasıyla 32,8±3,6 ve 48,4±5,2'ye karşı 54,4±3,7, p<0,001). Pearson iki değişkenli korelasyon analizi, betatrofinin total kolesterol (r=0,303, p=0,029), LDL-kolesterol (r=0,339, p=0,014) ve trigliserid (r=0,562, p=0,015) düzeyleri ile pozitif korelasyon gösterdiğini ortaya koydu.

Sonuç: Dolaşımdaki betatrofin seviyeleri diyabetik hastalarda diyabetik olmayan kontrollere göre azaldı ve lipit parametreleriyle pozitif korelasyon gösterdi, ancak glukoz seviyeleri ile göstermedi.

Anahtar kelimeler: ANGPTL8; betatrofin; Diabetes mellitus; glukoz; lipidler

Introduction

Diabetes mellitus (DM) is a complex disease characterized by chronic hyperglycemia that has become increasingly prevalent during the last decades (1). It is broadly classified into type 1 and type 2 DM; while type 1 diabetic patients do not produce insulin due to the autoimmune destruction of the pancreatic beta cells, type 2 DM is a result of metabolic syndrome and insulin resistance (2). However, recent discoveries regarding the molecular pathophysiology of DM advocate a more stringent disease classification based on patient genotypes and phenotypes (2, 3). Both types are associated with a gradual loss of pancreatic beta cell function, which

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Received: 27/09/2018 **Received in revised form:** 03/12/2018 **Accepted:** 16/12/2018 **Available online:** 20/03/2019

DOI: 10.25179/tjem.2018-63011

may persist for several decades (4), and cannot be attenuated by the current therapies (5). In addition, DM is also associated with serious complications such as atherosclerosis, retinopathy, and nephropathy, as well as high mortality (1).

Betatrophin, also known as lipasin, refeeding induced in fat and liver (RIFL) and angiopoietin-like protein 8 (ANGPTL8), is a 22 kDa hormone produced mainly in the liver and adipose tissues. Its levels increase in the liver after feeding and decrease upon fasting (6). Since betatrophin stimulates the proliferation of pancreatic beta cells (7), it is a promising option for regenerative therapy against diabetes (8). Betatrophin is also closely associated with lipid metabolism via inhibition of lipoprotein lipase activity (9), and its overexpression in mice increases triglyceride levels by modulating hepatic VLDL secretion (6, 10, 11). However, the betatrophin levels in either type 1 or type 2 DM patients are still ambiguous. While some studies have reported an increase in betatrophin concentration in both type 1 and type 2 diabetic patients (12-14), Gómez-Ambrosi et al. found lower betatrophin levels in patients with type 2 DM (15). Some studies on the other hand report lack of any correlation between betatrophin concentration and glucose tolerance (10, 16).

Moreover, only a few studies have evaluated the effects of anti-diabetic treatments on the circulating betatrophin levels. For example, Espes et al. found that diabetic patients treated with metformin had higher serum betatrophin levels compared to the controls (14). Another study on a Japanese cohort demonstrated that betatrophin concentration was elevated in type 1 diabetic patients receiving insulin therapy and in type 2 diabetic patients receiving oral hypoglycemic agents (17). However, Fenzl et al. found that type 2 diabetic patients treated with metformin, either alone or in combination with a sulfonylurea, showed similar circulating betatrophin levels compared to the normal controls (16). Taken together, any potential correlation between betatrophin concentration and anti-diabetic therapies is at present unclear (18).

In this study, the circulating betatrophin levels in diabetic patients treated with either insulin injection or with oral hypoglycemic agents, and in healthy controls were measured. In addition, the correlation between betatrophin levels with that of glucose and lipids were tested.

Material and Methods

Subjects

A total of 52 Egyptian subjects aged 40-60 years (24 males, 28 females) were included in this study, of which 36 subjects were diagnosed with DM and 16 were age- and sex-matched healthy controls. The average disease duration was 7.5±1.8 years, and the patients were classified into the insulin-treated (N=18), and oral hypoglycemic-treated (N=18) groups based on the respective monotherapies. In addition, 15 patients received metformin monotherapy and 3 were treated with metformin in combination with the sulfonylurea glimepiride. The subjects with liver, heart, and lung diseases or Cushing syndrome were excluded. The Ethics Committee of the Faculty of Medicine, Beni-Suef University (FM-BSU-REC) approved the study (declaration no: FWA00015574, approval date: 22/01/2017), which was performed in accordance with the declaration of Helsinki principles revised in 2000. All subjects provided written informed consent.

Evaluation of serum biochemical indices

Blood samples were drawn from all subjects after overnight fasting, and 2 h after a meal. The sera were separated and fasting/postprandial blood glucose, total cholesterol, HDL-cholesterol and triglycerides were measured (19) using commercially available kits (Bio-diagnostics, Giza, Egypt). The LDL-cholesterol levels were calculated as total cholesterol–HDL cholesterol–(triglycerides/5).

Betatrophin measurement

Serum betatrophin content was measured after overnight fasting by enzyme linked immunosorbent assay (human Betatrophin ELISA kit, Cat. No E3381Hu, Bioassay Technology Laboratory, China) according to the manufacturer's instructions. Briefly, 40 µL of serum samples was added to each well of a 96-well plate, and then 10 µL biotin-conjugated anti-human betatrophin antibody and

50 µL streptavidin-HRP were added. After incubating for 60 min at 37°C, the wells were washed 5 times for 5 min each and 50 µL each of the substrate solution A and solution B were added each well. Following a 10 min incubation at 37°C, 50 µL stop solution was added and the absorbance of the blue colored solution was measured at 450 nm by an ELISA plate reader. The sensitivity of the kit was 0.23 ng/mL, inter-assay precision CV<10%, and intra-assay precision was CV<8% (CV%= SD/mean × 100).

Statistical analysis

The statistical analysis was conducted using SPSS software version 22 (SPSS Inc., Chicago, Illinois). Normal distribution of the data was determined by the Kolmogorov-Smirnov test. Multiple groups were compared using the one way analysis of variance (ANOVA) followed by Tukey's post Hoc test. Correlations between betatrophin and other variables were determined using Pearson's bivariate correlation analysis followed by linear regression analysis. Data are expressed as mean±SE, and *p* values <0.05 were considered statistically significant.

Results

Demographic and biochemical characteristics

Out of the 36 diabetic patients, 18 were insulin-treated (7 males and 11 females, mean age 51.1±3.6 years) and 18 received oral hypoglycemics (10 males and 8 females, mean age 55.8±3.3 years). In addition, 16 age- and sex-matched non-diabetic controls (7 males and 9 females, mean age 49.6±5.3 years) were also included. There were no significant differences between the two patient subgroups in terms of age or disease duration. However, the insulin-treated patients showed a mean disease duration of 7.4±2.1 years and mean age of 51.1±3.6 years, which largely excludes the possibility of type 1 DM. Interestingly, no significant differences were observed in the age, and in the HDL-cholesterol, LDL-cholesterol, total cholesterol and triglyceride levels between the patients and controls, regardless of the therapeutic regimen in the former ($p>0.05$). In fact, all subjects showed high LDL-cholesterol levels (158.3±9.7 for normal, 127±9.7 for insulin-treated patients and 146±12.3 for oral hypoglycemics-treated patients), and the controls had borderline high total cholesterol (205.5±11.1 mg/dL), indicating dyslipidemia among all subjects. Furthermore, none of the established parameters of glucose or lipid metabolism showed significant differences between the two therapeutic subgroups among the diabetic patients, except HDL-cholesterol that was significantly lower in the oral hypoglycemics-treated patients compared to the insulin-treated patients (45.9±1.2 vs. 54.4±2.6, $p=0.027$). The biochemical characters of all groups are summarized in Table 1.

Table 1. The biochemical characters of the studied groups.

	Normal non-diabetics	Insulin-treated diabetics	Oral hypoglycemics-treated diabetics
M: F	7:9	7:11	10:8
Age (y.)	49.6±5.3	51.1±3.6	55.8±3.3
Disease duration (y.)	-	7.4±2.1	7.3±1.7
Fasting blood glucose (mg/dl)	83.8±2.5	158.6±18.3*	188.1±17 *
2 h. postprandial blood glucose (mg/dl)	98.2±1.2	226.6±18.3*	257.5±17.5 *
Total cholesterol (mg/dl)	205.5±11.1	181.4±9.5	191.9±12.3
HDL- cholesterol (mg/dl)	47.1±2.9	54.4±2.6	45.9±1.2[a]
LDL- cholesterol (mg/dl)	133.9±9.9	105.2±8.8	124.2±11.3
Triacylglycerol (mg/dl)	122.3±11.8	113.3±14.6	119.9±6.4

M: Male; F: female; y.: year.
Data are presented as mean±SE. One Way Analysis of Variance (ANOVA) followed by Tukey's post Hoc test was used to compare the differences among groups.
*: significant value when insulin-treated diabetics or oral hypoglycemics-treated diabetics were compared to normal non-diabetics ($p<0.001$).
[a]: significant value when insulin-treated diabetics were compared to oral hypoglycemics-treated diabetics ($p<0.05$).

Circulating betatrophin levels

The serum betatrophin levels were significantly lower in the diabetics compared to the normal subjects (40.61±3.3 vs. 54.4±3.7, p=0.02). Furthermore, both insulin-treated (32.8±3.6) and oral-hypoglycemics-treated (48.4±5.2) patients had lower betatrophin levels compared to the healthy controls, and that in the insulin-treated subgroup was significantly lower compared to the oral hypoglycemic subgroup (p=0.016). However, the hormone levels were similar between male and female patients (p=0.76). The results are summarized in Figure 1.

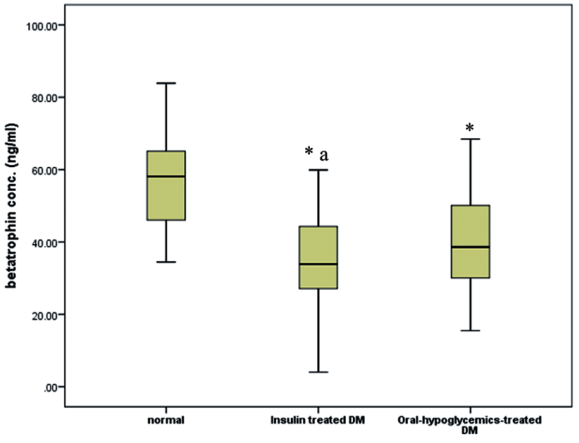

Figure 1: The circulating betatrophin concentrations among the studied groups.
Box plot shows the serum betatrophin concentrations in normal, insulin-treated DM and oral hypoglycemics-treated DM subjects. Box represents the first and third quartiles and median. Whiskers represents minimum and maximum values.
*: significant value when compared to normal subjects (p<0.001).
a: significant difference when insulin treated patients compared to oral hypoglycemic treated patients (p<0.05)

The correlation between betatrophin and lipid variables

The relationship between the circulating betatrophin levels and various biochemical parameters were evaluated by Pearson's bivariate correlation analysis. Betatrophin levels showed a significant positive correlation with total cholesterol (r=0.303, p=0.029) (Figure 2A) and LDL-cholesterol (r=0.339, p=0.014) (Figure 2B) levels in all subjects. However, no significant correlation was observed between betatrophin and fasting glucose, postprandial glucose, triglycerides, HDL-cholesterol or age (p>0.05). In the oral hypoglycemics-treated group, the betatrophin levels were positively correlated with that of triglycerides (r=0.562, p=0.015; Figure 2C). Multiple linear regression analysis showed that total cholesterol (B=0.131; SE=0.058; β=0.303, t=2.248, p=0.029), LDL-cholesterol (B=0.148; SE=0.058; β=0.39, t=2.5; p=0.014) and triglyceride (B=0.452; SE=0.166; β=0.562; t=2.7; p=0.015) levels are independent factors affecting circulating betatrophin levels.

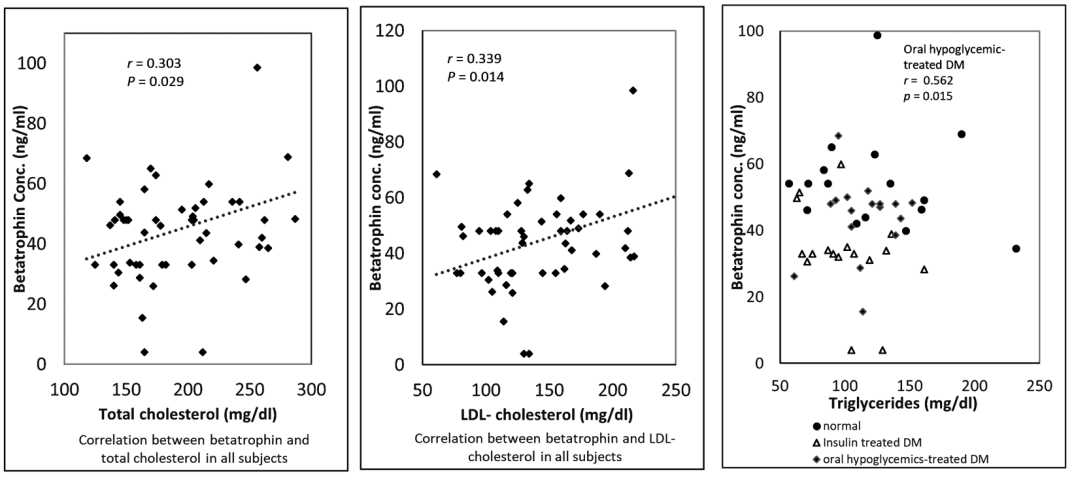

Figure 2: The correlations between circulating betatrophin and lipid parameters.
Scatter diagram shows the significant correlations between betatrophin and lipid parameters.
(A) Correlation between betatrophin and total cholesterol in all subjects; **(B)** Correlation between betatrophin and LDL-cholesterol in all subjects; **(C)** Correlation between betatrophin and triglycerides in normal, insulin treated DM and oral hypoglycemics- treated DM groups. Pearson's correlation coefficient and p values are indicated in each figure.

Discussion

This study found that circulating betatrophin levels were significantly lower in diabetic patients regardless of the therapeutic regimen, and positively correlated with various lipids but not glucose. The reports on betatrophin levels in DM are ambiguous at present (20). For example, several studies have shown higher concentrations in only type 1 (12), only type 2 (13, 14, 21, 22), and both type 1 and 2 DM (17), as well as in pregnant women with gestational diabetes (23, 24). Gómez-Ambrosi et al. measured the serum betatrophin levels in non-diabetic, glucose intolerant and type 2 diabetic obese individuals, and found an overall 40% reduction in the obese subjects compared to the non-obese controls, which was further reduced in those with impaired glucose tolerance or type 2 DM by 59% and 70%, respectively (15). However, Fenzl et al. found no difference in betatrophin levels between type 2 diabetics and non-diabetics, or between lean and morbidly obese individuals (16). Furthermore, Guo et al. found that excess body weight, and not diabetes was associated with high betatrophin levels in a cohort of type 2 DM patients (25). It is noteworthy that most studies that showed increased betatrophin levels in DM were conducted in animal models, and therefore might not be applicable to humans. For instance, Jiao et al. found that human pancreatic beta cells did not expand *in vitro* in the presence of murine betatrophin (26).

The relevance of the increased betatrophin levels in diabetes is still unknown (12). Obesity is a major driving factor of type 2 DM, with most patients exhibiting hypertriglyceridemia (27). Betatrophin has a regulatory role in triglycerides metabolism, and *Angptl8* knockout mice showed decreased plasma triglyceride levels after re-feeding (10). In addition, several studies have shown that betatrophin levels are positively correlated to that of triglycerides, HDL-cholesterol (1, 15), apolipoprotein B, total cholesterol, and LDL-cholesterol in type 2 diabetic patients (16). This study found a significant positive correlation of betatrophin with a lipid variable only in the oral hypoglycemics-treated patients. Based on these findings, the increase in betatrophin levels is likely a compensatory mechanism against hyperlipidemia and acts by reducing the plasma lipoprotein levels (15). In contrast, our and others' studies show lack of any correlation between betatrophin and lipid variables and/or glucose metabolic parameters (12, 25). In addition, Wang et al. found no change in glucose metabolism or insulin levels in *Angptl8* knockout mice (10). Finally, this study found no significant differences in betatrophin levels between males and females, in contrast to the findings of Gómez-Ambrosi *et al.,* (15).

To summarize, betatrophin levels were significantly lower in both insulin and oral hypoglycemics-treated patients compared to the normal controls, and not correlated with blood glucose levels. There were several limitations in the present study, such as the small cohort and lack of newly-diagnosed diabetic patients. In addition, the postprandial betatrophin levels should also be measured since it is known to be a nutritionally regulated factor and thereby affected by fasting/re-feeding, along with the HbA1c levels.

Conclusion

The circulating betatrophin levels were reduced in the DM patients compared to non-diabetic controls regardless of the therapy, and was positively correlated with total cholesterol, LDL-cholesterol, and triglycerides but not with the blood glucose level. Therefore, betatrophin is a potential clinical parameter for both diabetes and obesity that needs to be further investigated.

Ethics

Research involving Human Participants and/or Animals: This study was approved by the Ethics Committee of Faculty of Medicine, Beni-Suef University (FM-BSU-REC), declaration no: FWA00015574. Approval date: 22/01/2017.

Informed consent: ll participants gave a written informed consent.

Funding: This research did not receive a fund.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Rasha M. Hussein designed the experiments, measured the biochemical indices, analyzed the data and wrote the manuscript.

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