Chronic Diseases Journal

DOI: 10.22122/cdj.v10i4.668 Published by Vesnu Publications

# Serum betatrophin level of newly diagnosed and chronic diabetic patients and its relationship with metabolic parameters

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Original Article

## Abstract

**BACKGROUND:** Angiopoietin-like protein 8 (ANGPTL8) that was originally called betatrophin is a novel growth factor which is mainly secreted by the liver and associated with diabetes. Hence, it can be considered as a clinical parameter for diagnosis or treatment of diabetes. However, there are more unknown features about its relationship with diabetes that need to be clarified. The aim of this study was to understand the role of progress of diabetes on betatrophin levels. Therefore, serum betatrophin level of newly diagnosed patients and patients with chronic diabetes and its relationship with metabolic parameters were investigated.

**METHODS:** In a cross-sectional study, previous diabetic patients were compared with new diabetic patients in terms of betatrophin and other parameters, and matching on age, gender, and body mass index (BMI) was performed. New cases were recognized by endocrinologist and chronic patients were selected in diabetes center of Tohid Hospital, Sanandaj, Iran, in 2015-2016. Background information including height, weight, waist circumference, abdomen circumference, and hip circumference were collected. Fasting blood sugar (FBS) was measured by glucometer and separated blood sera were used for insulin and betatrophin measurement by enzyme-linked immunosorbent assay (ELISA) method.

**RESULTS:** The mean values of the betatrophin in the new and chronic diabetic individuals were 10.30 ± 6.31 and 10.71 ± 6.31 ng/ml, respectively, and no significant differences were seen between two groups. There was no significant relationship between betatrophin with metabolic parameters; however, a positive correlation with FBS in the newly diagnosed and a negative correlation with FBS in the chronic patients were observed.

**CONCLUSION:** Betatrophin is closely associated with glucose metabolism. It seems that chronic diabetes does not alter betatrophin levels.

**KEYWORDS:** Betatrophin; Diabetes Mellitus Type 2; Enzyme-Linked Immunosorbent Assay

***Date of submission:*** 20 Dec. 2021, ***Date of acceptance:*** 01 June 2022

**Citation:** Vakili N, Hosseini F, Ghaderi E, Saed L, Nikkhoo B, Shakiba N, et al. **Serum betatrophin level of newly diagnosed and chronic diabetic patients and its relationship with metabolic parameters.** Chron Dis J 2022; 10(4): 196-202.

## Introduction

Diabetes prevalence is dramatically increasing all over the world.[1] According to the most recent International Diabetes Federation (IDF) data, 537 million of all people aged 20-79 in the world were living with diabetes as of 2021 and it is expected to rise to 643 million by 2030 and to 783 million by 2045. It is estimated that 240 million of those who have diabetes are

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undiagnosed.[2] Although many factors have been proposed in the pathogenesis of diabetes, there is still room to reveal all involved mechanisms in different types of diabetes, and people are waiting for better and more effective treatment. Betatrophin, also known as angiopoietin-like protein 8 (ANGPTL8) and lipasin, is a newly identified hormone mainly secreted by the liver and adipose tissue. Betatrophin is a possible beta cell regenerative factor[3] and a glucose tolerance improvement agent.[4] This hormone has a vital role in lipid metabolism.[5] Investigations have shown that betatrophin levels were higher in patients with type 1 diabetes mellitus (T1DM),[6] type 2 diabetes mellitus (T2DM),[7] obesity,[8] and metabolic syndrome[9] than in normal people. Furthermore, the betatrophin level may be a predictive factor in the first stage of diabetes.[10] Conversely, some opposite results have been reported. For instance, betatrophin expression was upregulated in the liver and adipose tissue of mice with T2DM, while it was downregulated in white adipose tissue (WAT) of mice with T1DM.[11] In another study, the circulating level of human betatrophin was higher in patients with T1DM, without any effect on insulin reduction,[10] while its level was lower in obese and insulin-resistant cases.[12] Besides, in Abu-Farha et al. study, betatrophin level was elevated in obese subjects, which was related to C-peptide level elevation, as a possible cause of insulin resistance.[13] Despite Tuhan et al.[12] and Abu-Farha et al.[13] research, Roth et al.'s study found no relationship between betatrophin and glucose tolerance, insulin resistance, and lipid metabolism in obese children.[14] The elevated levels of betatrophin in obese adults decreased after physical activities,[15] but lower levels of betatrophin were reported in obese subjects in the Maurer et al. study, and its concentration was restored after weight loss.[8] Moreover, higher plasma betatrophin levels were reported in anorexic patients than in obese subjects.[16]

Based on the mentioned results obtained from different studies, wide variations and contradictory results can be seen regarding the serum levels of betatrophin and diabetes, indicating that various factors influence plasma betatrophin concentration in patients with diabetes. Furthermore, the betatrophin levels were higher in newly diagnosed patients with T2DM than in normal individuals based on some studies.[17-19] Regarding the reported association of betatrophin with diabetes stages,[20] betatrophin level and its relationship with metabolic parameters can be likely applied to understand some important points in diagnosis or treatment of diabetes. In addition, the previous studies have compared betatrophin levels between normal people and patients with obesity or diabetes. Therefore, for the first time, the present study was designed to compare betatrophin concentration between new individuals with diabetes and patients with chronic T2DM to assess the role of diabetes progression in serum betatrophin levels. Therefore, serum betatrophin level of newly diagnosed patients and patients with chronic diabetes and its relationship with metabolic parameters were investigated.

## Methods

This cross-sectional study recruited two groups of new patients with diabetes (80 cases) that were diagnosed by endocrinologist and patients with chronic diabetes (82 cases) who had visited the physician in the diabetes center of Tohid Hospital in Sanandaj, Iran, in 2015-2016. Referred chronic cases to the center were matched with new previously-diagnosed cases in terms of age, gender, and body mass index (BMI). The unmatched cases were excluded and the matched cases were selected for further investigation in this study. This method was used until all samples were collected. Written consent was obtained from

the patients who participated in this survey. Exclusion criteria were gestational diabetes, couching syndrome, corticosteroid consumption, liver failure, acromegaly, and lupus erythematous. Data included hip, abdomen, and waist circumferences, weight, height, and other background information. The waist-to-hip ratio (WHR) was obtained, and BMI was calculated by dividing the weight by the height squared (kg/m$^2$). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated by multiplying fasting blood glucose by fasting insulin content, divided by 405.[21] Since betatrophin levels are influenced by different factors, the two groups were matched in terms of age, gender, and BMI.

*Laboratory analysis:* Fasting blood glucose was measured by glucose oxidase method after 12-hour overnight fasting using glucometer (Nova Max Plus, USA). Blood samples were taken from 8:00-9:00 a.m. every day. After leaving blood samples at room temperature for 30 minutes, the serum was separated and transferred to a -80 °C freezer (centrifugation at 3000 rpm). When all samples were collected, betatrophin was measured by an enzyme-linked immunosorbent assay (ELISA) kit provided by ZellBio Company (ZellBio GmbH, Cat. No. ZB-13381S-H9648, Germany) with 0.2 ng/ml sensitivity. Insulin was measured using Monobind Inc. ELISA kit (Product Code: 5825-300, USA).

*Statistical analysis:* Data were reported as mean ± standard deviation (SD), frequency, and percentage. Statistical analysis was performed using SPSS software (version 19, SPSS Inc., Chicago, IL, USA). Differences in qualitative variables between the groups were determined by the chi-square test, and quantitative variables were compared using an independent samples t-test. Furthermore, the correlation between two quantitative variables was analysed using Pearson's coefficient. The Ethics Committee of Kurdistan University of Medical Sciences, Sanandaj, approved the study (MUK.REC.1394.153).

## Results

Figure 1 shows the standard curves for betatrophin and insulin, indicating that the procedure was standard.

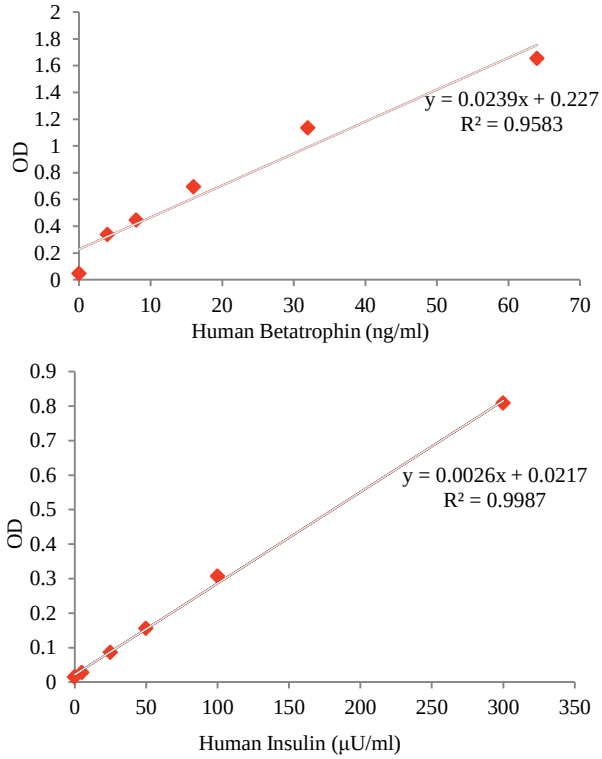


**Figure 1. Standard curve for betatrophin and insulin**

This study enrolled 80 new and 82 chronic cases, including 75 male patients (46.3%). There was no statistically significant difference between the new and chronic case groups regarding gender, age, and BMI. However, mean waist circumference, WHR, and HOMA-IR were significantly higher in new cases than in chronic cases (Table 1).

The mean betatrophin levels in the new and chronic case groups were 10.30 ± 6.31 and 10.71 ± 6.31 ng/ml, respectively (P = 0.652).

There was no statistically significant correlation between betatrophin levels and insulin, age, BMI, and HOMA-IR (Table 2).

**Table 1. Comparison of some variables between the two groups of diabetes**

| Variables | New cases | Chronic cases | P |
|---|---|---|---|
| Sex | | | |
| Male | 39 (48.8) | 36 (43.9) | 0.536 |
| Female | 41 (51.3) | 46 (56.1) | |
| Complication | | | |
| Cardiovascular | 13 (72.2) | 4 (26.7) | |
| Renal | 2 (11.1) | 8 (53.3) | |
| Ocular | 2 (11.1) | 3 (20.0) | |
| Other | 1 (5.6) | 0 (0) | |
| Family history of diabetes | | | |
| Yes | 16 (21.9) | 31 (37.8) | 0.032† |
| No | 57 (78.1) | 51 (62.2) | |
| Underling diseases | | | |
| Hypertension | 15 (65.2) | 1 (100) | |
| COPD | 6 (28.5) | 0 (0) | - |
| Cataract | 2 (6.3) | 0 (0) | |
| Age (year) | 52.04 ± 10.88 | 51.15 ± 10.76 | 0.603 |
| Height (cm) | 164.71 ± 8.53 | 162.59 ± 8.84 | 0.129 |
| Weight (kg) | 74.57 ± 11.31 | 73.62 ± 10.69 | 0.589 |
| Waist circumference (cm) | 98.00 ± 8.87 | 94.82 ± 7.58 | 0.011†† |
| Abdomen circumference (cm) | 107.71 ± 7.74 | 105.28 ± 8.67 | 0.069 |
| Hip circumference (cm) | 108.52 ± 7.36 | 109.18 ± 7.79 | 0.589 |
| BMI ($kg/m^2$) | 27.43 ± 3.20 | 27.80 ± 2.90 | 0.457 |
| WHR | 0.90 ± 0.05 | 0.86 ± 0.04 | < 0.001†† |
| HOMA-IR | 6.21 ± 7.00 | 3.96 ± 4.47 | 0.015†† |
| BUN (mg/dl) | 26.96 ± 17.53 | 34.45 ± 10.26 | 0.074 |
| FBS (mg/dl) | 170.36 ± 57.03 | 164.27 ± 98.15 | 0.629 |
| Insulin level (µU/ml) | 14.48 ± 3.82 | 11.14 ± 6.05 | 0.052 |
| Betatrophin (ng/ml) | 10.30 ± 6.31 | 10.71 ± 6.31 | 0.652 |

Data are reported as mean ± standard deviation (SD) or number and percentage
†Statistically significant difference using chi-square test; ††Statistically significant difference using independent t-test
COPD: Chronic obstructive pulmonary disease; BMI: Body mass index; WHR: Waist-to-hip ratio; HOMA-IR: Homeostatic model assessment of insulin resistance; BUN: Blood urea nitrogen; FBS: Fasting blood sugar

**Table 2. Correlation between betatrophin and quantitative variables**

| Variables | New cases [r (P)] | Chronic cases [r (P)] |
|---|---|---|
| FBS | 0.125 (0.271) | -0.150 (0.191) |
| Insulin | 0.046 (0.683) | -0.012 (0.923) |
| Age | 0.025 (0.829) | 0.002 (0.983) |
| Height | 0.127 (0.277) | -0.116 (0.916) |
| Weight | 0.014 (0.906) | -0.014 (0.902) |
| Waist circumference | -0.080 (0.502) | 0.067 (0.563) |
| Abdomen circumference | -0.150 (0.204) | 0.077 (0.507) |
| Hip circumference | -0.152 (0.199) | 0.066 (0.566) |
| Cholesterol | 0.059 (0.691) | -0.097 (0.526) |
| HDL | -0.233 (0.154) | 0.017 (0.915) |
| BMI | -0.105 (0.371) | 0.107 (0.356) |
| WHR | 0.073 (0.541) | 0.023 (0.842) |
| HOMA-IR | 0.074 (0.516) | -0.158 (0.169) |

There was no statistically significant correlation between the variables and betatrophin level. r: Pearson correlation coefficient; FBS: Fasting blood sugar; HDL: High-density lipoprotein; BMI: Body mass index; WHR: Waist-to-hip ratio; HOMA-IR: Homeostatic model assessment of insulin resistance

The correlation between fasting blood sugar (FBS) and betatrophin was positive in the new case group and negative in the chronic group; however, this correlation was not statistically significant (Figure 2).

## Discussion

Betatrophin is a liver-derived hormone playing a crucial role in the pathophysiology of diabetes. This study aimed at determining any differences between serum betatrophin level of new patients and patients with chronic diabetes and also its relationship with metabolic criteria, leading to use of betatrophin as a diagnostic or therapeutic factor.

Based on this goal, results of the present study illustrated that the mean waist

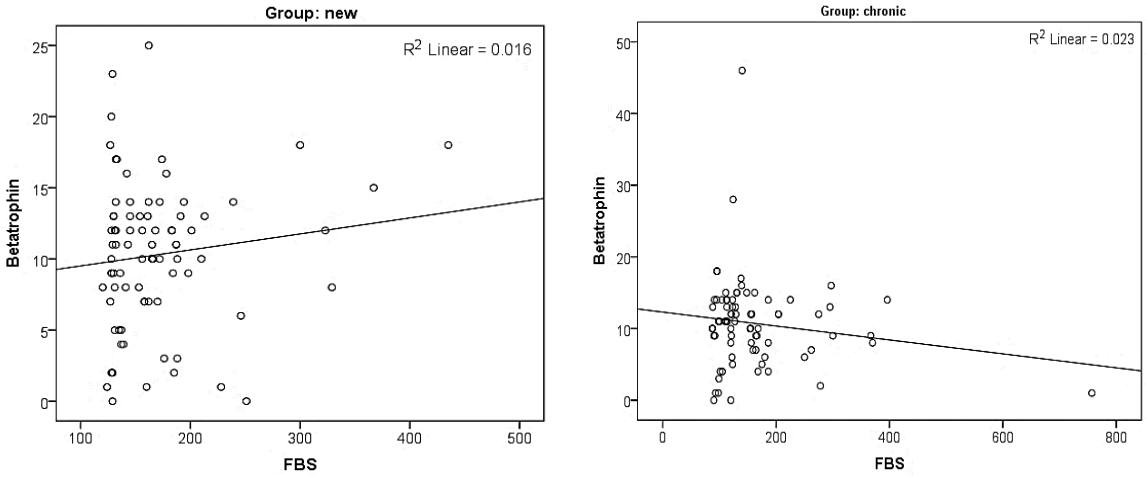


**Figure 2. The correlation between fasting blood sugar (FBS) and betatrophin**

circumference, WHR, and HOMA-IR were significantly higher in new cases than in chronic patients. These factors had probably changed since the chronic group was under long-term treatment for hyperlipidemia and high blood glucose. No difference has been observed in serum betatrophin levels between individuals with a new diagnosis of diabetes and patients with chronic diabetes. Similarly, Fenzl et al.'s research found no differences in plasma betatrophin levels between diabetic and normal people.[22] In this study, different parameters, including age, gender, and BMI that impact betatrophin levels, were matched between the two groups. Some studies showed the contrary results such as Gomez-Ambrosi et al. that revealed the higher levels of betatrophin among patients with T2DM,[5] while its lower levels were reported in obese individuals and insulin resistance condition by Liu et al.[23] In the present study, it seems that the dispersion of glucose concentration in the two groups was almost constant; this factor had likely influenced betatrophin levels. On the other hand, since the diabetes prevalence has risen, the new cases have probably had diabetes for a long period without treatment, which impressed the betatrophin level.

In addition, no correlations were seen between betatrophin levels and metabolic indices. Fenzl et al.'s report obtained the same results in patients with insulin resistance.[22] Although betatrophin concentration was higher in patients with diabetes in Wang et al. study, they similarly reported no relationship between betatrophin and fasting blood glucose, hemoglobin A1c (HbA1c), and insulin resistance in previously-diagnosed patients with T2DM.[24] It is also similar to the results of the Abu-Farha et al.'s study among patients with T2DM.[25] In the study by Guo et al., serum betatrophin levels were elevated in overweight but not in obese individuals or those with T2DM. Betatrophin concentrations were not associated with gender, glucose status, or diabetes in this research.[26] Betatrophin is strongly associated with FBS and insulin resistance in non-diabetic individuals. However, FBS and insulin resistance are not correlated to betatrophin in subjects with T2DM.[25] These results are similar to the present study as well.

The mean waist circumference, WHR, and HOMA-IR were significantly higher in new cases than in chronic subjects, but there were no correlations between the betatrophin levels of new and chronic diabetic groups. Although the correlation between FBS and betatrophin was not significant in this survey, its relationship was positive in the new case

group and inversely negative in the chronic group. Similarly, Abu-Farha et al. showed the correlation between betatrophin and fasting glucose in patients with T2DM.[25] Although a positive correlation has been reported in some studies, plasma betatrophin has been negatively correlated with weight, BMI, glucose, and insulin and positively correlated with high-density lipoprotein (HDL).[16] In Liu et al.'s study, there was no relationship between serum betatrophin and the first phase of glucose-stimulated insulin secretion.[23] Besides, there were no higher betatrophin levels in new cases in the present study.

The limitation of the study was finding the new cases of diabetes. Since the duration of diabetes may influence the level of betatrophin, diagnosis of new patients is a crucial factor. Suggestions for further studies include the comparison of plasma betatrophin levels between normal individuals, newly-diagnosed patients, and patients with chronic diabetes. In addition, betatrophin relationship with diabetic medications can be investigated in human or animal models.

## Conclusion

Although betatrophin has been known as a biomarker for onset of diabetes, the present study for the first time showed that the concentration of serum betatrophin does not change in lapse of time in chronic diabetes as compared to newly-emerging diabetes.

## Conflict of Interests

Authors have no conflict of interests.

## Acknowledgments

The authors thank all subjects for their participation in this study and the lab technicians for their assistance.

## Financials support and sponsorship

The study was funded by Kurdistan University of Medical Sciences (Grant No. 94-77).

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