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Correlation of serum betatrophin levels with disease severity and the emergence of insulin resistance in cirrhotic patients

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Abstract

Background: Insulin resistance (IR) is frequently associated with chronic liver disease. There has been an increased interest in betatrophin protein and its involvement in the compensatory response to IR. We aimed to investigate the correlation of serum betatrophin levels with disease severity and the emergence of IR in cirrhotic patients. This study included 27 cirrhotic patients and 30 healthy participants who served as a control group. IR was assessed by the Homeostasis Model Assessment (HOMA-IR). Serum insulin and betatrophin levels were measured using Enzyme-Linked Immunosorbent Assay (ELISA).

Results: IR was existing in 74% of cirrhotic patients ($p < 0.001$). Subjects with IR had higher serum betatrophin levels than those without IR ($p = 0.04$). Serum betatrophin levels were significantly higher in cirrhotic patients than controls ($p < 0.001$). In addition, Child-Pugh class C patients had higher serum betatrophin levels than those with Child-Pugh class B cirrhosis ($p = 0.01$). Moreover, the highest serum betatrophin levels were detected in patients with tense ascites followed by those with moderate and mild ascites ($p = 0.01$). In the cirrhosis group, serum betatrophin levels correlated positively with fasting blood glucose levels ($p < 0.001$), fasting insulin levels ($p = 0.006$), HOMA-IR ($p = 0.006$), Child-Pugh score ($p = 0.023$), MELD score ($p < 0.001$), and INR ($p = 0.005$), and correlated negatively with platelets count ($p = 0.01$).

Conclusion: Cirrhotic patients have higher serum betatrophin levels; moreover, these levels are positively correlated with disease severity as well as the emergence of insulin resistance.

Keywords: Liver cirrhosis, Insulin resistance, Human betatrophin protein

Background

The liver plays a vital role in glucose homeostasis; consequently, chronic liver disease results in disturbances in glucose metabolism. Insulin resistance (IR) is common in cirrhotic patients; it was reported that 57% of cirrhotic patients had IR [1]. This phenomenon was observed in cirrhotic patients even before the disturbance of glucose tolerance became prominent. Compensation for this hormonal resistance occurs by increasing the secretory capacity and β-cell mass [2, 3].

Factors accounting for IR in the context of cirrhosis remain mostly undefined, although there has been evidence that there is a circulating factor associated with insulin-resistant states [4, 5]. The identification of betatrophin hormone was reported by Douglas A. Melton's group [6]. It is a member of angiopoietin-like gene family (known as angiopoietin-like 8 (ANGPTL8)/Lipasin/refeeding-induced fat and liver protein (RIFL) [7]. It is secreted under insulin-resistant conditions mainly from adipose tissue and liver [8, 9]. There has been an increasing interest in serum betatrophin to better understand its role in human disease. It was reported that serum betatrophin levels were altered under specific physiological states such as the postprandial state [10]

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and pathological conditions such as type 1 and 2 diabetes [9–14].

The role of betatrophin in cirrhosis is still unknown, and scarce studies demonstrated the correlation of serum betatrophin levels and liver cirrhosis of different severities [15]. This study aimed to investigate the correlation of serum betatrophin levels with disease severity and the emergence of insulin resistance in cirrhotic patients.

Methods

This case-control study was carried out at Ain Shams University Hospitals. The participants were recruited from the outpatient clinic and in-patient ward of gastroenterology and hepatology unit of the internal medicine department, during the period from May 2017 till May 2018. Approval was obtained from the Ethics Committee of Faculty of Medicine, Ain Shams University. Informed written consent was obtained from each participant before enrollment in the study. This study was performed in accordance with the 1975 principles of the Declaration of Helsinki and its appendices.

Twenty-seven cirrhotic patients and 30 healthy controls with matched age and sex were consecutively enrolled in the study. Cirrhosis was diagnosed based on clinical, biochemical, ultrasonographic, or histological criteria. The exclusion criteria were patients who had undergone previous surgery for portal hypertension, patients suffering from bacterial infection or gastrointestinal bleeding, patients receiving vasoactive drugs within 14 days before the study, patients receiving medications known to affect body composition or lipid or glucose metabolism (e.g., thyroid medications, thiazolidinediones, metformin), and patients with renal disease, diabetes mellitus, renal failure, hypothyroidism, or Cushing's disease.

All participants were subjected to a detailed history taking, a thorough clinical examination, pelvi-abdominal ultrasound, and laboratory investigations including complete liver function tests, complete blood count, kidney function tests, fasting glucose level, and international normalized ratio (INR). The severity of cirrhosis was classified according to the Child-Pugh classification and the model for end-stage liver disease (MELD) scores [16, 17].

Serum insulin levels were measured using a recombinant human insulin Enzyme-Linked Immunosorbent Assay (ELISA) kit (Calbiotech®, CA, USA) with a standard detection range of 6.25–50 μIU/mL and a sensitivity of 6.25 μIU/mL. IR was defined as a HOMA-IR score of greater than 2 according to Matthews et al. [18].

Human active betatrophin level was analyzed by a specific quantitative sandwich ELISA kit (Aviscera Bioscience® AB, CA, USA). The sensitivity of the assay was 0.4 ng/mL, and the intra and inter-assay reproducibility were < 6% and < 10%, respectively.

Table 1 Comparison between liver cirrhosis patients and controls

Variable	Liver cirrhosis (n = 30)	Control (n = 30)	p value
Age (years)	58 (50–65)	52.5 (43–60)	0.032
Fasting glucose level (mg/dL)	103 ± 21.70	81 ± 22.55	0.008
Fasting insulin level (μIU/mL)	16 (10–37)	3.25 (2–9)	< 0.001
HOMA-IR	4.3 (0.5–9.65)	0.65 (0.3–1.9)	0.003
Insulin resistance	n = 20 (74%)	n = 7 (23.3%)	< 0.001
Serum betatrophin level (ng/mL)	20 (15–30)	8 (4–12)	< 0.001

Data are shown as median (IQR), mean ± SD, or number and percentage (n & %)

Statistical analysis

Data were analyzed using Stata® version 14.2 (StataCorp LLC, College Station, TX, USA). Normally distributed numerical data were presented as mean ± SD, and intergroup differences were compared using Student's t test. Non-normally distributed numerical data were presented as median and interquartile range (IQR), and intergroup differences were compared using the Mann-Whitney U test or Kruskal-Wallis test, as appropriate. Categorical data were presented as number and percentage, and differences were compared using Fisher's exact test (for nominal data) or the chi-squared test (for ordinal data). Multivariable binary logistic regression analysis was used to examine the relation between betatrophin and other variables as adjusted for possible confounding factors. Correlations were tested using the Spearman rank correlation. Receiver-operating characteristic (ROC) curve analysis was used to examine the diagnostic value of betatrophin in the prediction of IR. p value < 0.05 was considered statistically significant.

Results

The current study included 27 cirrhotic patients and 30 controls with a median age of 58 (50–65) and 52.5 (43–60) years, respectively (Table 1). All of them were males with hepatitis C virus (HCV)-related liver cirrhosis. The cirrhosis group included 12 (44.4%) and 15 (55.5%) patients with Child-Pugh class B and C, respectively. Eight, 12, and 7 cirrhotic patients had tense, moderate, and mild ascites, respectively.

Table 2 Determinants of insulin resistance

Variable	p value	Odds ratio	95% CI	
			Lower	Upper
Age	0.808	0.97	0.82	1.16
Cirrhosis	< 0.001	9.38	2.80	31.38
Serum betatrophin level	0.663	1.06	0.80	1.41

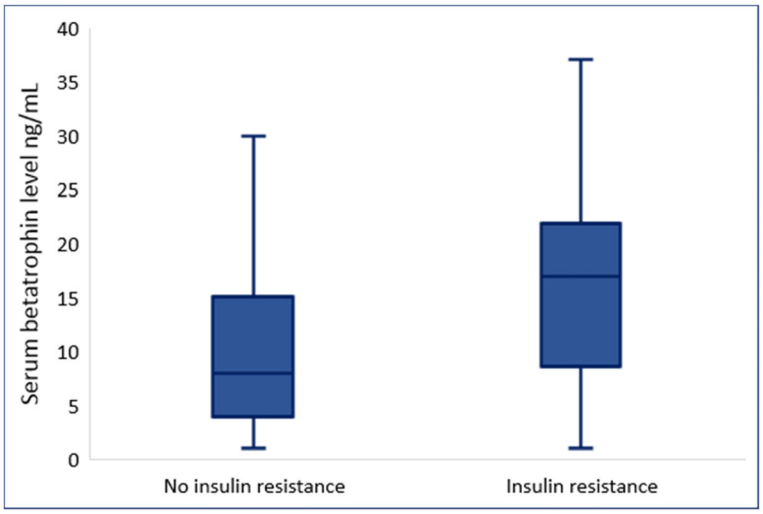

Fig. 1 Box plot showing serum betatrophin levels in subjects with and without insulin resistance

IR was observed in 20 (74%) cirrhotic patients, whereas only 7 (23.3%) controls had IR (Table 1). Moreover, Child class C patients had higher HOMA-IR than those with Child class B cirrhosis; however, this difference was statistically insignificant [5.4 (1.15–12.3) vs 3 (0.5–7.22), respectively, p = 0.07]. Determinants of IR are shown in Table 2. Subjects with IR had higher serum betatrophin levels than those without IR with a median level (IQR) of 20 (12–30) *vs* 8 (4–15) ng/mL, respectively ($p < 0.001$) (Fig. 1).

There was a statistically significant difference between cirrhotic patients and controls regarding serum betatrophin levels, fasting glucose levels, fasting insulin levels, HOMA-IR, and the prevalence of IR (Table 1, Fig. 2). Moreover, Child-Pugh class C patients had a significantly higher serum betatrophin levels than those with Child-Pugh class B cirrhosis [22.25 (15.50–53.75) vs 15.50 (8.87–21.37) ng/mL, respectively, p = 0.01] (Fig. 2). Additionally, the highest serum betatrophin levels were detected in patients with tense ascites followed by moderate and mild ascites [37 (15–60) vs 17.50 (15–30) *vs* 13 (2.5–22.5) ng/mL, respectively, p = 0.01] (Fig. 3).

In the cirrhosis group, serum betatrophin levels correlated positively with Child-Pugh score, MELD score, INR, fasting glucose levels, fasting insulin levels, and HOMA-IR and correlated negatively with platelets count (Table 3).

By ROC curve analysis, serum betatrophin cut-off level of > 16 ng/mL discriminated IR with AUROC of 0.759, 95% CI = 0.631–0.860, $p < 0.001$, 64.3% sensitivity, and 90.6% specificity (Fig. 4).

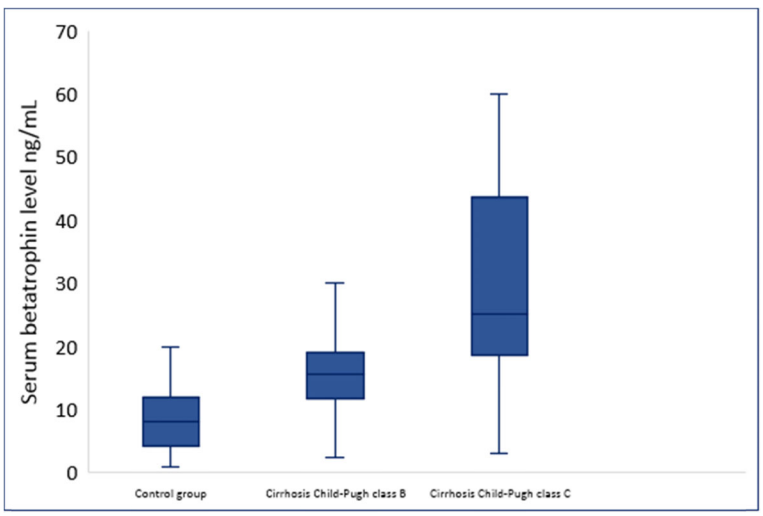

Fig. 2 Box plot showing serum betatrophin levels in patients with Child B and C cirrhosis and controls

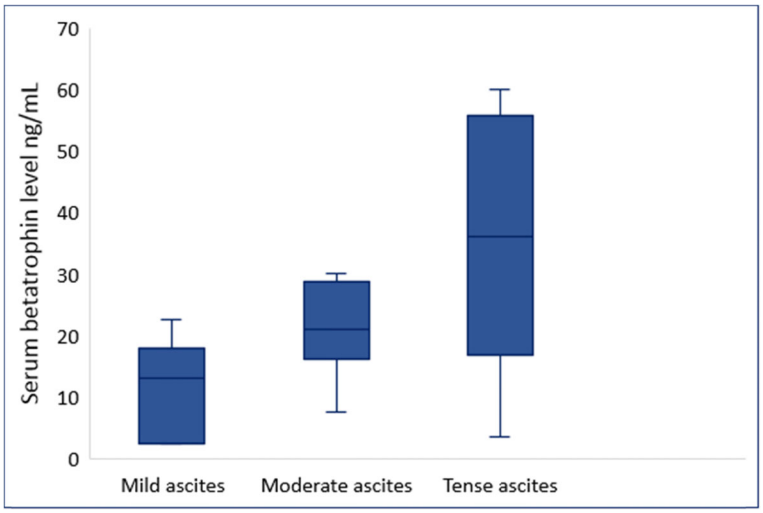

Fig. 3 Box plot showing serum betatrophin levels according to the degree of ascites

Discussion

Betatrophin is a novel protein that can enhance pancreatic islet β-cell mass resulting in improvement of glucose tolerance in mice models with IR [19]. Moreover, a correlation between serum betatrophin levels and IR indices was detected [4, 7, 13].

It was postulated that IR may have a role in the early stages of hepatic disease progression [20]. There is evidence that insulin has a contribution to the pathogenesis of hepatic fibrosis and clinically significant portal hypertension through inducing the proliferation of hepatic stellate cells and modulating the endothelial synthesis of nitric oxide and endothelin [21–24]. Furthermore, IR was independently associated with significant fibrosis [25, 26], an increased risk of death, liver transplantation [27], and the development of hepatocellular carcinoma in chronic HCV patients [28].

Table 3 Correlations between serum betatrophin level and other variables in the cirrhosis group

Variable	Serum betatrophin level	
	Correlation coefficient	*p* value
Hemoglobin	− 0.003	0.985
Total leucocytic count	− 0.215	0.280
Platelets count	− 0.468	0.013
Serum albumin	− 0.041	0.837
International normalized ratio	0.522	0.005
Total bilirubin	0.294	0.136
Child-Pugh score	0.435	0.023
MELD score	0.593	0.001
Fasting blood glucose level	0.589	0.001
Fasting insulin level	0.511	0.006
HOMA-IR	0.508	0.006

The present study aimed to investigate the relationship between serum betatrophin levels and IR in cirrhotic patients and its relevance with the severity of the disease.

The present study confirms that IR is a frequent phenomenon in cirrhotic patients, and it is found in 74% of our patients. Also, there was a statistically significant difference in fasting blood glucose and insulin concentrations between cirrhotic patients and healthy controls. These findings agree with previous reports [1, 3, 15, 24]. Additionally, in accordance with previous results [11], this study proves by using a multivariable binary logistic regression analysis the relationship between IR and cirrhosis.

Several mechanisms of hyperinsulinemia in chronic liver disease have been postulated. The most pronounced is an insufficient insulin clearance due to reduced hepatocellular function. Hyperinsulinemia will be aggravated with disease progression due to further impairment of hepatic function and portal hypertension-related portosystemic shunting of insulin [29, 30]. However, Greco et al. [31] suggested that increased serum insulin level is the result of increased β-cell sensitivity to glucose, whereas hepatic insulin extraction did not seem to contribute significantly in this condition. Chronic hyperinsulinemia then leads to insulin resistance via the desensitization and downregulation of insulin receptors.

In agreement with previous results [7, 9], candidates who had IR had higher serum betatrophin levels than candidates without IR. Moreover, serum betatrophin levels correlated positively with IR indices and the HOMA-IR model.

Serum betatrophin levels were higher in cirrhotic patients compared to those of controls as reported previously [15]; moreover, Child-Pugh class C patients had higher serum betatrophin levels than Child-Pugh class B

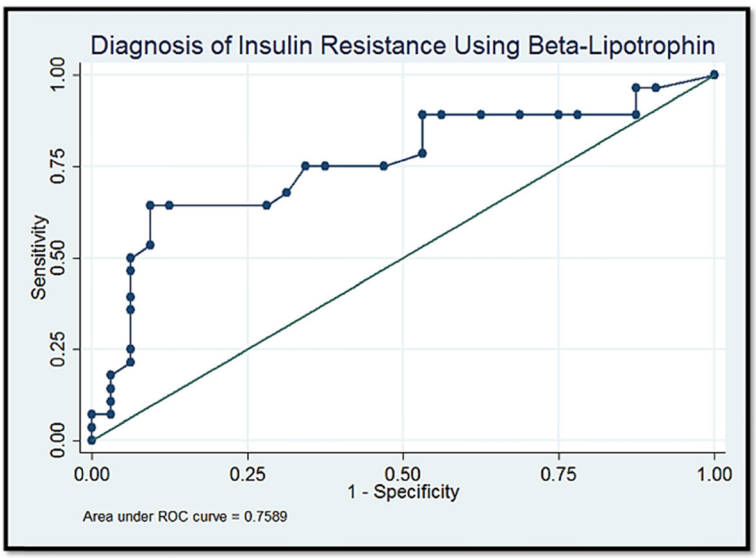

Fig. 4 ROC curve demonstrating the diagnostic performance of serum betatrophin in the prediction of insulin resistance

patients. Additionally, the present study is the first study to correlate between ascites and betatrophin levels; patients with tense ascites had the highest serum betatrophin levels followed by patients with moderate and mild ascites.

In the cirrhosis group, serum betatrophin levels correlated positively with Child-Pugh score, MELD score, and INR, and correlated negatively with platelets count. This is consistent with Arias-Loste et al. [15]. All the previous findings suggest that the serum betatrophin level is correlated with the severity of liver disease, and this may suggest that impaired clearance of betatrophin could contribute to increased serum betatrophin levels. Another possibility is that the increased betatrophin expression in liver and fat tissue in cirrhotic patients could be attributed to IR [14, 19, 32]. These preliminary results indicate that betatrophin may counterbalance, at least in part, IR in cirrhotic patients. Further studies are needed to confirm this possibility and to investigate the exact pathophysiology and the clinical application of this conclusion.

The present study is limited because we did not investigate the causal relationship between serum betatrophin and IR in cirrhotic patients, and only an association between both variables can be concluded.

Conclusion

Cirrhotic patients have higher serum betatrophin levels; moreover, these levels are positively correlated with disease severity as well as the emergence of insulin resistance. Further studies are needed to clarify the ultimate clinical utility of serum betatrophin in liver cirrhosis and glucose homeostasis.

Abbreviations

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; INR: International normalized ratio; IR: Insulin resistance; MELD: Model for end-stage liver disease

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Authors' contributions

MS contributed in the conception and design of the work and the revision of the manuscript. WK contributed in the revision of the work and language polishing of the manuscript. SH contributed in the collection of data and in performing the statistical part of the work. GM contributed in the writing of the manuscript, revision of the work, and the publication process. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Approval was obtained from the Ethics Committee of Faculty of Medicine, Ain Shams University (FWA 000017585). Informed written consent was obtained from each participant before enrollment in the study. This study was performed in accordance with the 1975 principles of the Declaration of Helsinki and its appendices.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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