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EVALUATION OF ADROPIN AND PHOENIXIN-14 LEVELS IN WOMEN WITH EUTHYROID HASHIMOTO'S THYROIDITIS

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ABSTRACT

Objective: *Hashimoto's thyroiditis (HT) is an autoimmune disease that is common worldwide and may result in metabolic disorders. Adropin and phoenixin (PNX) are newly discovered peptides that play a role in the regulation of metabolic homeostasis in many tissues. The aim of the study was to investigate peripheral circulating levels of these two peptides in women with euthyroid HT and their relationship with the clinical parameters of these patients.*

Material and Method: *This case control study performed in 40 euthyroid women with HT and 38 age-matched healthy controls. Serum adropin and PNX-14 levels were determined by ELISA method.*

Results: *We found that there was no statistically significant difference between the patient and control groups regarding AST, ALT, fasting serum glucose, fasting insulin, triglyceride, HDL-C, HOMA-IR, free T3, free T4, TSH, adropin and PNX levels ($p>0.05$). LDL-C, total cholesterol, BMI values were higher than those of controls ($p<0.05$, $p<0.005$, $p<0.001$ respectively). AntiTPO, AntiTG levels were significantly higher than those of controls ($p<0.001$). There was no correlation between PNX-14 and adropin values and clinical parameters.*

Conclusion: *In this study, we found that there were no significant differences in PNX-14 and adropin concentrations between the euthyroid HT and control groups. Our study is important in that is the first study to examine adropin and pnx-14 levels in euthyroid HT.*

Keywords: *Adropin, phoenixin-14, Hashimoto's thyroiditis.*

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ÖTİROİD HASHİMOTO TİROİDİTLİ KADINLARDA ADROPİN VE PHOENİXİN-14 DÜZEYLERİNİN DEĞERLENDİRİLMESİ

ÖZET

Amaç: Hashimoto tiroiditi (HT) dünya çapında yaygın olarak görülen, metabolik bozukluklara yol açabilen otoimmün bir hastalıktır. Adropin ve phoenixin (PNX), birçok dokuda metabolik homeostazın düzenlenmesinde rol oynayan yeni keşfedilen peptitlerdir. Çalışmanın amacı, ötiroid HT'li kadınlarda bu iki peptidin periferik dolaşımdaki düzeylerini ve bu hastaların klinik parametreleri ile ilişkilerini araştırmaktır.

Materyal ve Metot: Bu vaka kontrol çalışması HT'li 40 ötiroid kadın ve yaşları eşleştirilmiş 38 sağlıklı kontrolde gerçekleştirildi. Serum adropin ve PNX-14 düzeyleri ELISA yöntemiyle belirlendi.

Bulgular: Hasta ve kontrol grupları arasında AST, ALT, açlık serum glukozu, açlık insülini, trigliserit, HDL kolesterol, HOMA-IR, serbest T3, serbest T4, TSH, adropin ve PNX açısından istatistiksel olarak anlamlı fark olmadığını tespit ettik (p>0,05). LDL kolesterol, total kolesterol, BMI değerleri kontrollerden yüksekti (sırasıyla p<0,05, p<0,005, p<0,001). AntiTPO, AntiTG düzeyleri kontrollere göre anlamlı derecede yüksekti (p<0,001). PNX-14 ve adropin değerleri ile klinik parametreler arasında korelasyon bulunmadı.

Sonuç: Bu çalışmada ötiroid HT ve kontrol grupları arasında PNX-14 ve adropin konsantrasyonları açısından anlamlı bir fark olmadığını bulduk. Çalışmamız ötiroid HT'de adropin ve PNX-14 düzeylerini inceleyen ilk çalışma olması açısından önemlidir.

Anahtar kelimeler: Adropin, phoenixin-14, Hashimoto tiroiditi.

INTRODUCTION

Hashimoto's thyroiditis (HT) is known as an autoimmune disorder characterized by lymphocytic infiltration, thyroid follicular cell destruction, and the presence of thyroid autoantibodies.[1] HT is common worldwide, with a higher incidence in low- and middle-income countries. Women are affected by this disease about 4 times more often than men.[2] Genetic, epigenetic and environmental factors are effective in the development of Hashimoto's thyroiditis.[1,2] Clinically, Hashimoto's thyroiditis presents with systemic manifestations due to hypothyroidism that develops with thyroid gland damage. However, early in the course of the disease, patients may show symptoms of hyperthyroidism or have normal laboratory findings. Diagnosis is difficult and often delayed. Basically, the diagnosis is made by the presence of antibodies to thyroid peroxidase (anti-TPO) and antibodies to thyroglobulin (anti-TG).[1,3] HT causes disorders in the functioning of the thyroid gland, whose hormones have important effects on other organs and tissues. Patients with autoimmune thyroid disease are prone to lipid metabolism, glocose metabolism disorder and inflamation.[4] Due to its high prevalence, it is necessary to carry out studies to evaluate risk factors that may lead to the development of HT and its metabolic complications.

Adropin and phoenixin (PNX) are newly discovered peptides that have a role in the regulation of metabolic homeostasis in many tissues.[5] Adropin is a peptide encoded by the energy homeostasis-associated gene (ENHO).[6] The clinical significance of adropine levels is being investigated in different patient groups. Circulating levels of adropin have been associated with metabolic disorders such as obesity, insulin resistance, metabolic syndrome, type 2 diabetes, and cardiovascular disorders.[7] Adropin increases insulin sensitivity. High adropin level is associated with lower BMI and better metabolic control.[7,8] PNX is a neuropeptide that has been shown to play a role in many biological events such as energy metabolism, food intake, reproduction, and inflammation.[9,10] However, their role in autoimmune thyroid diseases is unknown. HT is the most common autoimmune thyroid disease worldwide. Patients with Hashimoto's show metabolic symptoms. We wanted to investigate adropin and PNX peptides as potential protective factors of metabolic complications in Hashimoto's thyroiditis.

MATERIAL AND METHOD

Patients

This case-control study performed in 40 female euthyroid Hashimoto's thyroiditis patients and 38 healthy controls who applied to our internal medicine outpatient clinic. Patients with normal thyroid function tests, positive anti-thyroid peroxidase (anti-TPO)/ anti-thyroglobulin (TGAb), and sonographic reports consistent with Hashimoto's disease were included in the study. In the healthy control, thyroid function tests, TGAb, TPOAb, and thyroid ultrasound examination were within the reference range. The reference range is defined as follows: FT3: 2.0-4.4 pg/ml, FT4: 0,93-1,7 ng/dl, TSH: 0.27–4.2 mIU/l, TPOAb: 0–34 IU/ml,

TGAb: 0–115 IU/ml. Exclusion criteria for all subjects included abnormal TSH, FT3, FT4 levels, receive anti-thyroid or replacement medication, pregnancy, other autoimmun disease, hypertension, diabetes mellitus (DM), cardiovascular disease (CVD), neoplasm, or any other comorbid illnesses. BMI of individuals was recorded by dividing weight in kilograms by the square of height in meters. This investigation protocol was approved by the local ethics committee and all participants in the study signed the informed consent form.

Biochemical Analyses

Fasting venous blood samples were taken in the morning (between 8.00 a.m. and 10.00 a.m.). After the blood specimens were coagulated, all of the samples were centrifuged at 3,000 rpm for 10 min and serum was extracted. Serum specimens were aliquoted in polypropylene tubes so that it can be tested further, and frozen at –80°C. Serum biochemical variables determinations were performed by standard photometric methods on the Roche Cobas c501 analyzer (Roche Diagnostics, Germany). Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula: LDL-C=Total cholesterol-(HDL-C+triglicerides/5). Hormonal parameters were measured via electrochemiluminecence immunoassay on the Roche Cobas e601 analyzer (Roche Diagnostics, Germany). The enzyme-linked immunosorbent assay (ELISA) kits were used for serum PNX-14 and Adropin assays by the manufacturer's instructions (Bioassay Technology Laboratory Human Elisa Kits, Korain Biotech, Shanghai, China). PNX-14 catalog number: E7481Hu, standard curve range: 2-3800 ng/L, sensitivity: 8.19 ng/L, intra-assay: CV<8%, inter-assay: CV<10%. Adropin catalog number: E3231Hu, standard curve range: 5-1000 ng/L, sensitivity: 2.49 ng/L, intra-assay: CV<8%, inter-assay: CV<10%. The optical density was determined at 450 nm on an ELx800 Absorbance Microplate Reader (Biotek, Winooski, VT, USA). HOMA-IR was determined by using the following formula: [fasting serum glucose [mg/dL] × fasting insulin [μU/mL])/405 and IR was defined as the HOMA-IR value>2.5.[11]

Statistical Analysis

We analysed data using SPSS v. 22.0 (SPSS Inc., IL, USA). One-sample Kolmogorov-Smirnov test was used to assess the normality of data. We used the Student's t-test to compare variables with normal distribution. We compared non-normally distributed variables with Mann–Whitney U test. Correlations were performed by Spearman's correlation test. Significance was accepted at $p<0.05$.

Table. Clinical and demographic characteristics of control and Hashimoto thyroiditis subjects.

	Hashimoto Thyroiditis Subjects (n=40)	Controls (n=38)	*p*-Value
Age (years)	42 ± 7.7	42 ± 6.8	0.738
AST, IU/L	18 (11-30)	16 (10-31)	0.108
ALT, IU/L	18 (10-33)	19 (8-39)	0.958
Fasting insulin,μIU/mL	10.4 (3.8-30.0)	10.1 (2.0-17.3)	0.244
Fasting glucose, mg/dL	94 (86-109)	93 (75-107)	0.085
Triglycerides, mg/dL	100 (57-254)	96 (52-196)	0.595
HDL-C, mg/dL	57.2 ± 13.0	58.3 ± 12.5	0.732
LDL-C, mg/dL	134.4 ± 30.6	116.0 ±25.3	<0.05*
Cholesterol, mg/dL	210.0 ± 36.7	180.3 ±27.3	<0.005*
BMI, kg/m2	26.1 ± 1.8	22.8 ± 2.0	<0.001*
HOMA-IR	2.4 (0.9-6.9)	2.3 (0.5-4.1)	0.195
FT3, pg/mL	2.9 (1.8-3.7)	2.8 (0.4-3.6)	0.759
FT4, ng/dL	1.3 (1.0-1.9)	1.2 (0.9-2.0)	0.069
TSH, μIU/mL	2.3 (1.0-4.3)	2.3 (1.4-4.7)	0.420
Anti-Tg, IU/mL	283.6 (73.6-942.9)	15.1 (10.0-102.1)	<0.001*
Anti-TPO, IU/mL	105.8 (18.5-560.0)	10.1 (8.9-28.9)	<0.001*
Adropin, ng/L	981.4 (176.3-7294.3)	1092.4 (453.7-1683.9)	0.377
Phoenixin-14, ng/L	727.6 (379.4-1629.2)	725.9 (379.4-3926.3)	0.455

BMI: Body mass index, **HDL-C:** high density lipoprotein-cholesterol; **LDL-C:** low density lipoprotein-cholesterol; **HOMA-IR:** homeostasis model assessment of insulin resistance ***:** *p*-values indicate statistical significance

RESULTS

This investigation was performed on 40 euthyroid Hashimoto's thyroiditis female patients and 38 controls. The demographic characteristics of the patients diagnosed with HT and controls are presented in Table. There was no statistically significant difference between groups for age, AST, ALT, fasting serum glucose, fasting insulin, triglyceride, HDL-C, HOMA-IR, free T3, free T4, TSH, adropin and PNX levels ($p>0.05$). LDL-C levels were remarkable higher than those of controls ($p<0.05$). Total cholesterol concentrations were higher than those of controls ($p<0.005$). BMI values were higher than those of controls ($p<0.001$). AntiTPO, AntiTG levels were remarkable higher than those of controls ($p<0.001$). Spearman's rho correlation analysis was done to investigate the association between measures of serum PNX-14 and Adropin values between BMI, AST, ALT, fasting serum glucose, fasting insulin, triglyceride, Total cholesterol, HDL-C, LDL-C, HOMA-IR, free T3, free T4, TSH, AntiTPO and AntiTG. No correlation was observed between parameters.

DISCUSSION

Many patients with HT have an increase in body weight and metabolic disorders even in the euthyroid state. This study focused on the role of adropin and PNX-14, which have multiple critical functions from regulation of food intake to energy homeostasis, in HT. As a result of our statistical analysis, there was no significant difference was detected in serum adropin and PNX-14 levels between euthyroid Hashimoto's thyroiditis patients and controls included in the study (p>0.05).

Obese patients have an increased risk to develop HT. Current evidence suggests a relationship between obesity, inflammation and thyroid autoimmunity.[12] It has been observed that women with HT have higher BMI values than healthy women.[13] It has been reported that adropin levels are lower in obese people than in healthy controls, and there is a negative correlation between adropin and BMI.[14] Serum adropin levels were lower in subjects with type 2 diabetes (T2DM) and fatty liver disease associated with metabolic dysfunction (MAFLD) than in the control group. Additionally, serum adropin levels were inversely proportional to BMI and blood lipids in the T2DM group.[15] In a study in women with gestational diabetes, higher adropin levels were associated with lower BMI and better metabolic control.[16] However, some studies have not found a significant relationship between obesity and adropin.[17] In our study, BMI values in the euthyroid HT subjects were higher than the control group, but no significant relationship was observed between adropin and BMI values. In the current study, both the HT group and the control group consisted of euthyroid women. Therefore, thyroid dysfunction should not be the cause of high BMI in HT patients.

In a study in rats, experimental hypothyroidism and hyperthyroidism were shown to cause changes in adropin levels.[18] In this study, all participants had normal thyroid functions and there was no difference in adropin levels between the patient and control groups. The role of adropin in these conditions requires further investigation.

PNX-14 is a neuropeptide associated with food intake, body mass and energy balance.[19] In a previous study, we observed that PNX-14 and PNX-20 levels were negatively correlated with BMI.[20] PNX-14 ameliorated high fat diet induced obesity and fatty liver.[21] We could not find any information about the role of PNX-14 in HT in the literature. We observed no differences in PNX-14 levels between the patient and control groups and no correlation with BMI values.

In a recent study, the association between thyroid autoimmunity and dyslipidemia has been reported in the euthyroid HT.[22] In another study, a relationship was observed between Anti-TPO positivity and metabolic syndrome markers.[23] In our study, we obtained findings of dyslipidemia in euthyroid HT patients, similar to previous studies. However, we did not observe the relationship between lipid parameters and adropin and PNX-14 levels. Previous studies have shown that adropin is a notable factor in improving glucose homeostasis and dyslipidemia.[24] In a retrospective study by Lei *et al*., serum thyroid hormone levels were shown to be related blood lipids, insulin resistance and inflammatory factors in HT patients.[4] In this study, we did not observe any difference in insulin resistance between the HT patient group and healthy controls.

The current investigation was limited by its small sample size. The strength of our study is that it is one of the first studies to give information about the function of PNX and adropin in HT. More extensive researchs should be done and the results of the studies will contribute to future treatment options.

CONCLUSION

In this study, we revealed the relationship between thyroid autoimmunity and metabolic parameters without thyroid dysfunction. However, we did not observe any significant change in the levels of adropin and PNX-14, which are neuropeptides associated with metabolic homeostasis. We believe that current study makes an important contribution to literature.

*The authors declare that there are no conflicts of interest.

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