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ORIGINAL ARTICLE

Evaluation of NLRP3 and Asprosin Levels in Hashimoto's Thyroiditis

Hashimoto Tiroiditinde NLRP3 ve Asprosin Düzeylerinin Değerlendirilmesi

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ABSTRACT

Aim: The aim of the study is to evaluate how NOD-like receptor pyrin domain-containing 3 (NLRP3) and asprosin change in Hashimoto's thyroiditis (HT), one of the autoimmune thyroid diseases.
Materials and Methods: A total of 88 people, 48 patients with a diagnosis of HT and 40 healthy volunteers who applied to the Internal Medicine and Endocrinology Polyclinics between May 2019 and September 2020, were included in the study. All data recorded in the study were evaluated using the SPSS 22.0 program.
Results: There were 41 (85.0%) women and 7 (15.0%) men in the HT group, with a mean age of 31.06±5.76 years. In the control group, there were 30 (75.0%) women and 10 (25.0%) men, and their mean age was 29.47±6.31 years. Asprosin level was found to be higher in the patient group than in the control group (52.74±25.31, 34.61±13.84, p<0.001, respectively). Likewise, NLRP3 levels were higher in the patient group (227.84±107.27) than in the control group (145.94±54.83, respectively, p<0.001). A significant positive correlation was found between NLRP3 and asprosin (r:0.443 p<0.001) and CRP (r:0.361 p<0.001).
Conclusions: It has been observed that NLRP3 and asprosin levels may influence glucose metabolism and inflammatory processes in patients with HT, and there is a need for more comprehensive studies that could improve diagnosis, follow-up, and treatment.

Keywords: Adipokines (Asprosin), autoimmune, inflammasomes (NLRP3), inflammation, thyroiditis

ÖZ

Amaç: Bu çalışmanın amacı, otoimmün tiroid hastalıklarından biri olan hashimoto tiroiditi (HT)'de NOD-like receptor pyrin domain-containing 3 (NLRP3) ve asprosinin nasıl değiştiğini değerlendirmektir.
Gereç ve Yöntemler: Mayıs 2019-Eylül 2020 tarihleri arasında Dahiliye ve Endokrinoloji Polikliniklerine başvuran 48 HT tanılı hasta ve 40 sağlıklı gönüllü bireylerden oluşan toplamı 88 bireyi araştırmamıza dahil ettik. Çalışmada kaydedilen tüm verileri SPSS 22,0 programı ile sonuçları analiz ettik.
Bulgular: HT grubunda 41 (%85,0) kadın ve 7 (%15,0) erkek vardı, yaş ortalaması 31,06±5,76 idi. Kontrol grubunda 30 (%75,0) kadın, 10 (%25,0) erkek vardı ve yaş ortalamaları 29,47±6,31 idi. Asprosin düzeyi hasta tarafında sağlam tarafa göre anlamlı (yüksek) bulundu (sırasıyla 52,74±25,31, 34,61±13,84, p<0,001). Benzer şekilde, NLRP3 seviyeleri hasta grubunda (227,84±107,27) kontrol grubuna göre (sırasıyla 145,94±54,83, p<0,001) daha yüksekti. NLRP3 ile asprosin (r:0,443 p<0,001) ve CRP (r:0,361 p<0,001) ikilisinde anlamlı derecede korelasyon bulundu(pozitif yönde).
Sonuçlar: NLRP3 ve asprosin düzeylerinin HT'li hastalarda glukoz metabolizması ve inflamatuar süreçlerde etkili olabileceği görülmüş olup tanı, takip ve tedavide yenilikler yaratabilecek daha kapsamlı çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Adipokinler (Asprosin), enflamasyon, inflammazomlar (NLRP3), otoimmün tiroidit

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Introduction

Hashimoto's thyroiditis (HT), which is characterized by thyroid cell destruction caused by cell and antibody-mediated immune processes [1]. Although the pathogenesis of HT as an autoimmune disease is not well understood, it is thought that abnormal innate and adaptive immunity may play a role in the disease process [2]. NLR family pyrin domain-containing 3 (NLRP3) is the most prominent inflammasome and links innate and adaptive immune responses, and an abnormal NLRP3 response has been identified in inflammatory autoimmune diseases [3-6].

HT is closely related to fat metabolism, glucose metabolism, insulin metabolism and inflammatory factors. It has been shown that an increasing trend in thyroid autoimmunity is associated with a high prevalence of obesity and diabetes [7-11]. However, it is unclear whether excess adipose tissue directly influences the activation of the inflammatory process in genetically predisposed individuals' thyroids. Thyroid hormones, on the other hand, can influence adipose tissue activity. As a result, a dual interaction between the thyroid and obesity can be assumed [12-14].

Asprosin hormone is released from the white adipose tissue and is a hormone formed by the separation of profibril from the C-terminal part. This hormone is regulated by fasting, exists at nanomolar levels in the blood, and causes hepatic glucose release in the liver. It has been shown that asprosin has possible effects on carbohydrate and lipid metabolism. Hormones such as norepinephrine, epinephrine, glucocorticoids and glucagon, are known to stimulate glucose release from hepatocytes. Asprosin has been shown to reduce obesity, diabetes, and infertility [15-17]. The aim of the study is to evaluate how NLRP3 and asprosin change in HT, one of the autoimmune thyroid diseases.

Materials and methods

Patient selection

A total of 88 people, 48 patients with a diagnosis of HT and 40 healthy volunteers, who prospectively applied to the Internal Medicine and Endocrinology Polyclinics between May 2019 and September 2020, were included in the study. During the study, detailed history, physical examination and demographic data of the patients were recorded. The blood pressure, height and weight of the patients were measured, body mass index (BMI) was calculated, hemogram and biochemistry parameters were evaluated.

Measuring NLRP3 and Asprosin Level

Blood samples were collected in two groups. The collected blood was transferred to the gel hemogram tube. The blood was then centrifuged at 4000 rpm for 10 minutes to separate the plasma. The aliquot was kept at -80 °C. On the day of the study, the plasmas were removed from the deep freezer and brought to room temperature (18-25 °C). The study was carried out using the ELISA method with human asprosin kits.

Statistical analysis

All data recorded in the study were evaluated using the SPSS 22.0 program (IBM Statistical Package for Social Sciences, IBM Corporation, NY, USA). Descriptive statistics were given as number, percentage, mean, standard deviation and median. The conformity of the variables to the normal distribution was evaluated with the Kolmogorov-Smirnov test. Independent group t test was used between two groups for normally distributed numerical variables. In cases where there was a statistically significant difference, post-hoc analyzes were performed using the Tukey test. Mann Whitney U test was applied between the two groups for numerical variables that did not fit the normal distribution. Nominal data were evaluated using the Chi-square test. Pearson correlation test was used for correlation analysis. Values below 0.05 ($p<0.05$) were considered statistically significant in the statistical analysis performed in the study.

Results

A total of 88 people, 48 patients with HT and 40 healthy volunteers, were included in the study. There were 41 (85.0%) women and 7 (15.0%) men in the HT group, with a mean age of 31.06±5.76 years. In the control group, there were 30 (75.0%) women and 10 (25.0%) men, and their mean age was 29.47±6.31 years. There was no statistically significant difference between two groups in terms of gender, age and BMI.

The mean TSH was 7.98±10.4 µUI/ml in the HT group and 1.71±0.95 µUI/ml in the control group, which was significantly higher ($p<0.001$); The mean of anti-TPO was 801.71±307.62 in the HT group and 6.29±2.24 in the control group ($p<0.001$). Asprosin level was found to be significantly higher in the HT group than in the control group (52.74±25.31, 34.61±13.84, $p<0.001$, respectively). Similarly, NLRP3 levels were found to be significantly higher in the HT group compared to the control group (227.84±107.27; 145.94±54.83, $p<0.001$, respectively). The detailed demographic and laboratory findings of the groups are shown in table 1.

Table 1: Comparison of patients' demographic and laboratory data

	Patient	Control	P-value
Age (years)	31.06 ± 5.76	29.47 ± 6.31	0.224
Gender			
Female	41 (85%)	30 (75%)	0.22
Male	7 (15%)	10 (25%)	0.20
BMI(kg/m²)	25.79 ± 2.60	25.22 ± 2.74	0.32
Glucose(mg/dL)	89.50 ± 14.08	89.52 ± 11.61	0.72
Creatinine(mg/dL)	0.71 ± 0.18	0.72 ± 0.13	0.75
ALT(U/L)	22.87 ± 16.95	21.20 ± 11.29	0.53
TSH(µUI/mL)	7.98 ± 10,4	1.71 ± 0.95	**<0.001**
fT4(ng/mL)	1.18 ± 0.26	1.19 ± 0.18	0.66
Anti-TPO(ng/ml)	801.71 ± 307.62	6.29 ± 2.24	**<0.001**
WBC(10^3/L)	6.63 ± 2.00	7.15 ± 1.61	0.07
Platelet(10^3/L)	295.9 ± 69.5	285.4 ± 58.36	0.50
Hematocrit(%)	41.56 ± 3.96	44.00 ± 4.55	**<0.01**
CRP(mg/L)	0.58 ± 1.12	0.37 ± 0.74	0.13
Sedimentation (mm/hour)	18.95 ± 11.2	8.28 ± 7.78	**<0.001**

Asprosin(ng/mL)	52.74 ± 25.31	34.61 ± 13.84	**<0.001**
NLRP3	227.84 ± 107.27	145.94 ± 54.83	**<0.001**

BMI: body mass index, ALT: alanine aminotransferase, TSH: thyroid stimulating hormone, fT4: free thyroxine, anti-TPO: anti-thyroid peroxidase, WBC: white blood cell, CRP: C-reactive protein, NLRP3: family pyrin domain containing 3

A significant positive correlation was found between NLRP3 and asprosin (r:0.443 p<0.001) and CRP (r:0.361 p<0.001). The available data are summarized in table 2.

Table 2. Correlation findings between laboratory and clinical data

	Glucose	TSH	Creatinine	ALT	CRP	Asprosin	NLRP3
Glucose		r:0.044	r:0.106	r:0.146	r:0.046	r:0.023	r:-0.036
		p:0.369	p:0.473	p:0.323	p: 0.756	p:0.876	p: 0.810
TSH	r:0.044		r:0.053	r:-0.043	r:-0.050	r:0.008	r:0.133
	p:0.369		p:0.720	p:0.769	p:0.737	p:0.958	p:0.369
Creatinine	r:0.106	r:0.053		r:0.034	r:-0.146	r:0.069	r:0.150
	p:0.473	p:0.720		p:0.820	p:0.322	p:0.640	p:0.309
ALT	r:0.146	r:-0.043	r:0.034		r:0.156	r:0.011	r:-0.116
	p:0.323	p:0.769	p:0.820		p:0.289	p:0.940	p:0.434
CRP	r:0.046	r:-0.050	r:-0.146	r:0.156		r:-0.190	r:0.361
	p: 0.756	p:0.737	p:0.322	p:0.289		p:0.195	p:0.012
Asprosin	r:0.023	r:0.008	r:0.069	r:0.011	r:-0.190		r:0.443
	p:0.876	p:0.958	p:0.640	p:0.940	p:0.195		p:0.002
NLRP3	r:-0.036	r:0.133	r:0.150	r:0.150	r:0.361	r:0.443	
	p: 0.810	p:0.369	p:0.309	p:0.309	p:0.012	p:0.002	

BMI: body mass index, ALT: alanine aminotransferase, TSH: thyroid stimulating hormone, fT4: free thyroxine, anti-TPO: anti-thyroid peroxidase, WBC: white blood cell, CRP: C-reactive protein, NLRP3: family pyrin domain containing 3

Discussion

One of the most common chronic endocrine conditions is hypothyroidism. HT is a disease that involves an inflammatory process and also affects body metabolism. In our study, we found significantly high levels of NLRP3 and asprosin, two molecules that are effective in inflammation and obesity.

HT is more common in women between the ages of 30 and 50. It has been reported that it is seen approximately 7.2 times more in women than in men (18). The mean age of the patients was 31.06±5.76 years and 85.4% were female, which was consistent with the literature. Many studies have reported that NLRP3 may be a potential marker for detecting inflammation (19-20). In another study conducted in rats with HT, it was shown that the amount of NLRP3 and caspase1 protein increased in heart tissue. This increase suggested that hypothyroidism may exert its destructive effects on heart tissue through sterile inflammation (21). Similarly, NLRP3 levels were positive in HT, and the positive correlation with CRP, an indicator of inflammation, shows that inflammation increases and supports the literature. These results suggest that HT is a chronic autoimmune and inflammatory process; suggested that NLRP3, which was elevated in inflammation due to the chronic inflammation and stress environment in the body, these are the findings that support its significance on the patient's side.

While the presence of asprosin, a protein hormone that regulates glucose homeostasis, preserves euglycemia in humans; its absence, on the other hand, results in partial lipodystrophy accompanied by decreased plasma insulin. (22). In addition, asprosin is a fasting-derived hormone that contributes to hepatic glucose production, and it has been shown to cause an urge to accumulate fat and body weight as a result of appetite stimulation (15). Duerrschmid et al. suggested that asprosin level increased with fasting and decreased with feeding, and accordingly, plasma asprosin levels could be suppressed by a glucose-dependent negative feedback loop (23). It is also known that thyroid hormone affects glucose homeostasis. It has been reported in many studies that there is a relationship between pancreatic ⊠-cell development and thyroid hormones, and that it affects glucose metabolism through a wide variety of organs, primarily fat and muscle tissue and liver. It is known that patients with HT are prone to metabolic syndrome, and insulin resistance develops in these patients, which leads to glucose metabolism disorders (24).

There is no study in the literature evaluating asprosin levels in HT. Together with all these data, in our study, asprosin levels were found to be significantly higher in patients with HT, suggesting that hypothyroidism may also play a role in glucose metabolism changes, and that it should be considered in early diagnosis or treatment strategies of glucose metabolism disorders. NLRP3 and asprosin levels are significantly higher in patients with HT. In addition to providing insight into pathogenesis, the positive correlation between these two molecules may be valuable in managing the disease, potentially regressing the chronic process and contributing to treatment strategies.

Conclusion

As a result, there is a need for more comprehensive studies to develop innovations in diagnosis, follow-up, and treatment, given that NLRP3 and asprosin levels can significantly influence glucose metabolism and inflammatory processes in patients with HT.. With the clarification of this situation, we think that agents that inhibit asprosin can be used as a treatment tool in the future.

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Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local Ethics Committee (protocol number 74059997-050.04.04 date:06/08/2019)

Conflicts of interest

The authors declare no conflict of interest.

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