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INVESTIGATION OF NEOPTERIN LEVELS IN CHILDREN WITH HASHIMOTO'S THYROIDITIS

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Research Article

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Abstract

Hashimoto's Thyroiditis is an autoimmune disease caused by genetic and environmental factors and is known as the most common cause of hypothyroidism in childhood. Neopterin has been measured in many autoimmune diseases and has been identified as a marker of cellular immune activation. The aim of this study was to investigate the autoimmune association of neopterin in patients with Hashimoto's Thyroiditis aged 5-18 years. First of all, 40 patients with Hashimoto's Thyroiditis and 40 individuals who were not related to this disease were included in the study, and blood serums were taken. Then serum Neopterin levels of the subjects were determined using Neopterin Human ELISA kit. Neopterin levels were compared among patients with Hashimoto's Thyroiditis and individuals who were not related to this disease. Neopterin levels in Hashimoto's Thyroiditis and control groups were 101.32 ± 9.75378 ng/ml and 86.4170 ± 3.59284 (p= 0.436; r= - 0,127), respectively. When we look at the participant age data, it is seen that the patients and controls are compatible with each other. In addition, it is seen that there is a significant positive correlation between the variables of age-neopterin levels (p= 0.039; r=0.391) and TSH-neopterin levels (p=0.016; r=-0.055) in Hashimoto's Thyroiditis groups. Neopterin Levels did not reduce in Hashimoto's Thyroiditis. However, when the individuals with Hashimoto's Thyroiditis between the ages of 5-18 were compared with the control group individuals in the same age range, no statistically significant difference (p>0.05) was found between the neopterin levels.

Key Words: Hashimoto's Thyroiditis; Neopterin; ELISA

Özet

Hashimoto Tiroiditi, genetik ve çevresel faktörlerin neden olduğu otoimmün bir hastalıktır ve çocukluk çağında hipotiroidizmin en yaygın nedeni olarak bilinir. Neopterin birçok otoimmün hastalıkta ölçülmüş ve hücresel bağışıklık aktivasyonunun bir belirteci olarak tanımlanmıştır. Bu çalışmanın amacı, 5-18 yaş arası Hashimoto Tiroiditi olan hastalarda neopterinin otoimmün ilişkisini araştırmaktır. İlk olarak Hashimoto Tiroiditi olan 40 hasta ve bu hastalıkla ilgisi olmayan 40 birey çalışmaya dahil edildi ve kan serumları alındı. Daha sonra Neopterin Human ELISA kiti kullanılarak deneklerin serum Neopterin seviyeleri belirlendi. Neopterin düzeyleri; Hashimoto Tiroiditi olan hastalar ve bu hastalıkla ilişkili olmayan bireyler arasında karşılaştırıldı**.** Hashimoto Tiroiditi ve kontrol gruplarındaki Neopterin seviyeleri sırasıyla 101.32 ± 9.75378 ng/ml ve 86.4170 ± 3.59284 (p= 0.436; r= - 0,127) bulundu. Katılımcı yaş verilerine bakıldığında hastaların ve kontrollerin birbiriyle uyumlu olduğu görülmektedir. Ek olarak, Hashimoto Tiroiditi grupları içinde yaş-neopterin düzeylerinin değişkenleri (p= 0.039; r=0.391) ile tsh-neopterin düzeyleri (p=0.016; r=0.055) arasında pozitif korelasyon olduğu görülmektedir. Hashimoto Tiroiditinde Neopterin Düzeyleri azalmadı. Bununla birlikte, 5-18 yaşları arasında Hashimoto Tiroiditi olan bireyler, aynı yaş aralığındaki kontrol grubu bireylerle karşılaştırıldığında, neopterin seviyeleri arasında istatistiksel olarak anlamlı bir fark (p>0.05) bulunamamıştır.

Anahtar Kelimeler: Hashimoto Tiroidit; Neopterin; ELISA

1. Introduction

Hashimoto's Thyroiditis is an autoimmune disease. The combination of this disease with autoantibodies was first demonstrated by Roitt and Doniach in 1956. The most accepted theory in the progression of the disease is the deteriorated immune response theory; the dysfunction of suppressor T (thymus) - lymphocytes leads to the production of autoantibodies, which are specific to thyroid tissue from B (bone) lymphocytes, which are stimulated by helper- thymus lymphocytes, resulting in the deterioration of thyroid cells and the event develops (Muthıay & Yeğin). Hashimoto's thyroiditis was reported to be between 0.3% and 9.6% in children and young age groups. The prevalence reported in the studies depends mainly on the diagnosis criteria of the population studied, ethnicity, iodine status, and age or gender (Zdraveska, 2012). Because neopterin, which we think is associated with this disease, is produced during the activation of the immune system, the response to cellular immunity is a suitable variable to monitor therapeutic

drug regimes that activate it. In addition to monitoring directly effective treatment regimes on the immune system, neopterin can also be used to evaluate treatment regimes that cause pathogenesis to increase neopterin levels, which are associated with neopterin level and disease activation, and indirectly change immune system activation (Baydar et al., 2009). Rheumatoid arthritis, glomerulonephritis, Sjogren's syndrome, systemic lupus erythematosus. in the case of ulcerative colitis, Crohn's disease, and Graves' disease, macrophage infiltration is the production of neopterin in the developing body fluid and tissues. In these autoimmune diseases, serum neopterin levels have been observed to increase due to the activity of the disease (Berdowska & Zwirska-Korczala, 2001). Elevated neopterin concentrations have been encountered in a variety of diseases associated with immune activation. High neopterin concentrations are originally found in the urine of patients with viral infections and cancer. In subsequent research, it was observed that the concentration of neopterin is elevated in different diseases that enable activation of the immune system (Hamerlinck, 1999). So far, there have been no studies on evidence between this age group with Hashimoto's Thyroiditis and Neopterin. In light of this information, for this reason, the main purpose of this study was to investigate the levels of neopterin in these age group patients with Hashimoto's Thyroiditis. For this purpose, it will be revealed in this unique study whether neopterin can be used as a biomarker for children with Hashimoto's Thyroiditis aged 5-18 years old.

2. Material and Methods

2.1. Working groups

This study was carried out on the basis of 5-18 years old, 40 patients diagnosed with Hashimoto's Thyroiditis who applied to Tokat Gaziosmanpasa University Hospital Paediatrics Clinic and 40 healthy individuals to be used for control purposes. This study was completed in 1.5 years, including the collection of blood serums from patients diagnosed with Hashimoto's Thyroiditis and healthy individuals. Control patients have applied for foot child polyclinic and have been examined for Hashimoto's Thyroiditis and thyroid autoantibodies (Anti-TPO and Anti-TG) have also been selected from normal detection of negative, TSH (Thyroid-Stimulating Hormone) and ST4 (Specialty Training 4) levels. The criteria for inclusion and non-inclusion of patients and individuals in the control group are given in Table 1. For this study, approval was obtained by registration number 17-KAEK-125, which was discussed on 12.09.2017 from the Clinical Research Ethics Board of Tokat Gaziosmanpaşa University Faculty of Medicine.

Table 1 Criteria for inclusion and non-inclusion of patients and individuals in the control group

Inclusion Criteria	**Inclusion Criteria for the Patient Group of the Research**
	1. Hashimoto's Thyroiditis Diagnosis
	2. Have signed the written informed consent form
	3. Be between 5-18 years old
	Inclusion Criteria for the Control Group of the Research
	1. Hashimoto's Thyroiditis has not been diagnosed
	2. Have signed the written informed consent form
	3. Be between 5-18 years old
	4. Fever, diabetes, and the absence of any hereditary diseases
Non-inclusion Criteria	**Non-Inclusion Criteria for the Patient Group of the Research**
	1. Hashimoto's Thyroiditis Not Diagnosed
	2. Not having signed the written informed consent form
	3. Outside the age range of 5-18
	Exclusion Criteria for the Control Group of the Research
	1. Not signing the written informed consent form
	2. Have been diagnosed with Hashimoto's Thyroiditis between the ages of 5 and 18
	3. Fever, diabetes, and any hereditary condition

2.2. Obtaining blood serums

A total of 80 blood samples were collected from individuals in the 5-18 age group, both patient and controlled. After the blood samples were centrifuged for 10 minutes at 3600 rpm, serum was obtained. The resulting serum was stored at -20°C for about 4 weeks. Then, at the end of 4 weeks, it was kept at -80 °C until the date of ELISA study.

2.3. Application of ELISA method

50 µl from standard tubes were added to the wells. The 40 µl samples that we obtained from the blood serum were added to the wells. Biotinated neopterin was added to all other samples except for the standards. All other wells except the standard 7 were added 50 Streptavidin-HRP

and the 37 °C set body was kept for 60 min. Wash Buffer was prepared, and the washing process was carried out. After the washing process was carried out, 50 µl substrates A and B solutions were added. After this procedure, the body was left to incubate for 10 minutes. It was observed that the blue colour turned yellow by placing 50 µl stops on each well. After the stop solution was added, a reading was made with an ELISA microplate reader with an absorbance value of 450 nm.

2.4. Data analysis methods

As a result of the work done with the standards prepared in series for the evaluation of neopterin concentrations, the standard graph and the equation belonging to this graph were determined with the help of the Excel program. Concentrations of unknown samples were calculated in terms of ng/L with the help of this graph and the equation of the graph. Independent t-testing or one-way analysis of variance was used to compare continuous, normally distributed data across groups. The Tukey test was used for multiple comparisons. To investigate the effect of the group on Neopterin levels, we adjusted for age while evaluating covariance. A p-value < 0.05 was used to indicate statistical significance. All statistical analyses were performed with the use of SPSS version 29.0.2.0 software.

3. Results and Discussion

When we analyzed the variables of age, neopterin concentrations, and TSH values in the control and patient groups, it was revealed in Table 2 that there was no statistically significant difference between the neopterin levels between the two groups ($p>0.05$). Table 2 shows the descriptive statistics of age, neopterin, and TSH values. The mean ages of HT patients and controls were 14.12 ± 3.03 and 12.98 ± 3.69 years ($p=0.169$), respectively. Neopterin levels in Hashimoto's Thyroiditis and control groups were 101.32 ± 9.75378 ng/ml and 86.4170 ± 3.59284 ($p= 0.436$; $r= - 0,127$), respectively. Table 2 also determined the degree of severity by statistically comparing the amounts of age, neopterin, and TSH in themselves to the control group of the patient group. As can be seen from the table, when the amounts of TSH in the patient group with Hashimoto's Thyroiditis were compared to the amounts of TSH in the control group, a statistically significant difference was observed ($p<0.05$; $r=-0.055$).

Table 2. SPSS analysis result of values of control and patient groups

	Group	Number of people	Median	Average Value (ng/ml)	Standard deviation	Standard error	Significance level
Age	Control	40	13.00	12,9760	3,69344	0,58398	0,169
	Patient	40	14.55	14,1267	3,03041	0,55328	
Neopterin	Control	40	86.42	86,4170	22,72314	3,59284	0,436
	Patient	40	84.55	101,3238	61,68835	9,75378	
TSH	Control	40	2.25	2,7517	2,59210	0,45123	0,006
	Patient	40	6.4950	7,6895	8,50338	1,60699	

Statistical correlation values within the patient group are given in Table 3. As can be seen from this table, there was a significant positive correlation between age-neopterin levels (p= 0.039; r=0.391). This result showed us that there is a very close relationship between the group of patients aged 5-18 years who have Hashimoto's thyroiditis and the level of this serum marker. In addition, if we evaluate the other situation, there is a significant relationship between the TSH value and Neopterin levels (p=0.016; r=-0.055). However, it was revealed that there is no link between TSH values and age to a degree of meaningfulness (p = 0.158; r=0.274)

Hashimoto's thyroid is a chronic autoimmune thyroid disease caused by an interaction between genetic factors and environmental conditions that are not yet fully understood (Liontiris & Mazokopakis, 2017).

As a chronic autoimmune disease of the thyroid gland, Hashimoto's Thyroiditis is the most common cause of hypothyroidism (Barić et al., 2017).

Neopterin, which we think is immunologically associated with this disease, is a pyrazine-pyrimidine compound belonging to the pteridine group. It is known to be a biochemical marker associated with cell-mediated immunity. It is produced by human monocytes/macrophages and dendritic cells from guanosine triphosphate (GTP) upon stimulation by interferon-gamma (IFNγ) released by activated helper T lymphocytes. Neopterin is a very important clinical parameter, but its physiological role has not been fully defined so far. Neopterin level reflects the activation phase of the cellular immune system, which is important in the pathogenesis and progression of various diseases (Michalak et al., 2017). In autoaggressive diseases such as rheumatoid arthritis, Crohn's

disease, ulcerative colitis, autoimmune thyroiditis, systemic lupus erythematosus, and early-onset autoimmune diabetes, neopterin levels are highest in the acute phase of the disease and are associated with the expansion of disease activity (Hamerlinck, 1999).

Table 3. Statistical values of patients with Hashimoto's Thyroiditis

		Age	TSH	Neopterin
Age	Significance level	1	0.158	0.039
	Number of people	40	40	40
TSH	Significance level	0.158	1	0.016
	Number of people	40	40	40
Neopterin	Significance level	0.039	0.016	1
	Number of people	40	40	40

In light of this information, it is obvious that there is a link between Hashimoto's Thyroiditis, which is an immunological disease, and neopterin level.

Wagner et al. investigated neopterin levels by comparing 24 control groups with 17 patients with autoimmune thyroiditis. As a result of their study, they revealed that patients with autoimmune thyroiditis did not have abnormal neopterin levels compared to normal controls with age and sex and that neopterin serum levels were not affected by hypothyroidism (Wagner et al., 1993). As can be seen in our study, it will be seen that there is no abnormal difference in terms of neopterin levels between patients aged 5-18 years with Hashimoto's Thyroiditis and controls in the same age range. Kondera-Anasz and Mertas compared serum neopterin levels by comparing 88 (64 female, 24 male) hypothyroid patients with an age range of 26 to 55 years, with an average age of 42.3, and a control group of 36 (20 females, 16 males) aged between 24 and 50, with an average age of 39.6. they have researched. As a result of their study, when the patients with thyroiditis were compared with the control group, they found significantly ($p<0.001$) high neopterin levels in patients with thyroiditis (Kondera-Anasz & Mertas, 1999).

Gulkesen et al. studied neopterin levels by comparing 33 (9 males, 24 females) patients with Rheumatoid Arthritis with an age range of 29 to 75 years and 24 healthy controls (11 males, 13

females) aged between 18 and 75 years. As a result of their research, they revealed that there was no statistical difference (0.078) between rheumatoid arthritis, an autoimmune disease, and neopterin levels. In our study, no statistically significant difference is found between Hashimoto's Thyroiditis, which is an autoimmune disease, and neopterin (Gülkesen et al., 2016).

Plata-Nazar et al. researched the use of serum neopterin by comparing the control group of 67 (36 girls, 31 boys) children with Juvenile Idiopathic Arthritis disease between the ages of 3.8 and 17.9 and a total of 105 children (47 girls, 58 boys) in close age. As a result of the study, it is stated that there is a positive correlation between neopterin and Juvenile Idiopathic Arthritis, which is an autoimmune disease, and that it may be a marker in this disease (Plata-Nazar et al., 2015).

Neopterin is released in reaction to cytokines generated by T-lymphocytes and natural killer cells, making it a sign of activation of cell-mediated immunity, including release by infections linked with activation of T-lymphocytes, natural killer cells, malignancies, and autoimmune disorders (Eisenhut, 2013).

We must also point out again; that Neopterin is an essential clinical marker, although its physiological role has yet to be fully defined. The amount of neopterin shows the state of stimulation of the cellular immune system, which is crucial in the development and progression of many diseases (Michalak et al., 2017). Neopterin may have an innate physiological significance as an innate regulator of stimulated macrophages' cytotoxic effector capabilities. The micromolar concentration range of neopterin is where the majority of its effects take place (Fuchs et al., 2009).

The above studies have also shown that there is a definite relationship between neopterin levels. and individuals with thyroid and autoimmune diseases. However, although the difference between neopterin levels between the patient and control groups was statistically visible in some studies, this difference could not be clearly demonstrated in some studies and our study. But Our study's strengths are that we emphasize that measuring Neopterin levels at the time of diagnosis may provide insight into the T- lymphocyte and macrophage cell-based mechanism of Hashimoto's Thyroiditis and that we are the first to measure Neopterin levels in children with Hashimoto's Thyroiditis.

4. Conclusion

This study is original. For the first time in this age range, the association of thyroid disease called Hashimoto's Thyroiditis with the structure called neopterin is investigated and the results is revealed. In this study when individuals with Hashimoto's Thyroiditis between the ages of 5

and 18 are compared with the control group individuals in the same age range, no statistically significant difference is found between neopterin levels. As a result of this study, it is understood that neopterin could not be used as a biomarker in children aged 5-18 years with Hashimoto's Thyroiditis. But the level of the patient group neopterin is not reduced. This shows us that although Hashimoto's Thyroiditis, an autoimmune disease, works for the first time in this age group in understanding the cell-based mechanism of T lymphocytes and macrophages, neopterin levels are an important factor.

Considering the mean age in this study, the mean age of the patients was 14.12 ± 3.03 Hashimoto's Thyroiditis is a disease that increases with age. It can also be considered as an increase in neopterin levels due to the age-dependent increase in the course of this disease. Considering this evaluation, it can be assumed as a reason why the neopterin levels in the patients were so low that there was no significant difference when compared to the control group.

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