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Research

Serum Zonulin Levels in Patients with Hashimoto's Thyroiditis

Hashimoto Tiroditli Hastalarda Zonulin Düzeylerinin İncelenmesi

Zehra Sucuoğlu İşleyen[1], Sedat Yıldırım[2], Erdal Gündoğan[2], Hakan Sarı[2], Suat Hayri Küçük[3], Ahmet Engin Atay[2]

[1]Bezmialem Vakif University Hospital, Clinic of Medical Oncology, İstanbul, Türkiye
[2]University of Health Sciences Türkiye, İstanbul Bağcılar Training and Research Hospital, Clinic of Internal Medicine, İstanbul, Türkiye
[3]University of Health Sciences Türkiye, İstanbul Bağcılar Training and Research Hospital, Clinic of Biochemistry, İstanbul, Türkiye

ABSTRACT

Objective: Hashimoto's thyroiditis, also known as chronic lymphocytic thyroiditis, is the most common autoimmune thyroid disease in children and adolescents. The exact mechanism that triggers autoimmunity in Hashimoto's thyroiditis is not fully known. Many pathologies, including the disruption of the intestinal epithelial barrier that causes the production of an inappropriate immune response by the interaction of submucosal immune cells with the antigens, have been accused. Zonulin is a protein that modulates the permeability of tight junctions between intestinal mucosal cells. Abnormal up-regulation of Zonulin causes increased intestinal permeability and is central to the development of autoimmune diseases.

Methods: A total of 53 adult patients with newly diagnosed Hashimoto's thyroiditis, 70 adult patients with previously diagnosed Hashimoto's thyroiditis, receiving thyroid hormone replacement therapy and 62 healthy controls were enrolled in the study. Patients with other autoimmune or systemic disorders were excluded. Hemogram parameters, biochemical variables and serum zonulin levels of the participants were examined.

Results: There was no significant zonulin elevation in the newly diagnosed Hashimoto's thyroiditis patient group or in the previously diagnosed Hashimoto's thyroiditis patient group currently receiving replacement therapy compared to the control group. Zonulin levels, which have an important place in the intestinal permeability modulation, can be affected by environmental factors, so they should be carefully evaluated. A statistically significant correlation was observed between zonulin levels and anti-thyroid peroxidase (anti-TPO) antibody levels in patient groups included in our study. There is also a significant correlation between zonulin levels and age, body mass index and cholesterol values.

Conclusion: A correlation between anti-TPO levels, an indicator of autoimmunity, and serum zonulin levels suggests that this molecule plays a role in the pathogenesis of Hashimoto's thyroiditis. The results of this study should be supported by larger-scale studies with longer follow-up periods.

Keywords: Zonulin, Hashimoto's thyroiditis, autoimmunity

ÖZ

Amaç: Hashimoto tiroiditi, diğer adıyla kronik lenfositik tiroidit, çocuk ve adölesanlarda en sık görülen otoimmün tiroid hastalığıdır. Hashimoto tiroiditinde otoimmünitenin nasıl tetiklendiği tam olarak bilinmemektedir. Bağırsak epitel bariyerindeki bozukluk submukozal immün hücrelerin antijenlere maruz kalmasına ve uygun olmayan immün cevabın oluşmasına sebep olur. Zonulin proteinin anormal up-regülasyonu bağırsak geçirgenliğinde artışa sebep olarak otoimmün hastalıkların oluşumunda önemli bir rol oynar. Tip 1 diabetes mellitus, çölyak ve Hashimoto tiroiditi ile ilgili yapılan çalışmalarda bağırsak geçirgenliğinin ve zonulin up-regülasyonun arttığını gösteren çalışmalar mevcut olmakla birlikte bağırsak geçirgenliği ve zonulin proteini ile hashimoto tiroiditi arasındaki bağlantıyı gösteren çok az çalışma mevcuttur.

Gereç ve Yöntem: Çalışmaya 53 yeni tanı Hashimoto tiroiditli erişkin hasta, 70 tiroid hormon replasman tedavisi alan erişkin hasta ve 62 sağlıklı kontrol alındı. Hashimoto tiroidit tanılı hastalar diğer otoimmün ve sistemik hastalıklar, kontrol grubu da herhangi otoimmün ve sistemik hastalıklar açısından dikkatli bir anamnez ile incelenerek çalışmaya dahil edildi.

Address for Correspondence: Zehra Sucuoğlu İşleyen, Bezmialem Vakif University Hospital, Clinic of Medical Oncology, İstanbul, Türkiye
E-mail: zehrasucuoglu@gmail.com ORCID ID: orcid.org/0000-0002-0045-8009

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Bulgular: Çalışmamızda yeni tanı ve tedavi alan Hashimoto hastalarında kontrol grubuna göre anlamlı zonulin yüksekliği görülmedi. Ancak zonulin up-regülasyonu etkileyen birçok çevresel faktör olması bu verinin dikkatli incelenmesi gerektiğini düşündürmektedir. Çalışmamıza dahil edilen hastalarda anti-tiroid peroksidaz (anti-TPO) ve zonulin düzeyleri arasında anlamlı bir korelasyon izlenmiştir. Bununla birlikte zonulin proteininin yaş, vücut kitle indeksi ve kolesterol düzeyleri ile etkilendiği sonucuna ulaşılmıştır.

Sonuç: Otoimmünitenin bir göstesgesi olan anti-TPO düzeyi ile serum zonulin düzeyleri arasında bir korelasyon izlenmesi, Hashimoto tiroiditinin patogenezinde bu molekülün rolü olabileceğini düşündürmektedir. Bu çalışmanın sonuçlarının daha geniş ölçekli ve daha uzun takip süreli çalışmalarla desteklenmesi gerekmektedir.

Anahtar Kelimeler: Zonulin, Hashimoto tiroiditi, otoimmünite

INTRODUCTION

Hashimoto's thyroiditis is the most common cause of hypothyroidism (HT) in adults in areas with adequate iodine intake (1). Inadequate production of thyroid hormones in Hashimoto's thyroiditis causes symptoms that affect the quality of life of the patients and long-term functional insufficiency of the thyroid gland poses a risk especially for cardiovascular diseases (2).

Hashimoto's thyroiditis is in the group of autoimmune diseases. Both cellular and humoral immunity play a key role in the development of this disease. The damage in the thyroid tissue is caused by complex mechanisms because of T-cell-mediated and antibody-dependent cellular cytotoxicity. It develops because of complement activation and/or antibody-dependent cellular cytotoxicity mediated by antibodies against thyroid peroxidase (anti-TPO) and thyroglobulin (anti-TG) due to the effect of cytotoxic T-cells or due to cytokines such as interferon-gamma and interleukin-1. Antibodies against antigens in the thyroid tissue are released from B-cells after activation of T helper cells. It is considered that environmental factors and genetic factors are effective in the etiology of autoimmune thyroiditis (3). Subclinical *Yersinia enterocolitica* infection in Türkiye has been shown to be associated with autoimmune thyroid disease (4).

Recently, it has been observed that the intestines, through the epithelial barrier, play a role in controlling the entry of environmental antigens into the body, in the digestion and absorption functions. (5). The tight junctions (TJs) in the intestine provide the balance between tolerance to and/or immune response against non-self antigens, particularly since they are responsible for the paracellular passage of macromolecules (6). It has been shown that the protein called zonulin reversibly regulates intestinal permeability by modulating TJs. Exposure to bacteria and exposure to gluten in the small intestine has been identified as factors that trigger zonulin release (5). In celiac disease, it has been shown that zonulin levels increase with exposure to gluten. And it's shown that after removal of gluten, zonulin levels decrease and the intestines begin performing normal barrier function and the autoimmune response ends (7). It has been shown that zonulin levels are increased by 50% in patients with type 1 diabetes mellitus (DM), and this is associated with increased intestinal permeability (8).

The relationship between autoimmune diseases such as type 1 DM and celiac disease and zonulin up-regulation suggests that zonulin may play a role in the pathogenesis of Hashimoto's thyroiditis. It has been shown by some studies that intestinal epithelial morphology is impaired and intestinal permeability is increased in Hashimoto's thyroiditis. Intestinal microbiota is thought to play a role in the pathogenesis of Hashimoto's thyroiditis, as in other autoimmune diseases. There are also clinical studies suggesting that up-regulation of zonulin protein, causing increased intestinal permeability, plays a role in the pathogenesis of Hashimoto's thyroiditis (9,10). Our aim was to determine zonulin levels in Hashimoto's thyroiditis and to determine whether there is a relationship between autoantibody and zonulin levels.

METHODS

Patients who were admitted to the Internal Medicine Outpatient Clinic of University of Health Sciences Türkiye, Bağcılar Training and Research Hospital between March 1, 2017 and December 31, 2017 were included in the study. The purpose of the study was explained to each patient included in the study and a consent form was signed.

The study consisted of three groups. The first group consists of patients who were previously diagnosed with Hashimoto's thyroiditis and are currently receiving thyroid hormone replacement and are being followed up. The second group consists of patients that are newly diagnosed with Hashimoto's thyroiditis and the third group consists of healthy individuals. Patients included in the study were included by discussing their demographic data and comorbidities with the patients and their relatives and by examining their records. Ethical approval was obtained from the University of Health Sciences Türkiye, Bağcılar Training and Research Hospital Local Ethics Committee. Ethics committee date and approval number: 08.02.2017 and 2017/543.

Hashimoto's thyroiditis was diagnosed with the help of medical anamnesis, physical examination findings, serum anti-TPO and/or anti-TG level, and ultrasonography findings. Patients without any diagnosis or signs/symptoms of autoimmune disease other than Hashimoto's thyroiditis were included in the study. All individuals in the control group were negative for thyroid autoantibodies. Physical examinations of the patients were performed by the same physician. Body mass indexes (BMIs) of the patients were calculated using the body weight/height^2 (kg/m^2) formula.

Blood samples were obtained from the antecubital vein after 10-12 hours of fasting. Blood samples were centrifuged at 3000 rpm for 10 min and stored at -80 degrees until analyzed. Hemogram analysis was studied in EDTA containing tubes using an automatic hemogram device (XE-5000; Sysmex Corp, Kobe, Japan). Biochemical parameters such as serum glucose, urea, creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase (ALT), lactate dehydrogenase, calcium, sodium, potassium, chlorine, total protein, albumin, total cholesterol, low-density lipoprotein-cholesterol (LDL-c), high-density lipoprotein-cholesterol (HDL-c), triglyceride were studied by photometric method on a Siemens Advia 1800 device (Siemens Healthcare Diagnostics, Kobe, Japan). The hormone parameters such as insulin, C-peptide, and thyroid-stimulating hormone (TSH) were studied in a Siemens Advia Centaur device (XE-5000, Sysmex Corp. Kobe, Japan) a chemiluminescence immunoassay method. Hemoglobin A1c test was studied using high-performance liquid chromatography method (ADAMS A1c HA-8180V Kyoto, JAPAN).

Serum zonulin concentrations were measured using the Abbkine, INC brand ELISA kit. Samples removed from -80 °C were taken first to -20 °C and then to + 4 °C, and serums were gradually dissolved. After adding 150 uL of Standard Diluent to each tube; 150 µL of the stock standard tube were dispensed by serial dilution. Equal distribution was ensured by pipetting before each stage. The 30X Wash solution was diluted 1X with distilled water and this solution was used in the washing steps.

Statistical Analysis

In this study, statistical analyzes were performed with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program. In addition to descriptive statistical methods (mean, standard deviation) in the evaluation of the data, One-Way analysis of variance in the intergroup comparisons of normally distributed variables, independent t-test in the comparison of paired groups, Kruskal-Wallis test in the intergroup comparisons of non-normally distributed variables, Dunn's multiple comparison test in subgroup comparisons. Mann-Whitney U test was used for the comparison of paired groups, the chi-square test was used for comparison of qualitative data, Pearson correlation test was used to determine the relationships of variables with each other. The results were evaluated at a significance level of $p<0.05$.

RESULTS

The patients included in our study were grouped as follows. Fifty three of them are newly diagnosed Hashimoto's thyroiditis patients, 70 of them are previously diagnosed Hashimoto's thyroiditis patients and are receiving thyroid hormone replacement therapy, 62 of them are in the healthy control group.

The mean age of the patient group who were previously diagnosed with Hashimoto's thyroiditis and are currently receiving treatment was significantly higher than the other 2 groups. No significant difference was observed between the groups in terms of other demographic data, such as BMI and gender distribution (Table 1).

Alkaline phosphatase (ALP) level, calcium, total cholesterol and LDL-c levels were significantly higher in the previously diagnosed and currently being treated patient group than in the other 2 groups (p=0.034 p=0.0001 p=0.0001 p=0.003 respectively) (Table 2).

The mean vitamin D, ferritin, and vitamin B12 levels of the groups were similar. The mean vitamin D, ferritin, and vitamin B12 levels of the patients in the previously diagnosed and

Table 1. Comparison of demographic data of Hashimoto individuals newly diagnosed and treated with the control group

		Control group n=62		Newly diagnosed group n=53		Treatment group n=70		p*
Age		37.32±11.13		36.51±11.22		42.76±13.17		0.006
Gender	Male	11	17.74%	4	7.55%	5	7.14%	0.098
	Female	51	82.26%	49	92.45%	65	92.86%	
BMI		27.31±6.44		27.25±5.91		29.37±6.89		0.105

*One-Way analysis of variance + chi-square test, BMI: Body mass index

treatment receiving group were nonsignificantly higher than those in the newly diagnosed group (Table 3).

Significantly higher zonulin levels were observed in the control group compared with the other two groups (p=0.007) (Table 4). The mean zonulin levels were similar between patients with different TSH levels [0.3-4.5 TSH, 4.5-10 TSH, and >10 TSH groups (p=0.833) (Table 5)].

We determined a significant correlation between zonulin levels and age, BMI, LDL, triglyceride and anti-TPO (respectively p=0.001, p=0.024, p=0.041, p=0.016, p=0.045) (Table 6).

No significant correlation was observed between zonulin values and ALT, albumin, ALP, iron, iron binding capacity, transferrin saturation, glucose, cholesterol, HDL-c, creatinine, potassium, sodium, phosphorus, calcium, urea, vitamin D, ferritin, anti TG, free T4, TSH, vitamin B12, hemoglobin, leukocyte, platelet values (p>0.05).

DISCUSSION

The common point of autoimmune diseases is that several pre-existing conditions initiate the autoimmune process. The first is that the host immune system has a genetic predisposition to recognize and possibly misinterpret an environmental antigen present in the gastrointestinal tract. And the antigen must be presented to the gastrointestinal immune system by passing through the intestinal lumen, which is normally protected by TJ integrity, into the intestinal submucosa (6).

The intact intestinal epithelial barrier prevents both pathogenic and non-pathogenic bacteria from entering the immunoreactive submucosa. Damage to the mucosal

Table 2. Comparison of the biochemical data of the control group and the newly diagnosed and treated Hashimoto individuals

	Control group n=62	Newly diagnosed group n=53	Tretment group n=70	p*
ALP	62.6±17.08	63.57±21.09	70.23±16.86	0.034
Cholesterol	177.33±41.06	183.28±41.43	207.07±38.79	0.0001
LDL-c	98.72±35.58	98.39±38.49	125.29±34.87	0.0001
Calcium	9.34±0.34	9.12±0.41	9.34±0.42	0.003

*One-Way analysis of variance, ALP: Alkaline phosphatase, LDL-c: Low-density lipoprotein-cholesterol

Table 3. Comparison of vitamin D, ferritin, and vitamin B12 levels of the groups

	Control group n=62	Newly diagnosed group n=53	Treatment group n=70	p*
Vitamin D	20.41±8.56	18.32±9.55	22.59±12.81	0.088
Ferritin	44.06±40.78	31.27±30.2	40.94±45.37	0.361
Vitamin B12	351.28±137.46	329.76±152.76	353.58±126.07	0.596

*One-Way analysis of variance

Table 4. Comparison of zonulin levels of the groups

		Control group n=62	Newly diagnosed group n=53	Treatment group n=70	p*
Zonulin	Mean ± SD	584.25±787.90	293.25±537.03	236.13±392.82	0.007
	Median (IQR)	96 (71.25-1241.75)	72 (60.5-192.75)	78 (61-116)	

*Kruskal-Wallis test. SD: Standard deviation, IQR: Interquartile range

Table 5. Averages of zonulin levels according to TSH levels

		0.3-4.5 TSH n=106	4.5-10 TSH n=47	>10 TSH n=20	p*
Zonulin	Mean ± SD	410.47±675.72	304.40±487.46	344.55±537.31	0.833
	Median (IQR)	80 (64.5-276.5)	76 (62-241)	90 (66-491)	

*Kruskal-Wallis test. SD: Standard deviation, IQR: Interquartile range, TSH: Thyroid-stimulating hormone

Table 6. Correlation analysis of zonulin level and demographic, biochemical and hormonal parameters

		Zonulin
Age	R	0.284
	p*	0.001
BMI	R	0.172
	p*	0.024
LDL-c	R	0.156
	p*	0.041
Triglyceride	R	0.184
	p*	0.016
Anti-TPO	R	0.153
	p	0.045

*Pearson correlation test. BMI: Body mass index, LDL-c: Low-density lipoprotein-cholesterol, Anti-TPO: Anti-thyroid peroxidase

barrier results in the exposure of submucosal immune cells to bacteria and dietary antigens, that may result in inappropriate immune response formation. Increased intestinal permeability has been shown to be a common feature in the pathogenesis of most of the autoimmune diseases. Increased permeability precedes the onset of the disease, and abnormalities in antigen presentation cause a multi-organ response and initiate the autoimmune process. As a result, this leads to the development of different autoimmune diseases (11,12).

There is evidence that many environmental factors, including infections, may trigger Hashimoto's thyroiditis in genetically susceptible individuals. Non-pathological symbiotic microorganisms in the gut may also affect the extra-intestinal immune response. For this reason, intestinal dysbiosis (microbial disorder) may cause a loss of tolerance to specific antigens (including thyroglobulin) and autoimmunity in the pathogenesis of Hashimoto's thyroiditis. Increased intestinal permeability and intraepithelial lymphocyte infiltration are risk factors for thyroid autoimmunity. However, there are few studies showing a correlation between the gut and Hashimoto's thyroiditis (13).

Although the mechanisms by which zonulin protein is stimulated and how it is secreted into the intestinal lumen have not yet been fully explained, it is thought that it binds to its receptor on the intestinal epithelial cell and opens TJs transiently and reversibly.

The potential role of zonulin in the pathogenesis of Hashimoto's thyroiditis was observed by Özışık (10). They found that serum zonulin levels in the patient group were significantly higher compared to control groups. However, they failed to determine a significant correlation between serum TSH, anti-TPO and anti-TG concentrations and zonulin levels in the patient group (10). Similarly, in our study, although no correlation was found between TSH and anti-TG levels and zonulin, instead a correlation was observed between anti-TPO levels and zonulin.

Many studies have proven that HT is a cause of secondary hyperlipidemia. The improvement in the lipid profile after treatment with HT is proof of this. Therefore, HT is considered a risk factor for atherosclerotic heart diseases (14). In some studies, while thyroid replacement therapy caused a significant decrease in LDL and total cholesterol levels, it did not cause a significant change in triglyceride and HDL levels (15). O'Brien et al. (14) performed a study on 268 patients with primary HT and 27 patients with secondary HT, and they observed that there was a significant correlation between hyperlipidemia and both primary and secondary HT, and there was a significant improvement in hyperlipidemia after HT treatment (14). We also determined higher LDL and total cholesterol levels in patients with Hashimoto's thyroiditis without finding a significant correlation between triglyceride and HDL levels.

Some studies have shown that iron deficiency anemia has effects on thyroid metabolism. Iron deficiency anemia lowers plasma total thyroxine (T4) and total triiodothyronine (T3) concentrations and may increase serum TSH levels (16-18). In a study by Beard et al. (16) on mice, blood transfusion to mice with anemia resulted in improvements in T3 and T4 levels. In another study by Beard et al. (19) on mice, it was shown that thyroid hormone metabolism was affected in iron-deficient mice. In this study, we established higher ferritin levels in the control group, compared to with patients newly diagnosed Hashimoto's thyroiditis. This can be explained by the decrease in iron absorption in Hashimoto's thyroiditis, or it can be attributed to the negative effects of iron deficiency on thyroid hormone metabolism (anemia and decreased oxygen transport). However, higher vitamin D and B12 levels are found in the patient group that were previously diagnosed with Hashimoto's thyroiditis and are currently receiving thyroid replacement compared with newly diagnosed Hashimoto's thyroiditis patients.

Regarding the studies examining the effect of intestinal permeability on autoimmune disorders, authors showed that increased bowel permeability exists before the development of autoimmunity, as shown in celiac disease (20,21). Meddings et al.'s (7) study with diabetes-prone biobreeding (BB) rats; showed that intestinal permeability increases before the development of autoimmune diabetes. Although it is difficult to experiment these findings on

human-beings; we know that even after successful treatment in celiac patients, the defect in intestinal permeability continues (22).

Moreno-Navarrete et al. (23) showed that zonulin levels are higher in obesity in their study on 33 obese and 90 nonobese patients. Similarly, obese children have higher zonulin levels in the study by Küme et al. (24). Moreover, Ohlsson et al. (25) determined a correlation between serum zonulin levels and obesity and hyperlipidemia. We also observed a correlation between serum zonulin level and BMI, LDL and triglyceride levels.

Although there is limited available data on the relation of zonulin level and age, Zhang et al. (26) in polycystic ovary syndrome and Moreno-Navarrete et al. (23) in obesity found no significant relation between age and zonulin. Contradictorily, we found a negative correlation between zonulin levels and age. The reason for this may be the fact that autoimmune diseases are more common at younger ages and their frequency decreases with age.

An unidentified trigger that is not discovered yet is thought to be responsible for zonulin secretion. Some studies suggest that dietary proteins may be the possible triggers. For example, it has been shown that the incidence of type 1 DM is reduced in BB diabetes prone mice fed with hydrolyzed food (7). High-fat diet is associated with metabolic disorders by changing the intestinal microbiota and permeability (27). There are many factors affecting intestinal permeability and zonulin up-regulation like dietary habits, showing us that the results of the study should be interpreted with caution. Our study suggests that there may be a relationship between the high zonulin level in healthy individuals and the dietary habits of the participants.

In some studies, deterioration in the morphology of the intestinal epithelium, intraepithelial lymphocyte infiltration and increase in intestinal permeability have also been shown in Hashimoto's thyroiditis, similar to type 1 DM (9,28). However, in our study, serum zonulin level was not increased in patients with Hashimoto's thyroiditis compared with healthy individuals. However, measuring intestinal permeability *in vivo* and revealing its relationship with tissue zonulin level may enable us to obtain more realistic results.

In a study, blood was drawn from celiac patients and healthy individuals at different times of the day, and zonulin and antibody levels against zonulin were measured. Because of the study, it was observed that zonulin levels fluctuated during the day, but IgA and IgG antibody levels against zonulin were stable (29). Based on this study, due to the fluctuation in zonulin levels, with the zonulin level, measurement of IgA and IgG levels against zonulin is also recommended. The fact that this fluctuation may be responsible for the low zonulin levels in patients with Hashimoto's thyroiditis in our study is another matter of discussion.

This study has some potential drawbacks. A low sample size was the main limitation which is related to the exclusion of cases with metabolic, renal or rheumatologic comorbidities. The reason for this is the exclusion of patients with concomitant pathologies such as DM, hypertension, chronic kidney disease or rheumatological diseases. Secondly, histopathological examination of tissue zonulin level can more clearly reflect zonulin activity and its relationship with other parameters, although it is an invasive procedure. This study was conducted on the Turkish population, and the lack of data on the dietary habits of the participants, which may alter serum zonulin levels limits us to generalize our results to other populations.

CONCLUSION

In conclusion, the relationship between the zonulin protein, which is responsible for TJ regulation, and autoimmune diseases is a topic that is currently and increasingly being researched. In this study, we predicted that zonulin levels would be higher in the patient groups compared with the control group, but in the study, no relationship was found between zonulin levels and patients with Hashimoto thyroiditis.

New and differently designed studies will increase our knowledge and experience in this subject. In addition to the increasing number of animal studies, humans studies may provide valuable data on the role of intestinal permeability in the development of autoimmune disorders, and may guide us for developing preventive medical approaches in the future.

ETHICS

Ethics Committee Approval: Ethical approval was obtained from the Bağcılar Training and Research Hospital Local Ethics Committee. Ethics committee date and approval number: 08.02.2017 and 2017/543.

Informed Consent: The purpose of the study was explained to each patient included in the study and a consent form was signed.

Authorship Contributions

Surgical and Medical Practices: Z.S.İ., E.G., Concept: H.S., A.E.A., Design: H.S., A.E.A., Data Collection or Processing: Z.S.İ., S.Y., E.G., S.H.K., Analysis or Interpretation: Z.S.İ., S.H.K., Literature Search: Z.S.İ., S.Y., Writing: Z.S.İ., A.E.A.

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REFERENCES

1. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002;87:489-99.
2. Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system: from theory to practice. J Clin Endocrinol Metab 1994;78:1026-7.
3. Fisfalen ME, Palmer EM, Van Seventer GA, Soltani K, Sawai Y, Kaplan E, et al. Thyrotropin-receptor and thyroid peroxidase-specific T cell clones and their cytokine profile in autoimmune thyroid disease. J Clin Endocrinol Metab 1997;82:3655-63.
4. Corapçioğlu D, Tonyukuk V, Kiyan M, Yilmaz AE, Emral R, Kamel N, et al. Relationship between thyroid autoimmunity and Yersinia enterocolitica antibodies. Thyroid 2002;12:613-7.
5. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev 2011;91:151-75.
6. Fasano A, Shea-Donohue T. Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. Nat Clin Pract Gastroenterol Hepatol 2005;2:416-22.
7. Meddings JB, Jarand J, Urbanski SJ, Hardin J, Gall DG. Increased gastrointestinal permeability is an early lesion in the spontaneously diabetic BB rat. Am J Physiol 1999;276:G951-7.
8. Sapone A, de Magistris L, Pietzak M, Clemente MG, Tripathi A, Cucca F, et al. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. Diabetes 2006;55:1443-9.
9. Sasso FC, Carbonara O, Torella R, Mezzogiorno A, Esposito V, Demagistris L, et al. Ultrastructural changes in enterocytes in subjects with Hashimoto's thyroiditis. Gut 2004;53:1878-80.
10. Özışık G. İntestinal Permeabilite Düzenleyici Zonulin'in Hashimoto Hastalığı Patogenezinde Potansiyel Rolü [dissertation]. Genel Kurmay Başkanliği Gülhane Askeri Tip Akademisi Haydarpaşa Eğitim Hastanesi Komutanlığı; 2006.
11. Macdonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. Science 2005;307:1920-5.
12. Vaarala O, Atkinson MA, Neu J. The "perfect storm" for type 1 diabetes: the complex interplay between intestinal microbiota, gut permeability, and mucosal immunity. Diabetes 2008;57:2555-62.
13. Mori K, Nakagawa Y, Ozaki H. Does the gut microbiota trigger Hashimoto's thyroiditis? Discov Med 2012;14:321-6.
14. O'Brien T, Dinneen SF, O'Brien PC, Palumbo PJ. Hyperlipidemia in patients with primary and secondary hypothyroidism. Mayo Clin Proc 1993;68:860-6.
15. Arem R, Escalante DA, Arem N, Morrisett JD, Patsch W. Effect of L-thyroxine therapy on lipoprotein fractions in overt and subclinical hypothyroidism, with special reference to lipoprotein(a). Metabolism 1995;44:1559-63.
16. Beard J, Finch CA, Green WL. Interactions of iron deficiency, anemia, and thyroid hormone levels in response of rats to cold exposure. Life Sci 1982;30:691-7.
17. Beard J, Tobin B, Green W. Evidence for thyroid hormone deficiency in iron-deficient anemic rats. J Nutr 1989;119:772-8.
18. Zimmermann M, Adou P, Torresani T, Zeder C, Hurrell R. Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in Côte d'Ivoire. Am J Clin Nutr 2000;71:88-93.
19. Beard JL, Brigham DE, Kelley SK, Green MH. Plasma thyroid hormone kinetics are altered in iron-deficient rats. J Nutr 1998;128:1401-8.
20. Hall EJ, Batt RM. Enhanced intestinal permeability to 51Cr-labeled EDTA in dogs with small intestinal disease. J Am Vet Med Assoc 1990;196:91-5.
21. Hall EJ, Batt RM. Abnormal intestinal permeability could play a role in the development of gluten-sensitive enteropathy in Irish setter dogs. J Nutr 1991;121(11 Suppl):S150-1.
22. Bjarnason I, Peters TJ. In vitro determination of small intestinal permeability: demonstration of a persistent defect in patients with coeliac disease. Gut 1984;25:145-50.
23. Moreno-Navarrete JM, Sabater M, Ortega F, Ricart W, Fernández-Real JM. Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance. PLoS One 2012;7:e37160.
24. Küme T, Acar S, Tuhan H, Çatlı G, Anık A, Gürsoy Çalan Ö, et al. The Relationship between Serum Zonulin Level and Clinical and Laboratory Parameters of Childhood Obesity. J Clin Res Pediatr Endocrinol 2017;9:31-8.
25. Ohlsson B, Orho-Melander M, Nilsson PM. Higher Levels of Serum Zonulin May Rather Be Associated with Increased Risk of Obesity and Hyperlipidemia, Than with Gastrointestinal Symptoms or Disease Manifestations. Int J Mol Sci 2017;18:582.
26. Zhang D, Zhang L, Yue F, Zheng Y, Russell R. Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation. Eur J Endocrinol 2015;172:29-36.
27. de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. Am J Physiol Gastrointest Liver Physiol 2010;299:G440-8.
28. Cindoruk M, Tuncer C, Dursun A, Yetkin I, Karakan T, Cakir N, et al. Increased colonic intraepithelial lymphocytes in patients with Hashimoto's thyroiditis. J Clin Gastroenterol 2002;34:237-9.
29. Vojdani A, Vojdani E, Kharrazian D. Fluctuation of zonulin levels in blood *vs* stability of antibodies. World J Gastroenterol 2017;23:5669-79.