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Relationship of zonulin levels with thyroid function and metabolic parameters in Hashimoto's Thyroiditis

Cigdem Damla Deniz[a], Gulsum Gonulalan[b], Kubra Sayin[c,*], Betul Kozanhan[d]

[a] University of Health Sciences, Konya Training and Research Hospital, Department of Biochemistry, Konya, Türkiye
[b] University of KTO Karatay, School of Medicine, Medicana Konya Hospital, Department of Endocrinology and Metabolism, Konya, Türkiye
[c] University of Necmettin Erbakan, Health Science Faculty, Department of Nutrition and Dietetics, Konya, Türkiye
[d] University of Health Sciences, Konya Training and Research Hospital, Department of Anaesthesiology and Intensive Care, Konya, Türkiye

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Abstract

Aim: Intestinal permeability is one of the factors responsible for the pathogenesis of many immune-related diseases, one of which is Hashimoto's-thyroid (HT). We used zonulin as a biomarker of intestinal permeability in this cross-sectional study. We aimed to determine serum zonulin levels in individuals diagnosed with HT and to investigate the relationship between zonulin and autoimmunity biomarkers, thyroid function and metabolic parameters.

Materials and Methods: The study was conducted with 43 women newly diagnosed with HT and a control group of 30 matched with them. The evaluated parameters were thyroperoxidase-antibody (TPO-AB), thyroglobulin-antibody (Tg-AB), thyroid hormone profile, metabolic parameters and zonulin levels.

Results: Higher levels of TSH, TPO-AB, Tg-AB, TG and TC were measured in the HT group compared to controls ($P < 0.005$). The means of zonulin in HT.patients were significantly higher than in the control group ($P < 0.005$).

Conclusion: The correlations between zonulin levels and TSH and TPO-AB among all subjects in this study support the idea that intestinal permeability is a distinguishing feature in the conditions causing the formation of immunological hypersensitivity in HT. More studies are needed to prove that impaired intestinal permeability is among the causes of decreased thyroid function.

Introduction

Hashimoto's thyroiditis (HT), one of the most extensive human thyroid autoimmune diseases, is considered a multifactorial disease [1, 2]. HT is a condition that occurs with progressive thyroid cell extinction [3]. Although the exact pathogenesis is not known, it is expected to find new ways of prevention as well as treatment [4]. Generally, autoimmune pathogenesis is explained by events of specific gene expression and exposure to environmental stimulators, to which recently impaired intestinal permeability has been added as a third element [5]. The impaired intestinal permeability refers to ineffective tight junctions that allow antigens to pass through the intestinal flora and force the immune system to initiate a process that can attack any organ or tissue [6-9]. In addition to celiac disease, increased intestinal permeability has an effective role in the pathogenesis of many other autoimmune diseases, including type 1 diabetes [10, 11], multiple sclerosis [12, 13] and rheumatoid arthritis [14].

Zonulin is a protein that initiates a signaling pathway that leads to the dissolution of tight connections between the cells of the small intestine [15]. Zonulin is currently the only known measurable blood protein that reflects intestinal permeability and its elevation in blood is thought to reflect disruption of the intestinal barrier. Increased zonulin level is seen in celiac disease, type 1 diabetes and obesity related insulin resistance [16, 17].

In conclusion, HT continues to be a disease whose pathogenesis is not fully known and new ways of prevention are needed [4]. However, zonulin levels are not known in HT patients who are seen as an autoimmune disease. In this context, the measurement of zonulin levels in newly diagnosed HT patients is important to determine both its importance in the pathogenesis of the disease and its effects on metabolic conditions caused by the disease. Therefore,

*Corresponding author:
Email address: kubrakucur@hotmail.com (Kubra Sayin)

the current cross-sectional study was conducted to assess the relationship between serum zonulin levels and thyroid hormone profile, TPO-AB, Tg-AB and metabolic parameters in HT patients.

Materials and Methods

The patient group was involved of 43 adults with newly-diagnosed HT who applied to Konya Training and Research Hospital Endocrinology Outpatient Clinic (July-2018 and February 2019). Exclusion criteria in the study were pregnancy, menopause status, and use of anti-thyroid medications in the last 6 months. Thirty adults without HT and autoimmune dysfunction were used as the control group.

Blood samples (5 mL) were immediately centrifuged after collection by venous puncture; plasma was frozen and stored at -80°C until assays are performed. Zonulin ELISA kit (Immundiagnostik AG, Bernsheim, Germany; inter-assay variation 10.6%) was used for serum zonulin measurements. Serum ALT, glucose, lipid parameters were determined by routine laboratory automated techniques. Insulin levels were determined by a chemiluminescent method with Siemens immulite 2000XPi immunoassay analyzer (Siemens Inc.). HbA1c was measured using high performance liquid chromatography (Trinity Biotech Premier 9210, USA). Serum free-thyroxine (fT4), free-triiodothyronine (fT3) and thyrotropin (TSH) levels were measured by enzyme immunoassay (EIA) to evaluate thyroid status.

The ethical suitability of this study was approved by the ethics committee of Necmettin Erbakan University, Meram Medical School, Turkey (permission date/number 2018/1349). Written informed consent was obtained from all patients before entering the study.

Data analysis was performed using SPSS software for Windows version 16 (SPSS Inc., Chicago, USA). Normality of data distribution was assessed with Kolmogorov–Smirnov test. The relationship between the patient and control groups was compared using the independent T test and Kruskal-Wallis. The correlation of zonulin with the other variables was evaluated using the Spearman coefficient or the Mann-Whitney test. Logistic regression anaysis used for determine the association between serum zonulin and Hashimoto's thyroiditis was analyzed with binary. logistic.-regression. The level of significance for statistical analysis was:5%.

Results

A total of 43 patients-with HT, 37 of who (86%) were women, were included in the study. There were:30 people in the control group, 26 of whom were women (86.6%). Laboratory and clinical characteristics of the participants are described in:Table 1. The patients in this study did not differ from the controls in terms of age, sex, weight and BMI (all $P > 0.05$) (Table 1).

There was no significant difference between levels of insulin, glucose, uric acid or HDL-C, when comparing the two groups. TSH, TPO-AB and TG-AB concentrations were higher and free T4 and free T3 were lower in patients compared to the control group as expected.

Table 1. Demographic and biochemical characteristics of study participants.

Parameters	Hashimoto thyroiditis (n=43)	Controls (n=30)	P-value
Age (years)	34.4±8.1	33.6±9.2	NS
Weight (kg)	168.6±8.9	161±7.6	NS
Height (cm)	78.6±15.2	72.4±12.8	NS
BMI (kg/m2)	29.7±5.0	27.5±5.3	NS
WC (cm)	96.4±12.7	88.2±12.1	.022
ALT (IU/L)	19.8±7.3	17.4±9.3	.033
Uric acid (mg/dL)	4.9±0.9	5.7±3.61	NS
TSH	25.57±25.15	2.11±1.00	.000
T4	1.03±0.27	1.24±0.13	.000
T3	2.80±0.54	3.25±0.40	.000
Anti TPO	0.62±0.29	0000	.000
Anti Tg	0.22±0.41	0000	.000
TG (mg/dL)	123.05±43.93	101.60±59.05	.005
TC (mg/dL)	193.2±32.2	170.2±24.1	.001
HDL-C.(mg/dL)	46.0±8.3	48.7±11.5	NS
LDL-C.(mg/dL)	121.0±30.7	101.6±23.9	.004
Insulin (mIU/L)	12.0±6.7	16.9±21.2	NS
Glukoz (mg/dL)	89.16±8.71	84.76±7.57	NS
HOMA-IR	2.6±1.6	3.9±5.4	NS
Zonulin (ng/ml)	1.35±3.17	0.24±1.28	.003

Data are represented as the mean±SD. BMI, body mass index; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment: insulin resistance; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; WC, waist circumference; OR, odds-ratio; CI, confidence-interval.

Among all participants a positive correlation was observed between zonulin and TSH, TPO-AB, Tg-AB (Table 2). There was no significant correlation between zonulin and metabolic parameters TG, TC, HDL-C, LDL-C insulin, glucose (Table 2). We also found correlation between zonulin and BMI and VAI among all participants. In the patient group, there was no correlation between zonulin levels and and any parameters (Table 2).

Binary Logistic regression was used to explore the association between Hashimato Thyroiditis' disease diagnosis and serum zonulin levels. [Table3] Table 3 shows the unadjusted data and the data for one model with gender and age confounders. In the unadjusted model, higher serum Zonulin was significantly associated with reduced risk of Hashimato thyroiditis, with odds ratios (ORs) 0.368.

Discussion

In this study, we used a series of biochemical tests to investigate the relationship between thyroid functional status and intestinal permeability as assessed by serum zonulin in newly-diagnosed and untreated HT-patients. A major observation from the present study is that mean zonulin levels in HT patients are significantly lower than in healthy controls. Another interesting measurement from the present study is zonulin levels showed a significant inverse correlation with TPO-AB and Tg-AB among all participants. Also TSH levels were strongly and positively

Table 2. Correlations between zonulin and metabolic and tiroid parameters.

	Among all participans		Among hashimatos thyroidis	
	r	p	r	p
Age. (years)	.203	.089	.273	.093
BMI. (kg/m^2)	.293	.023	.307	.057
WC. (cm)	.285	.030	.221	.182
ALT. (IU/L)	.040	.241	.117	.540
Uric acid.(mg/dL)	.120	.317	.059	.933
TSH	.406	.001	.273	.144
T4	-.172	.148	-.137	.404
T3	-.110	.158	-.110	.562
Anti-TPO	.331	.004	.160	.311
Anti-Tg	.262	.026	.179	.275
TG.(mg/dL)	.175	.102	.186	.214
TC.(mg/dL)	.086	.314	.222	.174
HDL-C.(mg/dL)	.089	.512	-.164	.486
LDL-C.(mg/dL)	.218	.093	.123	.516
Insulin. (mIU/L)	.060	.615	.257	.119
Glukoz. (mg/dL)	.167	.170	.221	.110
HOMA-IR.	.143	.201	.109	.508

BMI, body mass index; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment: insulin resistance; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

Table 3. Logistic regressions between zonulin and Hashimato thyroiditis

	Unadjusted			Adjusted model 1		
Hashimato thyroiditis	OR.	95% CI.	P.	OR.	95% CI.	P.
Zonulin	.368	.400-1.146	.047	.310	.462-1.165	.075

Adjusted model 1 included two additional confounders (gender, age). OR, odds-ratio; CI, confidence-interval.

correlated with zonulin levels. On the other hand, no correlations were seen between zonulin levels and metabolic parameters –total cholesterol, triglycerides, insulin, glucose, LDL-C, HDL-C- among all participants.

In recent years, the relationship between microbiota composition and thyroid autoimmunity has attracted attention, as in other autoimmune diseases. It has been documented that impaired microbiota involves in the pathogenesis of autoimmune diseases such as rheumatoid arthritis and type 1 diabetes [18].There are several mechanisms that link an impaired microbiota to the onset of autoimmune disease, such as molecular mimetic, subsequent activation, and determinant spread [19-21].

In humans, it has been reported that increased zonulin levels are closely related to increased intestinal permeability that caused by genetic overexpression of intestinal tight junction proteins [22]. Zonulin, which is overexpressed in autoimmune diseases in which tight junction dysfunction have a role, such as celiac disease [24, 25] and type-1 diabetes [22], actively participates in innate immunity in the gut [23]. In this context, circulating zonulin can be considered as a practical biomarker of-intestinal permeability [26, 27].

In recent years, changes in the intestinal microbial content have been reported in patients suffering from HT. For example, Sasso (2004) found that the diagnosis of leaky gut in patients with-HT was higher than in the control group, and morphological differentiations of distal duodenal enterocytes were detected in patients with-HT [28].

Increased-intestinal-permeability allows antigens, toxins and bacterial metabolites to pass from the intestine into the blood circulation, so this will play a role in the onset of autoimmune-thyroid disease. [29]. There are two articles evaluating the composition of the microbiota in fecal samples of HT patients and showing that there is deterioration in the microbiota in this group [30, 31]. There is also an opposite interaction, changes in thyroid-function can modify the gut microbial population. For example, Lauritano's (2007) study showed that subjects with hypothyroidism were more likely to overgrow bacteria in the small intestine [32]. This bacterial excess seems to enhance the deterioration of gastrointestinal. neuromuscular-function. As detected by Zhou et al. (2014), hyperthyroidism caused a significant decrease in Bifidobacterium and Lactobacillus and an increase in Enterococcus strains in fecal samples, which seems to indicate that even hyperthyroidism contributes to the impaired gut microbiota [33].

As the relationship between thyroid-autoimmunity and intestinal permeability has been systematically less explored, this article aims to provide evidence on the possible association between intestinal permeability and this common autoimmune disorder. While intestinal permeability has not been found to be a useful predictor of autoimmune disorders because its difficulty to identify, we investigated whether zonulin could help identify the risk of HT.

In conclusion,,this study has disclosed that intestinal permeability as evaluated by zonulin levels is considerably increased in individuals with HT. In addition, serum zonulin levels are significantly and positively correlated with TSH, TPO-AB, Tg-AB. More studies with larger samples are needed to clarify the effect of zonulin in the etiology of inadequate thyroid-function. Further understanding the causal relationships that lead to decreased thyroid function may enable researchers to offer new therapeutic options, such as dietary changes or microbiome modifications through the use of probiotics, to restore thyroid function. There is a need for studies examining the effect of impaired intestinal barrier function, which can be measured with zonulin, on metabolic diseases.

Ethics approval

This investigation was approved by the ethical committee at the Necmettin Erbakan University Meram Medical School, Turkey (permission date/number 2018/1349). Written informed consent was obtained from all patients before entering the study.

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