

DOI: 10.4274/jcrpe.galenos.2020.2019.0186

Short Communication

Children with Hashimoto's Thyroiditis Have Increased Intestinal Permeability: Results of a Pilot Study

Short title: Intestinal permeability in thyroiditis

Banu Kucukemre Aydin¹, Melek Yildiz¹, Abdurrahman Akgun¹, Neval Topal¹, Erdal Adal², Hasan Onal¹
¹Health Sciences University, Kanuni Sultan Suleyman Training and Research Hospital, Pediatric Endocrinology and Metabolism Unit, Istanbul, Turkey
²Medipol University Faculty of Medicine, Department of Pediatric Endocrinology and Metabolism, Istanbul, Turkey

Abstract

Increased intestinal permeability (IIP) precedes several autoimmune disorders. Although Hashimoto's thyroiditis (HT) is the most common autoimmune disorder, the role of IIP in its pathogenesis had received little attention. Zonulin plays a critical role in IIP by modulating intracellular tight junctions. Rise of serum zonulin levels were shown to indicate IIP in human subjects. In this case-control study, we examined the hypothesis that patients with HT have IIP. We studied 30 children and adolescents with HT, and age, gender and body mass index (BMI) matched 30 patients with congenital hypothyroidism (CH). Serum zonulin levels, free thyroxine (fT4), thyroid stimulating hormone (TSH), anti-thyroglobulin antibody and anti-thyroid peroxidase antibody were measured. Zonulin levels were significantly higher in patients with HT than patients with CH (59.1±22.9ng/mL vs. 43.3±32.9ng/mL, p=0.035). In patients with HT, zonulin levels were positively correlated with weight (r= 0.406, p=0.03), BMI (r= 0.486, p=0.006) and levothyroxine dose (r= 0.463, p=0.02). In patients with CH, zonulin levels were positively correlated with age (r= 0.475, p=0.008), weight (r= 0.707, p<0.001), BMI (r= 0.872, p<0.001) and levothyroxine dose (r= 0.485, p=0.007). After adjusting for age, weight, TSH and fT4 levels, serum zonulin was only associated with levothyroxine dose in patients with HT (R²=0.36, p=0.05). In patients with CH, only weight was associated with zonulin levels (R²=0.62, p<0.001). In conclusion, higher zonulin levels in children and adolescents with HT suggested IIP in these patients. Additionally, the association between zonulin levels and levothyroxine dose might imply a relationship between serum zonulin and disease severity.

Keywords: Autoimmune thyroiditis; congenital hypothyroidism; zonulin; increased intestinal permeability.

What is already known on this topic?

- Increased intestinal permeability (IIP) was shown to precede the onset of several autoimmune disorders.
- Although Hashimoto's thyroiditis (HT) is the most common autoimmune disorder, the role of IIP in its pathogenesis had received little attention.
- Exact disease mechanisms are poorly elucidated in HT.

What this study adds?

- Children and adolescents with HT had increased zonulin levels compared to their age, gender and body mass index matched peers with congenital hypothyroidism.
- Higher zonulin levels indicated IIP in these patients.

Corresponding author: Banu Kucukemre Aydin, M.D., Health Sciences University, Kanuni Sultan Suleyman Training and Research Hospital, Pediatric Endocrinology and Metabolism Unit, Istanbul, Turkey

phone: +90 212 404 15 00

e-mail: bkucukemre@yahoo.com

Submitted: 15-Nov-2019

Accept: 23-Jan-2020

0000-0003-0859-1565

Introduction

Both genetic predisposition and environmental factors serve as the trigger of Hashimoto thyroiditis (HT), but the exact mechanisms are still not fully understood. Increased intestinal permeability (IIP) was shown to be a

constant and early feature of several autoimmune disorders (1). Although HT is the most common autoimmune disorder worldwide, the role of IIP in its pathogenesis had received little attention.

Human zonulin regulates intestinal permeability by disassembling the tight junctions (TJ) (2). Zonulin was shown to play a key role in the IIP, when up regulated (3). Increased zonulin levels were reported to be correlated with IIP in vivo and changes in claudin-1, claudin-2, and myosin IXB genes expression (4). Higher zonulin expression was reported in the intestinal tissues of patients with many autoimmune disorders (4). Increased serum zonulin levels were detected in human subjects during the pre-diabetic stage and preceded the onset of type 1 diabetes (4). On a rat model, zonulin-dependent IIP was shown to precede the onset of type 1 diabetes by 2–3 weeks (3). Moreover, oral administration of the zonulin inhibitor (AT-1001) to these rats blocked autoantibody formation, zonulin-mediated IIP, and finally reduced the incidence of diabetes (3). AT-1001 competitively blocks the apical zonulin receptor and prevents the opening of TJ (2).

In this study, we examined the hypothesis that patients with HT have IIP. To this end, we examined blood zonulin levels in patients with HT and used patients with congenital hypothyroidism (CH) as a control group. To the best of our knowledge this is the first study which examines zonulin levels in patients with HT.

Materials and Methods

This was a case-control study on a group of 30 consecutive children and adolescents with HT, and age, gender and BMI matched 30 patients with CH. The diagnosis of HT was based on positive anti-TPO (antibodies against thyroid peroxidase), anti-TG (antibodies against thyroglobulin) levels and typical thyroiditis ultrasound pattern. Obese patients and patients with acute or other chronic diseases were excluded from the study. Informed consent was obtained in accordance with a protocol approved by the Institutional Review Board of Sadi Konuk Training and Research Hospital (2017-207).

Anthropometric measurements

Heights were measured in the standing position with bare feet, using Harpenden equipment, and an electronic scale sensitive to 0.1 kilogram was used for weight measurements. Body mass index (BMI) was calculated according to standard formula. Height, weight and BMI z scores (SDS) were calculated according to national standards (5). Obesity was defined as a BMI SDS ≥ 2 SDS.

Laboratory evaluation

Venous blood samples were collected for zonulin, free thyroxine (fT4), thyroid stimulating hormone (TSH), anti-TPO, and anti-TG levels. Samples were immediately processed and sera were stored at -80°C for subsequent batch analysis. Zonulin levels were measured by human zonulin enzyme-linked immunosorbent assay kit (ELISA, Elabscience, Houston, Texas, USA). Levels of fT4, TSH, anti-TPO and anti-TG were measured by electrochemiluminescence immunoassay (ECLIA, Cobas e602, Roche Diagnostics, Basel, Switzerland). Thyroid ultrasound results were retrieved from the patient's files.

Data analyses

Data were presented as the mean \pm SDS. Comparisons were made by using Independent Samples t Test or X^2 Test. We used Pearson analysis to determine correlations between zonulin levels and other clinical parameters. We created separate linear regression models to examine the associations of blood zonulin levels in patients with HT and CH. All statistical analyses were conducted with SPSS version 15.0 (SPSS Inc, Chicago, IL). Statistical significance was defined as $p \leq 0.05$.

Results

Patients' Characteristics

Serum zonulin levels were significantly higher in patients with HT than patients with CH (Table 1 and Figure 1). Age, gender, weight SDS, height SDS, BMI SDS and daily levothyroxine dose were not different between the groups (Table 1). Serum fT4 levels were higher in patients with CH, but TSH levels were not different between the groups (Table 1). Anti-TPO and Anti-TG levels were higher in patients with HT (Table 1). Thyroid ultrasonography in patients with HT revealed heterogeneous parenchyma in 17 patients, as well as, heterogeneous and pseudo-nodular parenchyma in 13 (Table 1). In patients with CH, ultrasonography showed agenesis of the thyroid gland in 15 patients, heterogeneous parenchyma in 3 and normal thyroid gland in 12 patients (Table 1).

Correlation Analyses

In patients with HT, zonulin levels were positively correlated with weight ($r = 0.406$, $p = 0.03$), weight SDS ($r = 0.377$, $p = 0.04$), BMI ($r = 0.486$, $p = 0.006$), BMI SDS ($r = 0.419$, $p = 0.02$) and levothyroxine dose ($r = 0.463$, $p = 0.02$). There was no significant correlation between zonulin levels and anti-TPO or anti-TG levels ($r = -0.174$, $p = 0.4$ and $r = 0.295$, $p = 0.1$, respectively).

In patients with CH, there were strong positive correlations between zonulin levels and age ($r = 0.475$, $p = 0.008$), weight ($r = 0.707$, $p < 0.001$), weight SDS ($r = 0.532$, $p = 0.002$), BMI ($r = 0.872$, $p < 0.001$), BMI SDS ($r = 0.681$, $p < 0.001$) and levothyroxine dose ($r = 0.485$, $p = 0.007$).

Regression Analyses

In patients with HT, serum zonulin was only associated with levothyroxine dose after adjusting for age, weight, TSH and fT4 levels ($R^2 = 0.36$, $p = 0.05$).

When we put the patients with CH in the same regression model, there was no significant association between zonulin level and levothyroxine dose ($p=0.4$). However, serum zonulin was strongly associated with only weight in these patients ($R^2=0.62$, $p<0.001$).

Discussion

Intestinal mucosa is the largest contact site for the host's immune system and exterior antigens, such as food antigens, bacteria, pathogens, and toxins (6). Increase in permeability can cause an exposure of sub mucosal immune cells to these various antigens and may lead to the development of autoimmune disorders (6,7). Indeed, previous studies have shown that the increased permeability is a key pathogenic component rather than an epiphenomenon of autoimmune disorders (3,4). It was hypothesized that, intestinal permeability is also increased in HT (8), but to the best of our knowledge, to date no study has addressed this issue in these patients. Only in a letter to the editor, C Sasso et al. (9), described ultrastructural morphological changes of distal duodenum enterocytes in four patients with HT. They also reported IIP, evaluated by lactulose/mannitol test, in these four patients (9). In our study, we showed that children and adolescents with HT had increased zonulin levels compared to their age, gender and BMI matched peers with CH.

Intestinal TJ create gradients for the optimal absorption of nutrients and control tolerance/immunity balance to nonself antigens (10). Zonulin, a physiological modulator of TJ, is involved in trafficking of macromolecules and, therefore, in balance between tolerance and immune response (2,11). Sturgeon et al. (12) identified zonulin as a master regulator of intercellular TJ. They showed that, there was an IIP in zonulin transgenic mice compared to wild-type mice, which was associated with upregulation of zonulin gene expression. Moreover, treatment with AT1001 reduced intestinal permeability both in vivo and ex vivo and reverted morbidity and mortality in these zonulin transgenic mice. Their data demonstrated that zonulin-dependent small intestinal barrier dysfunction is an early step leading to the break of tolerance with subsequent development of colitis (12). Similarly, Arrieta et al. (13) reported that, IL10 gene-deficient mice treated with AT-1001, showed a marked decrease in small intestinal permeability and a clear reduction of colitis. In addition, several clinical trials reported a potential beneficial effect of AT-1001 (Larazotide acetate) in patients with celiac disease (14). In a study of 339 type 1 diabetic patients and their first degree relatives, Sapone A et al. (4) showed that patients with type 1 diabetes and their relatives have elevated serum zonulin levels that correlate with IIP associated with altered genetic expression of intestinal TJ proteins. Zonulin upregulation was shown to precede the onset of diabetes, providing a possible link between IIP, environmental exposure to non-self antigens, and the development of autoimmunity in genetically susceptible individuals for type 1 diabetes (4). Our finding of increased zonulin levels in patients with HT would trigger future studies that may lead to potential new therapies for HT.

Increased zonulin levels have been reported with aging and obesity (15). It has been suggested that, IIP may play a critical role in the development of age-related and obesity related inflammation and comorbidities. Similarly, we showed that, serum zonulin levels were strongly correlated with age, weight and BMI, in our study group. Serum zonulin levels were also positively correlated with levothyroxine dose both in patients with CH and HT. Moreover, in patients with HT, zonulin level was only associated with levothyroxine dose after adjusting for age, weight, TSH and fT4 levels. By contrast, there was no such association in CH patients. This finding might imply a relationship between serum zonulin levels and disease severity in HT, because in patients with CH, levothyroxine dose is mainly based on the amount of residual thyroid tissue/function, age, body weight, and thyroid hormone levels (16). However, in patients in HT, there is another associate of the levothyroxine dose; degree of the thyroid damage by autoimmune mechanisms (17). We did not find a significant correlation between anti-TPO or anti-TG antibodies and serum zonulin levels. But the contribution of anti-TPO antibodies to thyroid damage compared to T cell and cytokine-mediated apoptosis is minor and anti-TG antibodies do not cause thyroid cell destruction (18,19). Thyroid damage in HT is mostly due to an apoptotic processes combined with CD8⁺ cell mediated cytotoxicity, changes in cell junctions, and complement activation (18,19).

Study Limitations

This was a pilot study with small numbers of children. In addition, we did not have a healthy control group to compare zonulin levels in children and adolescents who were not on levothyroxine therapy.

Conclusion

Higher zonulin levels in children and adolescents with HT suggested IIP in these patients. Further research is needed to expand these results in larger cohorts to gain more insight into the pathogenesis of HT and to lead potential new treatments.

Acknowledgments

This study was funded by the Scientific Research Projects Coordination Unit of Health Sciences University of Turkey (grant Number: 2017/022 [to HO]).

Authorship contribution

BKA; Designed and performed the experiments, analyzed the data, drafted the manuscript, provided final approval of the version to publish and agreed to be accountable for all aspects of the work.

MY; Performed the experiments, analyzed the data, provided critical revision of the manuscript, provided final approval of the version to publish and agreed to be accountable for all aspects of the work.

AA; Performed the experiments, provided critical revision of the manuscript, provided final approval of the version to publish and agreed to be accountable for all aspects of the work.

NT; Performed the experiments, provided critical revision of the manuscript, provided final approval of the version to publish and agreed to be accountable for all aspects of the work.

EA; Designed the experiments, supervised the research, provided critical revision of the manuscript, provided final approval of the version to publish and agreed to be accountable for all aspects of the work.

HO; Designed the experiments, supervised the research, provided critical revision of the manuscript, provided final approval of the version to publish and agreed to be accountable for all aspects of the work.

References

1. Arrieta MC, Bistriz L, Meddings JB. Alterations in intestinal permeability. *Gut* 2006;55:1512-1520.
2. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. *J Cell Sci* 2000;113 Pt 24:4435-4440.
3. Watts T, Berti I, Sapone A, Gerarduzzi T, Not T, Zielke R, Fasano A. Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats. *Proc Natl Acad Sci USA* 2005;102:2916-2921.
4. Sapone A, de Magistris L, Pietzak M, Clemente MG, Tripathi A, Cucca F, Lampis R, Kryszak D, Carteni M, Generoso M, Iafusco D, Prisco F, Laghi F, Riegler G, Carratu R, Counts D, Fasano A. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. *Diabetes* 2006;55:1443-1449.
5. Neyzi O, Bundak R, Gokcay G, Gunoz H, Furman A, Darendeliler F, Bas F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. *J Clin Res Pediatr Endocrinol* 2015;7:280-293.
6. Virili C, Centanni M. Does microbiota composition affect thyroid homeostasis? *Endocrine* 2015;49(3):583-587.
7. Macdonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. *Science* 2005;307(5717):1920-1925.
8. Virili C, Fallahi P, Antonelli A, Benvenega S, Centanni M. Gut microbiota and Hashimoto's thyroiditis. *Endocr Metab Disord* 2018;19(4):293-300.
9. Sasso FC, Carbonara O, Torella R, Mezzogiorno A, Esposito V, Demagistris L, Secondulfo M, Carratu R, Iafusco D, Carteni M. Ultrastructural changes in enterocytes in subjects with Hashimoto's thyroiditis. *Gut* 2004;53:1878-1880.
10. Fasano A. Zonulin, regulation of tight junctions, and autoimmune diseases. *Ann N Y Acad Sci* 2012;1258:25-33.
11. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev* 2011;91(1):151-175.
12. Sturgeon C, Lan J, Fasano A. Zonulin transgenic mice show altered gut permeability and increased morbidity/mortality in the DSS colitis model. *Ann N Y Acad Sci* 2017;1397:130-142.
13. Arrieta MC, Madsen K, Doyle J, Meddings J. Reducing small intestinal permeability attenuates colitis in the IL10 gene-deficient mouse. *Gut* 2009;58:41-48.
14. Khaleghi S, Ju JM, Lamba A, Murray JA. The potential utility of tight junction regulation in celiac disease: focus on larazotide acetate. *Therap Adv Gastroenterol* 2016;9:37-49. Epub 2016/01/16
15. Kume T, Acar S, Tuhan H, Catli G, Anik A, Gursoy Calan O, Bober E, Abaci A. The Relationship between Serum Zonulin Level and Clinical and Laboratory Parameters of Childhood Obesity. *J Clin Res Pediatr Endocrinol* 2017;9:31-38. Epub 2016/12/23
16. Duntas LH, Jonklaas J. Levothyroxine Dose Adjustment to Optimise Therapy Throughout a Patient's Lifetime. *Adv Ther* 2019;36(Suppl 2):30-46.
17. Singh R. Does One Size Fit Everyone? Replacement Dose of Levothyroxine in Long-standing Primary Hypothyroidism in Adults. *Indian J Endocrinol Metab* 2017;21:404-409. Epub 2017/05/30
18. Zaletel K, Gaberšček S. Hashimoto's Thyroiditis: From Genes to the Disease. *Curr Genomics* 2011;12(8):576-588.
19. Fröhlich E, Wahl R. Thyroid Autoimmunity: Role of Anti-thyroid Antibodies in Thyroid and Extra-Thyroidal Diseases. *Front Immunol* 2017;9:8:521. doi: 10.3389/fimmu.2017.00521. eCollection 2017.

Table 1. Comparison of the clinical and laboratory parameters of the patients with Hashimoto Thyroiditis and with congenital hypothyroidism (All values are means±SDs, except if otherwise stated)

	Hashimoto thyroiditis n=30	Congenital hypothyroidism n=30	P value
Age (years)	12.6±2.7	11.3±3.4	0.11
Gender (F/M)	25/5	22/8	0.35
L-thyroxine dose (µg/d)	64.6±27.5	75.8±37.9	0.22
Weight SDS	-0.32±1.3	0.01±1.1	0.30
Height SDS	-0.40±1.1	-0.20±1.1	0.42
BMI SDS	-0.11±1.2	0.09±1.3	0.54
fT4 (ng/dL)	1.28±0.2	1.45±0.2	0.004
TSH (mIU/L)	6.5±5.9	7.7±6.3	0.37
Anti-TPO (IU/mL)	268.2±201	11.4±3	<0.001
Anti-TG (IU/mL)	641.4±1076	12.3±3.1	<0.001
USG			<0.001
Normal	0	12	
Agenesis	0	15	
Heterogeneous	17	3	
Heterogeneous and Pseudo-nodular	13	0	
Zonulin (ng/mL)	59.1±22.9	43.3±32.9	0.035

Anti-TG: Anti-thyroglobulin antibody; Anti-TPO: Anti-thyroid peroxidase antibody; BMI: Body mass index; F: Female; fT4: Free thyroxine; L-thyroxine: Levothyroxine; M: Male; SDS: Standard deviation score; TSH: Thyroid stimulating hormone; USG: Thyroid ultrasonography.

Figure 1. Serum zonulin levels (ng/mL) in patients with Hashimoto thyroiditis and congenital hypothyroidism

