

The Link between Zonulin and Hashimoto's Thyroid Disease. A Cross-Sectional Study

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ABSTRACT

Background: Hashimoto's thyroiditis (HT) is the most prevalent autoimmune thyroid disorder globally. Emerging evidence suggests a role of increased intestinal permeability—commonly referred to as "leaky gut"—in the pathogenesis of autoimmune diseases. Zonulin, a regulator of intestinal tight junctions, has been proposed as a marker of intestinal permeability. Its relationship to HT and gut microbiota, especially *Lactobacillus acidophilus*, remains understudied. **Objective:** To evaluate the association between serum zonulin levels and the presence of *Lactobacillus acidophilus* in stool with the occurrence and severity of HT. **Methods:** This cross-sectional study included 80 Egyptian adults (20–60 years), comprising 60 patients with HT and 20 controls. HT patients were subcategorized into euthyroid, subclinical, and overt hypothyroid groups. Serum zonulin, thyroid function tests, thyroid peroxidase antibodies (TPO Ab), and stool culture for *Lactobacillus acidophilus* were assessed. **Results:** Zonulin levels and *Lactobacillus acidophilus* prevalence were significantly elevated in HT patients, especially in the hypothyroid subgroup. Zonulin showed positive correlation with TPO Ab and TSH, and a negative correlation with FT4. A zonulin level >17 ng/ml predicted TPO Ab positivity with 71.67% sensitivity and 100% specificity (AUC=90.2). Patients with positive *Lactobacillus acidophilus* cultures exhibited higher zonulin, TPO Ab, and TSH levels compared to those with negative cultures. **Conclusions:** Elevated serum zonulin and increased intestinal colonization by *Lactobacillus acidophilus* are significantly associated with HT, suggesting a potential link between intestinal permeability and thyroid autoimmunity. These findings support a role for gut-thyroid axis dysregulation in HT pathogenesis.

Keywords: Hashimoto's Thyroiditis; Zonulin; Leaky Gut Syndrome; *Lactobacillus Acidophilus*; Thyroid Autoimmunity; Intestinal Permeability

INTRODUCTION

Hashimoto thyroiditis (HT) is the most common cause of hypothyroidism in developed countries. It is an autoimmune disease in which antithyroid antibodies attack thyroid cells destroying them with subsequent fibrosis [1]. Zonulin is a protein released under the effect of gluten and intestinal bacteria is accused of being a part of the pathogenesis of leaky gut [2]. *Lactobacillus acidophilus* is a beneficial strain of bacteria that occurs naturally in the intestine [3]. The relation between autoimmune thyroid disease and intestinal permeability is not clear [4]. Leaky gut is a condition in which bacteria and toxins leak through the intestinal wall to blood due to loosening of its tight junctions leading to widespread inflammation and possibly autoimmune disorders [3].

Aim of the Work

Our target was to investigate the association of each zonulin and *lactobacillus acidophilus* in stool with HT.

Patients and Methods

We conducted a cross-sectional study on 80 participants from Egypt recruited from Ain Shams University hospitals endocrinology outpatient Clinic. Ethics Committee of Faculty of Medicine, Ain Shams University approved our work. A written and/or oral consent was obtained from all participating subjects. The 80 participants aged from 20 to 60 years and included 60 newly diagnosed patients with HT with TPO Ab more than 34IU/ml (Group1) and 20 subjects without history of autoimmune thyroid disease and no anti-thyroid antibodies (Group 2). Group 1 was subdivided into three groups: Group 1a: 20 euthyroid patients with TSH and FT4 within normal range. Group 1b: 20 patients

with sub-clinical hypothyroidism with TSH more than 5.2mIU/ml & less than 10mIU/ml) FT4 within normal range (0.9 to 2.3 ng/dl). Group 1c: 20 patients with Hashimoto's thyroiditis and overt primary hypothyroidism (TSH more than 10mIU/ml) FT4 normal or low (0.9 to 2.3 ng/dl). We excluded patients with Inflammatory bowel diseases, end stage renal disease, chronic liver disease, heart failure, patients under stressful conditions like ICU admission or catabolic states, acute infections, sepsis or chronic inflammatory states, pregnancy & lactation, other autoimmune diseases (e.g., systemic lupus erythematosus, Rheumatoid arthritis, Multiple sclerosis, Type1 diabetes mellitus), anti-inflammatory drugs or Immunomodulators, and infective diarrhea or antibiotics usage 48 hours before stool culture for *lactobacillus acidophilus*. All individuals were subjected to detailed history taking, full clinical examination. We measured TPO Ab, thyroid profile (TSH, FT4, FT3) and zonulin levels in serum by enzyme-linked immunosorbent assay (ELISA). All participants underwent stool culture for *lactobacillus acidophilus* in stool as a marker for leaky gut using De Man, Rogosa and Sharpe Agar.

Statistical Analysis

Data was collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. Quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric. Also, qualitative variables were presented as number and percentages. Then the appropriate statistical analyses were applied. The confidence interval was set at 95% and the margin of error accepted was set at 5%. P-value was interpreted as P-value >0.05: Non-

significant (NS); P-value <0.05: Significant (S); P-value < 0.01: highly significant (HS)

Results

Subjects with and without HT were matched regarding age and gender distribution with no statistically significant difference between

them. Most participants with HT were females (85%) (Table 1). On comparing each of the HT patients' subgroups and controls, the hypothyroid subgroup showed significantly higher BMI (Table 2), and significantly higher TPO Ab and TSH and a significantly lower ft4. There was no statistically significant difference as regards FT3 (Table 3).

Table 2: Comparison of Age and Gender between HT Patients (group1) and Controls (Group 2)

		Group 1 (HT Patients)		Group 2 (Controls)	Test value	P-Value	Sig.
		No. = 60	No. = 20	No. = 20			
Age (Years)	Mean $\pm$ SD	36.35 $\pm$ 9.02	37.25 $\pm$ 7.81		-0.200*	0.842	NS
	Range	20 – 60	27 – 60				
Gender	Female	51 (85.0%)	16 (80.0%)		0.276*	0.600	NS
	Male	9 (15.0%)	4 (20.0%)				

*: Chi-Square Test; •: One Way ANOVA Test

Table 2: Comparison of BMI between HT Patients' Subgroups and Controls

BMI (Kg/m2)	Group I (HT patients)			Group 2	Test value	P-Value	Sig.
	Group1a (Euthyroid)	Group1b (Subclinical)	Group1c (Hypothyroid)	(Control)			
	No. = 20	No. = 20	No. = 20	No. = 20			
Mean $\pm$ SD	22.84 $\pm$ 4.23	31.86 $\pm$ 10.10	32.25 $\pm$ 6.54	23.37 $\pm$ 2.70	12.593*	0.000	HS
Range	18 – 31	19 – 50	23 – 49	19 – 29.8			

Table 3: Comparison of Thyroid Profile and of TPO Ab Levels between HT Patients' Subgroups and Controls

		Group I (HT Patients)			Group 2	Test Value	P-Value	Sig.
		Group1a (Euthyroid)	Group1b (Subclinical Hypothyroid)	Group1c (Hypothyroid)	(Control)			
		No. = 20	No. = 20	No. = 20	No. = 20			
TSH (mIU/ml)	Mean $\pm$ SD	2.83 (2.51 - 3.41)	6.9 (6.09 - 7.82)	17.5 (10.5 - 31.7)	2.26 (1.42 - 3)	67.874‡	0.000	HS
	Range	0.9 – 4.1	5 – 9.2	10 – 100	0.9 – 3.74			
FT4 (ng/dl)	Mean $\pm$ SD	1.37 $\pm$ 0.32	1.01 $\pm$ 0.61	0.74 $\pm$ 0.42	1.60 $\pm$ 0.56	12.049*	0.000	HS
	Range	0.9 – 2	0.3 – 3.2	0.04 – 1.5	0.9 – 2.3			
FT3 (pg/ml)	Mean $\pm$ SD	1.68 $\pm$ 0.54	1.75 $\pm$ 0.59	1.53 $\pm$ 0.77	1.82 $\pm$ 0.65	0.732*	0.536	NS
	Range	0.95 – 2.76	1.02 – 2.88	0.22 – 2.8	0.9 – 2.76			
Anti TPO (IU/ml)	Median (IQR)	67 (47 - 77)	250 (175 - 445)	400 (282.5 - 1000)	6.5 (4.5 - 10)	66.702‡	0.000	HS
	Range	40 – 90	56 – 1000	150 – 1000	1.4 – 15			
	Mean $\pm$ SD	22.84 $\pm$ 4.23	31.86 $\pm$ 10.10	32.25 $\pm$ 6.54	23.37 $\pm$ 2.70	12.593*	0.000	HS
	Range	18 – 31	19 – 50	23 – 49	19 – 29.8			

•: One Way ANOVA test; ‡: Kruskal Wallis Test

Zonulin levels were significantly higher in participants with HT (Group 1) compared to those without (Group 2) (Table 4). On comparing subgroups there was also a significant difference as regards Zonulin level being the highest among hypothyroid patients (group 1c) followed by subclinical hypothyroid patients (group 1b) and then

patient group were euthyroid Hashimoto's thyroiditis (group 1a) and least in patients without thyroiditis (group 2) (43 ng/ml, 38 ng/ml, 5 ng/ml and 3 ng/ml respectively). Moreover on post hoc analysis each of the three HT patient's subgroups had significantly higher zonulin levels compared to the subjects without HT (Table 5).

Table 4: A Comparison of Zonulin Levels in Patients with and without HT

Zonulin (Ng/ml)	Group 1 (Patients)	Group 2 (Control)	Test Value‡	P-Value	Sig.
	No. = 60	No. = 20			
Median (IQR)	26.5 (6.5 - 43)	3 (2.75 - 5)	-5.369	0.000	HS

‡: Mann Whitney Test

Table 5: Comparison of Serum Zonulin Levels between HT Patient's Subgroups and Controls

Zonulin (ng/ml)	Group I (HT Group)			Group 2	Test Value ‡	P-Value	Sig.
	Group1a (Euthyroid)	Group1b (Subclinical)	Group1c (Hypothyroid)	(Control)			
	No. = 20	No. = 20	No. = 20	No. = 20			
Median (IQR)	5 (3.15–6.5)	38 (18 - 44)	43 (34 - 50)	3 (2.75 - 5)	61.047	0.000	HS
Range	1.4 – 10	10 – 84	19 – 69	1.5 – 7			

‡: Kruskal Wallis Test

Table 5(b): Post Hoc Analysis by Mann Whitney Test

Post Hoc Analysis by Mann Whitney test						
	P1	P2	P3	P4	P5	P6
Zonulin (ng/ml)	0.000	0.000	0.024	0.175	0.000	0.000

P1: Group1a (Euthyroid) Vs Group1b (Subclinical), P2: Group1a (Euthyroid) Vs Group1c (Hypothyroid), P3: Group1a (Euthyroid) Vs Group 2 (Control), P4: Group1b (Subclinical) Vs Group1c

(Hypothyroid), P5: Group1b (Subclinical) Vs Group 2 (Control), P6: Group1c (Hypothyroid) Vs Group 2 (Control).

On studying the correlation between serum zonulin and anti-TPO we found a positive correlation in patients with HT collectively (Group

1) but no correlation in each of the HT patients' subgroups nor in subjects without HTs (Group 2) (Table 6).

Table 6: Correlation between Zonulin and TPO Ab in the Different Groups

TABLE 3: Correlation between Zonulin and TPO in the Different Groups										
	Anti TPO (IU/ml)									
	Group 1 (HT) Collectively		Group I Subgroups (HT Subgroups)						Group 2 (Control)	
			Group1a (Euthyroid)		Group1b (Subclinical)		Group1c (Hypothyroid)			
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
Zonulin (ng/ml)	0.781**	0.000	-0.019	0.938	0.422	0.064	0.365	0.113	0.084	0.724

In patients with HT (Group 1) ROC curve analysis showed that the best cut off point for zonulin to predict the positivity of TPO Ab was found > 17 ng/ml with sensitivity of 71.67%, specificity of 100.0% and area under curve (AUC) of 90.2 as shown in Fig. (1).

In patients with HT (Group 1) zonulin was positively correlation to TSH, negatively correlated to FT4 and not correlated to FT3. On

Addressing the subgroups of patients with HT zonulin in the hypothyroid subgroup (group1c) showed a negative correlation with FT4 but no correlation with TSH and FT3.

In addition, among patients without HT (group2), there was no correlation between zonulin level and each of TSH, FT4 & FT3. (Table 7).

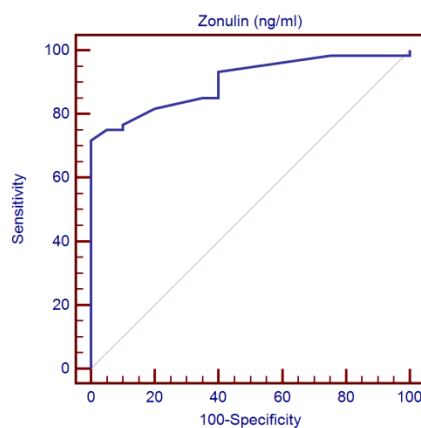


Figure 1: Sensitivity and Specificity of the Cut Off Value of Zonulin in Predicting TPO Ab Positivity

Table 7: Correlation between Zonulin Level and Thyroid Function Tests Among the Different Groups

TABLE 11: Correlation between Zonulin Level and Thyroid Panel: Results among the Different Groups										
Patients Group			Zonulin Level (ng/ml)							
			Group I (HT Group)						Group 2 (Control)	
			Group1a (Euthyroid)		Group1b (Subclinical)		Group1c (Hypothyroid)			
r	P-value	r	P-value	r	P-value	r	P-value	r	P-value	
TSH	0.732**	0.001	0.148	0.532	0.235	0.318	0.161	0.499	0.058	0.807
FT4	-0.445**	0.000	-0.133	0.575	-0.373	0.105	-0.577**	0.008	0.090	0.705
FT3	0.100	0.448	-0.027	0.910	0.179	0.449	0.316	0.174	0.087	0.716

r: Spearman Correlation Coefficient

The distribution of growth of Lactobacillus acidophilus in Stool culture was significantly higher in patients with HT than those without. (Table 8).

A statistically significant difference in the prevalence of lactobacillus acidophilus positive cases was detected among subgroups

with hypothyroid cases showing the highest prevalence and the control group showed the least prevalence. Patients with Subclinical hypothyroid and Hypothyroid cases showed statistically significant higher prevalence of positive lactobacillus acidophilus stool culture compared to control and euthyroid cases. (Table 9).

Table 8: Distribution of Lactobacillus Acidophilus Growth in Subjects with and without HT

Lactobacillus Stool Culture	Group 1(HT)		Group 2(Control)		Test value*	P-value	Sig.
	No.	%	No.	%			
Positive	41	68.3%	6	30%	9.095	0.003	HS
Negative	19	31.7%	14	70%			

*: Chi-Square Test

Table 9: Distribution of Lactobacillus Acidophilus Growth in HT Patient's Subgroups and Controls

Lactobacillus Acidophilus Stool Culture	TABLE 1: Distribution of Lactobacillus Acidophilus Growth in HT Patient Subgroups and Controls								Test Value*	P-Value	Sig.
	Group I (HT Group)						Group 2				
	Group1a (Euthyroid)		Group1b (Subclinical)		Group1c (Hypothyroid)		(Control)				
	No.	%	No.	%	No.	%	No.	%			
Positive	10	50.0%	14	70.0%	17	85.0%	6	30.0%	14.184	0.003	HS
Negative	10	50.0%	6	30.0%	3	15.0%	14	70.0%			

*: Chi-Square Test

As regards zonulin, patients with HT (group 1) who showed positive stool culture for lactobacillus acidophilus had higher statistically significant zonulin levels compared to those with negative lactobacillus acidophilus stool culture.

However, on addressing the patient's subgroups, this significance persisted among the hypothyroid subgroups (group 1c). But not

among euthyroid (group 1a) and subclinical hypothyroid (group 1b). (Table 10). As regards Anti TPO, there was significantly higher levels of Anti TPO in patients with positive stool culture compared to those with negative stool culture for lactobacillus acidophilus among patients with HT collectively (group 1) and among each of HT patients' subgroups (Table 11).

Table 10: Comparison of Zonulin in Lactobacillus Acidophilus Negative and Positive Stool Culture Among the Different Groups

Zonulin (ng/ml)		Lactobacillus Acidophilus Stool Culture		Test Value $\ddagger$	P-Value	Sig.
		Negative	Positive			
Patients Group (no.= 60)	Median (IQR)	7 (3.5 – 33)	37 (16 – 43)	-2.457	0.014	S
	Range	1.4 – 69	3 – 84			
Group1a (Euthyroid) (no.= 20)	Median (IQR)	4.25 (3.2 – 5.5)	5.5 (3 – 9)	-0.836	0.403	NS
	Range	1.4 – 7	3 – 10			
Group1b (Subclinical) (no.= 20)	Median (IQR)	41 (14 – 55)	36 (18 – 43)	-0.248	0.804	NS
	Range	10 – 69	16 – 84			
Group1c (Hypothyroid) (no.= 20)	Median (IQR)	27 (19 – 33)	43 (38 – 51)	-2.285	0.022	S
	Range	19 – 33	21 – 69			
Group 2 (Control) (no.= 20)	Median (IQR)	3 (3 – 5)	4 (2.5 – 5)	-0.254	0.800	NS
	Range	1.5 – 6.5	2 – 7			

$\ddagger$: Mann Whitney test

Table 11: Comparison of TPO Ab in Lactobacillus Acidophilus Negative and Positive Growth Among the Different Groups

Anti TPO (IU/ml)		Lactobacillus Acidophilus Stool Culture		Test Value $\ddagger$	P-Value	Sig.
		Negative	Positive			
Patients Group (no.= 60)	Median (IQR)	62 (45 – 200)	300 (114 – 470)	-4.279	0.000	HS
	Range	40 – 400	65 – 1000			
Group1a (Euthyroid) (no.= 20)	Median (IQR)	47 (45 – 60)	77 (72 – 80)	-3.712	0.000	HS
	Range	40 – 69	65 – 90			
Group1b (Subclinical) (no.= 20)	Median (IQR)	175 (99.3 – 220)	370 (210 – 470)	-2.477	0.013	S
	Range	56 – 220	114 – 1000			
Group1c (Hypothyroid) (no.= 20)	Median (IQR)	200 (150 – 238)	420 (350 – 1000)	-2.463	0.008	HS
	Range	150 – 238	220 – 1000			
Group 2 (Control) (no.= 20)	Median (IQR)	7.5 (5 – 10)	4.1 (3 – 10)	-1.293	0.207	NS
	Range	1.5 – 15	1.4 – 10			

$\ddagger$: Mann Whitney Test

As regards thyroid function tests, patients with HT (group 1) had significantly higher TSH levels in those with positive stool culture for lactobacillus acidophilus compared to those with negative lactobacillus acidophilus stool culture. But there was no difference as

regards Ft4 and Ft3 as shown in tables 12,13 and 14 respectively. There was no statistically significant difference among patients' subgroups (group1) between those with positive and negative lactobacillus acidophilus stool culture as regards TSH, ft4 and ft3

Table 12: Comparison of TSH in Lactobacillus Acidophilus Negative and Positive Growth Among the Different Groups

TSH		Lactobacillus Acidophilus Stool Culture		Test Value $\ddagger$	P-Value	Sig.
		Negative	Positive			
Patients Group (no.= 60)	Median (IQR)	4.10 (2.8 – 7.35)	7.85 (5 – 11)	-2.265	0.023	S
	Range	1.77 – 32.5	0.9 – 100			
Group1a (Euthyroid) (no.= 20)	Median (IQR)	2.8 (2.52 – 3.22)	3.05 (2.5 – 3.5)	-0.189	0.850	NS
	Range	1.77 – 4.1	0.9 – 4.1			
Group1b (Subclinical) (no.= 20)	Median (IQR)	6.8 (5.64 – 7.35)	7 (6.3 – 7.85)	-0.660	0.509	NS
	Range	5.12 – 8	5 – 9.2			
Group1c (Hypothyroid) (no.= 20)	Median (IQR)	21 (20 – 32.5)	14 (10 – 30.89)	-0.854	0.393	NS
	Range	20 – 32.5	10 – 100			
Group 2 (Control) (no.= 20)	Median (IQR)	1.88 (1.34 – 2.52)	2.89 (2.5 – 3)	-1.488	0.137	NS
	Range	0.9 – 3.74	1.34 – 3.2			

$\ddagger$: Mann-Whitney Test

Table 13: Comparison of FT4 in Lactobacillus Acidophilus Negative and Positive Growth Among the Different Groups

FT4		Lactobacillus Acidophilus Stool Culture		Test Value $\bullet$	P-Value	Sig.
		Negative	Positive			
Patients Group (no.= 60)	Mean $\pm$ SD	1.16 $\pm$ 0.46	0.98 $\pm$ 0.55	1.250	0.216	NS
	Range	0.33 – 2	0.04 – 3.20			
Group1a (Euthyroid) (no.= 20)	Mean $\pm$ SD	1.48 $\pm$ 0.29	1.27 $\pm$ 0.33	1.538	0.142	NS
	Range	1.18 – 2	0.9 – 1.9			
Group1b (Subclinical) (no.= 20)	Mean $\pm$ SD	0.92 $\pm$ 0.35	1.04 $\pm$ 0.7	-0.420	0.680	NS
	Range	0.33 – 1.3	0.3 – 3.2			
Group1c (Hypothyroid) (no.= 20)	Mean $\pm$ SD	0.6 $\pm$ 0.2	0.76 $\pm$ 0.44	-0.604	0.553	NS
	Range	0.4 – 0.8	0.04 – 1.5			
Group 2 (Control) (no.= 20)	Mean $\pm$ SD	1.58 $\pm$ 0.57	1.62 $\pm$ 0.61	-0.143	0.888	NS
	Range	0.9 – 2.3	0.9 – 2.3			

Table 14: Comparison of FT3 between Lactobacillus Acidophilus Negative and Positive Stool Culture Among the Different Groups

FT3		Lactobacillus Acidophilus Stool Culture		Test Value •	P-Value	Sig.
		Negative	Positive			
Patients Group (no.= 60)	Mean $\pm$ SD	1.67 $\pm$ 0.57	1.65 $\pm$ 0.68	0.098	0.923	NS
	Range	0.9 – 2.76	0.22 – 2.88			
Group1a (Euthyroid) (no.= 20)	Mean $\pm$ SD	1.71 $\pm$ 0.57	1.65 $\pm$ 0.54	0.273	0.788	NS
	Range	0.99 – 2.76	0.95 – 2.22			
Group1b (Subclinical) (no.= 20)	Mean $\pm$ SD	1.77 $\pm$ 0.62	1.74 $\pm$ 0.6	0.130	0.898	NS
	Range	1.1 – 2.67	1.02 – 2.88			
Group1c (Hypothyroid) (no.= 20)	Mean $\pm$ SD	1.28 $\pm$ 0.49	1.58 $\pm$ 0.82	-0.594	0.560	NS
	Range	0.9 – 1.83	0.22 – 2.8			
Group 2 (Control) (no.= 20)	Mean $\pm$ SD	1.99 $\pm$ 0.61	1.42 $\pm$ 0.62	1.934	0.069	NS
	Range	0.9 – 2.76	1.03 – 2.66			

Discussion

Many studies showed a link between increased intestinal permeability and autoimmune disorders and its specific link with HT has been explored only in a small number of studies [5]. Zonulin is mainly released due to an increase in intestinal permeability leading to the presence of activated immune cells in the mucosa of the gut which could migrate to distant organs leading to autoimmune disorders or chronic inflammation [6]. In our study serum zonulin was significantly higher in patients with HT compared to those without HT. Our findings agree with Cayres et al. [7] and Demir et al. [8] supporting the link between leaky gut with HT, one of the prevalent autoimmune diseases. Our results were also in line with Küçükemre et al. [9] who showed higher zonulin in HT patients compared with those having congenital hypothyroidism. Our results disagreed with Deniz et al. [10] who reported lower zonulin levels in HT and did not alien with Isleyen et al. [11] who elicited no difference of significance which could be due to different study designs. In our work zonulin was positively correlated to TPO Ab in patients with HT which agrees with Isleyen et al. [11], Demir et al. [8] and Halawa et al. [12]. The positive correlation supports the suggestion that zonulin may play a role in the pathogenesis of HT [11]. We reported a preliminary cut off point for zonulin to predict the positivity of TPO Ab in HT above 17 ng/ml with sensitivity of 71.67%, specificity of 100.0% and area under curve (AUC) of 90.2. Although our work supports the relationship between zonulin and TPO Ab, our findings were unlike Deniz et al. [10] who reported an inverse relation of zonulin with TPO Ab and unlike the study conducted by Küçükemre et al. [9] that showed no significant relation between serum zonulin and TPO Ab, which was a pilot study. Our work showed that there was a significantly higher distribution of growth of Lactobacillus acidophilus in stool culture in patients with HT compared to those without in agreement with Ishaq et al. [13] who showed that HT patients have intestinal microbial dysbiosis which was reflected in the abundance of microbiota of which was Bifidobacterium Lactobacillus. Considerable alterations in composition of the intestinal bacteria were observed in HT patients [14]. Our results also agreed with Kiseleva et al. [15]. Our findings disagreed with Liu et al. [16] who found that patients with HT have decreased intestinal microbiota richness and diversity, and this could be attributed to differences in study controls, detection methods, and the diversity of Lactobacillus species especially in the era of widespread antibiotic use. It is hypothesized that gut dysbiosis can lead to zonulin release with the subsequent passage of luminal contents through epithelial barrier precipitating the release of pro-inflammatory

cytokines [6]. Another explanation is antigenic cross-reactivity where immune system attacks both the gut bacteria and thyroid. For example, Lactobacillus has components that resemble thyroid peroxidase (TPO) developing TPO Ab [15, 17].

We also reported a significantly higher zonulin level among hypothyroid patients with HT compared to those with HT patients with euthyroidism which agrees with Deniz et al. [10]. Our results showed that zonulin was positively correlated to TSH, negatively correlated to FT4 and not correlated to FT3 in patients with HT. These results disagreed with Aydin et al. [18] and Tomov et al. [19] who found no correlation between zonulin and thyroid hormones or autoantibodies in HT patients. Moreover, we found that among patients with HT, hypothyroid participants showed a statistically higher distribution of growth of Lactobacillus acidophilus than those with euthyroidism. This is in line with other studies' findings Ishaq et al. [13], Zhao et al. [20] and Liu et al. [16] who on comparing the gut microbiota between euthyroid subjects and hypothyroid patients found bacterial overgrowth and dysbiosis in the hypothyroid patient group. Moreover, Su et al. [21] revealed significant differences in diversity and richness of the gut microbiota, in HT untreated primary hypothyroidism, compared to healthy controls. Subjects with HT who showed positive stool culture for lactobacillus acidophilus in our study had higher statistically significant zonulin levels, TPO Ab and TSH compared to those with negative lactobacillus acidophilus stool culture.

Limitations

Because our study was cross-sectional in nature we could not claim a causative relationship between zonulin and HT. And on proposing a preliminary zonulin cut-off value to predict TPO Ab positivity larger studies are needed to explore its clinical significance.

Conclusion

We support the relationship between HT and each of zonulin and Lactobacillus acidophilus which are indicators of leaky gut with more studies needed to support a causal effect relationship.

Declarations

Ethics Approval and Consent to Participate

our ethics committee at Ain Shams University Faculty of Medicine gave approval to the study.

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