Original Research

Comparison of immunoglobulin E anti-thyroid peroxidase antibodies in patients with Hashimoto thyroiditis and chronic spontaneous urticaria

Songül Çildağ[1], Çiğdem Yenisey[2], Mustafa Ünübol[3],Taşkın Şentürk[1]

1) Department of Immunology and Allergy, Adnan Menderes University, Turkey

2) Department of Biochemistry, Adnan Menderes University, Turkey

3) Department of Endocrinology and Metabolism, Adnan Menderes University, Turkey

Abstract

Background and aim. Chronic spontaneous urticaria (CSU) is a disease of unknown etiology and autoimmunity has been thought to be an etiological factor. Immunoglobulin E (IgE)-anti-thyroid peroxidase antibodies (anti-TPO) may play a role in the pathogenesis of certain cases of urticaria. The aim of this study is to investigate IgE-anti-TPO in patients with chronic spontaneous urticaria and in patients with Hashimoto's thyroiditis.

Methods. A total of 175 subjects were included in this study. 59 patients had chronic spontaneous urticaria without history of Hashimoto's thyroiditis, while 58 patients had Hashimoto's thyroiditis without history of urticaria. The control group consisted of 58 participants without history of Hashimoto's thyroiditis and urticaria. Serum IgE-anti-TPO levels were analyzed by site-directed IgE capture Enzyme-Linked Immunosorbent Assay technique. We used this technique by modifying it.

Results. IgE-anti-TPO antibodies were detected in all three groups and in all subjects. There was no significant difference between the three groups in terms of IgE-anti-TPO levels. Although total IgE and IgE-anti-TPO levels were higher in the IgG-anti-TPO positive chronic spontaneous urticaria, there was no significant difference.

Conclusions. IgE-anti-TPO antibodies do not play a pathogenic role in the majority of patients with chronic spontaneous urticaria.

Keywords: chronic urticaria, immunoglobulin E, thyroiditis, thyroid peroxidase

Introduction

Chronic urticaria is a continuous or intermittent period of urticaria lesions lasting longer than six weeks [1-3]. Chronic spontaneous urticaria is a disease of unknown etiology which is frequently observed in women and more common in adults. It is not usually related to external factors [1,2,4,5]. Autoimmunity has been reported to be an etiological factor in 40 to 60% of patients with chronic spontaneous urticaria, particularly in those with thyroid autoimmune disorders such as Hashimoto's thyroiditis [6-8]. There is an increased incidence of anti-thyroid antibodies in CSU both immunoglobulin G (IgG) anti-thyroid peroxidase (anti-TPO) and IgG anti-thyroglobulin (anti-Tg) with an incidence of about 25% [9]. Some authors have suggested that immunoglobulin E (IgE) anti-thyroid peroxidase antibodies (IgE-anti-TPO) may play a role in the pathogenesis of certain cases of urticaria [10-12].

In this study, we aimed to investigate IgE-anti-TPO levels in patients with CSU and in patients with Hashimoto's thyroiditis using the site-directed IgE capture Enzyme-Linked Immunosorbent Assay (ELISA) technique.

Methods

Study population

A total of 175 -subjects -were included in this cross-sectional study. - 59 patients had CSU without a history of Hashimoto's thyroiditis, while 58 patients had Hashimoto's thyroiditis

DOI: 10.15386/mpr-1598

Manuscript received: 18.02.2020
Received in revised form: 27.07.2020
Accepted: 30.09.2020

Address for correspondence:
songulcildag@yahoo.com

without a history of urticaria. The control group consisted of 58 participants without any history of autoimmune diseases, Hashimoto's thyroiditis and urticaria. Patients with CSU who received anti-IgE, corticosteroid and/or immunosuppressive therapy were excluded.

All participants were informed about the nature of the study and written informed consent was obtained. For this study, approval was obtained from the Ethics Committee for Non-invasive Clinical Studies at the University School of Medicine (No:2017/1083). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Detection of IgE-anti-TPO and IgG-anti-TPO levels

Serum IgG-anti-TPO levels were measured using the chemiluminescence immunoassay *(*ARCHITECT i1000SR-Abbott*)*.

Serum IgE-anti-TPO levels were assessed by a site-directed IgE capture ELISA method modified as below [11]. We used a 96-wellplate and coated the well using anti-human IgE (clone: MHE-18, Cat. No. 325502, 0.5 mg/mL, Biolegend, CA, USA) in such a way as to 1.6 µg/mL antibody in 10mM sodiumcarbonate (Na_2CO_3) at pH 9.0 solution. After adding 100 µL anti-human IgE solution, the plates were incubated overnight at 4°C without shaking. We used cell culture plates (CorningCostar, Cat No. CLS3595, USA). After 24 hours of incubation, the plates were blocked with 2% fetal bovine serum (FBS) (Sigma-Aldrich, Cat No. F2442, Germany) for 4 hours. At the end of the blocking procedure, we poured FBS solution and did not wash the plates. The samples (serum, 100 µL/well) were added in the well (four replicates) and incubated for 2 hours at 37°C. The plates were then washed with washing buffer and 100 µL TPO-biotin (1:1000 dilution) was added to the well. As no commercial TPO-biotinis was available, biotinylated (biotin-XX Microscaler Protein Labeling Kit, Cat No. B30010, Invitrogen/ThermoFisherScientific, USA) recombinant human-TPO (RSR-TPO-SF9, Ltd, Cardiff, UK) was used. The plates were incubated for 2 hours at 37°C without shaking and the plates were washed three times with washing buffer. Then, 100 µL/1:2000 diluted HRP-streptavidin (Biolegend, Cat No. 405210, CA, USA) was added to all wells. After incubation for 1 hour at 37°C, the plates were washed three times with washing buffer. Subsequently, 100 µL TMB substrate (Biolegend, Cat No. 421501, CA, USA) was added to each well and the plates were incubated at least 1 hour at 37°C (depending on the chloride content) with shaking at 100 rpm. Finally, the reaction was stopped by using 50 µL/3M NaOH. The yellow color absorbance was measured at 450 nmviaBioTek ELX800 microplatereader.

Since we were unable to reach the SP2/Sp1,4 cells transfected mouse myeloma cells as positive control and standard, we used different controls. For the control group, two lines of the plate were not coated with anti-human IgE-antibody, and four wells were coated with TPO-biotin directly (1:1000 in Na_2CO_3); four wells with HRP-streptavidin (1:2000), four wells with anti-TPO IgG (Cat No: M00320, Boster, CA, USA) and the rest of the wells with anti-IgGFc antibody (clone, HP6017, Cat. No. 409301, Biolegend, CA, USA). After the coating procedure, we blocked and treated the controls as the other wells. As previously described, Altrichter et al. [13] used streptavidin alkaline phosphatase (AP) and its substrate p-NPP, rather than HRP-streptavidin/TMP, and they measured the absorbance at 405 nm.

The TPO-biotinand HRP-streptavidin gave the maximal signal as 2.0 to 2.5.

We calculated the optic density (OD) in U/mL as follows: in a 96-well plate, measuring one OD is equal to one U/mL with HRP-streptavidin and TMB substrate. We had an OD of approximately 0.1 TMB blank. Then we subtracted 0.1 from the sample absorbance and 100 µL is ten times 1.0 mL, thus we multiplied ten times the OD.

For the first time we modified the determination of IgE-anti-TPO methods as follows;

1. After four-time detection we shortened the incubation period with IgE-anti-TPO relative to overnight measurement. For incubation we used 37°C, rather than 4°C for 6 hours.

2. We shortened the blocking period with FBS (or human serum albumin) at 37°C to 4 hours.

3. We used the HRP-streptavidin/TMP substrate rather than AP.

Assessment of Urticaria Activity Score (UAS)

The UAS-7 is a calculated patient-reported outcome measure derived by summing the score for the number of wheals and intensity of pruritus (0: none, 1: mild, 2: moderate, 3: severe) per day, for seven consecutive days [2].

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 25.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in number and frequency and median (min-max) values. All data were analyzed using the Kolmogorov-Smirnov test and Shapiro-Wilk test for normal distribution. The Kruskal-Wallis test was used to compare more than two groups, while the Mann-Whitney-U test was used for binary comparisons. Categorical variables were compared using the chi-square test. A p value of <0.05 was considered statistically significant.

Results

The IgE-anti-TPO antibodies were detected in all three groups, and in all subjects; however, there was no significant difference in the IgE-anti-TPO levels among the groups. Demographic characteristics of the patients and IgE-anti-TPO levels are shown in table I.

Table I. Comparison of IgE-anti-TPO levels and demographic characteristics of study groups.

	Hashimoto's	CSU	Control	Total	p
Number	58	59	58	175	-
Female, %	96.6	76.3	67.2	80	0.000
Age (years)*	40 (20-76)	42 (18-67)	29 (19-70)	37 (18-76)	0.000
IgE-anti-TPO Abs*	0.17 (0.06-1.37)	0.16 (0.06-1.21)	0.14 (0.06-0.39)	0.16 (0.06-1.37)	0.136
IgE-anti-TPO U/ml*	1.76 (0.65-13.73)	1.62 (0.65-12.19)	1.52 (0.68-3.92)	1.69 (0.65-13.73)	0.189

CSU, chronic spontaneous urticaria; TPO, Thyroid peroxidase; Abs, Absorbance; *Data are expressed as median (min-max).

The clinical and laboratory characteristics of CSU patients are summarized in table II.

Table II. Clinical and laboratory characteristics of CSU patients.

	CSU n=59
Age (years)*	42 (18-67)
Female, %	76.3
Duration of disease, months*	16 (2-240)
Total serum IgE IU/ml*	86.8 (2-1175)
Total serum IgE>100 IU/ml, %	44.1
UAS-7*	18 (8-32)
IgG-anti-TPO, %	23.7
IgE-anti-TPO Abs*	0.16 (0.06-1.21)
IgE-anti-TPO U/ml*	1.62 (0.65-12.19)

CSU, chronic spontaneous urticaria; UAS-7, urticarial activity score for 7 consecutive days; TPO, thyroid peroxidase; Abs, Absorbance;*Data are expressed as median (min-max).

When patients with CSU were further divided into two subgroups according to the IgG-anti-TPO positivity, the IgE-anti-TPO levels and total serum IgE levels were found to be higher in the IgG-anti-TPO positive group, although it did not reach a statistical significance. In addition, there were no significant differences in age, sex, duration of disease and UAS-7 among the groups (Table III).

Discussion

Chronic spontaneous urticaria is a chronic disease which is thought to be involved in the etiology of autoimmunity. In the literature, there are several studies investigating Type 1 and Type 2 autoimmunity in CSU pathogenesis and it has been suggested that it may be involved in the pathogenesis in certain patients [13]. The IgE type antibodies such as IgE-anti-TPO, IgE-anti-double stranded DNA (dsDNA), higher levels of total IgE in patients with urticaria with IgE-anti-TPO and a clear response to anti-IgE treatment indicate that Type 1 autoimmunity is involved in the pathogenesis of CSU [10-15]. Detection of IgG-type antibodies against IgE or FcεR1 [16-19], accompanied by autoimmune diseases such as autoimmune thyroiditis [9,20], autologous serum test positivity [19,21], the effectiveness of immunosuppressive treatments [22-25], and the fact that some patients respond more slowly to anti-IgE treatment suggest that Type 2 autoimmunity is also involved in the pathogenesis of the disease [13].

Autoimmune thyroid disease is one of the most common autoimmune diseases in patients with chronic urticaria [20,26]. Thyroid autoantibodies such as IgG-anti-TPO and IgG-anti-Tg have been investigated in several studies and found to be higher in CSU patients [27]. In addition, IgE-anti-TPO has been considered as one of the autoantibodies which is involved in the etiopathogenesis of the disease. Studies have shown controversial results; however, the discrepancy in the results can be attributed to the different methodological approaches used in the studies [10-12,28,29].

Table III. Comparison of clinical and laboratory characteristics of IgG-anti-TPO (+) and IgG-anti-TPO (-) CSU patients.

	IgG-anti-TPO (+) CSU n=14	IgG-anti-TPO (-) CSU n=45	p
Age (years)*	46.5(23-67)	40(18-65)	0.075
Female, %	85.7	73.3	0.48
Duration of disease, months*	24(2-36)	16(2-240)	0.58
UAS-7*	17.5(10-32)	18(8-32)	0.63
Total serum IgE IU/ml*	123.3(12.5-385)	78.6(2-1175)	0.91
IgE-anti-TPO Abs*	0.22(0.07-0.92)	0.15(0.06-1.21)	0.28
IgE-anti-TPO U/ml*	2.22(0.72-9.23)	1.5(0.65-12.1)	0.31

CSU, chronic spontaneous urticaria; UAS-7, urticarial activity score for 7 consecutive days, TPO, thyroid peroxidase; Abs, Absorbance; *Data are expressed as median (min-max).

In the literature, Sera et al. were the first to investigate the role of IgE-anti-TPO in the etiology of CSU [10]. The authors found IgE-anti-TPO antibodies in a patient with chronic urticaria using the direct ELISA method. In a later study, Tedeschi et al. were unable to detect IgE-anti-TPO in 38 patients with IgG-anti-TPO positive or negative patients with CSU and healthy controls using radioimmunoassay and concluded that IgE-anti-TPO did not play a pathogenic role in most cases with chronic urticaria [28]. In another study, Concha et al. examined IgE-anti-thyroid antibodies in 20 patients with IgG-anti-TPO positive chronic urticaria and in patients with Hashimoto's thyroiditis using the direct ELISA method. The authors found IgE-anti-thyroid antibodies in two patients with chronic urticaria, and none in those with Hashimoto's thyroiditis. One of these two patients had IgE-anti-TPO while the other patient had IgE-anti-Tg. The authors concluded that IgE-anti-TPO might play a causal role in the subset of urticaria patients [29].

To the best of our knowledge, the largest series of studies on IgE-anti-TPO is the study of Altrichter et al [11]. In this study, a significant level of IgE-anti-TPO was detected (54.2%) in 478 CSU patients compared to healthy controls with a cut-off value of 5 IU / mL. A site-directed human IgE capture ELISA was used in this study which showed that IgE-anti-TPO positivity in patients with CSU was associated with the IgG-anti-TPO positivity, lower C4, and lymphocytosis. However, in the afore mentioned study, patients with Hashimoto's thyroiditis were not evaluated as another group. In another study including aspirin-intolerant acute and chronic urticaria patients, the basophil activation test was used for the first time, and it was suggested that IgE-anti-TPO could induce aspirin-intolerant urticaria [12]. A recent study by Sanches et al. showed that IgE-anti-TPO levels were higher in CSU patients than healthy controls and those with autoimmune thyroid diseases.- In this study, the basophil activation test was used for the second time and skin tests with TPO were performed [30].

In our study, patients with CSU without a history of Hashimoto's thyroiditis, patients with Hashimoto's thyroiditis without a history of urticaria, and a healthy population without a history of Hashimoto's thyroiditis or urticaria were evaluated together using the site-directed IgE capture ELISA (previously described by Sabine Altrichter in 2011). We made a slight modification related to the incubation time and incubation temperature. Also, we used HRP-enzyme substrate/TMB solution instead of streptavidin alkaline phosphatase/p-NPP solution for detection.

In our study, the IgE-anti-TPO antibodies were detected in all three groups. There was no significant difference among the three groups in terms of IgE-anti-TPO levels, and, therefore the cut-off value was unable to be evaluated. In addition, in the present study the IgG-anti-TPO positivity was found to be 23.7% in CSU patients, consistent with the literature [7]. When CSU patients were further divided into two subgroups according to IgG-anti-TPO positivity, total IgE and IgE-anti-TPO levels were also found to be higher in the IgG-anti-TPO positive CSU group, although it did not reach a statistical significance. Also, total IgE levels were higher than 100 IU/mL in 44% of the patients with CSU.

Conclusions

According to our study, IgE-anti-TPO antibodies were detected in similar rates in patients with CSU, Hashimoto's thyroiditis and healthy population. These findings suggest that IgE-anti-TPO antibodies do not play a pathogenic role in the majority of patients with chronic spontaneous urticaria.

Acknowledgements

We thank Sabine Altrichter (Department of Dermatology and Allergy, Allergie-Centrum-Charité/ ECARF, Charité - Universitatsmedizin Berlin, Berlin, Germany) for her contribution to the construction of IgE-anti-TPO.

References

1. Bernstein JA, Lang DM, Khan DA, Craig T, Dreyfus D, Hsieh F, et al. The diagnosis and management of acute and chronic urticaria: 2014 update. J Allergy Clin Immunol. 2014;133:1270-1277.
2. Zuberbier T, Aberer W, Asero R, Bindslev-Jensen C, Brzoza Z, Canonica GW, et al. The EAACI/GA(2) LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. Allergy. 2014;69:868-887.
3. Abajian M, Schoepke N, Altrichter S, Zuberbier T, Maurer M. Physical urticarias and cholinergic urticaria. Immunol Allergy Clin North Am. 2014;34:73-88.
4. Powell RJ, Leech SC, Till S, Huber PA, Nasser SM, Clark AT; British Society for Allergy and Clinical Immunology. BSACI guideline for the management of chronic urticaria and angioedema. Clin Exp Allergy. 2015;45:547-565.
5. Sussman G, Hébert J, Gulliver W, Lynde C, Waserman S, Kanani A, et al. Insights and advances in chronic urticaria: a Canadian perspective. Allergy Asthma Clin Immunol. 2015;11:7.
6. Najib U, Sheikh J. The spectrum of chronic urticaria. Allergy Asthma Proc. 2009;30:1–10.
7. Levy Y, Segal N, Weintrob N, Danon YL. Chronic urticaria: association with thyroid autoimmunity. Arch Dis Child. 2003;88:517-519.
8. Leznoff A, Josse RG, Denburg J, Dolovich J. Association of chronic urticaria and angioedema with thyroid autoimmunity. Arch Dermatol. 1983;119:636-640.
9. Kikuchi Y, Fann T, Kaplan AP. Antithyroid antibodies in chronic urticaria and angioedema. J Allergy Clin Immunol. 2003;112:218.

10. Bar-Sela S, Reshef T, Mekori YA. IgE antithyroid microsomal antibodies in a patient with chronic urticaria. J Allergy Clin Immunol. 1999;103:1216-1217.
11. Altrichter S, Peter HJ, Pisarevskaja D, Metz M, Martus P, Maurer M. IgE mediated autoallergy against thyroid peroxidase--a novel pathomechanism of chronic spontaneous urticaria? PLoS One. 2011;12;6:e14794.
12. Shin YS, Suh DH, Yang EM, Ye YM, Park HS. Serum specific IgE to thyroid peroxidase activates basophils in aspirin intolerant urticaria. J Korean Med Sci. 2015;30:705-709.
13. Kolkhir P, Church MK, Weller K, Metz M, Schmetzer O, Maurer M. Autoimmune chronic spontaneous urticaria: What we know and what we do not know. J Allergy Clin Immunol. 2017;139:1772-1781.e1.
14. Hatada Y, Kashiwakura J, Hayama K, Fujisawa D, Sasaki-Sakamoto T, Terui T, et al. Significantly high levels of anti-dsDNA immunoglobulin E in sera and the ability of dsDNA to induce the degranulation of basophils from chronic urticaria patients. Int Arch Allergy Immunol. 2013;161 Suppl 2:154-158.
15. Chang TW, Chen C, Lin CJ, Metz M, Church MK, Maurer M. The potential pharmacologic mechanisms of omalizumab in patients with chronic spontaneous urticaria. J Allergy Clin Immunol. 2015;135:337-342.
16. Tong LJ, Balakrishnan G, Kochan JP, Kinét JP, Kaplan AP. Assessment of autoimmunity in patients with chronic urticaria. J Allergy Clin Immunol. 1997;99:461-465.
17. Sabroe RA, Fiebiger E, Francis DM, Maurer D, Seed PT, Grattan CE, et al. Classification of anti-Fcepsilon RI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. J Allergy Clin Immunol. 2002;110:492-499.
18. Hidvégi B, Nagy E, Szabó T, Temesvári E, Marschalkó M, Kárpáti S, et al. Correlation between T-cell and mast cell activity in patients with chronic urticaria. Int Arch Allergy Immunol. 2003;132:177-182.
19. Sun L, Erxun K, Li J, Yang J, Han C. Correlations between anti-mast cell autoantibodies and chronic idiopathic urticaria. Ann Dermatol. 2014;26:145-149.
20. Confino-Cohen R, Chodick G, Shalev V, Leshno M, Kimhi O, Goldberg A. Chronic urticaria and autoimmunity: associations found in a large population study. J Allergy Clin Immunol. 2012;129:1307-1313.
21. Aktar S, Akdeniz N, Ozkol HU, Calka O, Karadag AS. The relation of autologous serum and plasma skin test results with urticarial activity score, sex and age in patients with chronic urticaria. Postepy Dermatol Allergol. 2015;32:173-178.
22. Neverman L, Weinberger M. Treatment of chronic urticaria in children with antihistamines and cyclosporine. J Allergy Clin Immunol Pract. 2014;2:434-438.
23. Chakravarty SD, Yee AF, Paget SA. Rituximab successfully treats refractory chronic autoimmune urticaria caused by IgE receptor autoantibodies. J Allergy Clin Immunol. 2011;128:1354-1355.
24. Perez A, Woods A, Grattan CE. Methotrexate: a useful steroid-sparing agent in recalcitrant chronic urticaria. Br J Dermatol. 2010;162:191-194.
25. Zimmerman AB, Berger EM, Elmariah SB, Soter NA. The use of mycophenolate mofetil for the treatment of autoimmune and chronic idiopathic urticaria: experience in 19 patients. J Am Acad Dermatol. 2012;66:767-770.
26. Kim YS, Han K, Lee JH, Kim NI, Roh JY, Seo SJ,et al. Increased risk of chronic spontaneous urticaria in patients with autoimmue thyroid diseases: a nationwide, population-based study. Allergy Asthma Immunol Res. 2017;9:373-377.
27. Pan XF, Gu JQ, Shan ZY. The prevalence of thyroid autoimmunity in patients with urticaria: a systematic review and meta-analysis. Endocrine. 2015;48:804-810.
28. Tedeschi A, Lorini M, Asero R. Anti-thyroid peroxidase IgE in patients with chronic urticaria. J Allergy Clin Immunol. 2001;108:467-468.
29. Concha LB, Chang CC, Szema AM, Dattwyler RJ, Carlson HE. IgE antithyroid antibodies in patients with Hashimoto's disease and chronic urticaria. Allergy Asthma Proc. 2004;25:293-296.
30. Sánchez J, Sánchez A, Cardona R. Causal Relationship Between Anti-TPO IgE and Chronic Urticaria by In Vitro and In Vivo Tests. Allergy Asthma Immunol Res. 2019;11:29-42.