

Chronic Spontaneous Urticaria: Epidemiological Characteristics Focusing on the Histocompatibility Profile and Presence of Antibodies

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Abstract: *Background:* Understanding the etiopathogenesis of chronic spontaneous urticaria (CSU) remains a challenge. The clinical and laboratory characteristics relating to its histocompatibility profile and autoimmunity are constant research topics.

Objectives: To analyze the clinical and laboratory characteristics of patients with CSU by means of a cross-sectional study, focusing on the histocompatibility profile, presence of antinuclear antibodies (ANA) and presence of antithyroperoxidase antibodies (anti-TPO).

Materials & Methods: Sixty-seven adults with CSU were analyzed. The autologous serum skin test (ASST), ANA and anti-TPO were performed in all cases and MHC classes I and II (loci A, B and DR) were evaluated in 49 patients.

Results: The factors that worsened urticaria included use of non-steroid anti-inflammatory drugs, emotional stress and physical stimuli, reported by 27%, 16% and 15% of these patients, respectively. The ASST test was positive in 49 patients (73%) and anti-TPO and ANA were present in 15 (22.4%) and 7 (10.5%), respectively. The OR (with 95% CI) for the association between ANA and anti-TPO was 5.94 (1.16-30.42), and thus statistically significant. There was a favorable association (with statistical significance) between HLA B*50 and patients with CSU, with OR (95% CI) of 2.96 (1.17-7.48).

Conclusion: A significant favorable association was found between these patients and HLA B*50, and between the presence of anti-TPO and ANA. The greater prevalence of HLA B*50 in these patients and the association between ANA and anti-TPO reinforce the possibility that an immunogenic mechanism may be the triggering factor for CSU.

Keywords: Autoimmune disease, ANA, anti-TPO, autoantibodies, chronic urticaria, histocompatibility, HLA, MHC.

INTRODUCTION

Recurrent chronic urticaria is still a challenge for physicians, in relation to both the etiological diagnosis and the treatment. This is a clinical condition that affects the skin and is characterized by formation of erythematous papules that are often pruriginous, occurring singly or in groups, and generally of fleeting and recurrent nature. These lesions result from vasodilatation and edema of the dermis, and when they affect the deeper regions of the skin (subcutaneous tissue and sub mucosa), they are termed angioedema, and are manifested through swelling, especially of the face. Urticaria and angioedema are common illnesses that affect between 12 and 22% of all individuals at some stage of life [1].

Urticaria is classified as acute or chronic according to the duration of the symptoms. In the acute form, the symptoms do not last for more than six weeks, while cases are classified as chronic if they persist for longer times [1-4]. Chronic urticaria has worldwide prevalence ranging from 0.27% to 2.1% [1].

Cases are described as idiopathic chronic urticaria if they present chronic nature but the cause remains obscure despite

clinical and laboratory investigations. The most recently accepted term for such cases is chronic spontaneous urticaria (CSU) [3, 4]. Currently, it is considered that around 40% of “idiopathic” spontaneous urticaria cases are of autoimmune etiology, consequent to the action of immunoglobulin G (IgG) autoantibodies directed against high-affinity IgE receptors (FcεRI) of cutaneous mast cells, or against IgE bonded to mast cells [5-11]. These autoantibodies predominantly have complement fixation properties in relation to subclasses IgG1 and IgG3 [9-11].

The human leukocyte antigen (HLA) system is formed by polymorphic glycoprotein molecules that are expressed on the surface of all the nucleated cells (HLA class I) and on the main cells involved in the immune response (HLA class II). The genetic locus involved in expression of HLA molecules is known as the major histocompatibility complex (MHC). The HLA molecules serve as “self” markers during the maturation of the thymus, which allows the immunological system to recognize antigens that are foreign to the organism, within the context of “self” HLA molecules. This system is directly involved in various aspects of the immune-mediated mechanisms, among which the following deserve to be highlighted: the organism’s defensive response against external infectious aggressors, the graft rejection process and the triggering of autoimmune diseases. Many autoimmune diseases are associated with specific alleles of

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the HLA system [12], but few studies in the literature deal with this issue in relation to chronic urticaria [13-20].

With regard to the issue of autoimmunity, another very interesting aspect is the possible connection between CSU and autoimmune diseases of the thyroid [21] or between CSU associated with thyroid disease and HLA [22].

The present study was a cross-sectional descriptive analysis that had the objective of investigating the main clinical and laboratory features and the antigen expression of HLA classes I and II of patients with CSU seen by a reference service in Brazil.

MATERIALS AND METHODS

The participants were 67 Brazilians of both genders with CSU. The study was coordinated by the Discipline of Allergy and Clinical Immunopathology, of the Marília Medical School.

A detailed history and physical examination was obtained from each patient, with emphasis on general use of medications and dietary characteristics. Cases of possible hypersensitivity to medications or dietary allergies were excluded, as were patients with clinical evidence of an underlying systemic disease or physical urticaria.

The following laboratory tests were requested: hemogram, erythrocyte sedimentation rate (ESR), ALT, AST, creatinine, serological tests for hepatitis B and C, VDRL, urine I, protoparasitological tests (three samples), total IgE. Antinuclear antibodies (ANA) were investigated by means of indirect immunofluorescence (HEp-2) and anti-thyroperoxidase (anti-TPO) by means of an enzymatic immunoassay. Patients with a diagnosis of hypothyroidism who were using levothyroxine were included, provided that their condition was clinically and laboratorially compensated. Likewise, ANA-positive patients were included, but only if they did not present other criteria for a possible diagnosis of collagenosis.

The genotyping of the HLA system (A, B and DR) was done on peripheral blood of 49 (73%) patients, using the PCR-SSO molecular biological method (kit from One Lambda Inc.), and the flow cytometry method (Labsan 100 apparatus with the SD-Luminex software).

The autologous serum skin test (ASST) was performed in all patients, in accordance with previous descriptions [2], being considered positive when the red weal was 1.5 mm greater than a normal saline control.

All the patients signed an informed consent approved by the ethics committee of Marília Medical School and the study was conducted in accordance with the recommendations of the Declaration of Helsinki.

The data were presented as means (M) and standard deviations (SD). We used the odds ratios (OR) with 95% confidence interval as a measure of association between the variable analyzed.

RESULTS

The general characteristics of the patients evaluated are summarized in Table 1. Sixty-seven adult patients of mean

age 41.5 years ($SD \pm 14.5$), ranging from 18 to 73 years were analyzed, of whom 46 (68%) were women. The mean time that had elapsed between the start of the crises and the medical consultation was 44.9 months ($SD \pm 92.6$). It should be noted that in the great majority of the cases, the patient had already been attended by other services. For 60 patients (90% of the cases), the frequency of the crises was reported as being daily, if the patient was not regularly using some type of antihistamine. We found that the main factors worsening the crises were the following: use of non-steroid anti-inflammatory drugs (NSAID) in 18 cases (27%); emotional stress in 11 cases (16%); and physical stimuli (including having a bath/shower, sports activities, any type of local pressure or heat) in 10 cases (15%). Seven patients (10%) reported that their urticaria worsened with ingestion of specific foods or food additives. It should be noted that the urticaria crises occurred spontaneously, even in the absence of the aggravating factors described above. However, as reported by the patients, there was a tendency for the crises to become more intense when such situations were present. In the majority of the patients (49 individuals; 73%), the clinical manifestation was composed of the presence of urticaria and angioedema (generally on the face) together, which sometimes presented concomitantly and sometimes separately, depending on the occasion. Clinical presentations consisting only of urticaria (i.e. without angioedema) were observed in 16 patients (24% of the cases), while angioedema as the only form of clinical presentation was seen in just two patients (3%).

Forty-nine patients (73%) presented positive ASST; anti-TPO were observed in 15 patients (22.4%) and hypothyroidism in 14 (20.8%) of the 67 patients analyzed. Among the patients who were positive for anti-TPO, seven presented hypothyroidism. Seven patients (10.5%) were positive for ANA. Odds ratio (OR) analysis (with 95% CI) for the association between the presence of anti-TPO and positive ASST test results was 1.62 (0.4-6.58) and between the presence of ANA and positive ASST test results was 2.37 (0.27-21.20). Thus, favorable associations were shown in both of these cases, although without statistical significance, because the value of one was present within both confidence intervals. Analysis on the OR (with 95% CI) of the association between positive findings for ANA and the presence of anti-TPO showed an OR of 5.94 (1.16-30.42), thus demonstrating a statistically significant association (the value of one wasn't present within the confidence interval).

Genotyping for HLA class I (loci A and B) and class II (locus DRB1) was performed for 49 patients (73%). A comparative analysis was done based on the frequencies found in the healthy population in the same region [23]. The frequency of HLA B*50 found in patients with CSU was 6.38% whereas in the healthy population it was 2.25%. We found a favorable association for HLA B*50 among the patients with CSU, with an OR (95% CI) of 2.96 (1.17-7.48), which was thus statistically significant. For the others alleles investigated found no statistical difference.

DISCUSSION

We found that the main factors worsening the crises were use of NSAID, emotional stress and physical stimuli however the urticaria crises occurred spontaneously, even in the absence

Table 1. General Characteristics of Patients: Demographic, Clinical and Laboratory

Total number of patients with CSU	N = 67
Age (years): mean ($\pm$ SD); Varying age (years)	41.5 ($\pm$ 14.5); 18 - 73
Gender (%)	M: 21 (32%); F: 46 (68%)
Number of patients which positive ASST	49 (73%)
Patients with positive antibodies anti TPO (%)	15 (22.4%)
Patients with both hypothyroidism and anti TPO positive	7 (10.5%)
Proportion of patients with hypothyroidism / anti TPO positive	7 / 15 (46.6%)
ANA positive among all patients evaluated (%)	7 (10.5%)
Patients with both positivity for ANA and anti TPO	4 (5.9%)
OR (95% CI): odds ratio with 95% confidence interval for the association between ANA and anti TPO	5.94 (1.16-30.42) †
Number of patients in which the HLA genotyping was performed (classes I and II: loci A, B and DRB1)	49 (73%)
Frequencies (%) of HLA-B*50 in CSU patients and in healthy population	CSU: 6,38%, healthy population: 2,25% ††
OR (95% CI): odds ratio with 95% confidence interval for the association between CSU and HLA B*50	2,96 (1,17-7,48) †

CSU: chronic spontaneous urticaria; SD: Standard deviation; M: male; F: female; ASST: autologous serum skin test; anti TPO: anti thyroperoxidase; ANA: antinuclear antibody; † with statistical significance; †† prevalence of these alleles obtained in a previous study on the healthy population in the same region [23].

of these aggravating factors. It would be interesting to highlight the worsening of the crises with emotional stress, which occurred in 16% of the patients. This feature, which is often seen in clinical practice, can be explained by the possible interrelations between the immunological, neuroendocrine and psychological systems. These relationships have been proven through field research on chronic urticaria, by means of analyses on fluctuations on the serum levels of cortisol, corticotrophin-releasing factor (CRF) and adrenocorticotrophic hormone (ACTH), as parameters indicative of the presence of stress, in relation to basophile activation, as assessed using CD63 expression [24].

With the aim of coming closer towards diagnosing autoimmune chronic urticaria, we performed the ASST, although we were aware that the accuracy of the ASST for diagnosing autoimmune etiology is limited [25]. In fact, ASST is considered to be an *in vivo* test that only given assurance of auto reactivity and does not necessarily define autoimmunity, although even so, it may indicate the possibility of activation of mast cells by autoantibodies [25]. The high prevalence of positive ASST tests (73%) may be explainable through the fact that this study was conducted at a referral service to which screened cases of difficult-to-control urticaria are sent, which are therefore more likely to be of autoimmune etiology.

We found that 15 patients (22.4%) were positive for anti-TPO, which was similar to the rate seen in the literature [21]. Among these, seven patients (approximately 50%) also presented hypothyroidism. Among the hypotheses corroborating the association between thyroid disorders and CSU, there is the possibility that similarities between thyroperoxidase and endogenous and exogenous structures might “confuse the immune system” and trigger an autoimmune reaction, sometimes named an “autotoxic” reaction [26]. Furthermore, the fact that we found a statistically significant reaction between positive findings for ANA and the presence of anti-TPO strengthens the possibility of an autoimmune mechanism in these cases.

Although it is known that associations exist between several autoimmune diseases and certain alleles of HLA [12], few studies have correlated the antigen expression of the MHC with chronic urticaria. Most of these studies have found that predisposition towards urticaria presents possible associations with the following alleles: HLA DRB1*04 [16, 17, 20]; HLA-Bw4 and DQ1 [18]; HLA-DRB1*01 and DRB1*15 [19]; and HLA-B44 [19, 20]; or an inverse (protective) relationship: HLA-DRB1*15 [16]; HLA-A24 [18]. In the present study, we analyzed the histocompatibility profile in 49 patients and found a significant association between these patients and HLA B* 50. Comparison between our study and these others allowed us to suspect that the differences in findings between them were possibly due to the ethnic and regional variations peculiar to each study. It is of interest to note that when the profile of HLA was studied only among ASST-positive, a strong trend towards an association for this allele was found, although without statistical significance [23]. On the basis of the current understanding of CSU, the patients could be divided into three subpopulations: autoimmune chronic urticaria, idiopathic chronic urticaria, and a subgroup that overlaps the two but are characterized with hypo-responsiveness of patients' basophils to anti-IgE and hyper responsiveness to serum [11]. Therefore, association between CSU and profile HLA may be more complex. These considerations may serve for future studies with larger sample the patients can be sub-divided according to those characteristics.

The action of T regulatory cells (Treg) in allergic and autoimmune diseases has recently been much studied [27, 28], in relation both to natural Treg cells, which develop during thymopoiesis, and to adaptive T reg cells, which are generated on the periphery consequent to a variety of stimuli (under conditions that are said to be tolerogenic). With a tolerogenic mechanism controlled by Treg cells, an escape of tolerance to the self could occur, since variations in the binding capacity of self antigens to the slot in the MHC may affect the process of T cell education in the thymus. There could also be a population of T reg cells that would be incapable of recognizing certain self molecules. This might also correlate with genetic polymorphism

of the HLA. In addition, the suppressive effect of certain cytokines like IL 10 and TGF- β , along with environmental factors, may act on adaptive T reg cells [27, 28] and contribute towards triggering allergies in a general manner. This reasoning can be applied to chronic urticaria.

One point that might be criticized in the present study is that the design used here (cross-sectional) is not ideal for this type of analysis, which would be better done using a case-control design. In the present study, the parameter used for comparisons with the HLA alleles investigated in the patients were the prevalences of these alleles obtained in a previous study on the healthy population in the same region [23]. Questions could also be raised because we did not evaluate other loci of HLA such as MHC class I (locus C) or class II alleles (loci DP and DQ). However, even with these limitations this study provides information that can help us in future research on the CSU. Therefore, this study allowed us to conclude that there is significantly greater HLA B*50 expression in this group of patients with CSU, and that there is a statistically significant association between positive findings of anti-TPO and ANA antibodies. These results suggest that genetic and autoimmune mechanisms are present in the pathogenesis of CSU. We believe that further research on this subject is necessary, reinforcing the need to expand the sampling.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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