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Immediate, Delayed and Dual-Contact Reactivity to Common Contact Urticariogens in Patients with Chronic Spontaneous Urticaria: A Study in Serbia

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Abstract

Background: Multiple studies have suggested that chronic spontaneous urticaria (CSU) may be an autoimmune condition occurred in a substantial proportion of cases, but it is important to identify the potential triggers of the disease. There is no study in which the authors tried to answer the question of whether patch testing to common contact urticariogens should be routinely done in patients with CSU.

Objectives: We assessed urticarial, eczematous, and dual contact reactivity in patients with CSU to common contact urticariogens, the frequency and etiology, and compared with patients suffering from atopic dermatitis (AD) and healthy non-atopic persons.

Methods: All consecutive patients with chronic urticaria (CU) or AD referred to us from Vojvodina province (Serbia) between November 2015 and May 2017, and healthy volunteers were recruited. Contact reactivity was defined as a positive patch-test to at least one of 15 well-known contact urticariogens.

Results: During the study period, 155 patients with CU and 100 patients with AD were referred, and 100 healthy control volunteers were also recruited. Among them, 90 patients with CU, 75 with AD and 70 healthy volunteers gave their written informed consent. Moreover, those who did not fulfill the proposed criteria were excluded. Finally, the patients were divided into three groups: the CSU group included 28 patients with current CSU selected from 73 patients with CU; the Group A included 60 persons with current extrinsic AD, and the Group C included 50 healthy non-atopic persons. In all groups, benzoin, cinnamic acid, benzoic acid, and Peru balsam were among the top five allergens in contact-urticarial response, whereas nickel and cinnamic acid were among the top three in eczematous response. The rates of urticarial, delayed, and dual contact reactivity to at least one allergen did not significantly differ [(χ^2-boot = 1.410; P = 0.480). (χ^2-boot = 1.341; P = 0.527) and (χ^2-boot = 0.316; P = 0.907), respectively] among different groups. The difference was detected to benzoin urticarial reactivity (χ^2-boot = 8.487; P = 0.016): in the CSU or AD groups, it was significantly higher than the C group (P = 0.025 and 0.010, respectively). A significant difference was detected in female urticarial reactivity to benzoin (χ^2-boot = 6.998; P = 0.031): in the group AD, it was higher than the C group (P = 0.018).

Conclusions: This could be of the first studies in which the researchers tried to answer the question of whether patch testing to common contact urticariogens should be routinely proposed in patients with chronic spontaneous urticaria. The authors suggest that more investigations have to be designed through multicentric research.

Keywords: Allergens, Atopic, Contact, Dermatitis, Eczema, Hypersensitivity, Patch Tests, Serbia, Urticaria

1. Background

According to the European Academy of Allergy and Clinical Immunology, the Global Allergy and Asthma European Network, the European Dermatology Forum, and the World Allergy Organization (EAACI/GA2LEN/EDF/WAO), chronic urticaria (CU) is classified into chronic inducible urticaria (CINDU) and chronic spontaneous urticaria (CSU) (1). The cause of CINDU remains unknown, except for the cases of contact urticaria, which may be chronic if the association with the triggering factor is not recognized (1-4). Multiple studies have suggested that CSU may be an autoimmune condition occurred in a substantial proportion of cases, but it is important to identify the potential triggers of the disease (5). There have been very few studies on contact reactivity in patients with chronic urticaria, particularly in patients with spontaneous chronic urticaria (6-11). The controversy over the obtained results published in the world literature may be partly explained by the fact that

patch testing in patients with CSU is not only quite challenging and time-consuming but also by the fact that patch testing in those studies was performed with the baseline standard series, which revealed in average only 60% of offending agents responsible for the development of allergic contact dermatitis. Although the world literature available to us is concerning, there is no study in which the authors tried to answer the question of whether patch testing to common contact urticariogens and substances capable of producing urticarial reaction after contact with the skin should be routinely done in patients with chronic spontaneous urticaria.

2. Objectives

We performed a prospective experimental clinical research study to assess the frequency of immediate, delayed, and dual reactivity to common contact urticariogens in patients with chronic spontaneous urticaria (CSU) in comparison with those suffering from extrinsic atopic dermatitis (AD) and healthy control persons. To the best of our knowledge, this is the first manuscript in which the authors have tried to answer the question of whether patch testing to common contact urticariogens should be routinely investigated in patients with chronic spontaneous urticaria.

3. Methods

3.1. Sampling Structure of the Study Subjects

All consecutive patients with chronic urticaria (CU) or atopic dermatitis (AD) referred to us from Vojvodina province (Serbia) between November 2015 and May 2017, and healthy volunteers who fulfilled the study criteria were included in this study after being routinely evaluated, diagnosed, and treated at our Department of Allergy and Clinical Immunology, at the University Clinic of Dermatovenereology Diseases in Novi Sad, Clinical Center of Vojvodina (the Province of Serbia), Faculty of Medicine, University of Novi Sad, Serbia. The participants were divided into three groups: CSU (patients with chronic spontaneous urticaria selected from those with CU), AD (patients with AD) and C (healthy volunteers).

This prospective clinical experimental study, registration number 172058/15, was performed in compliance with the relevant laws and institutional guidelines and approved by the Institutional Review Board of the Clinical Center of Vojvodina (the Province of Serbia), and the Ethics Committee of the University of Novi Sad, Serbia on March 2, 2015 (No. 00-05/124). All participants signed informed consent forms before the participation in the study, after being informed about the aims of this research.

3.2. Inclusion and Exclusion Criteria

The CSU group included patients with current CSU. The disease was defined with regard to the recommended classification, including the spontaneous appearance of wheals with average hive duration less than 24h, angioedema, or both, present or documented by referring physician, at least twice per week, within the last six weeks (1). The exclusion criteria were any type of physical, aquagenic, cholinergic, contact, or occupation related urticaria, food- and drug-induced urticaria and urticaria related to other autoimmune disorders. Thus, in all patients with CSU, any serious inflammatory/auto-inflammatory disease (using erythrocyte sedimentation rate, C-reactive protein, CBC, and differential) and potential triggering drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) were excluded. Based on patient history, specific triggers for CINDU were excluded after provocation testing. If urticaria lasted more than three months, potential causes, such as food allergy/intolerance, and chronic focal infections, were excluded or alleviated, respectively. Extended laboratory investigation, including thyroid function tests, antinuclear, anti-thyroglobulin and anti-peroxidase antibodies, complement components C3, C4 served to rule out autoimmune disorders. The Group AD included patients with current extrinsic AD with no history of intolerance to conventional topical treatment or occupation-related symptoms. All patients with AD fulfilling Hanifin and Rajka criteria had positive skin test(s), the presence of IgE antibodies specific to a mixture of inhalant allergens and/or specific IgE to at least one ubiquitous inhalant allergen in their sera. The Group C included healthy non-atopic subjects with no history of skin diseases and no chronic drug intake.

3.2.1. Diagnostic Tests for Subtypes of Chronic Urticaria

Provocation tests for exclusion of CINDU were done according to the EAACI/GA2LEN/EDF guidelines (1, 12). Cold urticaria was excluded by placing an ice cube in a thin plastic bag for 5 - 20 minutes on the volar forearm and waiting for wheal at the test site within 15 minutes after testing. Heat urticaria was excluded by the application of closed test tube containing hot water 40 - 45°C that was wrapped with a layer of cotton wool and attached to the volar forearm by a bandage for five minutes with no wheal at the test site within 15 minutes after testing. Urticaria factitia was assessed using a dermographometer (HTZ Ltd Vulcan Way, New Addington, Croydon, Surrey, CR0 9UG, UK), with a pressure of 36 g/mm^2 with no induction of linear itchy wheals within 10 minutes after testing. Delayed pressure urticaria was excluded using 100 g/mm^2 pressure on the upper back skin for 70 seconds with no wheal induced 4 - 6 hours after testing. Solar urticaria was excluded 10 minutes after exposure to UVA 6 J/cm^2 and UVB

60 m J/cm^2 (Therapy unit UV 801 KL (PUVA/IB21) and UV/PDT metar/VARIOCONTROL Waldmann Medical Division, Germany) and/or natural sunlight. Cholinergic urticaria was excluded by checking for wheal appearance after moderate exercise (exercise suitable for patient's years and general condition, e.g. wearing warm clothes in a warm room that makes it easy to test the quick walk and climbing the stairs) which has been performed until active sweating occurred; if positive, the test proceeded after > 24 hours by 42°C bath with monitoring body temperature up to15 minutes after body temperature has increased by ≥ 1°C over baseline; wheals were evaluated during the test, immediately, and 10 minutes after the end of test. Aquagenic urticaria was excluded by wearing wet clothes at body temperature (at 37°C) for 20 minutes, or immersion of a body part into water, or by placing wet towels for a few minutes onto the most affected area of the skin. Screening for food allergy was done by means of history and skin testing with a panel of common food allergens (Torlak®, Beograd, Serbia). The patients were instructed that avoidance of type I-allergens clears urticaria within 24 - 48 hours if the relevant allergen has been eliminated rapidly, but in pseudo-allergy, a diet should be maintained for at least three weeks. When drug allergy was suspected, the drug was omitted or replaced if indispensable. The patients with a history of atopic manifestations and/or presence of IgE antibodies specific to a mixture of inhalant allergens in their sera, and/or skin prick test ≥ 2+, and/or the presence of elevated serum level of specific IgE to at least one common inhalant allergens (≥ 0.35 kU/L -class 1), and skin prick test 1+ were excluded from the CSU group.

3.2.2. Determination of Specific IgE Levels in Serum

Phadiatop® EIA-Enzyme immunoassay (Pharmacia AB, Uppsala, Sweden) was used as a method that provides the differential determination of IgE antibodies specific to a mixture of inhalant allergens in human serum. The specific IgE to at least one common inhalant allergen was determined by the Phadezym RAST®-Radioallergosorbent test or ImmunoCAP Specific IgE 0 - 100® system with a completely automated platform. Both tests were routinely conducted at the Immunology Department at the University Institute of Pulmonary Diseases in Sremska Kamenica, Faculty of Medicine, University of Novi Sad, Serbia, according to the manufacturer's instructions (Pharmacia AB, Uppsala, Sweden).

3.3. Patch Testing

3.3.1. Exclusion Criteria for All Groups at Baseline Patch Testing (Day D0)

Excluded subjects were those with ≥ 5 hives; with ≥ 1 hive on test areas (the skin on the upper back and the extensor aspect of the upper arm); with hive > 1 cm in diameter; with signs of dermatitis; pregnant or lactating women; taking oral immunosuppressants; users of cytotoxic or immunosuppressive agents, including UV-therapy, or sun exposure in the last two months; taking systemic corticosteroids or potent topical corticosteroids within the last one month; with systemic use of antihistamines for at least 3 days prior to testing.

3.3.2. Patch-Test Allergens

All were tested with: camphor 10% pet; vanillin 10 pet; vanilla (ethanol extract) 10% acet; balsam of Peru 25% pet; benzoin tincture 10% alc; benzoyl peroxide 1% pet; paraben mix 15% pet; cinnamic acid 5% pet; benzoic acid 5% pet; cobalt chloride 1% pet; nickel sulphate 5% pet; formaldehyde 1% aq; ammonium persulphate 2.5% pet; sorbic acid 2% pet; sodium benzoate 5% pet: supplied from Hermal-Trolab®, Reinbek, Germany or Chemotechnique Diagnostics®, Vellinge, Sweden; vanilla and benzoin tincture were prepared at the Department of Chemistry, Faculty of Natural Sciences.

3.3.3. Immediate-Urticarial Contact Reactivity

The PTs were applied to the extensor aspect of the upper arm for 20 minutes, using (Chemotechnique Diagnostics, Vellinge, Sweden). Readings were made at 20, 40, and 60 minutes after test removal as follows: +, stage 1 or typical urticaria on the test site; ++, stage 2 or generalized, or urticaria at sites distant from test area; +++, stage 3 or systemic symptoms (asthma, rhinoconjunctivitis, oropharyngeal or gastrointestinal symptoms) with/without urticaria; ++++, stage 4 or anaphylaxis/anaphylactoid reactions. Reactions ≥+ were considered positive (2).

3.3.4. Delayed Contact Reactivity

The PTs were applied to the back for two days, using IQ Chambers® (Chemotechnique Diagnostics, Vellinge, Sweden). Readings were made on days D2 and D3, if indicated on D5 - D8, according to the International Contact Dermatitis Research Group (ICDRG) (13). Reactions ≥ + (palpable erythema) were considered positive and - allergic (AR). Reactions recorded only on D2 were considered irritant (IR) and excluded.

All readings were conducted in our patch test clinic by the same, highly experienced observer (Jovanovic M). We had no missing value in our study, and shortly before the experiments, all instruments and equipment were calibrated and checked.

3.4. Standardization of Sensitization Rates

Crude sensitization rates (number of positive per 100 tested) were standardized following the recommenda-

tion of the population-adjusted frequency of sensitization (PAFS): age standardization was done based on 9 age groups of a 10-year sequence, whereas sex standardization was based on standard distribution of 40% of males and 60% of females (14).

3.5. Statistical Analysis

Descriptive statistics, including the frequency, mean and standard deviation, median and IQR (interquartile range) were used in this study. The analysis of variance (ANOVA) test was used to test the hypothesis that the mean ages differed in the groups. Where F test was significant, Scheffe's post hoc analysis was used to show which means were significantly different. Normality assumptions were evaluated by the Kolmogorov-Smirnov test. If normal distribution of the variables was not estimated, Kruskal-Wallis one-way ANOVA by Ranks was used to test the hypothesis that the mean ages differed in groups. Where H test was significant, Multiple Comparisons analysis of mean ranks was used to show which means were significantly different. The Pearson Chi-square test (χ^2) was used to examine the differences among the three-groups regarding the standardized reactivity rates to the allergens applied. In order to improve the reliability of statistical inference, Monte Carlo simulations with 10,000 replications were carried out, yielding the simulated P values for each of Chi-square tests. The same procedure was applied to the testing of differences in proportions (standardized reactivity rates) between two groups. Cohen's h estimates of effect sizes, as well as the statistical power for the respective analyses, were additionally calculated. Within-group differences regarding the reactivity to particular allergens were examined by pair-wise proportion tests.

The hypotheses were that there might have been differences in reactivity: between all groups; between male and female subjects in each group; and between male and female subjects among different groups. Two-tailed P values less than 0.05 we considered statistically significant.

All statistical analyses were conducted with the "TIBCO" statistical analysis software Inc. (2018) Statistica (data analysis software system) version 13, as well as with the packages included in the «R» statistical software (15).

4. Results

During the study period, among those referred to our Department of Allergy and Clinical Immunology, there were 155 patients with CU urticaria, present or documented by referring physician, at least twice per week, within the last six weeks, 100 were diagnosed as having AD, and 100 healthy control volunteers were recruited. Among them, 90 patients with UC, 75 with AD, and 70 healthy volunteers gave their written informed consent. However, those who did not fulfill the proposed criteria were excluded. Finally, a total of 138 adult persons were tested. This cohort of patients remained after additional exclusions were made because of clinically significant affective or other psychic problems that might have affected the study procedures. The patients were divided into three groups: CSU (patients with CSU), AD (patients with AD), and C (healthy volunteers) (Table 1). The CSU group included 28 patients with current CSU selected from 73 patients with CU after the exclusion of 10 patients with CINDU and 35 patients with hypersensitivity reactions to drugs. In this group, four patients had atopy, 15 focal infections, five patients had autoimmune diseases (three had systemic lupus erythematosus, and two had thyroid disease). The mean duration of CSU was 16.85 months (range 3 - 60 months). The Group A included 60 persons with current extrinsic AD: 43.4% had rhinitis and 15% asthma. The Group C included 50 healthy non-atopic subjects with no history of skin diseases and no chronic drug intake.

After all exclusions were made, there were statistically significant differences in age distribution between the groups (ANOVA: F (3. 179) = 11.332, P < 0.001). Scheffe's post hoc analysis showed that the CSU and C groups were significantly older than the AD group (for both differences P < 0.001), while the CSU and C groups did not differ significantly (P = 0.470) (Table 1). Since age variables were not distributed normally in one (AD) group (K-S d = 0,22053, P < 0,01; the AD group), all groups were also compared using Kruskal-Wallis ANOVA by Ranks, which showed that there were statistically significant differences in age distribution between the groups (Kruskal-Wallis test: H (2, N = 138) = 29,375 P < 0.001). Post hoc Multiple Comparisons analysis of mean ranks used to show which means were significantly different, demonstrating that the CSU and C groups were significantly older than the AD group (for both differences P < 0.001), while the CSU and C groups did not differ significantly (P = 0.420).

There were no significant differences in gender distribution among the groups (Pearson χ^2 (2) = 0.470; P = 0.790), but there were significantly more females in all three groups (P < 0.001). The reactivity rates were standardized in order to avoid differences biased by age and sex (14).

4.1. Contact Reactivity to at Least One Allergen

4.1.1. Immediate-Urticarial Contact Reactivity

All positive contact urticarial reactions were stage 1 +, with typical urticaria on the test site. The rates of urticarial contact reactivity (UCR) to at least one allergen did not significantly differ (χ^2-mc = 1.410; P = 0.480) (Table 2) among

Table 1. Demographic Characteristics of Groups After Exclusions[a,b]

Group	No.	Males	Mean ± SD	95% CI	Range	Median	IQR
CSU	28	17.86	39.64 ± 10.35	35.06 - 44.22	21 - 60	39.50	60 - 21
AD	60	25.00	26.17 ± 12.64	23.04 - 29.29	12 - 61	20.00	37 - 17
C	50	20.00	35.32 ± 10.79	31.89 - 38.74	17 - 67	33.00	43 - 28

[a] AD, atopic dermatitis; C, healthy controls; CSU, chronic spontaneous urticarial; IQR, interquartile range.
[b] Males are reported in percent, mean age in years, and median age in years.

different groups. The rate of UCR for those with CSU was not significantly higher than rates for those with AD, or healthy controls (P = 0.999 and 0.503, respectively); the rate for the healthy controls was not significantly lower than those for patients with AD or CSU (P = 0.381 and 0. 0.503, respectively). There were no significant sex-related differences regarding UCR in the CSU, AD, and C groups [(χ^2-mc = 0.078; P = 1.000), (χ^2-mc = 1.015; P = 0.311) and (χ^2-mc = 0.159; P = 0.668), respectively]. In males, UCR reactivity did not significantly differ (χ^2-mc = 0.586; P = 0.824) among different groups; thus there were no significant differences between the rates for healthy males and males with CSU or AD, or between the rates for males with CSU and AD, (P = 0.941, 1.000 and 0.990, respectively). In females, UCR reactivity did not significantly differ (χ^2-mc = 1.813; P = 0.401) among different groups; thus, there were no differences between the rates for healthy females and females with CSU or AD, or between the rates for females with CSU and AD (P = 0.909, 0.277 and 0.669, respectively).

4.1.2. Delayed Contact Reactivity

The rates of delayed contact reactivity (DCR) to at least one allergen (Table 2) did not significantly differ (χ^2-mc = 1.341; P = 0.527) among different groups. The rate of DCR for those with AD was not significantly higher than the rates for those with CSU or healthy persons (P = 0.999 and 0.369, respectively); the rate for the healthy controls was not significantly lower than the rates for those with CSU or AD (P = 0.673 and 0.369, respectively). There were no significant sex-related differences regarding DCR in the CSU, AD, and C groups [(χ^2-mc = 0.502; P = 0.545), (χ^2-mc = 1.423; P = 0.269), and (χ^2-mc = 0.988; P = 0.172), respectively]. In males, DCR reactivity did not significantly differ (χ^2-mc = 1.315; P = 0.664) among different groups, thus there were no differences between the rates for healthy males and males with CSU or AD, or between the rates for males with CSU and AD (P = 1.000, 0.707 and 1.000, respectively). In females, DCR reactivity did not differ (χ^2-mc = 0.384; P = 0.842) among different groups, thus there were no differences between the rates for healthy females and females with CSU or AD, or between the rates for females with CSU and AD (P = 0.999, 0.710, and 0.999, respectively).

4.1.3. Dual Contact Reactivity

Rates in dual contact reactivity (DUCR) to at least 1 allergen (Table 2) did not significantly differ (χ^2-mc = 0.316; P = 0.907) among different groups. The rate of DUCR for those with CSU was not significantly higher than the rates for those with AD or healthy persons (P = 0.942, 1.000, respectively). The rate for those with AD was not significantly lower than the rates for patients with CSU or healthy controls (P = 0.942 and 1.000, respectively). There were also no significant sex-related differences in the CSU, AD, and C groups [(χ^2-mc = 0.786; P = 0.392), (χ^2-mc = 0.453; P = 0.579) and (χ^2-mc = 1.049; P = 0.080), respectively]. In males, DUCR reactivity did not significantly differ (χ^2-mc = 0.373; P = 1.000) among different groups; thus there were no significant differences between the rates for healthy males and males with CSU or AD, or between the rates for males with CSU and AD (P = 1.000, 1.000 and 1.000, respectively). In females, DUCR reactivity did not significantly differ (χ^2-mc = 0. 785; P = 0.762) among different groups, thus there were no significant differences between the rates for healthy females and females with CSU or AD, or between the rates for females with CSU and AD, (P = 0.920, 0.990, and 0.651, respectively).

4.2. Rates of Contact Reactivity to Each Allergen

4.2.1. Rates of Immediate-Urticarial Contact Reactivity to Each Allergen

Regarding UCR to the same allergen (Table 3) among different groups, a significant difference was detected to benzoin reactivity (χ^2-mc = 8.487; P = 0.016). Benzoin reactivity in the CSU or AD groups was significantly higher than the group C (P = 0.025 and 0.010, respectively). The significant difference was detected in female reactivity to benzoin (χ^2-mc = 6.998; P = 0.031). Benzoin reactivity in the group AD was higher than the C group (P = 0.018).

Regarding UCR to allergens in the same group, the highest rates in patients with AD and healthy persons belonged to benzoin and cinnamic acid, respectively. In addition, the differences were significant in comparison with all other allergens in each group (P < 0.05).

Table 2. Delayed Urticarial and Dual Contact Reactivity Rates (%) to at Least 1 Allergen Standardized for Age and Sex and Rates in Females and Males Both Standardized for Age[a]

Group	Crude	Standardized			
		Age			Age/Sex
		Total	Females	Males	
Delayed Contact Reactivity Rates					
Chronic spontaneous urticaria					
Rate	42.86	23.88	25.89	11.11	19.97
No.	28	28	23	5	28
Atopic dermatitis					
Rate	48.33	26.81	28.48	12.34	22.01
No.	60	60	45	15	60
Healthy controls					
Rate	40.00	17.80	22.59	0.00	13.55
No.	50	50	40	10	50
Urticarial Contact Reactivity Rates					
Chronic spontaneous urticaria					
Rate	53.57	20.09	27.12	33.33	29.60
No.	28	28	23	5	28
Atopic dermatitis					
Rate	50.00	33.76	33.53	19.75	29.22
No.	60	60	45	15	60
Healthy controls					
Rate	34.00	20.21	22.43	16.66	20.12
No.	50	50	40	10	50
Dual Contact Reactivity Rates					
Chronic spontaneous urticaria					
Rate	10.71	5.58	13.93	0.00	8.35
No.	28	28	23	5	28
Atopic dermatitis					
Rate	11.66	6.21.	7.25	2.46	5.33
No.	60	60	45	15	60
Healthy controls					
Rate	12.00	7.24	9.68	0.00	5.83
No.	50	50	40	10	50

[a] No., number of tested.

4.2.2. Rates of Delayed Contact Reactivity to Each Allergen

Regarding the rates of DCR for the same allergen among different groups, no significant difference was found for any allergen (Table 4).

4.2.3. Rates of Dual Contact Reactivity to Each Allergen

Regarding the rates of DUCR to each allergen among different groups, no significant difference was found for any allergen (Table 5).

5. Discussion

Mast cells in the skin play a central part in the pathogenesis of the CSU, which encompasses autoimmune, allergic or idiopathic mechanisms (1, 16). The former may occur

Table 3. Urticarial Contact Reactivity Rates (%) Crude and Standardized for Age and Sex and Rates in Females and Males Both Standardized for Age[a]

Group	CSU (N = 28)					AD (N = 60)					C (N = 50)				
Allergen	CR	A	WA	MA	AS	CR	A	WA	MA	AS	CR	A	WA	MA	AS
Camphor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vanillin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vanilla	0.00	0.00	0.00	0.00	0.00	1.66	0.39	0.58	0.00	0.35	0.00	0.00	0.00	0.00	0.00
Balsam of Peru	7.14	4.81	12.34	0.00	7.40	10,00	2.84	3.45	1.23	2.56	2.00	0.69	0.00	5.55	2.22
Benzoin	35.71	20.92	21.19	22.22	21.59	31.6	25.50	26.80	13.60	21.50	6.00	4.16	5.29	0.00	3.17
Benzoyl peroxide	0.00	0.00	0.00	0.00	0.00	5.00	2.18	2.17	1.23	2.31	0.00	0.00	0.00	0.00	0.00
Paraben mix	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cinnamic acid	25.00	11.65	16.40	11.11	14.28	23.30	10.40	10.60	2.47	7.40	32.0	21.50	20.90	22.20	21.40
Benzoic acid	10.71	6.20	13.93	0.00	8.36	16.60	7,40	8.15	3.70	6.37	4.00	3.43	4.44	0.00	2.66
Cobalt chloride	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nickel sulphate	7.14	2.50	2.82	0.00	1.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Formaldehyde	3.57	1.11	1.23	0.00	0.74	1.66	0.39	0.00	1.23	0.49	0.00	0.00	0.00	0.00	0.00
Ammonium pers	3.57	1.11	1.23	0.00	0.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorbic acid	7.14	4.81	12.34	0.00	7.40	1.66	0.85	0.00	3.70	1.48	0.00	0.00	0.00	0.00	0.00
Sodium benzoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] Ammonium pers, Ammonium persulphate; n, number of tested; CSU, chronic spontaneous urticaria; AD, atopic dermatitis; C, healthy controls; CR, crude rate; A, age-standardized rate; WA, age-standardized rate in females; MA, age-standardized rate in males; AS, total rate standardized for age and sex.

Table 4. Delayed Contact Reactivity Rates (%) Crude and Standardized for Age and Sex and Rates in Females and Males Both Standardized for Age

Group	CSU (N = 28)					AD (N = 60)					C (N = 50)				
Allergen	CR	A	WA	MA	AS	CR	A	WA	MA	AS	CR	A	WA	MA	AS
Camphor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vanillin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vanilla	3.57	3.70	0.00	5.50	2.22	1.66	0.39	0.00	1.23	0.49	0.00	0.00	0.00	0.00	0.00
Balsam of Peru	3.57	3.70	11.11	0.00	6.66	6.66	2.50	3.39	0.00	2.03	8.00	3.19	4.07	0.00	2.44
Benzoin	3.57	1.11	1.23	0.00	0.74	3.33	1.25	1.11	1.23	1.15	8.00	3.81	6.35	0.00	3.81
Benzoyl peroxide	0.00	0.00	0.00	0.00	0.00	6.66	3.04	2.43	4.94	3.43	6.00	2.08	2.38	0.00	1.43
Paraben mix	3.57	3.7	0.00	5.55	2.22	1.66	0.85	0.00	3.70	1.48	0.00	0.00	0.00	0.00	0.00
Cinnamic acid	7.14	4.81	1.23	5.55	4.82	16.66	8.85	9.42	6.17	8.11	14.00	8.48	11.53	0.00	6.91
Benzoic acid	0.00	0.00	0.00	0.00	0.00	6.66	2.50	1.11	6.17	3.13	6.00	3.16	4.49	0.00	2.69
Cobalt chloride	3.57	1.85	2.22	0.00	2.60	5.00	1.65	1.69	1.23	1.50	0.00	0.00	0.00	0.00	0.0
Nickel sulphate	25.0	12.41	10.07	5.55	8.26	11.66	10.40	11.90	0.00	7.12	16.00	5.93	7.19	0.00	4.31
Formaldehyde	3.57	1.38	1.59	0.00	0.95	13.33	5.08	4.65	4.94	4.77	0.00	0.00	0.00	0.00	0.00
Ammonium pers.	10.71	4.35	5.04	0.00	3.02	1.60	0.85	1.11	0.00	0.66	0.00	0.00	0.00	0.00	0.00
Sorbic acid	0.00	0.00	0.00	0.00	0.00	3.33	1.78	2.17	0.00	1.30	0.00	0.00	0.00	0.00	0.00
Sodium benzoate	6.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] Ammonium pers, Ammonium persulphate; n, number of tested; CSU, chronic spontaneous urticaria; AD, atopic dermatitis; C, healthy controls; CR, crude rate; A, age-standardized rate; WA, age-standardized rate in females; MA, age-standardized rate in males; AS, total rate standardized for age and sex.

Table 5. Dual Contact Reactivity: Rates (%)s Crude and Standardized for Age and Sex and Rates in Females and in Males Both Standardized for Age[a]

Group	CSU (N = 28)					AD (N = 60)					C (N = 50)				
Allergen	CR	A	WA	MA	AS	CR	A	WA	MA	AS	CR	A	WA	MA	AS
Balsam of Peru	3.57	3.70	11.11	0.00	6.66	1.66	1.38	1.58	0.0	0.94	0.00	0.00	0.00	0.00	0.00
Cinnamic acid	0.00	0.00	0.00	0.00	0.00	8.33	4.96	6.14	1.23	4.17	10.00	6.55	8.86	0.00	5.31
Benzoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.69	0.79	0.00	0.47
Nickel sulphate	7.14	2.50	2.82	0.00	1.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Formaldehyde	3.57	1.38	1.59	0.00	0.95	1.66	0.39	0.58	0.00	0.34	0.00	0.00	0.00	0.00	0.00

[a] ACSU, chronic spontaneous urticaria; AD, atopic dermatitis; C, healthy controls; CR, crude rate; A, age-standardized rate; WA, age-standardized rate in females; MA, age-standardized rate in males; AS, total rate standardized for age and sex.

through the presence of serum histamine-releasing factors (HRFs), such as self-reactive IgE against auto-allergens or/and auto-antibodies against immunoglobulin E (IgE) or high-affinity Fc receptors (5, 16-19). Intradermal injection

of autologous serum was found to cause a wheal and flare reaction in some patients with CSU, indicating the presence of serum HRF. Not all patients with a positive intradermal serum test have auto-antibodies, implying the presence of other serum HRF. Many patients with CSU have a negative intradermal serum test; therefore, there are other pathways to cutaneous mast cell activation, as yet unknown (20). Multiple studies have suggested that CSU may be an autoimmune condition in a substantial proportion of cases, but it is important to identify potential triggers of disease (5). Immunologic studies indicate that CD4+ and CD8+ T cells are capable of stimulating histamine release from mast cells, particularly in the presence of allergen. Recently, it has been theorized that HRFs are involved in the pathway by which contact allergens after systemic absorption mediate a type IV (T cell-mediated) urticaria (6). All well-known contact urticariogens that we tested have been previously considered potentially systemic (2, 4, 17). The following allergens were considered potentially systemic: cobalt, nickel, potassium dichromate, fragrance mixes, balsam of Peru, parabens, and formaldehyde. All of these allergens are well-known contact urticariogens included in our study.

Our patients' demographics were within the range of means encountered in the previous studies on DCS in patients with CSU (the mean age of 39.6 years with 82.1% females in our study and the mean age varying between 31 - 46 years with 36.6% - 95.3% females, in the previous studies) (6-11). A significantly higher prevalence of chronic urticaria in females than males can be partially explained by a higher incidence of autoimmune diseases in females than males (21). The overall (crude) frequency of DCS in our study was 42.9%. Data literature has shown the prevalence between 13.3% and 42.9% (7-11), only in one study where beyond basic, specific batteries were tested, 95.6% of patients had at least one positive PT (6). After standardization, which application on the prevalence of contact sensitization is highly recommended (12), the frequency of positive PTs found in the CSU in the present study (19.9%), was not significantly different from patients with AD or healthy control persons. We also included patients with AD, as another Th-cell-dependent chronic inflammatory skin diseases with a debatable risk for contact sensitization (22). The difference between patients with AD and healthy controls was not significant, which was in accordance with our previous results (23). Moreover, in a recent meta-analysis, no significant association was shown between AD and contact sensitization, while a statistically significant positive relationship was found only between Compositae contact sensitization and AD status, which was in line with our previous study (22, 24).

The peculiarity of the study lies in the fact that all inclusion criteria were made on academic level, regarding all criteria for setting the diagnosis, particularly of chronic spontaneous urticaria and atopic dermatitis, as well as patch testing and evaluation of positive immediate patch tests which were regarded as positive only if urticarial but not simple erythema appeared. Before patch testing, CU should be treated until spontaneous remission occurs, intermittent attempts at medication withdrawal should be made to identify spontaneous remission (17). During patch testing, all measures have to be taken into account with respect to the classification and recommended practical guides, in order to alleviate false positive and negative patch test results, from the definition up to the withdrawal of symptomatic therapies such as antihistamines (1, 4, 13). Apart from this study, to our knowledge, these were only partially considered in few studies (6-9). The application of population-adjusted frequency of sensitization (PAFS) on the prevalence of contact sensitization is highly recommended since remaining differences cannot be longer attributed to age and sex, but to other factors e.g., the percentage of male patients, occupational cases, atopy, hand or leg dermatitis (e.g., MOAHL index), and genetic susceptibility of the tested population. Therefore, exclusions from the study that were made at the baseline were followed by standardization of the frequency of positive patch tests, in this study the crude sensitization rates (number of positive per 100 tested) were standardized following the PAFS recommendation, which as far as we know, has never been done in the previous studies on contact sensitivity in patients with chronic urticaria.

When investigating urticarial contact reactivity of substances naturally present in foods or commonly used in topical preparations, Lahti recorded UCR in 44% of patients with CU and in 31% of atopic subjects; however, the difference was not significant; benzoic acid, cinnamic acid, and balsam of Peru elicited CUR most frequently (25). The rate of CUR in our patients with CSU was not significantly higher than in patients with AD or healthy controls; benzoin, cinnamic acid, benzoic acid, and balsam of Peru were among the top five allergens in all three groups, which is in line with the study conducted by Lahti (25). Immunogenetic studies have demonstrated that polymorphism of inflammasome, an innate immunity guardian, participates in dual, skin urticarial, and contact dermatitis hypersensitivity reactions (26). After standardization, dual reactivity rate among those with CSU was the highest (8.3%), but once again, there were no significant differences between the groups.

The weak points of the study may be the small sample size, as well as the limitation to the population in Vojvodina. In order to overcome these limitations, as well as to validate and expand obtained findings, the authors

suggest that more investigations have to be designed and conducted with different populations through multicentric research.

The authors believe that this study is worth publishing, since it strives to strengthen connections between research and practice, thereby enhancing professional development and improving practice within the field of medicine.

5.1. Conclusions

This might be the first study in which the researchers tried to answer the question of whether patch testing to common contact urticariogens should be routinely proposed in patients with chronic spontaneous urticaria. The obtained results showed that the overall rates of urticarial, delayed, and dual patch test reactivity in patients with chronic urticaria did not differ significantly from patients with atopic dermatitis or healthy controls.

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Footnotes

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