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N. Santos[1], V. Iraola[2], J.L. Plácido[1]

Tetranychus urticae allergy in a population without occupational exposure

[1]Serviço de Imunoalergologia, Centro Hospitalar São João, E.P.E., Porto, Portugal
[2]Departamento de Investigación y Desarrollo, Unidad de Alergia, Laboratorios Leti

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Tetranychus urticae*; spider mite; conjunctival provocation test; allergy*

Corresponding author
Natacha Santos
natachalsantos@gmail.com
Serviço de Imunoalergologia
Centro Hospitalar São João, E.P.E.
Alameda Prof. Hernâni Monteiro
4300 Porto, Portugal

Summary
Background. Tetranychus urticae *is a phytophagus mite found in the leaves of numerous plants. High sensitization rates have been demonstrated, however, provocation tests have only been performed in an occupational setting.* ***Objective.*** *To assess accuracy of skin prick tests and clinical relevance of* T. urticae *sensitization by means of conjunctival provocation tests (CPT) in a population without occupational exposure and to evaluate possible environmental risk factors for* T. urticae *allergy.* ***Methods.*** *Patients ≥ 18 years old sensitized to* T. urticae *(n = 12) and a non-sensitized control group (n = 12) were invited to perform CPT with* T. urticae *and fulfill a questionnaire including demographic data, questions on environmental exposure to* T. urticae *and allergy symptoms/diagnosis. A single-blinded placebo-controlled CPT with* T. urticae *(Leti®) was performed with increasing concentrations (0.002, 0.02, 0.2 and 2 mg/mL) and considered positive if conjunctival hyperemia, palpebral edema or lacrimation were observed in the tested eye.* ***Results.*** *Of* T. urticae *sensitized patients (mean wheal 4.4±1.5 mm), 9 had a positive CPT, including 3 monosensitized. A good diagnostic accuracy was found for skin prick tests: AUC = 0.952, sensitivity = 100%, specificity = 80%, positive likelihood ratio = 5 and negative likelihood ratio = 0 for a 3 mm wheal. No differences were found between allergic and non-allergic subjects regarding atopy, allergic disease or farming activities.* ***Conclusions.*** *A high prevalence of allergy to* Tetranychus urticae *was found in the north of Portugal. Future studies with a larger number of patients are needed to evaluate its relation to clinical symptoms and the impact of environmental factors.*

Introduction

Tetranychus urticae, commonly known as red spider mite or two-spotted spider mite, is a macroscopic phytophagus mite of the *Acari* subclass and *Trombidiformes* order, found in the leaves of numerous plants and an important plague in agriculture, especially in association with sulfur pesticides utilization, to which it is specially resistant (1).

A high sensitization rate to this mite has been shown both in occupational (2) and non-occupational exposure (3), and both species specific and cross-reactive allergens with house dust mites have been identified (4,5).

In Portugal, predominantly in the North of the country, viticulture, farming and greenhouses are important economic activities, and we have previously reported that almost 40% of patients followed in the Immunoallergy Department of our University Hospital were sensitized to *T. urticae* (6). However, the clinical relevance of this sensitization was not assessed.

Allergy to *T. urticae* has been associated with symptoms of rhinitis in the summer and autumn, asthma, contact dermatitis and

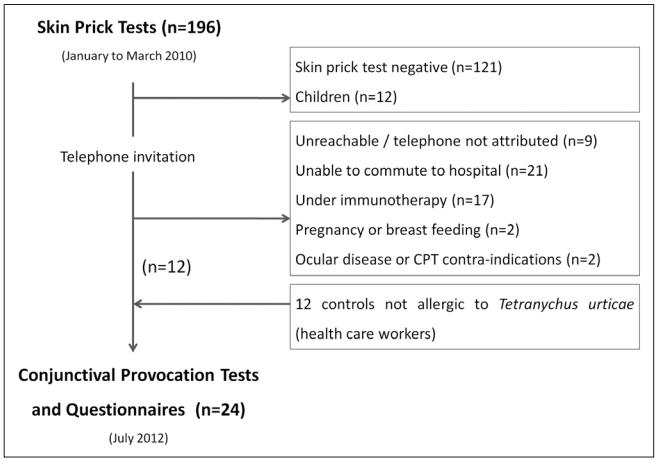
urticaria (3,7,8), and was previously evaluated with bronchial (9,10), and environmental provocation (8), but not with other safer and simpler target organ provocations, namely nasal or conjunctival provocation tests.
Our aims were to assess accuracy of skin prick tests and clinical relevance of *T. urticae* sensitization by means of conjunctival provocation test (CPT) in a population without known occupational exposure to this mite and to evaluate possible environmental risk factors for *T. urticae* allergy.

Methods

Study design and participants

Subjects aged 18 years or older who participated in a previous study to determine sensitization frequency to *T. urticae* (6) and had a positive skin prick test, were eligible (**figure 1**).

***Figure 1** - Study protocol and patients excluded.*



Subjects were contacted by telephone and invited to participate in the study, consisting on a skin prick test to *T. urticae* to assess wheal size, a CPT and a questionnaire on allergy symptoms and risk factors for *T. urticae* exposure.
Those to whom the phone number was no longer attributed, who did not answer the telephone after 3 attempts, who could not commute to the hospital in one of the four days set to perform the study, who were under current or previous allergen specific immunotherapy with mites, who were pregnant, breastfeeding or presented any contra-indication for CPT, were excluded.
A control group of medical, nursing and technical personal of the Immunoallergy Department of Centro Hospitalar São João, E.P.E., consisting of atopic and non-atopic subjects not sensitized to *T. urticae* was also recruited.
24 patients were included, 20 (83.3%) female, mean (SD) age 32.8 (10.9) years old, ranging from 19 to 54 years old.
This study was approved by the ethics committee of Centro Hospitalar São João, E.P.E. Written informed consent was obtained from all subjects included.

Skin prick tests

Skin prick tests were performed with an extract of *T. urticae* bodies (Leti©) 2 mg/mL, histamine 10 mg/mL and a saline solution as negative control. The procedure was performed by the investigator. Extracts were applied in the volar surface of the forearm and pricked with an allergen prick lancet (Heinz Herenz Medizinalbedarf GmbH©), according to international recommendations (11). The mean wheal size was measured and registered. Subjects were considered sensitized if a mean wheal diameter ≥ 3mm for *T. urticae* was observed after 15 minutes.
Other sensitizations, as for house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), storage mites (*Lepidoglyphus destructor*, *Tyrophagus putrescentiae* and *Acarus siro*), cat and dog dander, pollen (*Platanus acerifola*, *Betula verrucosa*, *Olea europea*, grass mix, *Parietaria judaica, Plantago lanceolata* and *Artemisia vulgaris*) and fungi (*Alternaria*, *Cladiosporum*, *Aspergillus* and *Penicillium*) were evaluated according to the results obtained in our previous study (6), using the same methodology.

Conjunctival provocation test (CPT)

A single-blinded placebo-controlled CPT with *T. urticae* was performed, according to the Allergy Department's protocol (12). Exclusion criteria were ocular infection, dry eye syndrome, autoimmune disorders and allergic conjunctivitis exacerbation. Subjects were instructed to stop any topical eye drugs 2 weeks before, oral or nasal corticosteroids 2 weeks before, oral antihistamines 1 week before and not to wear contact lenses on CPT day. Subjects who could not stop medication were also excluded.
Four concentrations of *T. urticae* were prepared from the original lyophilized 2 mg/mL extract (0.002, 0.02, 0.2 and 2 mg/mL) according to manufacturer's instructions and used in the 24 hours following preparation.
One single drop of increasing concentration was applied in the conjunctival eye sac, while a control solution of diluent was applied in the opposite eye. The eyes were evaluated after 10 minutes of each concentration.
The CPT was considered positive if objective signs of conjunctival hyperemia, palpebral edema or lacrimation were observed in the tested eye. Patients with positive tests did not proceed to the next concentration and were treated with a topical antihistamine (opatanol 1 mg/mL) and eventually topical corti-

costeroid (rimexolone 10 mg/mL) until full recovery. The test was negative if no reaction was observed 10 minutes after the last concentration. Patients were instructed to inform if any late reaction occurred.

Questionnaire

Subjects filled in a questionnaire including demographic data, questions on hypothesized risk factors for environmental exposure to *T. urticae* and allergy symptoms/diagnosis.
Questions on environmental exposure included housing type (apartment or dwelling house, with or without a garden), region (rural or urban), distance to farming fields or greenhouses, and farming activities, either professional or recreational (mean hours per week spent, mean years, type of cultures exposed to and pesticide use).
In patients presenting with allergy symptoms, a relation with either exposure or removal from those factors was asked and the most symptomatic seasons of the year were also questioned.

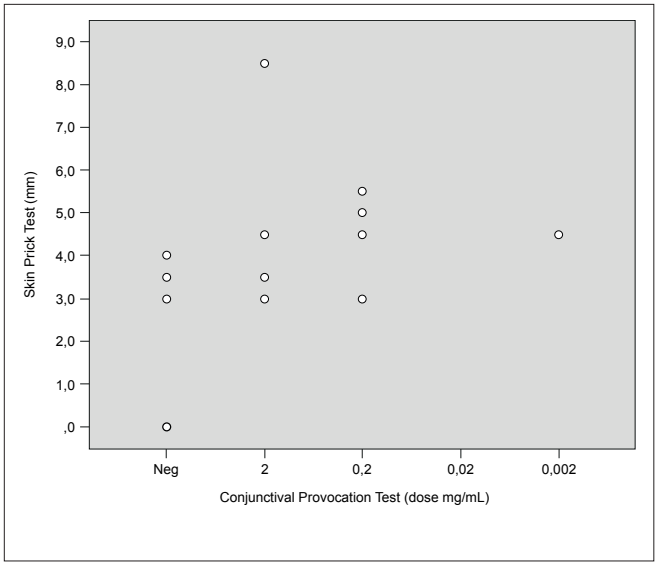
Statistic analysis

Categorical variables were described using absolute and relative frequencies; comparisons were performed with Qui-square or Fisher's exact tests. Continuous variables were described using mean with standard deviation (SD); comparisons were performed with independent samples T-test. A p-value of < 0.05 was considered statistically significant.
Diagnostic accuracy of the *T. urticae* skin prick test was presented as sensitivity, specificity and positive and negative likelihood ratios with 95% confidence intervals. Spearman correlation test was used to test association between mean wheal size and the CPT result. The Area Under Curve (AUC) of the test was calculated.
Data analyses were performed using SPSS® version 18.0 for Mac (IBM SPSS, Chicago, USA) and VassarStats® online calculator (13) for diagnostic accuracy tests.

Results

Among the 24 subjects included, 12 were sensitized to *T. urticae* and 12 were controls; their clinical and demographical characteristics are presented in **table 1**.
The sensitized subjects had a mean (SD) wheal size of 4.4 (1.5) mm and included 3 monosensitized patients.
Figure 2 shows the relation between wheal size in mm and CPT result, including the dose that elicited the positive response. The Area Under Curve (AUC) for the skin prick test with *T. urticae* was of 0.952. For a wheal size ≥ 3 mm, the test presented a sensitivity of 100% (63-100%), specificity of 80% (52-95%), positive likelihood ratio of 5 (1.8-13.8) and negative likelihood ratio of 0 (0-not applicable). Three false positive patients had positive skin prick tests to *T. urticae* (wheals measuring 3-4 mm) but negative CPT to *T. urticae*, were co-sensitized to mites, 2 had rhinitis and the other referred asthma and urticaria symptoms. Considering a wheal size ≥ 4.5 mm, the false negatives were excluded, giving a specificity of 100% (75-100%). The correlation between wheal size and CPT eliciting dose was not significant ($r = -0{,}112$. $p = 0{,}774$). In all patients, positive CPT symptoms resolved within 30 minutes after topical antihistamine treatment, and none reported late reactions.

Figure 2 *- Scatter plot showing the relation between skin prick test wheal size and conjunctival provocation test result, including eliciting dose.*



No differences were found between subjects positive and negative to the CPT regarding atopy, allergic disease, housing type, region type, distance from farming fields or greenhouses, and farming activities (**table 2**). None worked in agriculture or other professions with known occupational exposure to *T. urticae*.
Seven patients with positive CPT to *T. urticae* referred respiratory symptoms: 6 reported symptoms in the spring (2 of these were co-sensitized to pollen), 2 in the summer, 4 in the autumn and 4 during winter; 5 referred an exacerbation of symptoms in the vicinities of farming fields (3 were co-sensitized to pollen); 1 monosensitized patient had rhinitis all year long, noticing however an exacerbation while in the countryside.
Two patients monosensitized to *T. urticae* reported isolated cutaneous symptoms (urticaria) in the spring, which was not related either to exposure or to removal from farming fields or greenhouses.

Sensitization to *T. urticae*	**Yes (n = 12)**	**No (n = 12)**	**p (< 0.05)[1]**
Age, mean (SD)	28.8 (9.9)	36.8 (10.8)	0.071
Gender, female (%)	10 (83)	10 (83)	1.000
Co-sensitization, mites (%)	9 (75)	2 (17)	0.012
Allergy symptoms, n (%)	12 (100)	9 (75)	
Rhinitis	9 (75)	6 (50)	0.400
Asthma	3 (25)	3 (25)	1.000
Cutaneous	3 (25)	3 (25)	1.000

[1]Fisher test

Table 1 *- Clinical and demographical data on subjects sensitized to* Tetranychus urticae *and controls.*

Conjunctival Provocation Test	**Positive (n = 9)**	**Negative (n = 15)**	**p (< 0.05)[1]**
Atopy, n (%)	6 (67)	8 (53)	0.678
Allergic symptoms, n (%)	9 (100)	12 (80)	0.266
Rhinits	7 (78)	8 (53)	0.389
Asthma	2 (22)	4 (27)	1.000
Cutaneous	2 (22)	4 (27)	1.000
Housing type, dwelling house (%)	6 (67)	5 (33)	0.206
Region type, rural (%)	6 (67)	5 (33)	0.214
Farming activity, n (%)	3 (33)	7 (47)	0.678
> 40 hours per week	0 (0)	1 (7)	1.000
< 20 hours per week	3 (33)	6 (40)	
Pesticide use	2 (22)	4 (27)	1.000
Distance to farming fields, n (%)			0.590
< 200 meters	5 (56)	6 (40)	
> 1 Kilometer	2 (22)	6 (40)	

[1]Fisher test

Table 2 *- Frequency of atopy, allergic symptoms and evaluated risk factors for* Tetranychus urticae *in subjects with positive and negative conjunctival provocation test.*

Discussion

This is the first study, to our knowledge, to report CPT with *T. urticae* and to perform a specific provocation test in patients without known occupational or environmental exposure to this mite. To evaluate allergy to *T. urticae*, we chose to perform CPT because it is standardized, easy to perform and safer for the patients, and because of our department's experience with the technique (12). CPT has been extensively used for ocular allergy diagnosis (14) but has also the potential to evaluate other allergic diseases, where eye is used as a target organ (15-17).

We were unable to perform a double-blinded CPT due to the difference in color of the higher concentration *T. urticae* solution in relation to the control solution. However, an effort was made to conceal the content from the subjects, and *T. urticae* drops were randomly applied either in the left or right eye of a given patient, so that subjects remained blinded. Also, the CPT was only considered positive when objective signs were observed and these were always found to be associated with pruritus only in the tested eye.

In our study CPT was positive in 9 out of 12 patients sensitized to *T. urticae*. Skin prick tests revealed a good accuracy to detect allergy (AUC = 0.952, sensitivity = 100% and specificity = 80% for a skin prick test wheal size ≥ 3 mm); all patients with wheal size ≥ 4.5mm had a positive CPT and therefore could be con-

sidered allergic without performing a specific provocation test.
In relation to other studies where a provocation test was performed, Astarita et al. (8) evaluated *T. urticae* allergy through a single-blinded specific exposure test, with symptoms and peak expiratory flow rate monitorization, in a pesticide-free oleander greenhouse specifically infested by *T. urticae*; it was positive in all 28 farmers sensitized to *T. urticae* who reported occupational symptoms, inducing the referred respiratory or cutaneous symptoms until 1 hour after exposure. Delgado et al. (9) and Jee et al. (10) evaluated suspected *T. urticae* induced asthma with a specific bronchial provocation test, which was positive in 12 out of 13 carnation greenhouse workers and 10 out of 16 asthmatics living near pear orchards, respectively. Almost half of them presented a late asthmatic response.
Most of our *T. urticae* allergic patients, even without pollen sensitization, reported nasal symptoms in the spring. Although *T. urticae* levels in different cultures peak between July and August and that Astarita et al. (8) reported that recurrent summer-autumn occupational rhinitis showed the best clinical correlation with *T. urticae* in Italian farmers, it has been described that *T. urticae* populations increase in spring in adventitious plants and ground cover vegetation, moving to the cultures in summer (18), which might explain the occurrence of spring symptoms even in patients without pollen sensitization.
Regarding housing type, region type, distance to farming fields or greenhouses, and farming activities, no differences were found between those with and without *T. urticae* allergy. However, we point out that allergy symptoms were self-reported and retrospective, and that assessed risk factors were not confirmed through objective measures of *T. urticae* exposure.
To conclude, a high prevalence of sensitization and conjunctival provocation test confirmed allergy to *Tetranychus urticae* was found in a non-occupational population from the North of Portugal. Future studies with a larger number of patients are needed to evaluate its relation to clinical symptoms and the impact of environmental factors.

The data in this article has been briefly presented in poster form in the EAACI-WAO Congress 2013: Santos N, Plácido JL. Sensitization to *Tetranychus urticae*: prevalence and clinical relevance. Allergy 2013; 68 (Suppl.97):463.

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