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Sensitization to tomato peel and pulp extracts in the Mediterranean Coast of Spain: prevalence and co-sensitization with aeroallergens

C. H. Larramendi*, A. Ferrer†, A. J. Huertas‡, J. L. García-Abujeta*, C. Andreu†, R. Tella§, M. T. Cerdà§, J. Bartra¶, J. R. Lavín||, J. A. Pagán**, M. A. López-Matas††, E. Fernández-Caldas†† and J. Carnés††

*Allergy Unit, Hospital Marina Baixa, Villajoyosa, Alicante, Spain, †Allergy Unit, Hospital Vega Baja, Orihuela, Alicante, Spain, ‡Allergy Unit, Hospital General Básico de la Defensa, Cartagena, Murcia, Spain, §Allergy Unit, Hospital Universitari de Girona Dr Josep Trueta, Spain, ¶Allergy Unit, Hospital Clinic de Barcelona, Barcelona, Spain, ||Allergy Unit, Hospital General Básico de la Defensa, Valencia, Spain, **Allergy Unit, Hospital Virgen de la Arrixaca, Murcia, Spain and ††Research & Development, Laboratorios LETI, S.L., Tres Cantos, Madrid, Spain

Clinical and Experimental Allergy

Summary

Background Tomatoes (*Lycopersicon esculentum*) are consumed world-wide. The prevalence of sensitization to tomatoes remains unknown.

Objective To determine the prevalence of skin test reactivity to tomato and to describe the characteristics of tomato-sensitized subjects.

Methods Individuals attending for the first time during the period of the study to six Allergy centres, located along the Mediterranean coast of Spain, reporting respiratory and/or cutaneous symptoms, were included. All patients were skin prick tested with a battery of inhalant allergens and with peel and pulp of Canary tomato extracts.

Results The study included 1734 individuals (757 males, 977 females; 31.9 ± 17.8 years old). The prevalence of sensitization to tomato was 6.52% (113 patients; 65 males, 48 females; 29.5 ± 13 years old). The peel extract was positive in 110 patients and the pulp extract in 47 patients; three patients were positive exclusively to pulp. Only 1.8% of individuals reported symptoms with tomato; 44% of them had skin test negative to both extracts. Among tomato-sensitized subjects, 16% reported symptoms with tomato, 97% were sensitized to inhalant aeroallergens, including 84% to pollens (mainly *Artemisia vulgaris* and *Platanus hybrida*), with differences between Northern and Southern centres.

Conclusions The prevalence found of skin test sensitivity to tomato is high. Peel extracts detected most of the sensitized subjects. Most of the sensitized subjects were asymptomatic and some patients reported symptoms without skin test sensitivity. Positive subjects were very frequently sensitized to pollens, suggesting allergen cross-reactivity. Regional differences may exist, possibly related to the pattern of sensitization to cross-reacting pollens.

Keywords epidemiology, food allergy, *Lycopersicon esculentum*, pollens, prevalence, skin prick test, tomato peel, tomato pulp

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Correspondence:

Jerónimo Carnés, Research & Development, Laboratorios LETI S.L., Calle del Sol, 5, 28760 Tres Cantos, Madrid, Spain.
E-mail: jcarnes@leti.com

Introduction

Tomato (*Lycopersicon esculentum*) is an important part of the Mediterranean diet. It is a fruit, albeit usually considered as a vegetable, which is widely consumed, either raw or cooked and used in salads, soups or sauces. In Spain, the mean consumption of tomato fruit per person and year in 2004 was 17.3 kg [1], equivalent to 90–100 pieces [2]. It is well established that fruits and vegetables are the most common foods involved in allergic reactions in Europe [3]. This fact has been related

to cross-reactivity between fruits and vegetables with pollens [4].

Several tomato allergens have been characterized including Lyc e 1 (profilin) [5, 6], Lyc e 2 (β -fructofuranosidase) [7] and Lyc e 3 (lipid transfer protein) [8]. Other proteins with capacity to bind IgE have also been described such as glucanase [9], peroxidase [10], polygalacturonase 2A [8, 11], pectinesterase [8, 11], superoxide dismutase [11], chitinase [12] and patatin [13].

Tomato is currently recognized as a food of allergenic importance. In recent years substantial efforts have been

made to render tomato fruits hypoallergenic [14–16], but further efforts are needed to characterize the whole spectrum of tomato allergens, their relationship to pollens and their clinical relevance.

Studies that evaluate the prevalence of allergy to a specific food and specifically to tomato in the general population are scarce or non-existent. In a study conducted by the European Community in several countries throughout the world, about 0.35% of the analysed individuals reported symptoms after tomato ingestion [17]. In a UK population, about 1.2–1.3% of the responders to a questionnaire about food intolerance reported symptoms after contact or ingestion with tomato [18]. In selected populations, the estimated rate of sensitization to tomato ranged from 1.7% to 39% in a group of Italian children who were 'monosensitized' to different inhalant allergens [19]. Sensitization to tomato was significantly greater in children allergic to grasses than to mites. Although tomatoes are widely consumed, severe allergic reactions are unusual, or rarely reported. Described allergic manifestations include urticaria/angioedema, dermatitis, oral allergy syndrome (OAS), rhinitis, exercise-induced anaphylaxis and abdominal pain [8, 20–22], whereas non-allergic food hypersensitivity, or food intolerance to tomato has also been reported [23], as well as asymptomatic sensitization. While there is great interest in the study of clinical allergy, the role and exact meaning of sensitization (IgE positivity to an allergen independently of its clinical significance) [24] has been less studied, although there is a rising interest in this area [25]. The prevalence and relative importance of sensitization to tomato in Spain is not well established.

The main objective of this study was to determine the prevalence of skin sensitization to tomato in a population of subjects attending Allergy Clinics in the Spanish Mediterranean coast. Other objectives were: to assess differences between peel and pulp tomato extracts, to analyse the clinical relevance of sensitization to tomato, and to describe the characteristics of tomato-sensitized subjects, including co-sensitization to inhalant allergens. This study intends to be the first step for further, broader and deeper studies on tomato sensitization and allergy in Spain.

Material and methods

Patient population and skin prick test

In this prospective observational study, all new patients attending the Allergy Clinics of the participating centres during the study period (September 2004–July 2005) were eligible; all those patients reporting respiratory and/or cutaneous symptoms were included. Skin prick tests (SPTs) were performed to all these patients with the same battery of aeroallergens, consisting of standardized extracts of

mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), pollens (*Olea europaea*, grass mix, *Artemisia vulgaris*, *Cupressus arizonica*, *Plantago lanceolata*, Chenopodiaceae, *Parietaria judaica* and *Platanus hybrida*), moulds (*Alternaria alternata* and *Cladosporium herbarum*) and animal dander (cat and dog). In addition, all patients were skin tested to peel and pulp tomato extracts. All extracts were kindly supplied by Laboratorios LETI, Spain.

A patient, consulting for respiratory and/or cutaneous symptoms, was considered atopic, according to the EAACI task force [26], when the tendency to produce IgE antibodies in response to low doses of allergens could be assessed by the positivity of SPT to at least one of the aeroallergens tested. Patients sensitized to different inhalants that those included in the study, were considered, for study purposes, as non-atopic. A SPT reaction ≥ 3 mm in the absence of a reaction in the negative control was interpreted as positive [27, 28].

Oral informed consent for skin testing and eventual blood sampling was obtained from patients during the allergy consult. Serum samples were obtained from 14 individuals with positive SPT to peel or pulp extracts. A serum pool was prepared by mixing equal amounts of serum from these patients. Specific IgE levels using the UniCAP system (Phadia, Uppsala, Sweden) to the manufactured peel and pulp Canary variety tomato extracts were determined with the pool of sera. Briefly, peel and pulp tomato extracts were biotin-labelled (Roche Diagnostics, Indianapolis, IN, USA) and individually incubated with Streptavidin ImmunoCAP[®]. After washing, specific IgE against tomato peel and pulp extracts was measured following the manufacturer's instructions (UniCAP[®] 100, Phadia). Specific IgE levels to latex were also individually determined.

The following centres (enumerated from north to south, with their area of influence in brackets) participated in the study: Hospital Universitari de Girona Dr. Josep Trueta (Girona province), Hospital Clínic de Barcelona (city of Barcelona), Hospital General Básico de la Defensa (city of Valencia), Hospital Marina Baixa (Marina Baixa county, Alicante), Hospital de la Vega Baja (Vega Baja county, Alicante) and Hospital General Básico de la Defensa (city of Cartagena, Murcia).

Tomato extracts

The Canary Island tomato variety was selected for the study because of its frequent consumption in Spain [1] and its high allergenicity [29]. Ripe tomatoes were purchased at a local market, washed in distilled water and carefully peeled; 217.33 g of peel and 360.8 g of pulp were used to manufacture two different extracts of peel and pulp following previously described methods [30]. Briefly, after homogenization in phosphate-buffered

saline/polyvinylpolypyrrolidone (PBS/PVPP) the raw materials were extracted under continuous magnetic stirring for 4 h at 4 °C, centrifuged and the supernatants collected, dialysed, sterile filtered and freeze-dried. Non-standardized SPT were prepared with each extract at 10 mg of freeze-dried material per millilitre.

The protein content was measured by the Lowry–Biuret method (Sigma, St Louis, MO, USA), expressing results in percentage of protein per milligram of dry weight.

Antigenic and allergenic characterization

Peel and pulp tomato extracts were characterized by SDS-PAGE and immunoblotting following previously described methods [30]. SDS-PAGE analysis was used to determine the protein profile of the extracts. Samples were run on SDS-PAGE gels. One hundred and fifty micrograms of freeze-dried material of peel and pulp tomato extracts were loaded per band. Samples were diluted in sample buffer, denatured at 100 °C for 10 min and centrifuged for 1 min at 16000 g. After electrophoresis, the gel was fixed for 45 min and silver stained (BioRad Laboratories, Hercules, CA, USA).

Allergenic profiling was carried out using a pool of sera made with 14 patients with positive SPT to peel and pulp tomato extracts. After electrophoresis, gels were electrophoretically transferred to P-Immobilon membrane (Millipore, Bedford, MA, USA). Afterwards, membranes were dried for 4 h, incubated overnight with the pool of sera diluted in PBS 0.01 M Tween 0.1%. Specific IgE binding was detected with peroxidase-conjugated monoclonal anti-human IgE (Ingenasa, Madrid, Spain).

Statistical analysis

Descriptive, parametric and non-parametric statistics were applied. Student's *t*-test and Fisher's exact test were used. χ^2 (Yates correction) and odds ratios (with 95% confidence interval), were calculated for comparison between the non-sensitized and sensitized individuals. Software SigmaStat 3.5 (Point Richmond, CA, USA) was used for the statistical analysis.

Results

Characteristics of the tomato extract

At the end of the process, 1.48 g of freeze-dried peel extract (yield = 0.68%) and 2.66 g of freeze-dried pulp extract (yield = 0.74%) were obtained. The peel and pulp extracts contained 84.9 and 37.5 µg of protein/mg of freeze-dried material, respectively.

The protein profile of the extracts revealed several bands with a molecular weight between 9 and 50 kDa in the peel extract. The most prominent bands had molecular

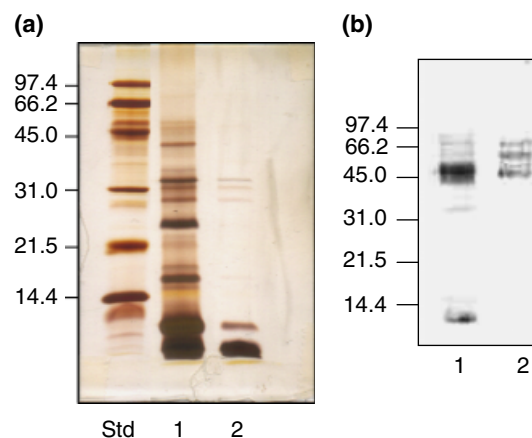


Fig. 1. (a) SDS-PAGE of the tomato extracts. Lane Std., standard low molecular weight; lane 1, peel extract; lane 2, pulp extract. (b) Immunoblot with a pool of sera from 14 patients positive to tomato (see text). Solid phase: lane 1, peel extract; lane 2, pulp extract.

weights of approximately 9, 16, 25, 28, 35 and 44 kDa. The pulp extract showed five bands (9, 16, 28, 31 and 35 kDa). The most prominent, in both cases, corresponds to the 9 kDa band (Fig. 1a).

In the immunoblot analysis (Fig. 1b), the pool of sera recognized bands with a molecular weight between 42 and 70 kDa in peel and pulp extracts. Allergens of 32, 15 and 9 kDa were also detected in the peel extract. No band was detected using a negative pool of sera as control.

Specific IgE levels of the serum pool was 13.7 kU/L for tomato peel extract and 7.45 kU/L for pulp Canary variety tomato extract.

Characteristics of the population

A total of 1734 individuals (757 males and 977 females; mean age 31.9 ± 17.8 years old; range 1–91, including 246 children under 15 years of age) were included. Sensitization to inhalant allergens is summarized in Table 1. Upper respiratory airway symptoms (rhinitis, conjunctivitis or both) were diagnosed in 1349 (77.8%), lower respiratory airway symptoms in 610 (35.2%) and skin symptoms (either immediate, such as urticaria and angioedema, or delayed, such as dermatitis) in 379 (21.9%).

Prevalence

The prevalence of sensitization to tomato in this population was 6.52% (113/1734 patients; 65 males and 48 females; mean age 29.5 ± 13 years; range 4–75; 11 subjects had <15 years of age). Tomato peel was positive in 110 patients (6.34%) and the pulp in 47 patients (2.71%). Three patients (0.17%) were positive to pulp and negative to peel. The prevalence of self-reported symptoms after tomato ingestion, or contact was 1.85%, and that of

Table 1. Description of the population and sensitization to aeroallergens

	Tomato-positive subjects	Tomato-negative subjects	OR (CI) χ^2 (P)
<i>n</i>	113 (6.52%)	1621 (93.48%)	
Age (years $\pm$ SD)	29.5 ($\pm$ 13)	32 ($\pm$ 18)	NS
Sex: male/female (%)	65/48 (57.5%/42.5%)	692/929 (42.7%/57.3%)	1.8 (1.2–2.7) <i>P</i> = 0.003
Atopy	110 (97.4%)	1014 (62.6%)	22 (7.3–108.4) <i>P</i> < 0.00001
Pollens	95 (84.1%)	659 (40.7%)	7.7 (4.5–13.3) <i>P</i> < 0.00001
Mites	68 (60.2 %)	653 (40.3%)	2.2 (1.5–3.4) <i>P</i> < 0.0001
Epithelia	43 (38.1%)	308 (19%)	2.6 (1.7–4) <i>P</i> < 0.00001
Moulds	19 (16.8%)	113 (7%)	2.7 (1.5–4.7) <i>P</i> < 0.001

Percentages calculated towards *n*. Atopy, presence of a positive SPT to at least one of the inhalant allergens tested.

OR, odds ratio; CI, confidence interval; SD, standard deviation; NS, non-significant; SPT, skin prick test.

self-reported symptoms with skin sensitivity to tomato extracts, 1.04%.

Sex and age

A positive SPT to tomato was found more frequently in males (*P* < 0.01). Even after excluding non-atopic subjects, more frequently females, the significance persisted (*P* = 0.04). In subjects reporting symptoms with tomato, males tended to be more frequently SPT positive (*P* = 0.06). In the whole population, males were also more sensitized to pollens, mites (*P* < 0.01) and moulds (*P* < 0.05) than females. No significant differences were observed in age between tomato SPT-positive and -negative individuals. Among subjects reporting symptoms with tomato, tomato SPT-positive subjects were younger (23.6 ± 11) than tomato SPT-negative subjects (32.1 ± 17.8), but without statistically significant differences. There were also no differences in sensitization to tomato between children and adults.

Atopic status

Differences in the rate of sensitization to inhalant allergens were evident for all the allergens tested in tomato-positive and tomato-negative patients (Table 1) and tomato positive subjects reported more asthma (44.3% vs. 34.6%; *P* < 0.05). One thousand one hundred and twenty-four individuals (64.9%) were considered atopic.

Three subjects sensitized to tomato (but not reporting symptoms) and not sensitized to any of the inhalant extracts tested, were considered as non-atopic (one male with a single episode of urticaria and anaphylaxis sensitized to multiple spices; another male with urticaria and anaphylaxis, sensitization to peach and positive challenge

with metamizol and one old female with perennial rhinitis, sensitized to peach and *Anisakis*, with previous history of immediate skin reactions with latex but current negative SPT and CAP to latex).

When non-atopic subjects were excluded from the analysis, differences between both populations were still evident for sensitization to pollens as a group, and individually, especially to *Artemisia* and *Platanus* (Fig. 2). In tomato-sensitized subjects, sensitization to *Artemisia* was fourfold higher than in tomato-negative subjects and for *Platanus* 2.5. For other pollen sensitization ranged from 0.3- to 1.2-fold. Among subjects sensitized to *Artemisia*, the prevalence rate of sensitization to tomato

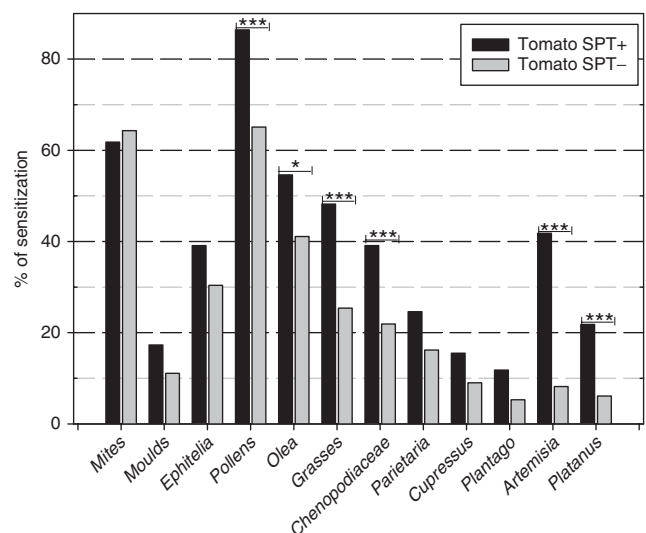


Fig. 2. Percentage of sensitization to inhalant allergens among atopic subjects (*n* = 1124); according to the positivity of the skin prick test (SPT) to tomato (tomato SPT+ : *n* = 110; tomato SPT- : *n* = 1014). **P* < 0.01; ****P* < 0.0001.

was 35.7% (46/129) and in those sensitized to *Platanus* of 27.9% (24/86). For other pollen the prevalence values ranged between 12.6% of *Olea* and the 19.4% of *Plantago*; among those sensitized to mites was 9.4%, to moulds 14.4%, to epithelia 12.3% and among subjects sensitized to at least one pollen 12.6%.

Also, atopic subjects sensitized to tomato reported more skin symptoms than those not sensitized to tomato (23.6% vs. 13.4%; $P < 0.01$) without statistically significant differences in other symptoms.

Symptoms with tomato

From the total patient population, 32 subjects (1.9%) reported symptoms either upon ingestion or contact with tomato; SPT to both tomato extracts were negative in 43.7% of these subjects.

Among the 113 tomato SPT-positive subjects, 18 patients (15.9%) reported symptoms, one of them with a negative SPT to tomato peel but positive to the pulp extract, and 6 with negative SPT to pulp but positive to peel. All these 18 patients were atopic and sensitized to pollens, mainly to *Artemisia* and *Platanus* (Fig. 3). Seven percent of the subjects sensitized to *Artemisia* and 9.3% of those sensitized to *Platanus* reported symptoms with tomato. For other pollens, the rate ranged between 1.5% to *Plantago* and 4.6% to *Cupressus*. For other inhalants, the prevalence rate was 0.8% in moulds sensitized individuals, 1.8% in mite, 2.6% in epithelia and 3.1% in subjects sensitized to any pollen. 83.3% of the symptomatic and SPT-positive individuals (15 out of 18) reported rhinitis, rhinoconjunctivitis and/or asthma.

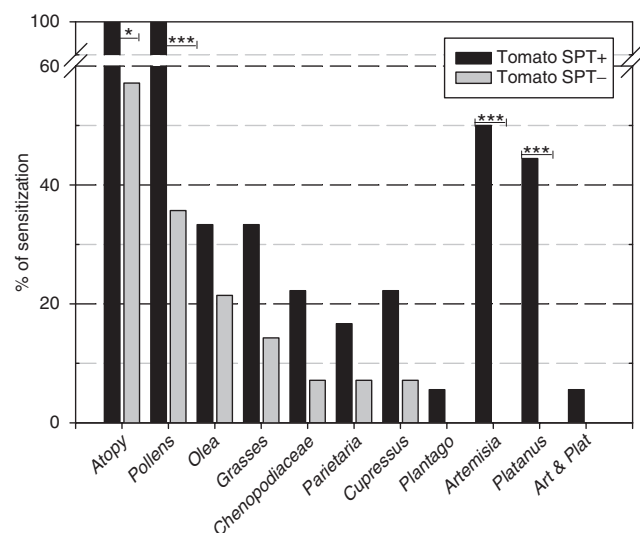


Fig. 3. Percentage of sensitization to pollens in patients reporting symptoms with tomato ($n = 32$) according to the positivity of the skin prick test (SPT) to tomato (tomato SPT+ : $n = 18$; tomato SPT- : $n = 14$). * $P < 0.01$; *** $P < 0.0001$. Art & Plat: patients sensitized to both *Artemisia* and *Platanus*.

Among the 1621 tomato-negative SPT subjects, 14 (0.9%), reported symptoms. Eight (57.1%) of them were atopic and five patients (35.7%) were sensitized to pollens (none to *Artemisia* or *Platanus*) (Fig. 3). Among symptomatic and SPT-negative subjects, 92.9% (13 out of 14) reported rhinitis, rhinoconjunctivitis and/or asthma.

There were great differences in the frequency of sensitization to pollens between SPT-positive and -negative individuals ($P < 0.0001$), but there were no differences among the sensitization to other inhalants (mites, moulds and epithelia) between both groups.

All tomato SPT-positive subjects who reported symptoms and 9 (64.2%) of the subjects with negative SPT to tomato, reported immediate oral or cutaneous symptoms (pruritus, erythema or hives) with tomato ($P < 0.01$). Digestive and delayed cutaneous symptoms (persistent itching, dermatitis, vesicles, desquamation ...) were more frequent among tomato SPT-negative subjects (7 vs. 2; $P < 0.05$).

Peel and pulp extracts

Peel extract was positive in 110 subjects and pulp extract in 47. Subjects sensitized to both tomato peel and pulp, reported less asthma-like symptoms (29.8%) than those sensitized only to peel (54.5%) ($P < 0.05$), but reported more symptoms with tomato (15.9% vs. 9.1%, $P < 0.05$). Also subjects positive to both peel and pulp were more sensitized to *Artemisia* (53.2% vs. 31.8%, $P < 0.05$) and *Platanus* (31.9% vs. 13.6%, $P < 0.05$). Non-statistical differences were observed with other pollens or symptoms.

Geographical areas

All subjects were recruited from six participating centres in the Mediterranean coast of Spain. The region could be divided into two areas: North, comprising three centres located in Valencia, Barcelona and Girona (425 patients) and South, comprising three centres located in Villajoyosa-Benidorm (Marina Baixa) and Orihuela (Vega Baja) both in Alicante and Cartagena, Murcia (1309 patients). These areas were established after observing different patterns of pollen sensitization between centres (Fig. 4). It is interesting to point out that 82% of the subjects sensitized to *Artemisia* lived in the south and 82% of the subjects sensitized to *Platanus* in the north. Overall, these discrepancies may reflect differences in the flora of these two regions. Subjects from the North area reported more skin symptoms (mainly immediate) as a group than individuals from the south ($P < 0.001$) without other statistical differences.

Prevalence by areas

The two areas differed slightly in their rate of sensitization to tomato (5.88% in the South and 8.47% in the North, $P = 0.08$). This difference was statistically significant for

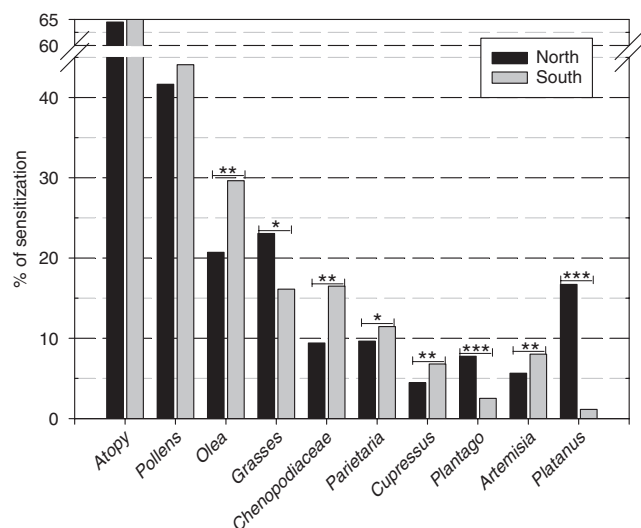


Fig. 4. Geographical differences in sensitization to pollens among the subjects included in the study ($n = 1734$). North: Girona, Barcelona and Valencia; $n = 425$. South: Villajoyosa-Benidorm, Orihuela and Cartagena; $n = 1309$. * $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$.

atopic subjects (8.71% in the South and 13.1% in the North, respectively; $P = 0.04$). Fifty-eight percent of tomato-sensitized subjects from the North were sensitized to *Platanus* but only 3.9% in the South. *Artemisia* was positive in 46.8% sensitized subjects living in the South and 27.8% on those living in the North. More than half of the tomato-sensitized subjects were sensitized, to at least one of these two pollens, but 78% of those positive to *Artemisia* lived in the South, and 90% of those sensitized to *Platanus* lived in the North. Among symptomatic patients, the pattern of sensitization was similar. Among the eight symptomatic patients from the South, seven (87.5%) were sensitized to *Artemisia*, whereas only two patients from the North (20%) were *Artemisia* positive; eight out of 10 symptomatic patients (80%) from the North were positive to *Platanus*, but none from the South. Non-statistical differences were observed between peel and pulp extracts in both areas.

Discussion

In this paper, the prevalence of sensitization to tomato extracts in a large population of subjects attending Allergy Clinics in the Mediterranean coast of Spain has been evaluated. The clinical characteristics of these individuals are also described. The prevalence of SPT reactivity to tomato extracts (6.5%) is comparable with that of some inhalant allergens, such as *Cupressus* (6.2%), *Artemisia* (7.4%) or moulds (7.6%) in the same patient population.

We are aware that our data can not be extrapolated to the overall population and that our results should be

compared very cautiously with other studies, but the prevalence found in this study is in the range of that obtained in other studies, which have evaluated the prevalence of positive SPT to tomato in different populations either as a primary objective or as secondary result. In the present study, the number of subjects 'monosensitized' to mites and SPT-positive to tomato (2.1%) matches closely the published number of 'monosensitized' children in Italy (1.9%) [19]. However, the Italian study differs greatly in the proportion of subjects 'monosensitized' to grasses and sensitized to tomato (7.1% in our study vs. 39% in the Italian study), but it should be taken in consideration that 'monosensitization' to grasses was infrequent (0.8%) in the present study. Most studies are not designed to calculate the prevalence of SPT reactivity to tomato, but it can be observed that in selected populations the prevalence can be quite high. When analysing subgroups of patients, sensitization to tomato varies greatly. It was 6.5% in the population studied, 9.8% in the atopic population, 28% in subjects sensitized to *Platanus* and 36% in subjects sensitized to *Artemisia*. These numbers are similar to those found in other selected populations, as the 39% in patients sensitized to grasses commented previously [19], suggesting local influences of different pollens in tomato-pollen cross-reactivity.

The rate of both sensitization and self-reported symptoms to tomatoes in our study is 1.04%, while the rate of self-reported symptoms (with or without sensitization) is 1.85%. This number is higher than those found in the general population in the UK (1.2–1.3%) [18] and throughout the world (0.35%) [17], but our population was not unselected. In selected populations, other studies have reported different prevalence rates of symptomatic sensitization to tomato such as 2% [31] and 5% [32] in latex allergic individuals, 9% in birch sensitized subjects from Central Europe [8] and 16% in patients with OAS from Northern Italy [33]. When considering only subjects sensitized to *Platanus* or *Artemisia* (9.3% and 7%, respectively), the rates of 'allergy' to tomato detected in our study are similar to those previously mentioned studies. Sensitization to tomato has also been frequently reported in latex allergic individuals, ranging from 28% in the USA [32] to 50% in a group of patients from Central Spain [34]. While latex was not routinely tested in the present study, specific IgE levels to latex were determined in the sera of 14 patients being slightly positive in 50% of them. In our opinion, these results should be carefully considered and could be related to cross-reactive carbohydrate determinants, frequently found in pollen-polysensitized subjects [35].

In general, there are discrepancies between the prevalence based on self-reported symptoms and that based on the number of cases confirmed with challenges, the latter being lower. As challenge tests were not routinely performed in the present study, the real figures of tomato

allergy may be even lower. In fact, nearly half of the subjects reporting symptoms upon contact or ingestion with tomato showed negative SPT to both extracts. Whether this fact reflects a non-IgE-mediated pathology (irritative or not), or a failure of the extracts tested remains beyond the scope of this paper, but the different profile of these subjects compared with those symptomatic and sensitized suggest a role for other mechanisms [36]. Only one patient reported anaphylaxis, while the most commonly reported symptom were OAS, or mild skin symptoms (pruritus and/or erythema while manipulating tomatoes). Two SPT-positive patients reported abdominal symptoms. This kind of symptoms, as well as delayed skin contact symptoms, was more frequent in non-sensitized subjects.

In the present study, most of sensitized tomato patients did not report clinical symptoms with tomato. The exact meaning of asymptomatic sensitization to allergens is not well understood [25] but in clinical practise, positive skin test results in patients who do not report symptoms, can be observed, not only after food ingestion but also with other allergens as pollens. The knowledge of the characteristics of sensitization to allergens, including asymptomatic sensitization, may have a great importance to further evaluate the clinical relevance of an allergen.

SPT are only a marker of sensitization and not of allergy, but they are widely accepted as the first step to allergy diagnosis. In the case of food allergy, SPT with foods can give both false negative results (subjects sensitized but not detected, frequently due to the loss of relevant allergens during the extraction process) and false positive results (usually due to clinically irrelevant cross-reactivity with other allergens). So, results of SPT should be cautiously interpreted. Food challenges are still mandatory to confirm the diagnosis of food allergy but pitfalls do exist too [37]. In our study we can only assess the sensitization rate (6.52%) and make an estimation of the upper confidence level of the 'allergy' to tomato (1.04%).

Several proteins have been described in tomato including some allergens such as Lyc e 1 a profilin of 14 kDa [6], Lyc e 2, a β -fructofuranosidase with 31 kDa and Lyc e 3 a lipid transfer protein with 9 kDa. Other proteins have also been described in tomato with capacity to bind IgE such as pectinesterase [11] with MW around 30–35 kDa and polygalacturonases with 45 kDa. In our study, the pool of sera-recognized bands, which could correspond to LTP, polygalacturonase, pectinesterase and β -fructofuranosidase. Profilin was hardly recognized suggesting that this protein is not highly relevant in tomato sensitization in our population. When pulp extract was used in the solid phase, only proteins with high molecular weight were recognized. According to our results, tomato pulp extract shows less capacity to identify sensitized patients than peel extract, reflecting the presence of more proteins and more allergenic bands in the peel extract, as described

with other fruits [38], but sensitization to pulp could be more related with the presence of symptoms.

About one-third of the patients consulting for respiratory symptoms in the present study, were not sensitized to any of the common aeroallergens tested. Tomato-sensitized subjects were highly atopic and more frequently males. An association between tomato-sensitized subjects and almost all allergens tested has been found, related greatly with the higher number of non-atopic subjects between the subjects non-sensitized to tomato. In order to avoid this distortion, calculations were performed eliminating non-atopic subjects from the analysis. Association with pollens but not with other inhalants allergens was observed; in some cases the association with specific pollens was high.

Our results cannot be extrapolated to the general or to other populations, because we have studied a selected population and because geographical differences of sensitization to aeroallergens between countries and within countries, as that found in the present study, are well known [39]. We have found a clear geographic distribution cut in the pattern of co-sensitizations to pollens, especially to *Artemisia* and *Platanus* among tomato-sensitized subjects living in our area. However, we cannot conclude that this co-sensitization reflects the clinical cross-reactivity and *in vitro* studies are necessary. Most of the tomato-sensitized subjects sensitized to *Artemisia* lived in the South, whereas most of those sensitized to *Platanus* lived in the North. These results correlate with the sensitization rates to both pollens in those areas. It is also interesting to point out that most of the *Platanus*-sensitized subjects lived in urban areas (cities of Valencia and Barcelona) because *Platanus* allergy has been considered as a particular problem for city dwellers [40]. A described allergen in tomato [8, 11] and *Platanus* [41] (Polygalacturonase 2A) could be implicated in the potential cross-reactivity between *Platanus* and tomatoes. In fact, in a previous report with part of this population [42], without the inclusion of subjects from Barcelona and Valencia (i.e. subjects mainly from southern and rural areas), *Platanus* sensitization was considered of minor significance. *Platanus* allergy has been related to food allergy [43] in one of the studied areas and cross-reactivity between *Artemisia* pollen and Solanaceae has been described in an insular region of Spain [44]. Sensitization to some pollens such as birch [45] and *Platanus* [46] is associated with sensitization to specific food allergens (Bet v 1 homologues, profilins, etc.) and this could be the situation with tomato.

In conclusion, sensitization to tomato extracts is high in the studied population. Most of the tomato-sensitized subjects (84%) were also sensitized to pollen and did not report symptoms with tomato. In combination with our clinical observations this suggests an important role for cross-reactivity, although cross-reacting pollen may

differ between geographical areas. Whether sensitization to *Artemisia* and *Platanus* in the Mediterranean coast of Spain reflects sensitization to different tomato allergens should be addressed in the future. Further *in vitro* studies are needed to demonstrate these observations and to elucidate the implicated allergens.

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