In our first study, which was published in 2005, the effectiveness of the Hacettepe Method was demonstrated in 84 analgesic-intolerant patients by a decrease in the number of test days from 264 to 148 days.[5] Subsequently, we performed a similarly designed study using the Hacettepe Method and 53 antibiotic-allergic patients, and again test days were reduced from 136 to 65.[6] The third of our studies was published in 2012, and the Hacettepe Method saved 32 test days in 42 patients diagnosed with antibiotic and analgesic intolerance/allergy, as compared to the conventional OPT method.[7] There were no serious adverse reactions in any patients in any of the three studies. Additional research on the Hacettepe Method is ongoing at our clinic.[8,9]

Based on the findings of our three studies, it is reasonable to conclude that the Hacettepe Method is a safe, cost-effective, time-effective, and manpower-effective method of identifying safe alternative drugs in drug–allergic–allergic/intolerant patients. Considering the difficulty of finding a new approach to an old problem, the Hacettepe Method is recommended for use in all adult allergy clinics worldwide.

Ethical disclosures

Confidentiality of data. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study have received sufficient information and have given their informed consent in writing to participate in that study.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Protection of human subjects and animals in research. The authors declare that no experiments were performed on humans or animals for this investigation.

References

1. Gamboa PM. The epidemiology of drug allergy-related consultations in Spanish Allergology Services: Alergológica-2005. J Investig Allergol Clin Immunol. 2009;19 Suppl. 2:45–50.
2. England RW, Ho TC, Napoli DC, Quinn JM. Inpatient consultation of allergy/immunology in a tertiary care setting. Ann Allergy Asthma Immunol. 2003;90:393–7.
3. Gomes ER, Demoly P. Epidemiology of hypersensitivity drug reactions. Curr Opin Allergy Clin Immunol. 2005;5:309–16.
4. http://www.tuik.gov.tr/PreHaberBultenleri.do?id=13425 [last viewed on 02.05.13].
5. Kalyoncu AF, Karakaya G, Bozkurt B, Artvinli M. A new method of oral drug provocation testing for determining safe alternatives for patients with non-steroidal anti-inflammatory drug intolerance: the triple test. Int Arch Allergy Immunol. 2005;138:319–23.
6. Karakaya G, Isik SR, Kalyoncu AF. Determining safe antibiotics for drug hypersensitive patients with the alternative method of double-triple test. Allergol Immunopathol. 2008;36:264–70.
7. Ozturk AB, Celebioglu E, Karakaya G, Kalyoncu AF. Determining safe alternatives for multidrug hypersensitive patients with the alternative triple antibiotic–analgesic test. Allergol Immunopathol. 2013;41:189–93.
8. Celebioglu E, Karakaya G, Kalyoncu AF. The safety of codeine in patients with non-steroidal anti-inflammatory drug hypersensitivity: a preliminary study. Allergol Immunopathol. 2013;41:163–8.
9. Karakaya G, Celebioglu E, Kalyoncu AF. Non-steroidal anti-inflammatory drug hypersensitivity in adults and the factors associated with asthma. Respir Med. 2013;107:967–74.

A. Fuat Kalyoncu

Hacettepe University School of Medicine, Department of Chest Diseases, Immunology and Allergy Section, Ankara, Turkey
E-mail address: kalyon@ada.net.tr

http://dx.doi.org/10.1016/j.aller.2013.08.002

Pomegranate anaphylaxis due to cross-reactivity with Peach LTP (Pru p 3)

To the Editor,

The pomegranate, the fruit of *Punica granatum*, belonging to Lythraceae family and commonly cultivated in the Mediterranean area, has been involved in some immediate reactions,[1,2] including severe symptoms such as anaphylactic shock and laryngeal oedema.[3,4] Cases of hypersensitivity reactions to pomegranate have been reported and the implication of 29-kDa[1,2,4] and 9-kDa[3] protein allergens has been described. Subsequent characterisation of the 9-kDa allergen demonstrated its belonging to the lipid transfer protein (LTP) family, a family widely distributed in fruits, vegetables and nuts, which has been suggested as being responsible for immunological cross-reactivity between fruits, nuts and/or pollens. Allergy cases involving LTPs from pomegranate and peach, hazelnut and peanut have been published.[1–4] Furthermore, a study of LTPs in pomegranate identified and isolated two LTP isoforms with different IgE binding capacities [LTP1a (9-kDa) and 1b (7-kDa)].[5]

We describe the case of an 18-year-old women, without history of allergic disease, who suffered from angio-oedema, generalised urticaria, glottis oedema, vomiting, abdominal pain and malaise five to ten minutes after ingesting a pomegranate. Three years before she had two episodes of angio-oedema and urticaria after apple ingestion and one episode of urticaria, abdominal pain and angio-oedema after drinking pear juice. The patient did not develop any kind of allergic symptoms after performing a *Rosaceae* and pomegranate free diet.

The patient underwent skin prick tests with a commercial extract panel of common inhalants, fruits, vegetables and nuts allergens (Leti laboratories, Spain) and the results were positive to *Dermatophagoides pteronyssinus*, grass pollen, mugwort pollen, peach, apple, pomegranate, strawberry, cherry, almond, peanut, tomato, bell pepper, green beans, black bean and soybean seed. Prick test with profilin and

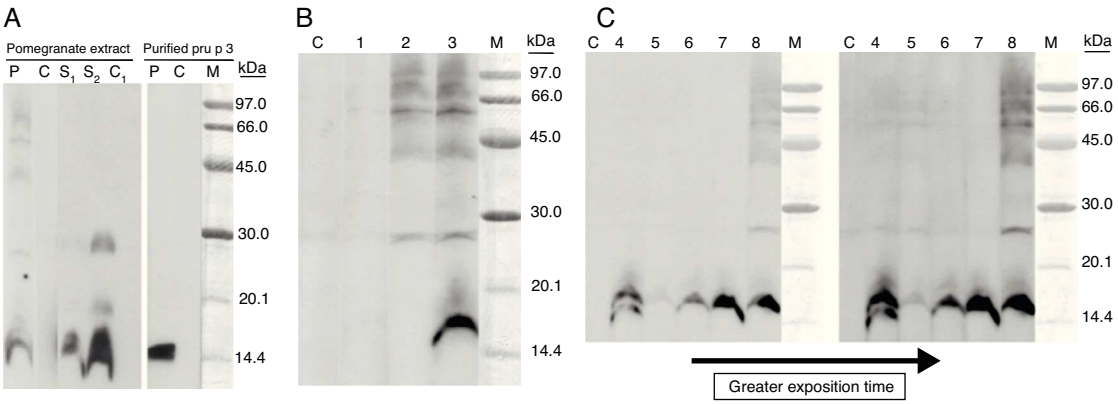

Figure 1 (A) SDS-PAGE IgE-immunoblotting of pomegranate extract (under non-reducing conditions). Lane P: patient serum; Lane C: control serum (pool from non-atopic subject sera); Lane S1 and S2: Anti-Pru p 3 rabbit serum (dilution 1/10,000–1/5000); Lane C1: serum from non-immunised rabbit (Dilution 1/5000); M, molecular mass markers. (B) SDS-PAGE IgE-immunoblotting-inhibition with pomegranate extract in solid phase. Lane 1: patient serum pre-incubated with pomegranate extract; Lane 2: patient serum pre-incubated with Pru p 3 (150 μg/ml); Lane 3: patient serum pre-incubated with BSA (150 μg/ml). (C) SDS-PAGE immunoblotting-inhibition with pomegranate in solid phase and pollen extracts as inhibitors: *Lolium perenne* (Lane 4), *Artemisia vulgaris* (Lane 5), *Olea europaea* (Lane 6), *Parietaria judaica* (Lane 7), lamb as negative control (Lane 8), molecular mass markers (Lane M).

LTP extract (Alk Abelló, Madrid, Spain) were positive. Prick-prick test with fresh pomegranate was positive. Specific IgE, performed with UniCAP® method, was positive to peach (10.3 kU/L), apple (8.45 kU/L), pear (6.61 kU/L), cherry (9.79 kU/L) and green bean (3.94 kU/L). Immuno solid-phase allergen chip (ISAC®) microarray was positive to nPru p 3 (12 ISU) and nSal k1 (2.5 ISU). Because of the systemic reactions and the positive test results with pomegranate, peach, apple and peanut, a possible involvement of LTPs and IgE-mediated allergy was expected. We performed a SDS-PAGE immunoblotting assay in non-reducing electrophoresis conditions (without 2-mercaptoetanol) with pomegranate extract in solid phase and using the patient serum and an anti-Pru p 3 serum from rabbit. Both sera revealed a clear 16 kDa-IgE reactive protein and some others faint ones with higher molecular masses. Identical assay, carried out with purified Pru p 3 and patient serum, showed an IgE binding band with the same molecular mass. The molecular weight of binding band protein detected is higher than that usually described for LTPs (7–9 kDa).[5] This apparent molecular weight difference is probably explained by the electrophoretic mobility shift that occurs under non-reducing conditions, as has already been shown by Zoccatelli.[5] We decided to perform the electrophoresis assay in non-reducing conditions in order to preserve the conformational structure of epitopes LTP and consequently their IgE-binding capacities. Cross-reactivity between the pomegranate IgE binding protein and Pru p 3 (peach LTP) was demonstrated and was then confirmed by immunoblotting inhibition assay.

Despite the absence of clinical inhalant allergy as positive skin prick tests results were detected with extracts from grass pollen, mugwort pollen and plane tree pollen, the possible cross-reactivity between pollen and pomegranate extract was studied. A very high level of IgE-binding inhibition on pomegranate band was detected when *Artemisia vulgaris* pollen extract was used as inhibitor, whereas partial inhibition was observed with *Olea europaea* pollen extract (Fig. 1).

The clinical history, the skin tests and the immunoblotting results led us to conclude that the allergy symptoms were probably caused by the presence of specific IgE for the 15/16-kDa protein completely inhibited by Pru p 3, and suggest that the primary sensitisation must be due to Rosaceae LTPs, greatly consumed in southern European countries. The inhalation of pollen LTP could have contributed to maintain the specific LTP IgE levels in patient serum. LTP appears to be the main factor associated with cross-reactivity with clinical allergy between pomegranate and botanically unrelated food from different families, such as nuts, namely hazelnut, and peanut.

Ethical disclosures

Confidentiality of data. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study have received sufficient information and have given their informed consent in writing to participate in that study.

Right to privacy and informed consent. The authors have obtained the informed consent of the patients and/or subjects mentioned in the article. The author for correspondence is in possession of this document.

Protection of human subjects and animals in research. The authors declare that no experiments were performed on humans or animals for this investigation.

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Gaig P, Bartolomé B, Lleonart R, Garcia-Ortega P, Palacios R, Richart C. Pomegranate anaphylaxis due to cross-reactivity with Peach LTP. Allergy. 1999;54:287–96.

2. Pomegranate (*Punica granatum*) allergy: clinical and immunological findings. Ann Allergy Asthma Immunol. 2009;103:178–80.
3. Enrique E, Utz E, Utaz M, Mateo JA, Castello JV, Malek T, et al. Allergy to lipid transfer proteins: cross-reactivity among pomegranate, hazelnut, and peanut. Ann Allergy Asthma Immunol. 2006;96:122–3.
4. Gangeni S, Mistrello G, Roncarolo D, Amato S, Minciullo PL. Pomegranate-dependent exercise-induced anaphylaxis. J Investig Allergol Clin Immunol. 2008;48:482–95.
5. Zoccatelli G, Dalla Pellegrina C, Consolini M, Fusi M, Sforza S, Aquino G, et al. Isolation and identification of two lipid transfer proteins in pomegranate (*Punica granatum*). J Agric Food Chem. 2007;55:11057–62.

E.M. Almeida[a,*], B. Bartolomé[b], E.G. Faria[a], N.G. Sousa[a], A.S. Luís[a]

[a] *Immunoallergology Department, Coimbra University Hospitals, Coimbra, Portugal*
[b] *R&D Department, Bial-Arístegui, Bilbao, Spain*

* Corresponding author.
E-mail address: eugeniamalmeida@gmail.com (E.M. Almeida).

http://dx.doi.org/10.1016/j.aller.2013.07.013

Sodium metabisulphite allergy with multiple food and drug hypersensitivities in a five-year-old child: A case report and literature review

To the Editor,

Among chemical products used as additives, sulphites are often used for their antioxidant properties in order to control bacterial growth and prevent discolouration by bleaching agents. They are mostly used with vegetables (i.e. potatoes and green salads) and fresh fruits, where they are added to prevent enzymatic and non-enzymatic browning. They can also be found in soft drinks, wine, beer, and dried foods, where they have antimicrobial effects. In addition, their agents, such as sulphur dioxide and sodium sulphite, are used as sanitisers for containers and equipment.[1] Among these agents sodium metabisulphite (SMB) is usually employed as a source of sulphur dioxide in applications where the handling of liquefied gas is inconvenient. It is moreover added to local anaesthetic solutions containing adrenaline, as its antioxidant property prevents oxidation of adrenaline itself, to topical medicaments, and eye drops containing sympathomimetics.[1]

The ubiquity of sulphites in foods and drugs makes it difficult to pinpoint the relevance of positive patch test reactions.[1]

Allergy to SMB is known to induce asthma and contact dermatitis, and these reactions have been widely described in adulthood, nevertheless this kind of allergy is rare in childhood and only two studies on SMB-induced asthma have been reported in this age group.[2,3] On the other hand, there is no paediatric report on urticaria and anaphylaxis caused by SMB allergy.

Herein the authors report the case of a five-year-old female child, admitted to our Pediatric Acute and Emergency Department for urticaria and anaphylaxis secondary to SMB sensitisation.

A five-year-old female, Caucasian child was admitted to our Pediatric Acute and Emergency Room, Policlinico-Vittorio-Emanuele University Hospital, University of Catania, Italy for daily incontinence after administration of oral cetirizine.

Her familial anamnesis was positive for allergic diseases, as her mother was allergic to common inhalants and to eggs, tomatoes and strawberries, as well as to various antibiotics (penicillin, amoxicillin, most cephalosporines and macrolides). The child's brother was allergic to mite dust antigens and parjetaria.

Since birth the child was affected by atopic dermatitis, secondary to cow's milk protein allergy (CMPA), which was treated with extensive hydrolysed formula. Moreover, she often suffered from bronchial asthma, allergic rhinitis and recurrent episodes of dermatitis. Her mother also referred that the child presented nausea and vomit after ingestion of egg (albumen and yolk), tomato, chocolate and wurstel. The peculiar thing was that the symptoms appeared with the ingestion of preserved food but not with fresh food.

When she was first admitted to our hospital, on November 2012, we performed routine blood analysis that gave a negative result, except for the total IgE dosage that was high (289 UI/l, normal value: 70–100 UI/l) and skin prick tests were positive for house dust mites and ambrosia, while food skin prick tests were all negative. The child was then discharged with an inhaled corticosteroid therapy and oral anti-leukotriene.

One month later, after the consumption of oral clarithromycin for fever and respiratory infection, the child had an episode of anaphylaxis and she was again admitted to our Paediatric Acute and Emergency Room. On that occasion, her mother referred that the same reaction had previously occurred with the consumption of other antibiotics and in particular with penicillin, amoxicillin and ampicillin, so that antibiotic treatment in this child was difficult to establish. Therefore, the child was hospitalised for four days, during which oral azithromycin was started. During the hospitalisation the child did not manifest any disturbance, but when she was discharged, a second assumption of the same drug provoked another allergic reaction, most likely referable as urticaria. Thus, the child was admitted a third time to our Pediatric Acute and Emergency Unit, and in particular she underwent a Pneumologic and Allergologic consult.

When the child came to our observation, her physical exam showed the presence of skin wheal erythematous manifestations, each of 2–3 cm in diameter, spread all over her body, and all over her face and trunk. These manifestations were itching and they appeared as pale red, raised bumps, lasting about one hour and resolving spontaneously. Dermographism was present. She also presented a harsh breath with whistles by the lung, associated with respiratory failure.