Tree nut and peanut allergy in a Portuguese pediatric cohort – clinical characterization and anaphylaxis predictors

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Abstract

Background: Tree nuts and peanuts (TN/P) are frequent causes of anaphylaxis in children. Aim: to characterize a Portuguese pediatric cohort with TN/P allergy and to assess skin tests (ST), specific IgE (sIgE) and molecular components (mcIgE), as well as sIgE/total IgE ratio’s utility in anaphylaxis prediction. Methods: Retrospective study (2017-2021) of pediatric patients with TN/P allergy, grouped according to reaction severity (anaphylaxis–G1 vs milder reaction–G2). ST mean papule diameter (MPD), sIgE (ImmunoCAP®), mcIgE (ISAC®) and sIgE/total IgE ratio were compared (SPSS®, p<0.05: statistically significant). Results: 98 patients, 64% male, 88% concomitant allergic disorder, 40% allergy family history. Major culprit nuts: peanut (63%), hazelnut (59%) and walnut (53%). Index reaction manifestations were mostly cutaneous (46%), followed by anaphylaxis (36%). Chestnut and cashew sensitizations were significantly associated with anaphylaxis (OR=5.023, p=0.002; OR=2.901, p=0.018). MPD was higher in G1 for almond, cashew and pistachio (p<0.05). sIgE was not a good severity predictor for any TN/P, however, a significantly higher value of sIgE/total IgE ratio was found in G1 for walnut (p=0.023). mcIgE was obtained in 49%; peanut Ara h2 and Ara h6 were more represented in G1 (2.8 vs 0 ISU-E, p=0.042; 1.3 vs 0 ISU-E, p=0.020). Conclusion: Peanut, hazelnut and walnut were the most frequent nuts. Anaphylaxis was the first manifestation in 36%, significantly higher in chestnut and cashew allergic children. MPD should be valued not only for diagnosis, but also for anaphylaxis risk prediction in almond, cashew and pistachio allergic patients. sIgE/total IgE ratio seems to be useful in anaphylaxis prediction.
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ABSTRACT
Background: Tree nuts and peanuts (TN/P) are frequent causes of anaphylaxis in children. Aim: to characterize a Portuguese pediatric cohort with TN/P allergy and to assess skin tests (ST), specific IgE (sIgE) and molecular components (mcIgE), as well as sIgE/total IgE ratio’s utility in anaphylaxis prediction.
**Methods:** Retrospective study (2017-2021) of pediatric patients with TN/P allergy, grouped according to reaction severity (anaphylaxis–G1 vs milder reaction–G2). ST mean papule diameter (MPD), sIgE (ImmunocAP®), mcIgE (ISAC®) and sIgE/total IgE ratio were compared (SPSS®, p<0.05: statistically significant).

**Results:** 98 patients, 64% male, 88% concomitant allergic disorder, 40% allergy family history. Major culprit nuts: peanut (63%), hazelnut (59%) and walnut (53%). Index reaction manifestations were mostly cutaneous (46%), followed by anaphylaxis (36%). Chestnut and cashew sensitizations were significantly associated with anaphylaxis (OR=5.023, p=0.002; OR=2.901, p=0.018). MPD was higher in G1 for almond, cashew and pistachio (p<0.05). sIgE was not a good severity predictor for any TN/P, however, a significantly higher value of sIgE/total IgE ratio was found in G1 for walnut (p=0.023). mcIgE was obtained in 49%; peanut Ara h2 and Ara h6 were more represented in G1 (2.8 vs 0 ISU-E, p=0.042; 1.3 vs 0 ISU-E, p=0.020).

**Conclusion:** Peanut, hazelnut and walnut were the most frequent nuts. Anaphylaxis was the first manifestation in 36%, significantly higher in chestnut and cashew allergic children. MPD should be valued not only for diagnosis, but also for anaphylaxis risk prediction in almond, cashew and pistachio allergic patients. sIgE/total IgE ratio seems to be useful in anaphylaxis prediction.

**KEYWORDS:** Anaphylaxis; Component-resolved diagnosis; Food allergy; Peanut allergy; Sensitization profile; Tree nut allergy.

**INTRODUCTION**

Tree nut (TN) and peanut allergy prevalence has been increasing over the last 20 years, particularly in pediatric age, as a presumable result of changes in recent eating habits.\(^1\)\(^-\)\(^3\) Allergy prevalence for each TN appears to vary in different parts of the world. A current systematic worldwide review of studies estimated the global prevalence of probable TN allergy to range from 0.05% to 4.9%, and peanut allergy between 0.5% and 2.5%.\(^1\)\(^-\)\(^4\)\(^,\)\(^5\)

These food allergies can be potentially life-threatening, accounting for a high number of fatal food-induced anaphylaxis, even when ingested in very small quantities or inadvertently, as occult allergens.\(^1\)\(^-\)\(^3\) Recent studies reported TN and peanut allergies as the responsible for 70–90% of deaths from food-induced anaphylaxis, with TN alone accounting for 18–40%.\(^6\)

TN and peanut allergies usually develop early in life and tend to persist into adulthood. According to previous published data, acquisition of natural tolerance to TN and peanut occurs in only 9%–20% of allergic patients.\(^7\)-\(^9\)

The constant need for caution when choosing food and the potential risk of anaphylaxis, frequently leading to diet and social activities restrictions, significantly affects both patient and family’s quality of life. Presently, and regardless of years of research, the management cornerstone of these patients remains strict avoidance of the incriminated nut, in addition to patient and family’s education on prompt recognition of anaphylaxis and immediate use of adrenaline.\(^10\),\(^11\) Other treatment possibilities have been largely explored, namely oral immunotherapy, for peanut and TN allergic patients, but their use is still limited.\(^12\)

Homology amongst nut proteins and cross-reactivity between their main allergens (namely 2S albumins, 7S and 11S globulins, lipid transport proteins [LTPs], and PR-10) leads to frequent co-sensitization in nut allergic patients, which does not always mean a true concurrent allergy to different nuts.\(^13\) As a result, it can be challenging to manage these patients and a distinction between cross-sensitization and clinically relevant cross-reactivity between different TN and peanut is critical, although it frequently requires multiple oral food challenges (OFC) with the associated risk of a possible anaphylaxis. For this fact, dietary restriction of all TN and peanut is a common practice. Deeper knowledge of sensitization patterns and investigation of possible anaphylaxis predictors would be of great value to establish a more precise diagnostic approach and individual dietary guidance for patients allergic to these foods.\(^1\)\(^-\)\(^5\)
In non-English-speaking countries, like Portugal, data on sensitization patterns and anaphylaxis predictors is sparse. We aimed to characterize a pediatric cohort with TN and peanut allergy followed in an Immunology department of a Portuguese tertiary hospital, and to assess the utility of skin tests (ST), specific IgE (sIgE) and molecular components (mcIgE), as well as ratio sIgE/total IgE in predicting the anaphylaxis risk.

METHODS

Study design and population

A five-year single-center, observational, retrospective study was conducted, including Portuguese children (0-18 years-old) referred to Food Allergy consultation for TN and/or peanut IgE-mediated allergy suspicion between January 2017 and December 2021. The study was approved by the hospital’s ethics committee and all patients’ parents/guardians gave an informed consent.

Data collection and interventions

Patients’ charts were reviewed for demographic, clinical and analytical data collection. Detailed information for the characterization of the index reaction (first nut reaction which motivated referral to Food Allergy Consultation) was obtained. Post-index nut reactions or nut allergies diagnosed during patients’ allergological investigation were also assessed. Patients were considered allergic to a specific nut if they had a suggestive clinical history and at least one positive test of the following: (1) skin prick/prick-prick tests (SPT/SPPT); (2) sIgE; (3) OFC. In the absence of previous contact or in cases of previous ingestion without reaction, and evidence of sensitization to a specific nut (positive SPT/SPPT and/or sIgE), only those with a positive OFC were considered allergic. Previous personal and family history of atopy was also recorded.

Allergological investigation included ST, serum analysis of total IgE, sIgE for TN and peanut and mcIgE, and OFC. For ST, mean papule diameter (MPD) ≥3mm above the negative control was considered a positive result. When SPT was negative or commercial extract was unavailable, SPPT was carried out. Regarding serum analysis, cut-off value for positive result for total IgE and sIgE (ImmunoCAP(r), Thermo Fisher Scientific) was >0.35 kU/L, while for mcIgE (ImmunoCAP(r) ISAC-112, Thermo Fisher Scientific) was ≥0.1 ISU-E. OFC was performed only in selected cases, as it is the gold standard for allergy confirmation or exclusion.

Anaphylaxis predictors assessment

Children were grouped according to reaction severity for anaphylaxis risk assessment. Patients with a reaction compatible with anaphylaxis (definition based on Muraro et al. criteria12,14) were included in group 1 (G1), while those with a milder reaction (oral allergy syndrome or systemic reaction without severity to fulfil anaphylaxis criteria) were included in group 2 (G2). The following variables were compared between groups: MPD of ST (for methodologic uniformization only SPPT were considered in this analysis), sIgE and mcIgE values, as well as ratio sIgE/total IgE.

Statistical analysis

Continuous variables were presented as means and standard deviations, or medians and interquartile ranges for variables with skewed distributions, and categorical variables as frequencies and percentages. Normal distribution was confirmed using Shapiro-Wilk test. Categorical variables were compared using Fisher’s exact test or the Chi-square test, as appropriate, while t-independent test and Mann-Whitney test were used to compare parametric and non-parametric independent samples, respectively. P-values <0.05 were considered statistically significant. Analyses were performed with the use of IBM SPSS software (version 25.0).

RESULTS

Clinical characterization
A total of 150 clinical records of children with suspected TN and/or peanut allergy were reviewed, of which 52 were excluded (38 for exclusion of nut allergy and 14 for incomplete data). As a result, 98 patients were included in this study, of whom 63 were male (64%).

The majority of patients (n=86, 88%) had a concomitant allergic disorder: 76 (78%) allergic rhinoconjunctivitis, 50 (51%) asthma, 45 (46%) another food allergy, including 30 (31%) with egg allergy, and 43 (44%) eczema. Thirty-nine (40%) patients had a family history of allergy (Table 1).

Major culprit nuts were peanut (n= 62, 63%), hazelnut (n=58, 59%), walnut (n=52, 53%), almond (n=37, 38%) and cashew (n=31, 32%), followed by chestnut (n=19, 19%), pistachio (n=18, 18%) and pine nut (n=6, 6%). Eighty-eight (86%) patients reported a single-nut reaction, although only 31 (32%) were monosensitized after allergological investigation was carried out (16 to peanut, 7 to walnut, 4 to cashew, 2 to hazelnut and 2 to pine nut). In 96 (98%), symptoms appeared within 30 minutes after exposure. The index reaction occurred before 2 years in 26% of patients and in the majority (59%) before the 5 years (Table 1).

Index reaction

Patients’ mean age of index reaction was 5.8 years (standard deviation (SD) 4.6, range 0.2-17), being significantly lower in patients describing peanut reactions (4.6 years (SD 3.5, range 0.2-17 years), comparing with patients describing TN reactions (6.7 years, SD 5.8, range 0.2-17 years) (p=0.02) (Table 1).

Index reaction manifestations were more frequently cutaneous (n=45, 46%), with urticaria/angioedema in 38 (84%) children and eczema exacerbation in 7 (16%). Thirty-five (36%) patients described a reaction compatible with anaphylaxis. Gastrointestinal symptoms were reported by 16 (16%) patients, of whom 9 (56%) oral allergy syndrome, 5 (31%) nausea and vomiting and 2 (13%) abdominal pain. Lastly, 2 (2%) patients presented shortness of breath (Figure 1).

Skin tests and laboratory parameters

More than half of the allergic population (n=50, 51%) had positive SPT results to various nuts and 38 (39%) had positive SPPT. MPD of SPT and SPPT to the respective nuts are represented in table 2, as well as whole extract sIgE. Mean value of total IgE was 1860 U/mL (SD 1984, range 17-6314 U/mL).

Molecular components were obtained for 48 (49%) patients. The most frequent allergens were from LTP family, predominantly Pru p 3, for which 20 (42%) patients showed positivity. Subsequently, the most relevant was Ara h 9 (n=17, 35%), followed by Jug r 3 (n=16, 33%), Cor a 8 (n=12, 25%) and Tri a 14 (n=4, 8%). The 2S albumin family was the second most documented, namely Jug r 1 (n=18, 38%), Ara h 2 (n=13, 27%) and Ara h 6 (n=11, 23%).

Oral Food Challenges

A total of 17 OFCs were performed, of which 15 were conducted to diagnose secondary nut allergy, with non-culprit nuts, and 2 to clarify index reactions to a specific nut. Of the first 15 OFCs, 9 were performed to other nut to which patients were sensitized, but not exposed to before, all of them with negative or low levels of sIgE (<2 kU/L) to the respective nut. In the remaining 6 OFCs, patients had negative skin tests/sIgE and an OFC was performed to confirm non-allergy and safety in nut introduction. Six (35%) OFCs were positive (4 with non-culprit nuts and 2 with the suspected nut). All these patients had mild cutaneous reactions (2 pruritus, without lesions, and 4 urticaria), 4 of them with spontaneous resolution. The remaining 2 had symptoms resolution after treatment with oral antihistamines.

Anaphylaxis Predictors

A significant association was found between anaphylaxis and allergy to chestnut (OR 5.023 [IC 95% 1.691-14.922], p=0.002) and cashew (OR 2.901 [IC 95% 1.184-7.107], p=0.018) (Figure 2A). Reaction severity was independent of the number of nut sensitizations (p=0.655). Considering SPPT, MPD was significantly higher in G1 for almond (6.5 vs 4mm, p=0.015), cashew (10 vs 5mm, p=0.049) and pistachio (8 vs 3.75mm, p=0.046) (Figure 2B). sIgE values were not good predictors of reaction severity for any nut (Figure
However, a significantly higher value of sIgE/total IgE ratio was found in G1 for walnut (0.0125 vs 0.0005, p=0.023) (Figure 2D). There was no significant association between symptoms severity and serum eosinophils (682.9 vs 596.6/μL, p=0.261) or total IgE (603.4 vs 1879.6 kUA/L, p=0.068). As for the mcIgE, peanut Ara h2 and Ara h6 were identified in more patients from G1 vs G2, with higher median mcIgE values (2.8 vs 0 ISU-E, p=0.042; 1.3 vs 0 ISU-E, p=0.020) (Figure 2E).

DISCUSSION
In this retrospective Portuguese pediatric study that included 98 patients, the most frequent nuts involved were peanut (63%), hazelnut (59%) and walnut (53%). Mean age at first reaction was 5.8 years, being younger in patients with peanut allergy comparing with other nuts (4.6 years versus 6.7 years, p=0.02). We assume that it is a possible consequence of a recent rising nuts consumption in pediatric population in our country, predominantly of peanut. In Spain, Haroun-Díaz E et al. reported these same three nuts (hazelnut, peanut and walnut) as the most frequent nuts eliciting allergy. Allergy prevalence for each TN seems to vary in different regions of the world: hazelnut allergy is the most frequent in continental Europe; peanut, brazil nut, walnut and almond are the most commonly reported in the United Kingdom, and walnut and cashew allergies in the United States. These differences are representative of the variations in nuts consumption in each country, leading to different sensitization patterns.8, 16,17

Most patients had history of atopy (n=86, 88%), including 44% with eczema and 31% with egg allergy. Cetinkaya PG et al. reported a higher frequency of these atopic conditions in patients allergic to nuts, with 72% having atopic dermatitis and 50% egg allergy, probably related to a higher number of involved children.18 The epithelial barrier dysfunction characteristic of atopic dermatitis is a confirmed risk factor for the development of allergic sensitization, food allergy and other allergic diseases. Many epidemiological studies, and recently also studies in animals, demonstrated the connection between skin and digestive tract. Damaged keratinocytes produce IL-33, that stimulates group 2 innate lymphoid cells (ILC2) in the small intestine. These in turn produce IL-4 and IL-13, which leads to the expansion of activated mast cells, resulting in an increment of intestinal permeability and consequent transmission of allergens that can trigger food allergy.19,20

Nut reactions may be severe on the first contact. About one third of our patients presented with anaphylaxis, and more than half were polysensitized to several nuts, which in accordance with recent data of international cohorts.1, 18 Avoidance of all nuts has been the rule for many years in patients allergic to one nut, but the possible introduction of other nuts has recently been investigated in several studies.21,22 In our study, 9 out of the 15 OFCs performed with non-culprit nuts to which patients were sensitized, but not exposed to before, were negative, favoring the above-mentioned idea of avoiding unnecessary nuts restrictions. Additionally, 31 (32%) were monosensitized after allergological investigation (16 to peanut, 7 to walnut, 4 to cashew, 2 to hazelnut and 2 to pine nut).

IgE sensitization to pan-allergen LTP were the most prevalent in our population, differently from a Spanish cohort that found the 2S albumin family of the seed storage proteins as the most frequent.1 It is, however, in line with recently published Portuguese data.23

In our pediatric cohort, anaphylaxis was more common in patients with chestnut and cashew allergies. Severe systemic reactions in patients sensitized to cashew have been frequently reported in Europe.9,12,14 A Portuguese study on TN anaphylaxis in preschool age children concluded that cashew was the major culprit, accounting for 11 of the 25 cases.24 Data on chestnut allergy is sparse, especially considering prevalence and reaction’s severity. Our results could be explained by Portuguese eating habits, with chestnut being one of the most appreciated nuts, typical of the colder months.

Allergological investigation proved to be useful in anaphylaxis risk prediction. MPD should be valued not only for diagnosis but also for anaphylaxis risk prediction. In our study, MPD was significantly higher for almond, cashew and pistachio in patients with anaphylaxis to these TNs. Other authors reported utility of ST in diagnosis prediction (MPD [?] 8mm), but not in anaphylaxis risk prediction.9,12 sIgE did not differ significantly between groups. However, it should be taken into account that there was limited availability
of whole extract sIgE for some of the nuts analyzed (namely cashew, pistachio, chestnut and pine nut) and that not all patients did sIgE measurements. In addition, as previously postulated, our study highlights the important adding value that sIgE/total IgE ratio could bring to clinical practice. For example, sIgE measurement for walnut was not a good predictor of anaphylaxis but, when integrated in sIgE/total IgE ratio, it reached statistical significance. Component-resolved diagnosis was used as a study complement, showing interest in risk stratification, particularly for peanut, with Ara h 2 and Ara h 6 being significantly higher in patients with anaphylaxis, which is in line with other reports.

The present study has some limitations, such as the small sample of patients, which limits extrapolation of the results, and its retrospective design, that could itself weaken our findings. However, it is the first Portuguese study which extensively characterizes a pediatric population with TN and peanut allergy, analyzing the clinical utility of ST, sIgE and mcIgE measurements and, importantly and sparsely reported, sIgE/total IgE ratio in anaphylaxis prediction, and so the authors believe that it could add significant value to clinical practice.

Impact Statement:

The present study characterizes a Portuguese cohort of 98 pediatric patients with tree nut and peanut allergy, pointing out the clinical utility of skin tests, specific IgE and molecular components in anaphylaxis prediction, as well as the interesting adding value of sIgE/total IgE ratio for nut allergy in clinical practice.

REFERENCES

14. Muraro A, Worm M, Alviani C, et al; European Academy of Allergy, Clinical Immunology Food Allergy,
TABLES AND FIGURES

Table 1. Demographic and clinical characterization of patients with tree nut and peanut allergy

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n=98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (%) - Male - Female</td>
<td>63 (64%)</td>
</tr>
<tr>
<td>Atopic comorbidities, n (%) - Allergic rhinoconjunctivitis - Asthma - Another food allergy - Eczema</td>
<td>86 (88%)</td>
</tr>
<tr>
<td>Family history of allergy, n (%)</td>
<td>39 (40%)</td>
</tr>
<tr>
<td>Culprit nuts, n (%) - Peanut - Hazelnut - Walnut - Almond - Cashew - Chestnut - Pistachio - Pine nut</td>
<td>62 (63%)</td>
</tr>
<tr>
<td>Single-nut reaction, n (%) - Monosensitized, n (%)</td>
<td>88 (86%)</td>
</tr>
<tr>
<td>Index reaction - Symptoms [? ] 30 minutes after exposure, n (%) - Mean age of index reaction, years [range]</td>
<td>96 (98%) 5.8</td>
</tr>
<tr>
<td>Age distribution of index reaction by ranges - 0-2 years - 3-5 years - 6-10 years - 11-18 years</td>
<td>25 (26%) 32 (33%)</td>
</tr>
</tbody>
</table>

SD: standard deviation.

Figure 1. Clinical manifestations of the index reaction among the studied population.
Table 2. Results of mean papule diameter of skin prick (SPT) and skin prick-prick tests (SPPT) and mean values of serum-specific IgE levels (sIgE) (ImmunoCAP®) to the respective nuts.

<table>
<thead>
<tr>
<th></th>
<th>SPT (mm) (Mean papule diameter ± SD)</th>
<th>SPPT (mm) (Mean papule diameter ± SD)</th>
<th>sIgE (kUA/L) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine (10 mg/mL)</td>
<td>5.6 ± 1.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Peanut</td>
<td>7.3 ± 3.4</td>
<td>9.1 ± 7.8</td>
<td>18.8 ± 17.2</td>
</tr>
<tr>
<td>Walnut</td>
<td>6.4 ± 3.5</td>
<td>9.3 ± 4.9</td>
<td>13.8 ± 9.3</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>4.3 ± 1.9</td>
<td>9.3 ± 4.2</td>
<td>10.8 ± 8.4</td>
</tr>
<tr>
<td>Almond</td>
<td>5.3 ± 2.4</td>
<td>6.9 ± 2.9</td>
<td>5.1 ± 4.9</td>
</tr>
<tr>
<td>Cashew</td>
<td>7.1 ± 3.6</td>
<td>11.2 ± 7.3</td>
<td>5.8 ± 7.1</td>
</tr>
<tr>
<td>Chestnut</td>
<td>5 ± 1.5</td>
<td>7.0 ± 4.2</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>Pine nut</td>
<td>8 ± 2.8</td>
<td>5.9 ± 3.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Pistachio</td>
<td>6.3 ± 3.4</td>
<td>8.8 ± 7.5</td>
<td>7.3 ± 8.1</td>
</tr>
</tbody>
</table>

The mean diameter of papules was expressed in millimeters (mm) and those with a diameter of 3 mm above the negative control were considered positive. SD - standard deviation.

Figure 2. Differences between anaphylaxis (G1) vs non-anaphylaxis (G2) groups regarding culprit nuts (A), mean papule diameter (MPD) of SPPT (B), sIgE (C), sIgE/total IgE ratio (D) and mcIgE (E). *p < 0.05