Original article

Assessment of pine nut tolerance in children with tree nut allergy: a case series

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SUMMARY

Introduction. Tree nut allergy is often a lifelong condition impacting children's quality of life due to avoidance recommendations. Despite low cross-reactivity between pine nuts and other tree nuts, many children are advised to avoid pine nuts without proper diagnostic evaluation.

Materials and Methods. This case series retrospectively analyzed 35 children with tree nut allergies from Imola's Hospital, focusing on the diagnosis of pine nut allergy. Patients were divided into two groups based on pine nut consumption. Diagnostic tests included prick-by-prick, specific IgE, and oral food challenges.

Results. The diagnostic process led to a significant increase in pine nut consumption, from 23% to 66% (p = 0.0006). Most children who introduced pine nuts into their diet did not experience any clinically significant reactions.

Discussion. The findings suggest that many children with tree nut allergies may tolerate pine nuts, highlighting the importance of specific diagnostics for pine nut allergy.

Conclusion. Assessing pine nut sensitization before exclusion from the diet is important for children with tree nut allergies.

KEYWORDS: pine nut, nut allergy, oral food challenges, food allergy management.

INTRODUCTION

Tree nut allergy can be a life-threatening disease with typically lifelong persistence, and affecting an increasing number of pediatric patients worldwide[1-3]. Frequently, after diagnosis of tree nut allergy, patients are advised to avoid all nuts due to the possibility of cross-sensitization between different nut allergens [4,5]. This can have a significant impact on the quality of life of the children and their relatives, due to the fear of possible severe reactions following the ingestion of nuts, even in minimal traces [6,7]. Growing evidence points to a beneficial effect of tree nut consumption on the health of the individual which has determined an increase in the consumption of nuts and consequently in the prevalence of allergy [1,8].

Pine nuts, the seeds of pine trees (genus *Pinus*), are a nutrient-rich food with a beneficial impact on human health. They are frequently used in raw or roasted form in the preparation of several dishes (such as pesto sauce or cakes)[9]. Cases of severe IgE-mediated reactions to pine nuts have been reported [10-14]. In an Italian study, pine nut was found to be responsible for 3% pediatric food-induced anaphylaxis [15].

The diagnosis of pine nut allergy relies on clinical history and specific testing using skin prick test (SPT), prick-by-prick test (PbP), serum specific IgE (sIgE), and oral food challenges (OFC) [16]. Two studies have investigated the accuracy of these tests in the diagnosis of pine nut allergy [17,18].

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The major allergenic components in pine nuts are 2S albumins and vicilins [19], (seed storage proteins-SSP), molecules known for resistance to digestion and heat stability. To this group also belong hazelnut Cor a 14 and Cor a 9, and walnut Jug r1 [4,20,21]. The presence of IgE cross-reactive allergens in nuts is a common finding as SPP often share parts of their amino acid sequences [4]. With regards to pine nuts, available evidence suggests that monosensitization to this nut is common [10-14,19,22,23] and IgE cross-sensitization seems to be low [4,19,24]. However, pine nuts are frequently excluded from the diet of children allergic to tree nuts without a real reason and without an accurate diagnostic process.

In this case series, we described a group of children with tree nut allergies who introduced pine nuts into their diet following a proper diagnostic process.

MATERIALS AND METHODS

Study population

Clinical charts of patients attending the Pediatric Allergy Service of Imola's Hospital from January 2019 to June 2024 with allergies to nuts or seeds were retrospectively examined. Nut allergy was diagnosed by OFC or clinically suggestive of IgE-mediated reaction and evidence of IgE-mediated sensitization [16]. The study is in compliance with the principles stated in the Declaration of Helsinki "Ethical Principles for Medical Research Involving 'Human Subjects", adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, and as amended most recently by the 64th World Medical Assembly, Fontaleza, Brazil, October 2013, and parents gave informed consent to the use of clinical laboratory data emerging during the diagnostic process. Children with peanut allergies were excluded from the study, as peanuts belong to the Fabeaceae family and are classified as legumes [21]. For each patient, we investigated regular pine nut consumption at baseline. In patients who did not eat pine nuts, allergy was assessed with a PbP test, sIgE, and OFC. Patients were then divided into two groups based on pine nut consumption: pine nut consumers (PNC) and pine nut non-consumers (PNNC). Information on culprit allergen, clinical reactions, age at diagnosis along with tests indicating sensitization (skin prick test, PbP, sIgE to nuts, and the available molecular components) were collected and compared between the two groups.

Diagnostic tests

SPT and PbP tests were performed on the volar surface of the forearm using commercial extracts (Lofarma, Milan, Italy) and food respectively, as per European standards [25]. At 15 minutes reading the test was considered positive if the wheal diameter was ≥ 3 mm. Histamine (10 mg/mL, Lofarma, Milan, Italy) and normal saline were used as positive and negative controls. In case both SPT and PbP were available for the index nut, the greatest weal diameter was taken into account. sIgE to nuts and molecular components (hazelnuts: Cor a9, Cor a14) were detected by ImmunCAP (Thermo Fisher Scientific, Uppsala, Sweden). The antibody titer was considered positive when > 0.1 kUa/L. Values above 100 were set by convention as 100.

An open-labelled OFC was performed in patients with IgE sensitization to pine nuts but no history of consumption, under an allergist's supervision through administration of incremental dose given every 20 min until the threshold for reaction occurred or upon reaching a cumulative dose of 15 pine nuts (overall 3 gm of pine nuts about 410 mg of protein).

Statistical analysis

Data were summarized using descriptive analyses. Categorical variables were expressed as counts and percentages, whereas continuous variables were reported as median and standard deviation (SD) or median and interquartile ranges (IQR). Qualitative variables were compared using Fisher's exact test, while quantitative variables were compared using the unpaired student t-test. Statistical significance was set at a p-value < 0.05. Statistical analysis was performed by using GraphPad Prism version 10 (California, USA).

RESULTS

A total of 35 patients with tree nut and seed allergies were included in the evaluation (23 boys [66%] and 12 girls [34%]). Of these, 33 children (94%) had a clinical history of allergic reactions (with a median age at first reaction of 3.9 years, SD 3.3) to one of the following tree nuts and seeds: walnut (21, 66%), hazelnut (17, 51%), cashew (5, 15%), pistachio (3, 9%), pecan nut (1, 3%), sesame (1, 3%) and pine nut (1, 3%). Also, 17 (52%) had a history of anaphylaxis, 14 (42%) had urticaria/angioedema, and 2 (6%) had Pollen Food Allergy Syndrome (PFAS). The other 2 subjects (6%) included in the analysis had either skin prick test or sIgE results indicating important sensitization to tree nuts but had never introduced them into the diet. One had of a history of severe atopic dermatitis, while the other had experienced a severe anaphylactic reaction to egg. In the study population, 15/35 patients (43%) were allergic to more than one nut. Patients' characteristics are summarized in Table I.

Assessment of pine nut consumption

Twenty-seven (77%) children were pine nut non-consumers at baseline. Of these, 15 (55%) introduced this nut into their diet after performing PbP (median 3 mm, IQR 0-4.5), sIgE (median 0.48 kUa/L, IQR 0-1.41) and/or OFC, which was performed in those with IgE sensitization to pine nuts but no history of consumption. In total, 6 children (40%) underwent an OFC for pine nuts, in this group the median PbP was 2 mm (IQR 0-4.5), while the median sIgE was 1.41 kUa/L (IQR 0.44-2.48). The other 9 (60%) children consumed and tolerated pine nuts at home. Of these, seven did not present IgE-mediated sensitization, while the other two presented some degree of sensitization (one had a sIgE titer 0.64 kUa/L, the other had a sIgE titer of 0.4 kUa/L and a wheal diameter at PbP test of 5 mm).

TABLE I. Clinical characteristics of patients with a tree nut or seeds allergy at baseline.

Patient n.	Age at first reaction (years)	Clinical symptoms	Culprit nut	Pine nut consumer
1	0.5	Atopic dermatitis	Walnut, hazelnut, almond	no
2	6	UA	Walnut	No
3	1	A	Walnut, hazelnut	No
4	4	A	Walnut	Yes
5	2	UA	Walnut, hazelnut	Yes
6	7	UA	Pistachio, cashew	No
7	7	A	Walnut	No
8	1	UA	Hazelnut	Yes
9	1.5	A	Cashew	No
10	6	A	Walnut, hazelnut	No
11	2	UA	Hazelnut	No
12	1	A	Hazelnut	no
13	1	Atopic dermatitis	Walnut, hazelnut	no
14	11	PFAS	Walnut	Yes
15	4	UA	Walnut, hazelnut	no
16	2	A	Walnut, hazelnut, pistachio, cashew	No
17	2	A	Walnut	No
18	1	A	Walnut	No
19	1	UA	Walnut	No
20	2	UA	Walnut	No
21	3	A	Hazelnut	No
22	3	A	Sesame	No
23	2	UA	Hazelnut	No
24	3	A	Hazelnut, cashew	No
25	13	A	Walnut, hazelnut	Yes
26	8	A	Hazelnut	No
27	2	UA	Walnut	Yes
28	3	UA	Walnut	Yes
29	3	PFAS	Pistachio, cashew, pine nut	No
30	0.7	UA	Walnut, hazelnut	No
31	7	UA	Walnut	No
32	3	UA	Walnut, hazelnut	No
33	3	A	Walnut, hazelnut	No
34	11	A	Walnut, pecan nut	Yes
35	1.3	A	Hazelnut	No

A: anaphylaxis; UA: urticaria/angioedema; PFAS: Pollen Food Allergy Syndrome.

TABLE II. Clinical and demographic characteristics of pine nut consumers *vs* pine nut non-consumers (at the end of study).

	Pinenut consumers (N = 23)	Pinenut NON consumers (N = 12)	P-value
Age at first clinical presentation of the index nut allergy (years) (mean, SD)	4 (3.6)	3.1 (2.5)	0.44
Age at last clinical evaluation (years) (mean, SD)	9.6 (4.1)	6.7 (3.5)	0.04
Clinical presentation of index allergy No (%)			
Anaphylaxis	12 (52)	5 (42)	0.72
Urticaria/angioedema	10 (43)	4 (33)	0.72
PFAS	1 (4)	1 (8)	1.00
Never introduced in the diet, because of important sensitization	0 (0)	2 (17)	
Culprit tree nut or seeds No (%)			
Walnut	16 (70)	5 (42)	0.15
Hazelnut	10 (43)	7 (58)	0.49
Cashew	3 (13)	2 (17)	1.00
Pistachio	1 (4)	2 (17)	0.54
Pecan nut	1 (4)	0 (0)	1.00
Sesame	1 (4)	0 (0)	1.00
Pine nut	0 (0)	1 (8)	
Allergy to more than one nut, No (%)	9 (40)	6 (50)	0.72
sIgE (kUa/L) to index tree nut or seeds (kUa/L) (median, (IQR))*			
Walnut	7.06 (1.7-39.9)	21.5 (3.06-62)	0.72
Hazelnut	9.48 (1.03-13.4)	22.9 (1.3-66.2)	0.18
PbP or SPT (mm) to index tree nut (median, (IQR))*			
Walnut	4 (2-14)	2 (1-4)	0.20
Hazelnut	9 (5-14)	5 (5-7)	0.10
Molecular allergens to hazelnut (kUa/L) (median, (IQR))*			
Cor a9	4.91 (1.03-10)	8.83 (1.47-24.9)	0.50
Cor a14	2.29 (1.32-3.69)	4.29 (1.21-16.62)	0.17

* Only patients with a positive clinical history were included in the analysis.

Twelve children (44%) continued to not consume pine nuts. Of these, 2 (17%) did not consume the nut under medical advice: one due to clinical symptoms (PFAS) while the other due to a significant sensitization (PbP 12 mm, sIgE 16.8 kUa/L) which recommended against its consumption. Five (42%) children never introduced pine nuts into the diet because of fear of consumption, despite the indication to perform an OFC (median sIgE 0.2 kUa/L, IQR 0-4.8) while 5 (42%) never agreed to undergo a diagnostic evaluation because of a lack of interest in introducing this nut in the diet.

Overall, 18/27 patients (67%) who did not consume pine nut at baseline, underwent evaluation of sIgE for pine nut, of which 11 (61%) introduced the nut into their diet afterward (median sIgE 0.48 kUa/L, IQR 0-1.41), while 7 (39%) did not (median sIgE 1.2 kUa/L, IQR 0-7.58). No significant difference was found when the median sIgE of the two groups were compared (p = 0.1076).

The diagnostic process for pine nut allergy led to a significant increase in the number of patients regularly consuming this nut, from baseline (8/35 vs 23/35; p = 0.0006).

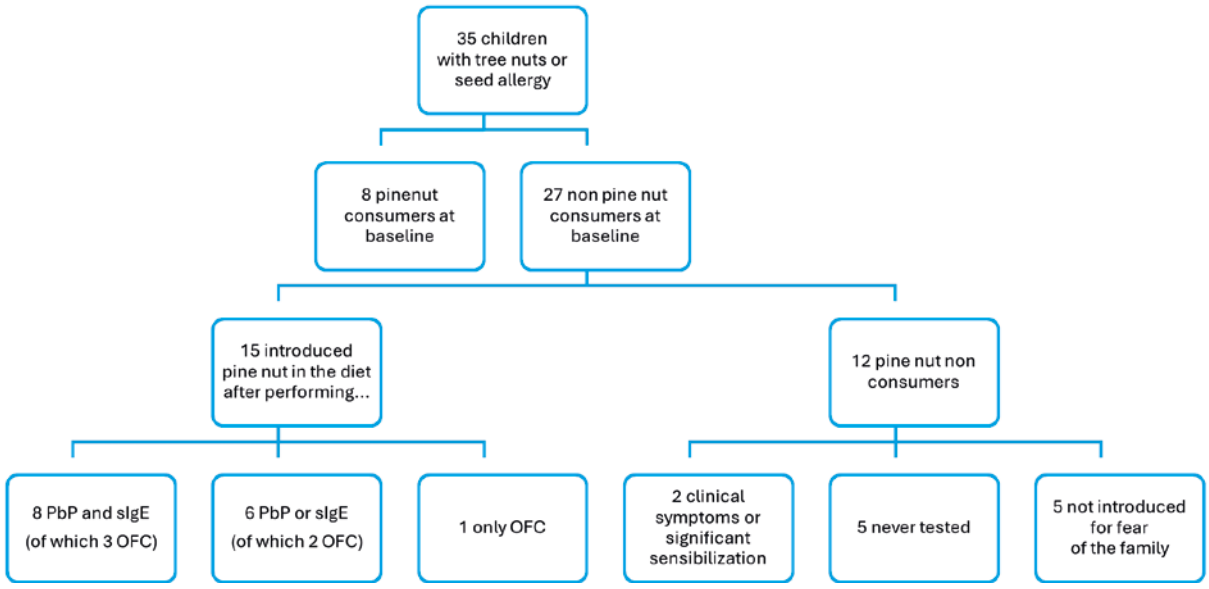

FIGURE 2. Flow-chart showing the diagnostic process of pine nut introduction in a group of children with tree nut allergies.

Comparison between the two groups

The 35 patients included in the analysis were divided into two groups according to pine nut consumption at the end of the study: *PNC* (23; 66%) and *PNNC* (12; 34%). Patients' clinical characteristics are summarized in Table II and the diagnostic process for pine nut allergy is described in Figure 1.

Age at first clinical presentation of the index tree nuts reaction was comparable between the two groups (p = 0.44), while age at last clinical evaluation was higher in the *PNC* group (9.6 ± 4.1 years *vs* 6.7 ± 3.5 years; p = 0.04).

At baseline for the index tree nut in the *PNC* group, 12 patients (52%) reported a history of anaphylaxis as compared to 5 (42%) in the *PNNC* group (p = 0.72). Also, rates of urticaria/angioedema and PFAS were comparable between the two groups (p =0.72 and p=1.00 respectively). Regarding the culprit allergen, hazelnut and walnut were the tree nuts most commonly responsible for nut allergy in both groups. No significant difference was found when the rate for hazelnut allergy (p = 0.15) and walnut allergy (p = 0.49) was compared. The frequency of allergy to more than one nut also did not differ among PNC and PNNC (p = 0.72).

The degree of sensitization to tree nuts was compared for the most common culprit tree nuts in the two groups. Firstly, walnut allergy was taken into account. Among patients allergic to walnut, 21 had available sIgE (16/16 pine nut consumers vs 5/5 pine nut non consumers) while 16 patients performed either PbP or SPT for the culprit nut (11/16 vs 5/5). No significant difference was found neither when median sIgE titer (p = 0.72), nor when median PbP or SPT values (p = 0.2) were compared. With regards to hazelnut allergy 14 children had available sIgE (8/10 *PNC vs* 6/7 *PNNC*) while 16 performed either PbP or SPT for hazelnut (10/10 vs 6/7). Again, no statistically significant difference was found neither for median sIgE titer (p = 0.18) nor for the median PbP or SPT values (p = 0.1).

A molecular analysis was performed in 31/35 (86%) eligible patients. The highest adherence was obtained among the 17 patients with hazelnut allergy, among which 10/17 (59%) performed assessment of Cor a 14 and Cor a 9 antigens. Of these 6 (60%) were *PNC*, while 4 (40%) were *PNNC*. No significant difference was found when the median sIgE value for Cor a 9 (p = 0.50) and Cor a 14 (p = 0.17) were compared. For the other molecular allergens, not enough data were available to perform a significant comparison.

DISCUSSION

After a diagnosis of tree nut allergy, patients are often advised to avoid all nuts due to the risk of possible cross-sensitization [4,5], with a potential impact on their quality of life [6-8]. Pine nuts, the seeds of *Pinus pinea*, are source of nutrients and are commonly used in various recipies [9]. While severe allergic reactions to this nut have been documented [10-15], evidence indicates that isolated sensitization to pine nuts is relatively frequent [10-14,19,22,23] and IgE cross-sensitization with other nut is generally minimal [4,19,24]. This series of cases investigated the rate of pine nut tolerance in a group of children with tree nut allergies.

A total of 35 children were enrolled. In our population tree nut and seed allergy were more common among males (66%), and the most common culprit allergens were hazelnut (51%) and walnut (66%), in

agreement with the data of a previous Italian study [26]. Only one patient was allergic to pine nut, though reported symptoms were mild (PFAS). The introduction of a diagnostic process to investigate pine nut allergy led to a significant increase in the number of patients regularly consuming this nut (from 23% to 66%, p = 0.0006). Among *PNNC*, only two excluded the pine nut from the diet under medical advice, while most of them were never investigated due to lack of interest or because of fear by the family.

Two studies in the literature have evaluated the relationship between pine nut sIgE levels, PbP and clinical symptoms [17,18]. The first was a Korean study which determined that the optimal cut-off level of pine nut sIgE to predict clinical reactivity is 0.40 kUa/L (specificity 77.8%, sensitivity 66.7%), while the positive decision point was set as 2.48 kUa/L (specificity 100%) [17]. An Italian study found PbP test to have a better diagnostic performance than sIgE for diagnosing pine nut allergy in children (cut-off > 7 mm, specificity 100%, PPV 100%) [18]. In our study the median sIgE titer was 0.48 kUa/L (IQR 0-1.41) among pine nut consumers, while 1.20 kUa/L (IQR 0-7.58) among pine nut non-consumers (p = 0.1076). Median PbP wheal diameter was not compared between the two groups as only one patient not eating pine nuts performed this test.

To identify differences in terms of clinical and demographic characteristics the two groups were compared. The rate of multi-tree nuts allergy and of anaphylaxis were comparable between *PNC* and *PNNC*. Also, the degree of sensitization to hazelnuts and walnuts (median sIgE titer, median PbP or SPT or titer of hazelnut molecular allergens Cor a14 and Cor a9) was comparable. This result indicates that no specific characteristics suggesting that a patient may tolerate pine nuts were found.

These data suggest that it may be useful to evaluate pine nut tolerance in children allergic to tree nuts. This may improve the quality of life of both pediatric patients and their families, as well as boost social interactions. Indeed, Italian children often play with pinecones and may consume this nut raw, so parents may be less inclined to let them engage with their friends in pine forests. In this case series, a possible improvement in quality of life was not quantified through a psychological assessment.

Regarding to demographic characteristics, a significant difference was found when age at the last evaluation was compared between *pine nut consumers* (9.6 $\pm$ 4.1 years) and *pine nut non-consumers* (6.7 $\pm$ 3.5 years). This result may indicate that the best time to try introducing pine nuts into the diet could be a few years after the tree nut allergy diagnosis, when the family has had time to process the information and may be more willing to introduce new foods into the child's diet.

Available evidence suggests a relatively low homology between pine nut Pin p1 and 2S albumins of other nuts [4,19,24]. The Molecular Allergology User's guide reports a sequence identity of 35% with hazelnut Cor a 14, of 33% with walnut Jug r1, and of 30% with both cashew Ana 3 and pistachio Pis v1 [4]. It was not possible to test molecular allergens for pine nuts.

The limitations of this study are its retrospective nature and the heterogeneous number of children who performed diagnostic tests to detect pine nut sensitization. Also, a small population referring to a single allergy unit was considered. On the other hand, its strength is that these data may boost current knowledge on clinical cross-reactivity between pine nuts and other tree nuts and may help guiding allergists' decisions in clinical practice.

CONCLUSION

In this cohort of children with tree nut allergy a large proportion consumed pine nuts without any clinically relevant reaction. These data suggest that it would be appropriate to investigate pine nut sensitization before excluding a priori this nut from the diet.

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Ethical consideration

The manuscript was in compliance with the principles stated in the Declaration of Helsinki "Ethical Principles for Medical Research Involving 'Human Subjects", adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, and as amended most recently by the 64th World Medical Assembly, Fontaleza, Brazil, October 2013 and the parents gave informed consent to the use of clinical laboratory data emerging during the diagnostic process.

Conflicts of interest statement

The authors declare no conflict of interest.

Author's contribution

AZ: conceived the manuscript and wrote the first draft. SB, MGM, CM collected the data and wrote the first draft. PB: conceptualized the project and conceived the manuscript. All authors revised the manuscript and approved the final version.

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