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Prevalence and clinical features of adverse food reactions in Portuguese adolescents

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ABSTRACT

Background & aims: The objective of the present study was to determine, for the first time, the prevalence and clinical features of food allergy in Portuguese adolescents.

Methods: Cross-sectional study performed in various secondary schools in central Portugal. Randomly selected adolescents replied to a validated food allergy questionnaire. Those who reported an adverse food reaction were seen at participating hospitals, where clinical history was taken, skin prick (SPT) and prick-prick skin (SPPT) tests were performed, and food allergen-specific IgE levels (sIgE) were determined. An open oral challenge was performed in selected cases. Cases of positive clinical history of immediate (up to 2 h after ingestion) reaction in association with positive food sIgE levels and/or SPT were classified as IgE-associated probable food allergy and as confirmed IgE-mediated food allergy if food challenges were positive. Cases of positive clinical history of delayed (more than 2 h after ingestion) and negative food sIgE levels independently of positive SPT or SPPT results, were classified as non-IgE associated probable food allergy.

Results: The prevalence of probable food allergy in Portuguese adolescents was 1.41% (95% CI: 0.90–2.03%), with fresh fruits, shellfish, nuts, and peanut as the most frequently implicated foods. IgE-mediated probable food allergy occurred in 1.23% (95% CI: 0.67–1.72%) of cases, with fresh fruits, shellfish, and nuts mainly involved. Cutaneous symptoms were most frequently reported.

Conclusions: The prevalence of probable food allergies in Portuguese adolescents is low, is mostly related to fresh fruits, shellfish, nuts, and peanut, and most frequently involves cutaneous symptoms.

Keywords: Adolescents, Adverse food reaction, Food allergy, Prevalence, Cutaneous tests, Open food challenge

INTRODUCTION

Food allergy is a relevant health problem which is associated with considerable morbidity, a non-negligible level of mortality (in cases of food-associated anaphylaxis), and lower quality of life.[1,2] In fact, the impact of food allergies on dietary habits and social integration of food allergic patients is obvious,[3,4] with many affected individuals having to undergo changes in their diet, including, in some cases, very restrictive

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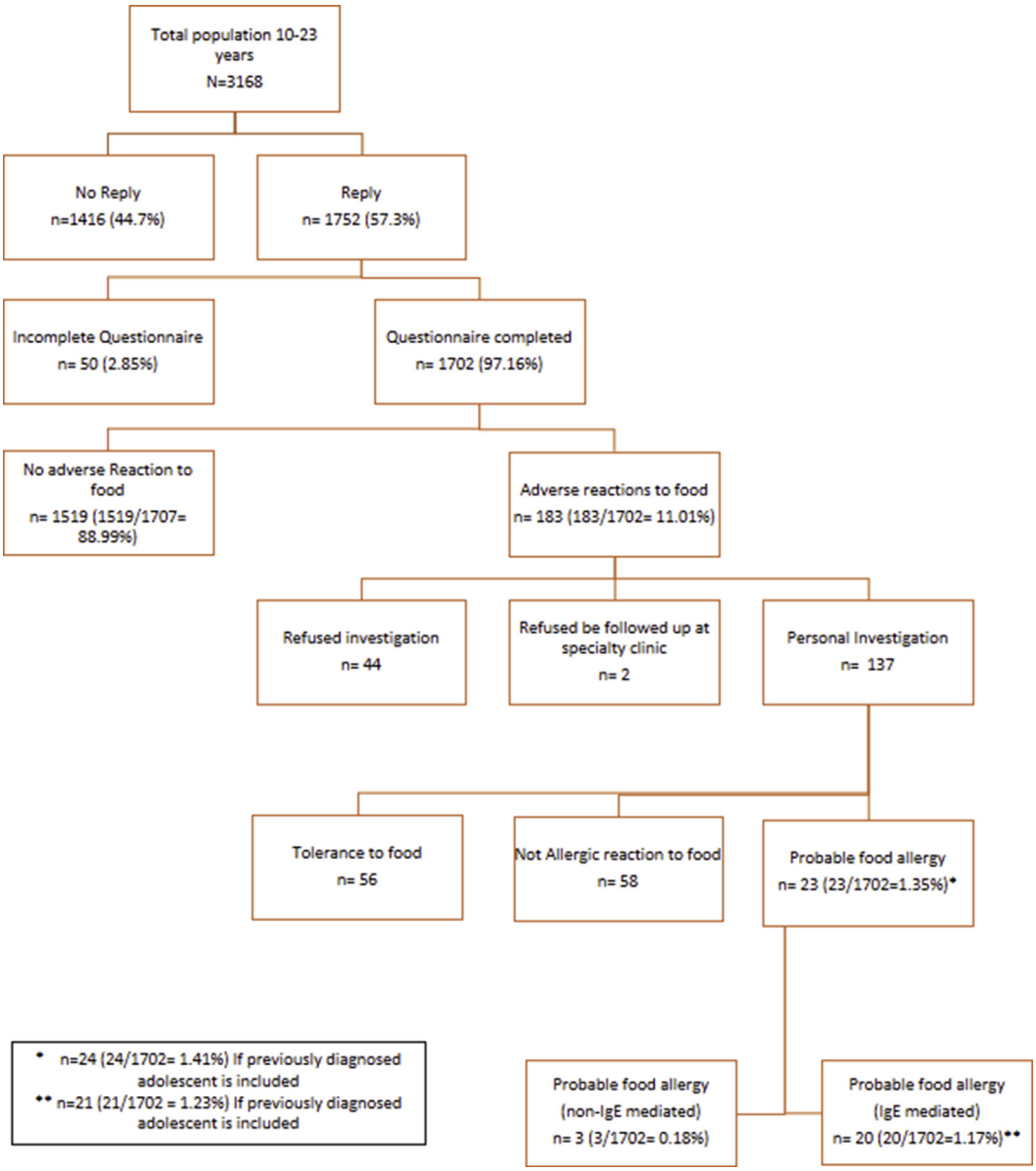

Fig. 1 Flowchart of the study design and investigations

diets, due to an adverse food reaction.[5] In addition, food allergies are also a clear economic burden, namely regarding work or school absenteeism,[6,7] with impact upon school performance[8] and quality of life.[9]

However, not all adverse reactions to foods are regarded as being an immunologically mediated "food allergy".[1,10,11] Partly for this reason, the prevalence values of food allergies in the general adolescent population are not well known. Various meta-analyses have estimated the prevalence of food allergies to any food in schoolchildren to be between 7% and 40% when only self-reported values are analysed,[9,12–15] and between 1% and 3% when studies include diagnostic tests.[14–18] As far as we know, besides a single study in children attending an allergy outpatient clinic,[19] the only actual population-based studies on the prevalence of food allergies carried out in Portugal were performed by our group in children[20] and adults,[21] but no studies in adolescents have been published. Thus, the objective of our study was to determine the prevalence of both self-reported and probable

food allergy, as well as to analyse the clinical features, involved foods, and associated factors in a general population of Portuguese adolescents.

METHODS

Population and sample

For this study, we took into account the fact that 3168 adolescents aged between 1023 years old (mean age: 14.3 ± 1.1; 51.7% female) were registered in 7 secondary schools of the cities of Castelo Branco, and Covilhã, in central Portugal. Based on an estimated prevalence of 4%,[13,22,23] and considering a 95% confidence interval and a margin of error of 2%, we calculated that we would need a representative sample of 399 adolescents (STATA Statistical Package®). Considering an expected reply rate of 40%, we reset the sample size to 779 adolescents.

Study design

This was a cross-sectional study performed in 2013–2015. A list of all students in each class of each school was obtained, and adolescents were selected by a simple randomisation process. A standardised screening questionnaire was given to each volunteer, and those who reported a previous adverse food reaction which was subsequently confirmed by telephone, were invited to an appointment at the outpatient allergy clinics of the participating hospitals, where a standardised food allergy-related clinical history was taken,[24] skin prick tests (SPT) and, when food was available, prick-prick skin tests (SPPT) were performed with a standardised technique, and blood was collected for determination of food allergen-specific IgE levels. In selected cases, an open oral challenge was performed. In these cases, if the patients did not exclude the suspected food from the diet, an elimination diet was followed for a minimum of 7 days prior to the food challenge. Patients with a positive clinical history of immediate (up to 2 h after ingestion) reaction in association with positive food sIgE levels and/or skin prick tests (with or without performance of a positive open challenge) were classified as IgE-associated probable food allergy. Cases of positive clinical history or delayed (more than 2 h after ingestion) and negative food sIgE levels independently of positive SPT or SPPT results, were classified as non-IgE associated probable food allergy.

Questionnaire

A 17-item, previously validated questionnaire on adverse food reactions[25] was given by hand to all volunteers. This questionnaire included demographic data, questions on the occurrence of previous episodes of adverse reactions to foods, types of foods involved, types of reactions, post-ingestion latency time until appearance of symptoms, date of latest reaction, need for medical assistance, and personal or family history of atopic diseases. Those adolescents who reported an adverse food reaction were subsequently contacted by phone by a trained allergist within the following 3 months (Fig. 1). Those who confirmed the previous self-report of an adverse reaction were invited to a full allergy screen at the participating hospitals.

Determination of allergen-specific IgE serum levels

In all individuals seen at the outpatient clinics, 5 ml of peripheral blood were taken for the determination of the levels of total serum IgE, aeroallergen-specific screening IgE (Phadiatop inhalant allergens®), and suspected food-specific IgE levels. No recombinant allergens or pan-allergens were used. A fluorometric (ImmunoCAP® 250 Phadia Diagnosis)-based technique was used (Phadia & Thermo Scientific, Uppsala, Sweden). Allergen-specific levels above 0.35 KU_A/L were regarded as positive.

Skin prick tests

In vivo studies included SPT (LETI Laboratories, Spain; Bial-Aristegui, São Mamede do Coronado, Portugal; Stallergènes, Antony, France) for aeroallergens (house dust mites; cockroach; *fungi*; latex; cat and dog dander; weeds, tree, and grass pollens) and suspected foods and, when available, SPPT with native suspect foods, since the sensitivity of the latter test is higher when compared with SPT using commercial extracts.[26] Tests were carried out in duplicate on the volar aspect of the forearms. A drop of each commercial extract was placed upon the skin, and each drop was pricked through using a metal lancet (Stallergènes, Antony, France). Histamine dihydrochloride as

positive and saline solution as negative controls were used respectively. The mean weal diameter was recorded after 15 min. Weals with a mean diameter at least 3 mm greater than that of the negative control were regarded as positive. SPPT tests used the same methodology, but fresh foods were used.

Oral challenge

Open oral challenges were performed in cases with positive clinical history, SPT and/or SPPT, and sIgE levels to suspect foods, and also in those cases in which clinical history was unclear and SPT results, as well as specific IgE levels, were negative or discrepant. Open challenge tests were carried out with suspect food,[22] in accordance with published guidelines.[10,11,27] In those cases in which individuals did not avoid the suspect foods, in spite of having symptoms, an elimination diet for at least 7 days before the oral challenge was carried out and monitored.[10,11,27-29] Oral challenges were performed at the hospitals, under direct clinical observation for 4 h after challenge. In all cases, volunteers were contacted by phone by the responsible allergist in the following 24 h. Volunteers who reported any symptoms were reassessed at hospital allergy services.

Statistical analysis

Data were analysed using the Software Package for Social Sciences (SPSS) version 20.0® (SPSS Inc., Chicago, IL, USA). Analysis of normality of distribution of variables was performed using the One Sample Kolmogorov-Smirnov test. Descriptive analysis was used for the characterization of the sample. Chi-Square test or Fischer's Exact Test were used in the case of nominal variables. Comparative analysis of quantitative variables was carried out using Student's *t*-test or Mann-Whitney *U* test depending on distribution of variables. Odds ratio values were calculated for analysis of possible risk factors for adverse for reactions. A *p* value of less than 0.05 was regarded as significant with all statistical tests.

RESULTS

Determination of prevalence and clinical features of self-reported food allergy

Of the 3168 questionnaires that were handed out (Fig. 1), 1752 were returned correctly filled in and with the written informed consent (57.3% reply rate). The questionnaire was properly completed by 1702 individuals (97.2% of the total of returned questionnaires; mean age: 14.9 ± 2.1 years; median age: 14 years; 61.9% female). Of these, 183 adolescents reported previous adverse reactions (total of 239 episodes) upon ingestion of at least 1 food (11.01%). These reactions had most frequently taken place 4 months to 5 years before (42.0% of the cases). Most adolescents reported symptoms with more than 1 type of food (50.2%; 92/183). Regarding episodes of adverse food reaction, most commonly implicated foods were fresh fruits (59/239 episodes– 24.7%; 73/239 episodes – 30.5% if latex-related fruits were included as well), seafood (32/239 episodes – 13.4%), milk (30/239 episodes – 12.5%), and nuts (15/239 episodes, excluding peanut – 6.3%) (Fig. 2).

Most frequently reported symptoms were cutaneous (urticaria/angioedema; 107 episodes – 44.7%), followed by abdominal (34 episodes – 14.2%), respiratory symptoms (18 episodes – 7.53%), or oral allergy syndrome (17 episodes – 7.1%). 49 episodes (20.5%), were difficult to define clinically (Fig. 3).

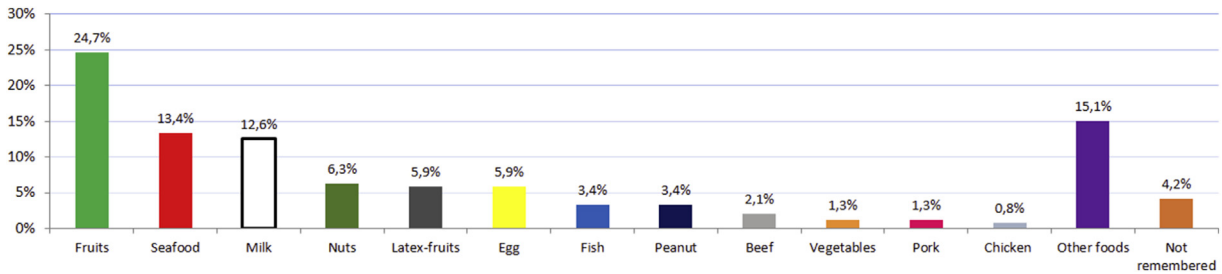

Fig. 2 Most frequently implicated foods by self-report (Values in %); number of episodes = 239

In most of the reported episodes (43.5%), symptoms developed up to 30 min after ingestion, and in 30.95% of the cases had a delayed onset (2–24 h) (Fig. 4).

Most of the 183 adolescents who reported a total of 239 episodes of AFR mentioned 2 to 5 reactions with the same food (48.6%; 89/183 individuals, reporting 116 episodes), with fresh fruits being the most frequent food in this group (38 out of 116 episodes, and in 47 out of 116 episodes if latex related fruits were included). No individuals with latex sensitisation were found.

In addition, 35 out of 183 adolescents (19.15%) reported 46 episodes of an AFR, with seafood being the most frequently associated food in this group (10/46 episodes) (Fig. 5).

About 56% (102/183) of the adolescents needed medical treatment: 67% of them (68 cases) at a hospital emergency department, 12.5% (13 cases) by a general practitioner, 13.5% (14 cases) by self-medication, and 7% (7 cases) by an allergy specialist.

Most individuals who reported reactions (59%) had not been diagnosed an adverse food reaction, and only 30% had been given such a diagnosis by an allergist.

Having a personal (OR: 3.00; 95% CI: 1.80–5.00) or family history (OR: 2.60; 95% CI: 1.53–4.32) of atopy were factors significantly associated with an increased risk of having an adverse food reaction.

Determination of prevalence and clinical features of probable food allergy

Of the 183 individuals who reported an AFR, 44 (24%) declined to continue the study, and 2 adolescents (1.1%) did not complete the study (1 of them had been studied thoroughly already) (Fig. 1). The remaining 137 adolescents (74.9% of the total number of AFR cases) were subsequently seen at an allergy hospital appointment. Of these, 56 (40.9%) reported absence of symptoms upon subsequent ingestion of the suspect food in the period between completion of the questionnaire and the hospital appointment, and were therefore not further studied. Thus, the remaining 81 adolescents under study (59.1% of the 137 adolescents seen at the hospitals) completed the full allergy study (clinical history, SPT/SPPT, food-specific IgE levels, and open oral challenge tests, in some cases). We performed 32 open oral challenges in 27 volunteers, which were clearly positive in 17 of them: isolated OAS in 5 cases; OAS in association with diarrhoea and colicky abdominal pain in 2 cases; vomiting and diarrhoea in 4 cases; isolated generalised urticaria in 2 cases; generalised urticaria and angioedema of lips in 1 case; generalised urticaria and mild dyspnea in 1 case. All of these cases occurred 15–30 min after the onset of the tests; finally, there were 2 cases of delayed reaction: 1 involving colicky abdominal pain and diarrhoea starting 9–12 h after the challenge, and 1 consisting of mild urticarial rash and itchy skin

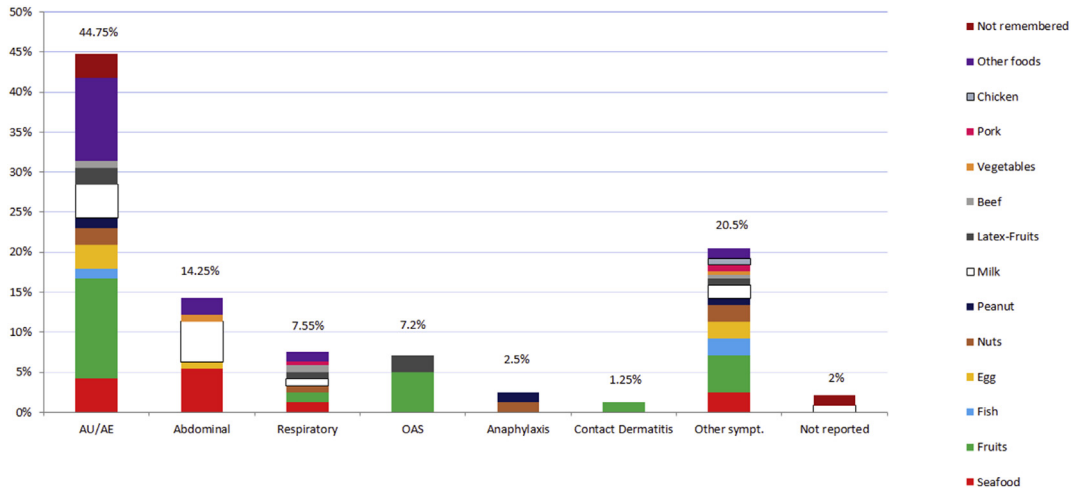

Fig. 3 Distribution of self-reported symptom frequency by food type (Values in %); number of episodes = 239

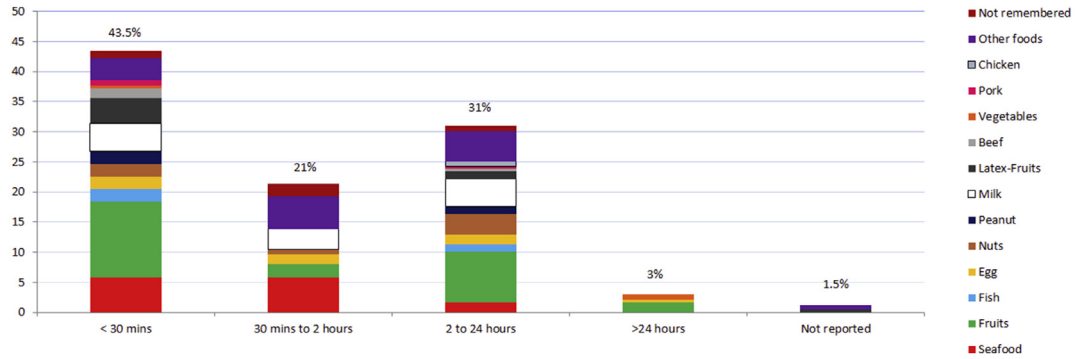

Fig. 4 Self-reported time until development of symptoms upon food ingestion (Values in %); number of episodes = 239

which started 12 h after the test. No cases of delayed anaphylaxis were identified.

Upon completion of the study, 24 adolescents (1 had already been diagnosed IgE-mediated milk allergy in another hospital) were diagnosed an AFR with an immunological basis (24/1702; 1.41% of the total number of adolescents that filled in the questionnaire; 95% CI = 0.90–2.03%; mean age: 15.1 years, median age: 15 years, 54.1% female), and a probable IgE-mediated mechanism was detected in 21 of them (21/1702; l.23%; 95% CI: 0.67–1.72%.) (Table 1 shows the results for the newly diagnosed adolescents).

Most frequently implicated foods were fresh fruits (30.8%). Most of these belonged to the *Rosaceae* family (80% of cases) – apple, pear, strawberry, and/or plum. Banana (*Musaceae*) and/or melon (*Cucurbitaceae*) were involved in 10% of cases, and orange and/or tangerines (*Rutaceae*) in 8% of cases; in the remaining cases, patients reported multiple sensitisations to these fruits. Other reported foods mostly included shellfish (26.9%, mainly crustaceans), nuts (23% walnut, cashew and hazelnut), peanut and milk (7.7% each), and egg (3.8% each). In the 20 cases in which an IgE-mediated association was newly found, specific IgE levels to implicated foods as well as Phadiatop were positive in all of them, and in addition, the mean total IgE serum levels were higher than compared with the group with non-IgE-mediated reactions (265.78 KU_A/L versus 63.93 KU_A/L, respectively; $p < 0.001$; Mann-Whitney *U* Test), Of the 3 adolescents in whom no IgE-associated mechanism was demonstrated, only 2 were atopic, 1 with a positive Phadiatop® test and 1 with positive SPT to aeroallergens. SPT performed with commercial food extracts were positive in 19 foods out of 22 in the group of adolescents with an IgE-associated mechanism and in 3 out of 9 foods tested in the non-IgE associated cases (general test sensitivity of 66.7%, specificity

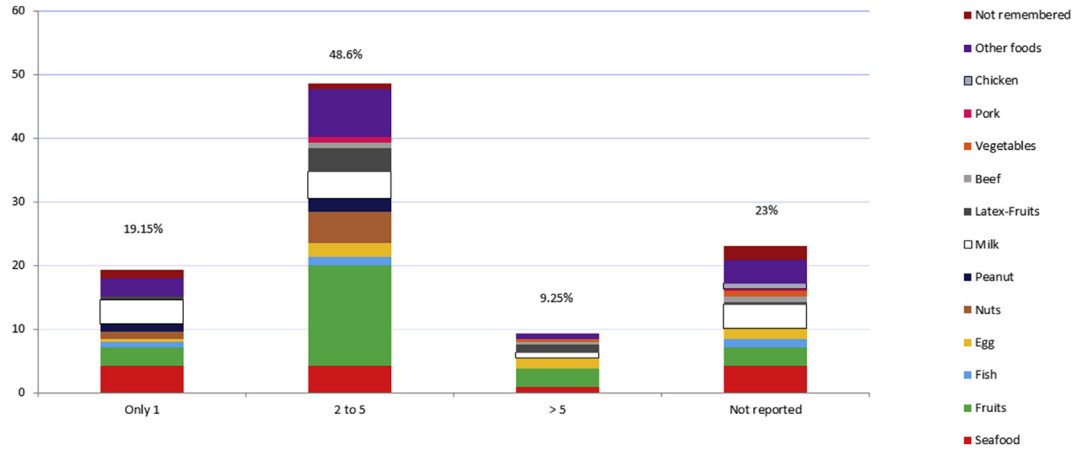

Fig. 5 Self-reported number of episodes induced by the same food (Values in %); number of adolescents (cases) = 183

Patient ID	Age	Sex	IgE levels (KUA)	Food Specific IgE	Personal History of atopy	Family History of atopy	SPT aero allergens	Sensitisation to more than one foodstuff	Foodstuff	Symptoms	Time to symptom development	Similar episodes with the same food	SPT with Commercial food extracts	Food Prick by Prick skin test	Open Food Challenge	Allergy mechanism
#1	12	M	269	POS	Yes	No	POS	No	Other tree nuts	Anaphylaxis	<30 mins	2 to 5	POS	POS	Not performed	IgE mediated
#2	12	M	69	Negative	Yes	No	Negative	No	Milk	Other symptoms	>24 h	>5	Negative	Not performed	POS	Non IgE mediated
#3	12	F	258	POS	Yes	Yes	POS	Yes	Peanut, Egg	UA/AE	<30 mins (peanut),	Only 1	POS (peanut), Negative (egg)	POS (peanut),	Not performed	IgE mediated (only for peanut)
											2-24 h (Egg)			Negative (Egg)		
#4	13	M	529	POS	Yes	Yes	POS	No	Fruits	OAS	<30 mins	>5	Negative	POS	Not performed	IgE mediated
#5	15	F	230	POS	Yes	Yes	POS	No	Seafood	UA/AE	<30 mins	2 to 5	Negative	POS	POS	IgE mediated
#6	15	F	92	POS	Yes	Yes	POS	No	Other tree nuts	Anaphylaxis	<30 mins	Only 1	POS	POS	Not performed	IgE mediated
#7	15	F	86	POS	Yes	Yes	POS	No	Fruits	OAS	<30 mins	>5	POS	POS	Not performed	IgE mediated
#8	18	F	114	POS	No	No	POS	No	Fruits	UA/AE	30 mins to 2 h	>5	POS	POS	POS	IgE mediated
#9	19	M	303	POS	Yes	Yes	POS	No	Other tree nuts	UA/AE	30 mins to 2 h	Only 1	POS	Not performed	Not performed	IgE mediated
#10	16	M	164	POS	Yes	Yes	POS	No	Fruits	OAS	<30 mins	2 to 5	POS	POS	POS	IgE mediated
#11	16	M	233	POS	Yes	Yes	POS	No	Fruits	OAS	<30 mins	2 to 5	POS	POS	POS	IgE mediated
#12	16	M	238	POS	Yes	No	POS	No	Fruits	OAS	<30 mins	>5	POS	POS	POS	IgE mediated
#13	14	F	112	POS	Yes	Yes	POS	No	Fruits	OAS	<30 mins	>5	POS	POS	POS	IgE mediated
#14	17	F	279	POS	Yes	Yes	POS	No	Seafood	Anaphylaxis	<30 mins	Only 1	POS	POS	POS	IgE mediated
#15	16	F	1686	POS	Yes	Yes	POS	No	Seafood	Anaphylaxis	<30 mins	Only 1	POS	POS	POS	IgE mediated

(continued)

Patient ID	Age	Sex	IgE levels (KUA)	Food Specific IgE	Personal History of atopy	Family History of atopy	SPT aero allergens	Sensitisation to more than one foodstuff	Foodstuff	Symptoms	Time to symptom development	Similar episodes with the same food	SPT with Commercial food extracts	Food Prick by Prick skin test	Open Food Challenge	Allergy mechanism
#16	16	M	88,5	POS	Yes	No	POS	No	Seafood	UA/AE	<30 mins	2 to 5	POS	POS	POS	IgE mediated
#17	15	F	112,7	POS	Yes	Yes	POS	No	Seafood	UA/AE	<30 mins	2 to 5	POS	POS	POS	IgE mediated
#18	17	M	131,2	POS	Yes	No	POS	Yes	Peanut, Seafood	Peanut: OAS Seafood: UA/AE	<30 mins	2 to 5	POS (peanut & seafood)	Not performed	POS (seafood) Negative (peanut)	IgE mediated (only for seafood)
#19	14	F	127,9	POS	Yes	Yes	POS	No	Seafood	UA/AE, Abdominal	<30 mins	2 to 5	POS	POS	POS	IgE mediated
#20	15	F	34,9	Negative	No	No	POS	No	Other tree nuts	Respiratory	2-24 h	2 to 5	POS	Negative	POS	Non IgE mediated
#21	14	F	114,4	POS	Yes	Yes	POS	No	Other tree nuts	UA/AE	<30 mins	2 to 5	POS	Not performed	POS	IgE mediated
#22	16	M	87,9	Negative	No	No	Negative	No	Other tree nuts	UA/AE	2-24 h	2 to 5	Negative	POS	POS	Non IgE mediated
#23	15	M	148	POS	Yes	Yes	POS	No	Fruits	OAS	<30 mins	>5	POS	Not performed	POS	IgE mediated

Table 1. (Continued) Characteristics of newly diagnosed Food Allergic patients

of 100%, PPV: 100%, NPV: 86.4%). No differences between commercial extracts were found. Fresh food SPPT were positive in 13 out of 15 cases in the group of adolescents with an IgE-associated mechanism and only 1 in the non-IgE associated cases (general test sensitivity of 87.5%; specificity: 100%, PPV: 100%, NPV: 91.7%).

The most prevalent symptoms in all studied cases were cutaneous (40% of cases), followed by OAS (32%) and anaphylaxis (16%), with the latter being associated with the ingestion of nuts and shellfish (2 cases each). Only in the 3 cases which were not IgE-associated were the symptoms delayed, appearing more than 2 h after ingestion, since in all cases with an IgE-association, symptoms appeared in less than 2 h upon ingestion.

Of all the adolescents who finished the study at the hospitals (81 individuals), 65 cases (80.2%) needed treatment for their symptoms, mostly at an emergency department. However, only 6 individuals with a diagnosis of probable food allergy (4 with anaphylaxis, 1 with respiratory symptoms, and 1 with cutaneous symptoms) sought medical attention. A high proportion of cases diagnosed with food allergy (either IgE- or non-IgE-associated) reported the presence of personal and/or family history of atopy.

No significant association factors were seen between sex, age, locality of origin (rural vs urban areas), type of food, and time elapsed since the latest reaction. In the same way, we found no significant association between severity of the food-induced reaction and total serum IgE levels.

DISCUSSION

Our study determined, for the first time in Portugal, the prevalence of probable food allergy, the type of implicated foods, types of symptoms, and other associated factors in an adolescent population. We have shown that the prevalence of probable food allergies in this population is low, is mostly related to fresh fruits and nuts, and most frequently involves cutaneous symptoms.

The initial, written questionnaire showed that the values of self-reported food allergy (11.01%) were within the range described in other population-based studies (3–40%).[9,12–16,22,30–33] Similarly, the value obtained in our study for the prevalence of probable food allergy (1.41%) is similar to that reported for adolescents in the United States (2,5%)[23,34] and Europe (0.5%–3.5%),[15,33] although the latter values were obtained after performance of single or double-blind oral challenge tests.[13,14,17,30,33,35]

This discrepancy in prevalence results between self-reported and medically confirmed data (using *in vitro* and *in vivo* tests and/or oral challenge) has been described. Previous studies have shown that self-reports tend to overestimate food allergies.[12–15,17,22,30,33,36–38] This discrepancy may be partly due to an information bias based upon an enhanced self-perception of symptoms which are wrongly ascribed to food ingestion. Cultural factors, health literacy, or accessibility to a medical diagnosis may be involved[15,33] (in our study, only 16% of the adolescents that reported food-associated symptoms had ever seen an allergist for that reason). Nevertheless, prevalence values across different studies are hardly comparable, given the heterogeneity of study designs and the types of population involved. In any case, the overestimation of self-reported food-related adverse food reactions may be worrying since it is frequently associated with inappropriate restriction diets with subsequent nutritional deficits.[3]

The implicated foods, both in self-reported allergies as well as in test-confirmed, probable food allergies, in our study are included in the so-called "big eight allergens"[37] and are similar to those found in other population-based studies using similar methodology in Europe,[9,13,14,16,17,22,30,39,40] Asia,[38,41] and the United States.[10,23,31,32,42,43] However, fresh fruits being the most frequently implicated foods, places our study in line with those performed in western and Mediterranean Europe,[16,17,22] but not with those from northern Europe, North America[9,10,13,14,23,30,31,43,44] or, surprisingly, the eastern Mediterranean Europe,[33] probably due to differences in study methodology. In fact, these differences may be partly due to different food habits[38] or concurrent pollen sensitisation, although we cannot exclude the possibility that the comparatively smaller size of our sample may have influenced our results. On the other hand, it is also fundamental to stress that, in contrast with

our study, OAS is often not regarded as a symptom of food allergy, since it is frequently associated with pollen-induced respiratory symptoms in the same patient, as happened in our study, and is therefore regarded as a "secondary allergy" by various research groups.[30,35,45]

It is also important to highlight the discrepancy between the panels of implicated foods when we compared self-reported results with those obtained upon completion of the allergy study. Whereas the self-reported panel mainly included fresh fruits, milk, and shellfish, the confirmed (post-tests) panel essentially identified fresh fruit and nuts. Other studies have also identified similar situations in adolescents in Europe[15,16,22,30,35] and this has been confirmed by meta-analyses,[12,13,45] having such discrepancies partly ascribed to differences in the concept of adverse food reactions between patients and specialist doctors. This highlights the need for an adequate diagnostic approach to food-associated symptoms, so that subsequent detrimental situations may be averted or better controlled.[46] These include inadequate diets,[3] difficulties in the reintroduction of the "culprit" food in case allergy was not confirmed,[47] stress and anxiety because of eventual accidental ingestion of suspected foods,[2,4,48] or even bullying at school.[49]

Cutaneous symptoms were the most prevalent ones in our study, both in self-report and in those adolescents who completed the full allergy workup. This is in agreement with results from most other groups.[10,11,13,16,22,23,30,35,37,43]

An interesting aspect of our work was the analysis of data obtained from the self-reported symptoms. We found several possible associations between the ingestion of certain foods and the development of certain symptoms (fresh fruits, milk, and egg in relation to cutaneous manifestations, shellfish in relation to abdominal symptoms, fresh fruits in connection with OAS, and nuts and peanuts in anaphylaxis), associations which, except for the latter 2, had not been previously reported. On the other hand, bearing in mind the timeframe for the appearance of symptoms, we found 2 predominant response patterns, previously identified by Osterballe:[30] an immediate type of reaction, arising in less than 30 min post-ingestion, and a more delayed, between 2 and 24 h post-ingestion, mainly associated with fresh fruits and milk, in both cases.

As far as adolescents with probable allergy are concerned, we also found an inverse relationship between symptom latency time and symptom severity. In addition, anaphylaxis cases were all associated with the ingestion of nuts and peanut. It should be stressed that the 3 non-IgE mediated cases had a latency time longer than 2 h and most IgE-mediated cases had developed within 30 min upon ingestion.

We also analysed eventual risk factors associated with food allergies. Multivariate analysis showed that a personal or a family history of atopy were significantly associated with a higher risk of having food allergies, as has been described in previous studies and metanalyses focusing on adolescents and children.[1,10,13,14,50]

One of the limitations of the present study was the fact that we could not perform double-blind, placebo-controlled food challenges, a test which is regarded as the "gold standard" for the final diagnosis of food allergy. In spite of this, the current report is the first population-based study in Portuguese adolescents. Furthermore, it yields information on probable food allergy in this population, based upon not only a positive clinical history/questionnaire, but also on diagnostic tests including SPT, food-specific IgE levels, and open oral food challenges (particularly in cases that were clinically less clear or in which there were diagnostic doubts), which makes it a very thorough study. In fact, a high proportion of population-based studies on food allergies performed in other countries only applied a questionnaire,[16,30,35,37,43] and others only performed SPT or determination of food-specific serum IgE in suspect cases of food allergy.[9,17,38]

Another possible limitation of our study concerns the fact that 25.1% of the adolescents who reported adverse food reactions did not complete the study, which is partly explained by the clear national increase in the "Healthcare service usage" fees, during the implementation of the study. In addition, an increase in unstable employment during the period of the study limited absences from work by parents accompanying the adolescents in hospital visits. A relatively high drop-out rate and low participation are indeed limiting

factors in population-based epidemiological studies, and this has been reported in multiple studies, with the reply rate being inversely associated with the magnitude of the study. The reply rate has varied between 40–50%[14,22,30,35] and 61–86%[9,15,16] when studies are only based upon questionnaires, and lower[22,35] when a more thorough assessment (skin tests, blood tests, food challenges) is involved, with a Turkish study being the only exception we found.[33] Nevertheless, our reply rate was quite acceptable for this type of study (57.3%). In spite of the relative limitations in our study, the size of our sample and the features of our work reached the predefined values in terms of statistical power, representativity, and proportionality for analysis. Relatively low reply rates may also lead to a selection bias, mainly with people who are more concerned about allergy problems and more prone to returning the questionnaire. However, if this were the case, we would expect to find high self-report prevalence rates, in comparison with other studies, and this was not the case. Finally, in order to more firmly extrapolate our results to other regions of Portugal, further studies carried out in other regions of the country, as well as a nationwide, multicentre study, are warranted.

CONCLUSIONS

The prevalence of probable food allergy in our sample of Portuguese adolescents was low. Fresh fruits, shellfish, nuts, and peanut were the main implicated foods, and the most frequently reported symptoms were cutaneous. There was a clear discrepancy between self-reported and probable food allergy, both in terms of prevalence and of the implicated foods.

This study is the first step towards a thorough study of food allergies and their repercussions in Portugal and may contribute towards a global characterization of food allergies in adolescents in Europe.

Abbreviations

AFR: Adverse Food Reactions; CI: Confidence Interval; IgE: Immunoglobulin E; NPV: Negative Predictive Value; OAS: Oral Allergy Syndrome; OR: Odds ratio; PPV: Positive Predictive Value; SPT: Skin prick test; SPPT: Skin prick-prick test

Consent for publication

All authors gave their written consent for publication.

Author' contributions

CLI and LTB conceived and coordinated the study and participated in its design. CLI prepared the first draft and performed the clinical approach. LTB helped, reviewed and translated to draft the manuscript. SMN performed the statistical analysis. AR and LTB performed *in vivo* and food oral challenges tests. PF performed *in vitro* tests. OL and AMF applied the questionnaires. All authors read and approved the final version of this manuscript.

Availability of data and materials

Please contact corresponding author for primary data requests.

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Ethical aspects

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committees of the Amato Lusitano Hospital, the Administrative Sub-Region of Health of Castelo Branco and the University of Beira Interior. It was also approved by the Ministry of Education (DGIDC, Reg. Nº 0266300001 from January 2012). All volunteers and their legal guardians/parents gave their written informed consent.

Declaration of Competing Interests

The authors declare no conflicts of interest.

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Appendix A.Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2020.100453.

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PROTOCOL SPECIFICALLY FOLLOWED FOR ORAL FOOD CHALLENGES IN PATIENTS INCLUDED IN OUR STUDY

Lozoya- Ibáñez C, et al. Prevalence and clinical features of adverse food reactions in Portuguese adolescentes

This protocol followed the protocol recommended by EAACI (Bindslev-Jensen et al, 2004), and endeavoured to reach a "standard portion" (Boyce et al, 2010)

- "Big-sized" fruits (apple, pear, banana, orange, peach, melon, watermelon…).
    - Standard portion: 1 full piece of fruit (for melon and watermelon: 1 "average" slice).
    - Time interval between administrations: 20 minutes
    - Administration protocol (1 = 1 full piece of fruit).
        - 1/8 - 1/8 – ¼ - ½
- "Middle-sized" fruits (strawberry, plum, appricot…)
    - Standard portion: 2-4 pieces
    - Time interval between administrations: 20 minutes
    - Administration protocol (1 = 1 full piece of fruit).
        - 1/8 - 1/8 – ¼ - ½ - 1 (2 pieces)
        - ½ - ½ -1 – 2 (4 pieces)
- "Small-sized" fruits (blueberry, raspberry, blackberry…)
    - Standard portion: 6-10 pieces
    - Time interval between administrations: 20 minutes
    - Administration protocol (1 = 1 full piece of fruit).
        - 1 – 2 – 3 (6 pieces)
        - 1- 3 – 6 (10 pieces)

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***
Lozoya-Ibáñez C, et al. Prevalence and clinical features of adverse food reactions in Portuguese adolescents

	Item Nº	Recommendation	Page Nº
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6
Bias	9	Describe any efforts to address potential sources of bias	4,5
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7-9
		(b) Give reasons for non-participation at each stage	7-9
		(c) Consider use of a flow diagram	Fig 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	9
Outcome data	15*	Report numbers of outcome events or summary measures	7-9

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7,8
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12,13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.