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Nickel allergy in lipid transfer protein sensitized patients: Prevalence and clinical features

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Abstract

Nickel (Ni), the main responsible for allergic contact dermatitis worldwide, is also involved in systemic condition called "Systemic Nickel Sulfate Allergy Syndrome (SNAS)." Likewise, IgE-mediated reactivity to Lipid Transfer Protein (LTP) represents the main cause of primary food allergy in adults of Mediterranean countries. We evaluated the prevalence of SNAS in LTP allergic patients and investigated patients' clinical features with double sensitization (LTP and Ni). A retrospective, single-center, observational study was conducted performing a complete allergological work-up including: (1) skin prick tests; (2) serum specific IgE for plant food allergens and rPru p3 (LTP); (3) patch test with 5% Ni sulfate in petrolatum. We enrolled 140 LTP allergic patients of which 36 patients (25.7% of sample) showed additional positivity to Ni patch test. Patients with double sensitization were more frequently females and reported fewer cutaneous symptoms. Higher values of sIgE for peach, apple, peanut, walnut, grain, corn, and garlic were found in LTP allergic patients, while higher values for hazelnut in the other subgroup. The prevalence of SNAS in the LTP allergic population is clinically relevant. Moreover, the clinical and immunological profiles of patients with double sensitization were different from patients monosensitized to LTP.

Keywords

food allergy, lipid transfer protein, Nickel, prevalence, rPru p3, systemic Nickel sulfate allergy syndrome

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Introduction

IgE-mediated reactivity to Lipid Transfer Proteins (LTP) are a group of highly conserved proteins primarily found in fruits. They represent the main cause of primary food allergy in adults of Mediterranean countries,[1,2] with a prevalence of 9.5% in Italy.[3] LTP is a highly conserved protein from a phylogenetic point of view[4] and widely diffused in plants for its involvement in the defense of plants against fungi and bacteria.[5] Therefore, the homologous form of the protein is found in a very wide spectrum of foods, even if botanically

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unrelated to each other[6,7]; but mainly in fruit peels of the *Rosaceae*,[8] and can be considered a "panallergen."[9–13]

LTPs are characterized by thermo- and gastrostability, due to their three-dimensional structure with 4 α-helices joined by 4 disulfide bridges,[14] which give the protein the ability to cause severe allergic reactions up to anaphylaxis.[15]

Clinical expression of allergic reactions to LTP sometimes requires cofactors: nonsteroidal antiinflammatory drugs (NSAIDs) intake,[16] alcohol ingestion, physical exercise,[17] or fasting.[18] It seems also that PR-10 and/or profilins contemporary sensitization could prevent severe reactions to LTP.[19]

Nickel (Ni) is a ubiquitous metal present in soil, water, air and is widely used for industrial purposes[20] owing to its high ductility, resistance to oxidation and corrosion, high melting point and low cost.[21] This highly sensitizing metal is the main allergen responsible for allergic contact dermatitis worldwide. Contact or cell-mediated allergy (type IV) to Ni Sulfate has an estimated prevalence of around 8% to 19% in adults with a strong predominance in women compared with men (4–10 times).[21]

About 20% of patients with Ni contact dermatitis can develop, after ingesting nickel containing foods, a clinical syndrome called systemic nickel sulfate allergy syndrome (SNAS),[22] which is characterized by systemic involvement causing urticaria, angioedema, erythema, generalized itching, gastrointestinal disorders, bloating, dyspepsia, abdominal colic, vomiting and/or diarrhea, gastroesophageal reflux symptoms, headache, and cough. Several Ni desensitization protocols have been adopted foreseeing oral administration of the metal with positive results on the reduction of SNAS symptoms and on the modulation of inflammatory parameters.[23] The variegated symptomatological expression of a systemic allergy to Ni creates problems of differential diagnosis with other pathological conditions, which mimic symptoms or are often associated with Ni allergy.[24–30]

In 2017, a study explored the associations between SNAS and Irritable Bowel Syndrome (IBS) underlining that IBS gastrointestinal disorders are similar to those caused by the ingestion of Ni-containing foods in SNAS patients (nausea, heartburn, bloating, abdominal pain, diarrhea, constipation).[31] Furthermore, growing evidence[32] suggests that IBS patients have reduced intestinal barrier function associated with a mild degree of inflammation of the mucosa. Similarly, Ni allergy is associated with a dysregulation of the immune system with a prevalent immunosuppressive action.[33,34]

According to our knowledge, no study investigated the association between Ni sulfate sensitization and LTP allergy, despite their high prevalence and common triggering effects represented by ingestion of plant foods.

The aim of this study was to evaluate the prevalence of Ni sensitization in LTP allergic patients and, secondly, to investigate clinical features of patients with double sensitization (LTP and Ni).

Methods

Study design

Primary outcome of this retrospective, single-center, observational study was to assess the prevalence of Ni sensitization in LTP allergic patients. Secondary outcomes were: (1) the description of clinical features of both LTP and Ni sensitized patients (type of adverse reactions to LTP containing plant foods: oral allergy syndrome, cutaneous symptoms such as contact urticaria, gastrointestinal and respiratory symptoms and anaphylaxis; plant foods mainly involved in adverse reactions; positivity of allergological exam to plant foods; patch test results); (2) evaluation of Ni as possible "cofactor" for the clinical expression of LTP allergic reactions.

Ethical approval

The Ethics Committee of our hospital approved this study. All enrolled patients signed the informed consent to participation in the study. From January to June 2019, we collected data from all patients who satisfied selection criteria.

Sample population

We adopted inclusion and exclusion criteria to select LTP allergic patients to reduce confounding effects related to medical conditions or drugs. We enrolled patients with: (a) suggestive clinical history of adverse reactions to cross-reacting LTP plant foods, b) confirmed diagnosis of LTP allergy. Exclusion criteria were: (a) age <18 years and >75 years, (b) other relevant systemic diseases, (c) concomitant

treatment with steroids and/or antihistamines, (d) pregnancy, lactation; (e) inability to give written informed consent.

We calculated a sample size of 138 patients, considering (1) an error estimate of 5%, (2) a confidence level of 95% and (3) a presumed prevalence of systemic allergy to Ni Sulfate of 10% in a patient cohort suffering from food allergy to LTP and a prevalence rate of 20% of SNAS in an Italian population.[35]

Skin test and double sensitization

All patients underwent a comprehensive allergological evaluation including: (1) skin prick tests (SPTs) performed with plant food allergens extracts—garlic, apricot, pineapple, peanut, orange, oats, banana, carrot, cabbage, chestnut, cherry, cocoa, kiwi, onion, strawberry, bean, wheat, lettuce, brewer's yeast, corn, almond, apple, hazelnut, walnut, barley, potato, pear, peach, pepper, pea, tomato, plum, rice, sunflower seeds, sesame, celery, soy and spinach (Lofarma, Milan, Italy)—and fresh plant foods—black cherry, asparagus, broccoli, khaki, raspberry, lemon, aubergine, melon and grapes—LTP extract (Alk-Abellò 30 µg/ml, Milan, Italy) with a positive (histamine 10 mcg/ml) and negative (saline solution) control on the volar surface of the forearm according to the EAACI recommendations[36]; (2) serum specific IgE for plant food allergens and rPru p3 (LTP), performed with the immuno-assay method (UniCAP System; Pharmacia, Uppsala, Sweden) considered positive for values greater than 0.35 U/ml; (3) patch test with 5% Ni sulfate ($NiSO_4$) in petrolatum (Hermal, Hamburg, Germany) according to International Contact Dermatitis Research Group guidelines.[37] Patch tests were evaluated 72 h after their application and were considered positive if an eczematous-vescicular reaction occurred at the contact site with the allergen. The intensity was assessed with the following criteria[38]: (1) ±, faint and non-palpable erythema; (2) +, palpable erythema; (3) ++, strong infiltrate, numerous papules, vesicles present, and strong reaction; and (4) +++, coalescing vesicles, bullae or ulceration extreme reaction erythema.

Statistical analysis

The socio-demographic and clinical characteristics of the studied population were reported as means and standard deviations for continuous variables and as frequencies and percentages for categorical variables.

The statistical analysis was performed by means of Chi-square test and multivariate analysis of the variance. $p < 0.05$ were considered significant. The statistical analysis was performed using SPSS version 16.0.

Results

Participants

We enrolled 140 patients with confirmed diagnosis of LTP allergy. Thirty-six (25.7%) patients showed positive Ni patch test. Whereas, the remaining 104 (74.3%) patients showed negative results. The first group represents the prevalence of Ni sensitized patients within total population of LTP allergic patients enrolled. The demographic and clinical findings of total population and two subgroups are described in Table 1.

The mean age of LTP allergic patients was 35 years (±12) with prevalence of female gender in LTP allergic patients with concomitant Ni sensitization (77.8% versus 55.8%, $p = 0.0194$, Chi-Square Test). Except for a higher prevalence of cutaneous symptoms in subgroup of LTP allergic patients (81.4% vs 63.9%, $p = 0.0326$, Chi-Square Test), the subgroups were homogeneous.

Symptoms

The predominant symptoms reported by study population were cutaneous, especially contact urticaria (76.8%) followed by dyspnoea and bronchospasm (40.3%), gastrointestinal disorders (diarrhea, nausea, vomiting (37.7%)) and oral allergic syndrome (35.3%).

Foods involved in adverse reactions

We evaluated retrospectively foods of plant origin most frequently associated with adverse reactions: peach (40%) and dried fruits: walnut 33.1%, peanut 29.5%, hazelnut 26.1% (Table 2). A comparative analysis of the two subgroups (Figure 1) showed that some foods were significantly more involved in adverse reactions in patients with concomitant sensitization to LTP and Ni: pineapple and corn (11.1% vs 2.9%, $p = 0.0509$, the Mann–Whitney U test), celery (5.6% vs 0%, $p = 0.0155$, the Mann–Whitney U test). Conversely, walnut was more frequently involved in adverse reactions in patients

Table 1. Demographic characteristics of the study population.

		Total Sample	LTP	LTP + Nickel	p value
Patients	[N°]	140	104	36	
Age	[M (years), SD]	35 (±12)	34 (±12)	39 (±12)	NS†
Sex (female)	[N°, %]	86 (61.4)	58 (55.8)	28 (77.8)	0.0194‡
Food related adverse reactions					
AD*	[N°, %]	10 (7.1)	7 (6.7)	3 (8.3)	NS‡
OAS^	[N°, %]	49 (35.3)	33 (32)	16 (44.4)	NS‡
Cutaneous symptoms	[N°, %]	106 (76.8)	83 (81.4)	23 (63.9)	0.0326‡
GI' symptoms	[N°, %]	52 (37.7)	37 (36.3)	15 (41.7)	NS‡
Respiratory symptoms	[N°, %]	56 (40.3)	43 (41.7)	13 (36.1)	NS‡
Anaphylaxis	[N°, %]	12 (8.6)	9 (8.7)	3 (8.3)	NS‡

AD*: atopic dermatitis; OAS^: oral allergic syndrome; GI': gastro-intestinal; †: Difference between two subgroups (TLP versus LTP + Nickel) was tested with Two Sample T Test.
‡Differences between two subgroups (LTP versus LTP + Nickel) was tested with Chi-Square Test.

Table 2. Foods involved in adverse reactions.

	Total Sample	LTP	LTP + Nickel	p value‡
	[N° of patients (% of sample/group)]			
Almond	26 (18.7)	21 (20.4)	5 (13.9)	NS
Apple	18 (12.9)	15 (14.4)	3 (8.3)	NS
Apricot	7 (5.0)	4 (3.8)	3 (8.3)	NS
Asparagu	1 (0.7)	1 (1.0)	0 (0.0)	NS
Aubergine	12 (8.6)	7 (6.7)	5 (13.9)	NS
Banana	14 (10.0)	13 (12.5)	1 (2.8)	NS
Barley	5 (3.6)	4 (3.8)	1 (2.8)	NS
Bean	5 (3.6)	2 (1.9)	3 (8.3)	NS
Black cherry	0 (0.0)	0 (0.0)	0 (0.0)	-
Blackberry	2 (1.4)	1 (1.0)	1 (2.8)	NS
Broccoli	1 (0.7)	0 (0.0)	1 (2.8)	NS
Cabbage	1 (0.7)	1 (1.0)	0 (0.0)	NS
Carrot	4 (2.9)	3 (2.9)	1 (2.8)	NS
Celery	2 (1.4)	0 (0.0)	2 (5.6)	**0.0155**
Cherry	13 (9.3)	10 (9.6)	3 (8.3)	NS
Chestnut	0 (0.0)	0 (0.0)	0 (0.0)	-
Corn	7 (5.0)	3 (2.9)	4 (11.1)	**0.0509**
Garlic	5 (3.6)	2 (1.9)	3 (8.3)	NS
Grape	10 (7.1)	8 (7.7)	2 (5.6)	NS
Hazelnut	36 (26.1)	26 (25.5)	10 (27.8)	NS
Khaki	0 (0.0)	0 (0.0)	0 (0.0)	-
Kiwi	14 (10.0)	10 (9.6)	4 (11.1)	NS
Lemon	0 (0.0)	0 (0.0)	0 (0.0)	-
Lettuce	16 (11.4)	9 (8.7)	7 (19.4)	NS
Melon	5 (3.6)	2 (1.9)	3 (8.3)	NS
Oat	1 (0.7)	0 (0.0)	1 (2.8)	NS
Onion	5 (3.6)	3 (2.9)	2 (5.6)	NS
Orange	4 (2.9)	3 (2.9)	1 (2.8)	NS
Pea	6 (4.3)	3 (2.9)	3 (8.3)	NS
Peach	56 (40.0)	41 (39.4)	15 (41.7)	NS
Peanut	41 (29.5)	32 (31.1)	9 (25.0)	NS
Pear	4 (2.9)	2 (1.9)	2 (5.6)	NS
Pepper	2 (1.4)	1 (1.0)	1 (2.8)	NS
Pineapple	7 (5.0)	3 (2.9)	4 (11.1)	**0.0509**
Plum	7 (5.0)	5 (4.8)	2 (5.6)	NS
Raspberry	3 (2.1)	3 (2.9)	0 (0.0)	NS
Rice	11 (7.9)	6 (5.8)	5 (13.9)	NS
Sesame	2 (1.4)	1 (1.0)	1 (2.8)	NS
Soy	3 (2.1)	3 (2.9)	0 (0.0)	NS
Spinach	6 (4.3)	5 (4.8)	1 (2.8)	NS
Strawberry	11 (7.9)	6 (5.8)	5 (13.9)	NS
Sunflower seeds	3 (2.1)	2 (1.9)	1 (2.8)	NS
Tomato	30 (21.7)	22 (21.6)	8 (22.2)	NS
Walnut	46 (33.1)	39 (37.9)	7 (19.4)	**0.0432**
Wheat	21 (15.1)	16 (15.5)	5 (13.9)	NS

(Continued)

Table 2. (Continued)

‡Differences between two subgroups (LTP versus LTP + Nickel) was tested with Chi-Square Test.
The data in bold are significant.

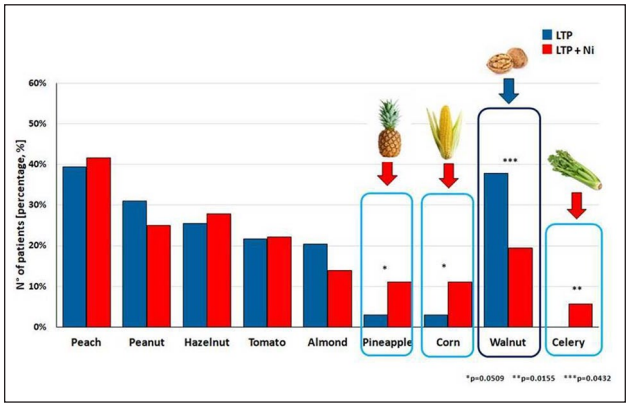

Figure 1. A comparative analysis of the two subgroups (LTP versus LTP + Ni): foods significantly more frequently involved in adverse reactions [the Mann–Whitney U test].

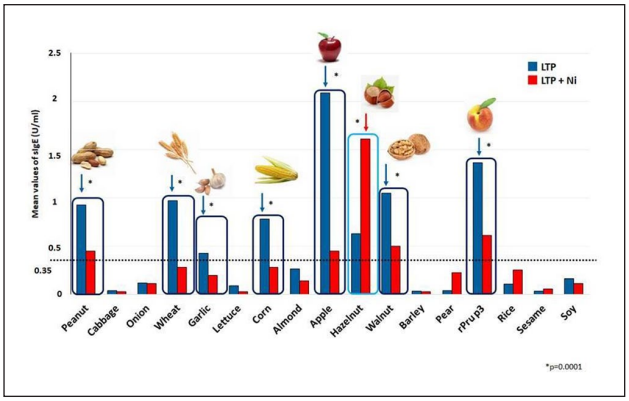

Figure 2. A comparative analysis between the two subgroups (LTP versus LTP + Ni) of mean serum levels of sIgE using Two Sample T Test and considering 0.35 U/ml as cut-off above which the value is positive.

Table 3. Serum specific IgE for plant food allergens.

	Total sample	LTP	LTP + Nickel	*p* value†
	[Mean, kUA/l]			
Apple	1.66	**2.08**	**0.44**	**0.0000**
Corn	0.65	**0.77**	0.27	**0.0000**
Garlic	0.36	**0.42**	0.19	**0.0000**
Hazelnut	0.88	**0.62**	**1.61**	**0.0000**
Oat	0.26	0.35	0.00	NS
Peach	1.17	**1.36**	**0.61**	**0.0000**
Peanut	0.80	**0.92**	**0.44**	**0.0000**
Tomato	0.88	1.08	0.31	NS
Walnut	0.91	**1.04**	**0.50**	**0.0000**
Wheat	0.79	**0.91**	0.28	**0.0000**

†Difference between two subgroups (LTP versus LTP + Nickel) was tested with Two Sample T Test.
The data in bold are significant.

monosensitized to LTP (37.9% vs 19.4%, $p = 0.0432$, the Mann–Whitney U test). Peach was the most frequently reported food in both subgroups (LTP group: 39.4%; LTP + Ni group: 41.7%) without significant differences in frequency.

In vivo and in vitro tests

Subsequently, we compared the results of skin prick test to plant food between the two subgroups with the Mann–Whitney U test, but no differences were found (data not shown).

Subsequently, we compared mean serum levels of sIgE between two subgroups using Two Sample T-Test considering 0.35 kUA/l as cut-off for positive values. Patients with single sensitization to LTP showed higher specific IgE values for the following foods: peanut (0.92 kUA/l vs 0.44 kUA/l, $p = 0.0000$), wheat (0.97 kUA/l vs 0.28 kUA/l, $p = 0.0000$), garlic (0.42 kUA/l vs 0.19 kUA/l, $p = 0.0000$), corn (0.77 kUA/l vs 0.27 kUA/l, $p = 0.0000$), apple (2.08 kUA/l vs 0.44 kUA/l, $p = 0.0000$), walnut (1.04 kUA/l vs 0.50 kUA/l, $p = 0.0000$) and peach (1.36 kUA/l vs 0.61 kUA/l, $p = 0.0000$). Conversely, the mean value of sIgE for hazelnuts was significantly higher in patients with concomitant sensitization to LTP and Ni (1.61 kUA/l vs 0.62 kUA/l, $p = 0.0000$) (Figure 2 and Table 3).

Discussion

Our data suggest: (1) a significant prevalence of SNAS in patients affected by LTP allergy (25% of our study population); (2) patients with concomitant sensitization to LTP and Ni are more frequently females, report fewer cutaneous symptoms (contact urticaria) compared with LTP allergic patients; (3) plant foods most frequently associated with adverse reactions, reported by patients with concomitant sensitization to LTP and Ni, are corn, pineapple and celery; (4) plant foods most frequently associated with adverse reactions, reported by LTP allergic patients, are walnuts; (5) there are significant differences of sIgE for LTP-containing foods between the two subgroups of patients with higher values for peach, apple, peanut, walnut, grain, corn and garlic in subgroup of LTP allergic patients and higher values for hazelnut in subgroup of patients with concomitant sensitization.

According to our knowledge, this is the first study exploring the prevalence of SNAS in LTP allergy. The high prevalence of SNAS in LTP allergic patient (25.7%) is clinically relevant, especially comparing it with prevalence of SNAS in patients with Ni contact dermatitis, which is around 20% according to literature.[35] In fact, it is well know that IV-type Ni allergy represents the first prerequisite to diagnose SNAS.

Additionally, several LTP-containing plant foods have a considerable proportion of Ni Sulfate (asparagus, cabbage, green bean, lentil, peanut, tomato and walnut)[39,40]; therefore, adverse reactions after ingestion of LTP foods might be "modulated" by the presence of Ni in the same food.

In fact, Ni may be considered a "cofactor" to determine clinical manifestations of LTP allergy with a possible "mitigating" or "amplifying" role,

similarly to already known cofactors described in literature: NSAIDs intake, alcohol ingestion, physical exercise and long fasting periods ("amplifying" factors),[41–43] PR-10 and/or profin sensitization ("mitigating" factors).[19,43,44]

Furthermore, the ingestion of Ni-rich foods in patients affected by SNAS determines histological modifications of gastrointestinal mucosa characterized by a massive CD4+ CD45R0+ T (memory) T lymphocytes infiltration and by a reduction of CD8+ T cells in the lamina propria and involves Th1 cytokines (INF-γ) and Th2 cytokines (IL-5 and IL-13) secretion in peripheral blood33,34. These data might suggest a dysregulation of the mucosal immune system. A similar modification of the cytokine pattern may affect the differentiation of CD4+ T lymphocytes in Th2 effector cells, determining clone-specific B lymphocytes ability to secrete IgEs to antigens. The high prevalence of female gender in LTP allergic patients with concomitant SNAS is consistent with literature reporting Ni contact dermatitis in the same gender.[21]

Our results stress the high frequency of gastrointestinal symptoms (meteorism, abdominal tension and pain, and disturbed defecation) in LTP allergic patients with concomitant SNAS. In fact, these clinical manifestations are peculiar for SNAS, which led us to study the association between LTP and Ni allergy. Curiously, our study showed that after the ingestion of vegetable foods, cutaneous symptoms, mainly contact urticaria, are more common in patients with a simple LTP allergy compared with patients with double sensitization to LTP and Ni. This finding is potentially unexpected, because it is known that in sensitized subjects, Ni is able to elicit cutaneous symptoms independent from direct skin contact, as demonstrated by some cases of generalized eczema and urticaria in patients with dental[45] and orthopaedic[46] protheses.

Regardless of the known widespread distribution of LTP[47] and Ni[48] in plants, LTP is a powerful inducer of serious systemic allergic reactions[41] because of its high thermal and gastro-resistance.[49–51] Furthermore, our data confirm that contact urticaria is one of most common manifestations of LTP hypersensitivity.[52,53]

To date, we can only speculate about a possible role of Ni as a "mitigating" factor determining clinical manifestations of LTP allergy.

Further data, supporting a possible "mitigating" role of Ni in patients with concomitant sensitization to LTP and Ni, derive from our observation of lower sIgE mean values for several LTP foods (peach, apple, peanut, walnut, grain, corn, and garlic) in patients sensitized to both LTP and Ni compared with LTP allergic patients.

Another equally unexpected result were higher mean levels of sIgE in LTP monosensitized patients compared with co-sensitized patients. Our data may help understanding the variability of symptoms in LTP allergy ranging from asymptomatic sensitization[54,55] to local oropharyngeal complaints and systemic reactions which might evolve to life-threatening anaphylaxis.[42,44]

Recently, Asero and coworkers[43] highlighted that sensitization profiles of LTP-allergic patients are extremely heterogeneous with possible high cross-reactivity among different LTPs. Furthermore, the levels of sIgE for non-*Rosaceae* foods are only partially predictive for clinical allergy due to a significant overlap between asymptomatic patients showing elevated levels and patients with a history of severe reactions showing rather low levels.[56]

On the other hand, the levels of IgE, specific for different food sources, follow a rather precise and predictable hierarchical order in LTP-allergic patients starting with peach, followed by apple, walnut, hazelnut, peanut, lentil, maize, soybean, tomato, kiwi, sesame, mustard, melon, and celery,[57] although clinical allergy symptoms are not necessarily parallel to corresponding foods.

This study has some limitations. First, the retrospective and monocentric nature of the study that describes a relevant cause of food allergy. Moreover, allergy diagnosis are not fully confirmed, because testing would require isolated administration of the suspected purified or recombinant LTPs. Moreover, up to now, challenges with LTP have not entered mainstream application mainly for practical reasons like unavailability of reliable and/or safe source material (purified or recombinant proteins).[58] Furthermore, due to the retrospective nature of the study, we were not able to consider confounding factors such as exercise, alcohol and NSAIDs or stress, menstruation or tiredness. Finally, it remains to be determined how Ni affects the reactivity towards offending foods in LTP allergic patients.

Conclusion

According to our knowledge, this was the first study in literature assessing prevalence, clinical manifestation and reactivity to culprit foods of LTP

allergic patients with concomitant sensitization to Ni in a large LTP allergic population.

Further prospective studies are necessary to explore possible mechanisms underlying this high prevalence of Ni sensitization in LTP allergy.

The prevalence of SNAS in LTP allergic population is clinically relevant. Moreover, the clinical and immunological profiles of patients with double sensitization were different from patients monosensitized to LTP.

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Authors' contributions

Conceptualization, A.R. and E.N.; methodology, A.R. and R.C.; formal analysis, R.I.; investigation, R.C. and V.C.; data curation, A.R. and R.C.; writing—original draft preparation, A.R., R.I. and F.P.; supervision, A.G., F.P. and E.N. Each author approved the version to be published and have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study was approved by Ethics Committee of the Hospital (Approval number: 2946).

Informed consent

All enrolled patients signed the informed consent to participation in the study. From January to June 2019, we collected data from all patients who satisfied selection criteria.

Setting

Setting: The study was performed at Allergology Unit of Fondazione Policlinico Universitario A. Gemelli IRCCS in Rome, Italy.

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