Title

Cross-sensitization between sesame and other seeds in children

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Running title

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Summary box

- A high rate of cross-sensitization among sesame and other seeds was described.
- Further studies are required to explore the clinical relevance in terms of cross-reactivity.

Main text

To the Editor,

Sesame is emerging as a relevant allergen also due to its increasing presence in our diet, linked to globalization (1). With a prevalence of 0.2%, sesame allergy is frequent in regions with greater exposure (2), namely the Middle East, Europe, and Japan. Specifically, seeds (e.g., sunflower, pumpkin, flax) have entered our habitual diet with new cases of allergic reactions to these compounds reported (3). Because sesame and other edible seeds are produced by phylogenetically-related plant lineages, allergenic proteins are often shared (4). Although cross-reactivity/cross-sensitization among

78 molecular components of nuts, legumes, and cereals is well described, there are few reports regarding
79 edible seeds (5), especially in children. This study aimed to describe the features of sesame-
80 allergic/sesame-sensitized pediatric patients and their cross-sensitization pattern with other seeds.
81 A retrospective study including children with a positive sesame prick-by-prick (PbP) test was
82 performed. PbP and serum specific IgE (sIgE) levels were assessed to detect cross-sensitization with
83 other seeds. Immunoblotting experiments with sera of allergic/sensitized patients were performed to
84 investigate also in-vitro cross-sensitization. Additional information about study methods and results
85 can be found at: https://zenodo.org/records/11094230.
86 From August 2014 to July 2021, 205 patients underwent PbP tests with fresh sesame at our Allergy
87 Unit. Patients with positive PbP were included (n=79, mean age 6.5 years, standard deviation, SD±4.2
88 years). Fifty-five (69.6%) subjects had a history of sesame allergy (six confirmed with oral food
89 challenge, OFC), while 24 (30.4%) had no history of previous ingestion and were avoiding sesame
90 due to PbP positivity. Concerning these, in five (20.8%) allergy was ruled out through a negative
91 OFC, while 19 (79.2%) did not undergo OFC. Allergic reactions to sesame were severe (anaphylaxis)
92 in 32 patients (58.2%) and mild-moderate (non-anaphylaxis) in 23 (41.8%). A higher PbP wheal
93 diameter was observed in patients with history of severe compared to mild-moderate reaction to
94 sesame ($p < 0.05$). No statistically significant difference was observed regarding sIgE levels to sesame
95 ($p > 0.05$).
96 Among sesame-sensitized patients, 68 (86.1%) underwent PbP/sIgE testing for flax, sunflower, and
97 pumpkin seeds. Two had a history of reaction, one with skin contact urticaria with flax seeds and one
98 with anaphylaxis to sunflower seeds. We found a high rate of cross-sensitization to seeds: 36 patients
99 (52.9%) were sensitized to flax, 35 (51.4%) to sunflower, and 31 (45.6%) to pumpkin seeds. Most
100 sesame-sensitized patients were pluri-sensitized. Sunflower-flax (odds ratio, OR 10.5, 95%
101 confidence interval, CI 3.42 - 32.23) and sunflower-pumpkin (OR 11.3, CI 3.57 - 35.54) were the
102 most likely combinations.
103 The potential cross-sensitization between sesame and seeds was also investigated by immunoblotting

104 experiments on patients with reported sesame allergy/sensitization and sensitization to flax, sunflower
105 and pumpkin seeds. **Figure 1** shows an SDS-PAGE and immunoblot referred to one of the screened
106 patients. Exploring the electrophoretic pattern of sesame, flax, sunflower, and pumpkin seed, some
107 common protein bands appear to intensively react against the patient's serum, especially that referred
108 to a 32 kDa protein attributed to 11 S globulin, thus confirming a potential cross-sensitization among
109 proteins bands. Specifically, highly reactive spots were detected in the region 20-50 kDa comprising
110 11S and 7S globulins, suggesting a high homology level in this family among the investigated plant
111 species.
112 Although Ses i 1 (2S albumin) is considered a harmful allergen in sesame-sensitized patients, the
113 main sesame protein component is a 11S globulin comprising Ses i 6 and Ses i 7 (6). While the 2S
114 albumins may explain the observed cross-sensitization among peanuts, tree nuts, and sesame seeds,
115 in the case of other seeds homology of other allergens including 11S globulin may account for the
116 reported cross-sensitization (7).
117 In our study a goat anti-human IgG secondary antibody was used; the results were nonetheless
118 replicated with an anti-human IgE, as in previously published studies (8). However, further proteomic
119 investigations and dedicated immunoblot-inhibition studies are needed to identify the specific IgE-
120 binding proteins involved in seed allergenicity.
121 This study describes one of the largest cohorts of pediatric patients with sesame allergy/sensitization.
122 We found significantly higher values of PbP wheal diameter in those investigated because of a history
123 of anaphylaxis compared to those without it, confirming the utility of PbP for sesame allergy
124 diagnosis and its potential association with reaction severity (9).
125 Our study has several limitations, including the retrospective design, small sample size, and selection
126 bias connected to the enrollment starting from patients with positive sesame PbP, including patients
127 with negative history of sesame exposure. Sesame allergy diagnosis was mainly based on the reaction
128 history and positive PbP/sIgE, but only a few patients underwent an OFC, which is fundamental to
129 establish the clinical value of sensitization. Moreover, we could not retrieve Ses i 1 levels in a large

group of patients, and this information is important in providing a more accurate definition of the molecular sensitization profile.

As a future prospect, it would be desirable to perform OFCs in sesame-sensitized patients, for sesame and other seeds. In patients with a history of reaction or no history of exposure, it would confirm or rule out allergy diagnosis, defining clinical cross-reactivity as well.

In conclusion, our study confirmed the severity of sesame allergy in children, exploring in vitro cross-sensitization and highlighting the role of sesame 11S and 7S globulins. A high rate of cross-sensitization among sesame and other seeds was described. However, further studies are needed in the area, e.g., to confirm the high rate of cross-sensitization among seeds and its clinical significance.

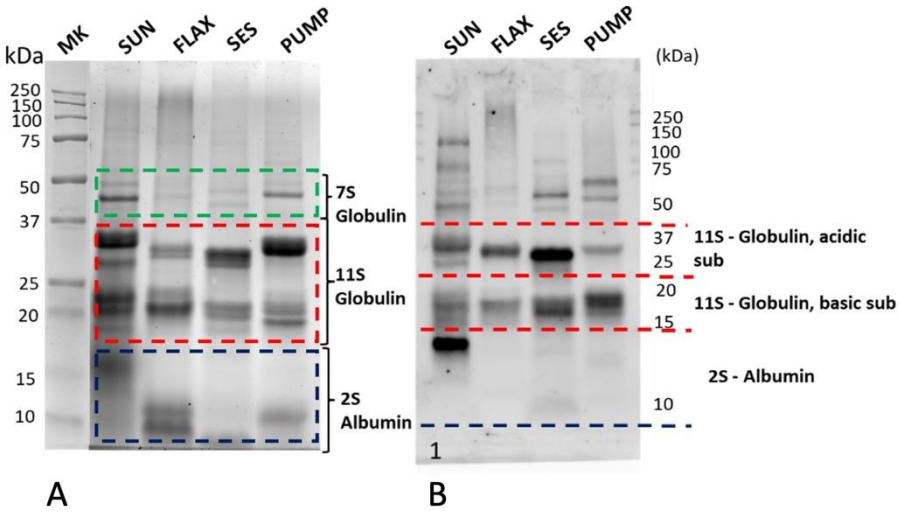

Fig. 1. A) SDS-PAGE of proteins extracted from sunflower (SUN), flax (FLAX), sesame (SES) and pumpkin (PUMP) seeds. In the green, red and blue boxes the bands putatively attributed to 7S globulin, 11S globulin and 2S albumin, respectively, are highlighted. B) Individual immunoblot of seed protein extracts of sunflower (SUN), flax (FLAX), sesame (SES) and pumpkin (PUMP) incubated with one serum from a patient allergic to sesame and sensitized to other seeds.

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