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Caso clínico

Occupational asthma due to spinach powder

Background and aims: Acting as inhaled antigens, foods can cause respiratory diseases. We report the case of a 45-year old male baker, with no past history of atopy, with symptoms of rhinoconjunctivitis and bronchial asthma in relation to the manipulation of dehydrated spinach powder used for the enrichment of whole-grain flours in the preparation of whole-grain bread. The patient tolerates the ingestion of spinach, bread and bakery and pastry products. *Methods:* Skin prick tests were performed with an extract of the dehydrated spinach powder (30 mg/ml), as well as a nasal challenge test with spinach powder extract (10 mg/ml) and specific IgE quantification by the HY-TEC-EIA and SDS-PAGE immunoblotting methods. *Results:* The prick test with the dehydrated spinach powder extract was positive, as was the nasal challenge test with a 1:1000 dilution of the 10 mg/ml extract, causing immediate clinical symptoms of rhinitis with increased nasal airflow resistance and a >50% drop in the total nasal airflow. The specific serum IgE level was 1.64 kU/ml, and the SDS-PAGE immunoblotting technique disclosed an IgE-binding protein band with a molecular weight of 29.6 kDa. *Conclusions:* A case of IgE-mediated immediate hypersensitivity to dehydrated spinach powder is reported, in a baker who evidenced rhinitis and asthma upon manipulating this material. The patient remains asymptomatic when avoiding contact with the allergen. Sensitisation to other vegetable antigens present in the bread dough may be the cause of occupational asthma caused by hidden and infrequent antigens.

Key words: Allergy. Asthma. Spinach. Occupational disease.

Asma ocupacional por polvo de espinaca

Antecedentes y objetivos: Los alimentos pueden ser causa de enfermedades respiratorias como antígenos inhalados. Se presenta un caso de un varón de 45 años, panadero, sin antecedentes de atopia, que presenta síntomas de rinoconjunctivitis y asma bronquial en relación con la manipulación de polvo de espinaca deshidratada, que utiliza para el enriquecimiento de harinas integrales en la fabricación del pan integral. El paciente tolera la ingesta de espinaca, pan y bollería. *Material y métodos:* Se realizó *prick test* con extracto de polvo de espinaca deshidratada (30 mg/ml) y prueba de provocación nasal con extracto de espinaca deshidratada, así como determinación de IgE específica mediante el método HY-TEC-EIA e SDS-PAGE-immunoblotting. *Resultados:* El *prick test* con extracto de polvo de espinaca deshidratada (30 mg/ml) fue posi-

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tivo. La prueba de provocación nasal con el extracto de espinaca deshidratada fue positiva a una dilución 1/1000 desencadenándose de forma inmediata síntomas clínicos de rinitis; se pudo observar aumento de las resistencias y disminución en el flujo total mayor del 50%. La IgE específica fue de 1,64 kU/L y mediante la técnica SDS-PAGE *immunoblotting* se determinó una banda proteica fijadora de IgE de 29,6 KDa. *Conclusiones:* Se describe la hipersensibilidad mediada por IgE frente a polvo de espinaca en un paciente panadero que presentaba síntomas de rinitis y asma con su manipulación. El paciente se encuentra asintomático al evitar su contacto. La sensibilización a otros vegetales utilizados en la masa del pan puede ser la causa de asma ocupacional por antígenos ocultos y poco frecuentes.

Palabras clave: Alergia. Asma. Espinaca. Enfermedad ocupacional.

Foodstuffs can be the cause of allergic respiratory disease when acting as inhaled antigens. The best-known example of such a condition is bakers' asthma, caused by exposure to the flour of grains or pulses such as soybean or vetch¹⁻³.

The aim of the present work is to demonstrate the relationship between clinical symptoms in a baker and a possible sensitisation to another vegetable product: spinach. The patient uses spinach in the course of his daily work for vegetable fibre enrichment of whole grain flours.

Spinach, *Spinacea oleracea*, is an herbaceous plant of the *Chenopodiaceae* family. The leaves are appropriate for human consumption when still tender. It is highly appreciated as a source of folates, magnesium, calcium and sodium, as well as the vitamins A, C and E.

Spinach is used, because of its laxative and diuretic properties, as a complement to the usual grain flours in the manufacture of whole grain bread.

CASE REPORT

A 42-year old male with no remarkable personal history and in particular with no personal or family history of atopy came to our Centre because of symptoms of rhinoconjunctivitis over the past three years; in the last year he had also presented cough, dyspnoea and wheezing.

The patient associated the occurrence of these symptoms to his occupational environment, and more specifi-

cally with the manipulation of dehydrated spinach powder which he uses for the enrichment of whole grain flours in the manufacture of bread.

The bakery where he works belongs to his family, and since childhood the patient has been present in the premises without developing symptoms until those reported first appeared. Dehydrated spinach has been used as a bakery complement since about five years.

When the patient first came to our Centre he worked basically in an administrative capacity and only sporadically co-operated in the actual manufacture of bread; over the last year he had presented symptoms of rhinoconjunctivitis and asthma when so doing. The same symptoms also occurred if the doors and barriers separating the administrative area from that where bread was manufactured were not carefully closed.

The symptoms were initially controlled with the occasional inhalation of salbutamol. However, six months after they first appeared the patient had an acute episode requiring emergency therapy, and his family physician initiated treatment with budesonide, 400 µg b.i.d. He was asymptomatic when he first consulted at our Centre.

METHODS

In vivo studies: skin tests and nasal challenge test

- Skin prick tests were performed with a panel of the airborne allergens that are common in our environment: household dust mites, animal epithelia, latex, grass, olive, plantain and cypress pollens, and herbaceous plant pollens including the *Chenopodiaceae* family. The habitual study for bakers was also carried out, with prick tests with wheat, barley, rye, oat and corn flours, enzymes (α -amylase, trypsin, chymotrypsin, papain), environmental and storage moulds, grains (wheat, barley, rye, oat, corn) and storage mites (*Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*), in all cases according to the EAACI recommendations. Prick tests were also performed with a commercially acquired spinach extract (CBF Leti, Barcelona, Spain), as well as with other leaf vegetables.

- The patient provided the dehydrated spinach he manipulated at the bakery, and an extract was prepared at a 30 mg/ml protein concentration (Bial-Arístegui Laboratories, Bilbao, Spain). Prick tests were performed with this extract on the patient and on atopic and non-atopic control subjects.

- The *in vivo* studies were completed with nasal challenge tests with a barley flour extract and with the dehydrated

spinach extract prepared by Bial-Aristegui (10 mg/ml concentration). A baseline rhinomanometry was performed prior to the test while the patient was asymptomatic and without medication, and the dehydrated spinach extract was then instilled at a 1:1000 dilution. Clinical and rhinomanometric (Rhinotest MP 500 rhinomanometer) were then carried out. Control tests were performed on both atopic and non-atopic subjects.

***In vitro* studies: specific IgE detection and SDS-PAGE**

- The total serum IgE levels, as well as the specific IgE ones to spinach, pollens, flours and grains were quantified by the Pharmacia CAP-System (Pharmacia, Uppsala, Sweden).

Specific IgE antibodies to the dehydrated spinach provided by the patient were detected using the HY-TEC-EIA procedure with reagent discs provided by Ifidasa-Aristegui Laboratories (Bilbao, Spain), with the allergen coupled (2 mg/disc) to BrCN-activated paper discs. The tests were developed with the HY-TEC-EIA specific IgE equipment (Hycor Biomedical, Inc.).

The SDS-PAGE procedure was carried out at Bial-Aristegui Laboratories using the discontinuous method described by Laemmli in 12% acrylamide gel, with protein staining with Coomassie blue R-250 or argentic stain as required, and with parallel application of a standard lane with protein markers of known molecular masses (Pharmacia Biotech): phosphorylase B (94.0 kDa), bovine serum albumin (67.0 kDa), ovoalbumin (43.0 kDa), carbonic anhydrase (30.0 kDa) and α -lactalbumin (14.4 kDa). The standard and the sample were applied at 10 μ /lane volume, with 20 μ g protein (Bradford) in each application when the Coomassie blue stain was used and with 1 μ g protein when the argentic stain was used. The argentic stain was carried out with the Silver Stain Plus apparatus (Bio-Rad), according to the manufacturer's instructions.

The proteins separated at the SDS-PAGE were electrotransferred to PVDF membranes (Immobilon P) and then incubated with the patient's serum. The IgE-binding bands were developed with an enzymatic system using an IgE-peroxydase conjugate that was developed with 4-Chloro-1-naphthol or with Lumigen PS-3 (chemoluminescence detection) as convenient. The molecular mass standard was developed with Amido black.

RESULTS

***In vivo* studies: skin tests and nasal challenge (Table I)**

- The skin prick tests with the standard allergen pa-

Table I. Results of the *in vivo* tests

	Prick-test (mm)	CAP-IgE (kU/l)	Nasal challenge test
Spinach	14x9	6,34	Positive
Barley flour	3x3	3.60	Negative

nels were negative but for barley flour (3 x 3 mm wheal). The prick test with the commercially acquired spinach extract was also positive (3 x 3 mm wheal).

The skin prick test with the dehydrated spinach extract prepared by Bial-Aristegui at 30 mg/ml concentration was clearly positive in the patient, with a 14 x 9 mm wheal, and negative in all the control subjects.

Even though the patient did not manipulate barley flour at his workplace, and considering the results of the skin tests, the *in vivo* study was completed with a nasal challenge test with barley flour extract (CBF Leti Laboratories), progressively increasing the instilled concentration up to a 1:1 dilution. This test was negative, with neither clinical nor rhinomanometric changes.

The nasal challenge test with the 10 mg/ml dehydrated spinach extract was positive at the 1:1000 dilution, with nasal pruritus, hydrorrhoea and obstruction, and the rhinomanometry disclosed a drop in the total nasal airflow by over 50%; the total nasal airway resistances increased over tenfold as compared to the baseline rhinomanometry value.

***In vitro* studies: specific IgE detection and SDS-PAGE**

- The total serum IgE level was 98.88 kU/l.
- The specific IgE quantitation (Pharmacia CAP-System) was positive for barley flour (3.40 kU/l, Class 2) and also for spinach (6.34 kU/l, Class 3).
- The specific IgE to dehydrated spinach (HY-TEC-EIA) was positive (1.64 kU/l, Class 2).
- The SDS-PAGE immunoblotting procedure disclosed an IgE-binding band with a molecular mass of 29.6 kDa. When the assay was carried out with an extract prepared from spinach tablets (prepared with the proteins that precipitate after dialysing the proteins extracted from the spinach powder), a 32-kDa protein band appeared. It is quite probable that both results are due to the same protein, which appears in slightly different forms in the two samples due to the precise nature of the sample itself (state of the protein and of other accompanying ones, other non-protein compounds in the sample, etc.) (Fig. 1).

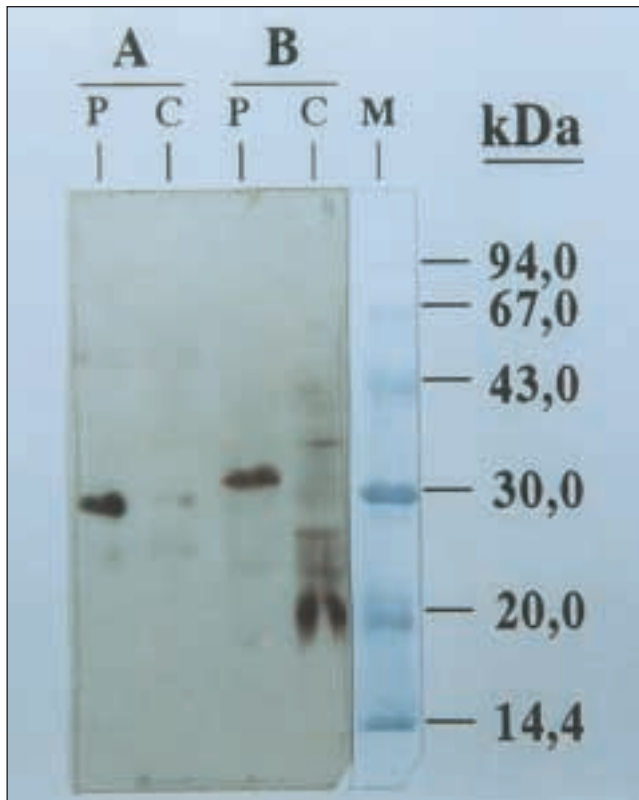


Fig. 1. SDS-PAGE Immunoblot. A: spinach powder extract; B: spinach powder tablet extract. Lanes "P": patient's serum; lanes "C": control serum (serum pool from non-allergic subjects); lane "M": molecular mass standard.

DISCUSSION

We report one case of sensitisation to dehydrated spinach powder in a baker who habitually manipulates that material at his workplace.

It has been known for decades that exposure to grain flours and dusts/powders may cause respiratory symptoms of rhinitis and bronchial asthma. The concept of bakers' asthma as an occupational disease was first introduced in 1929 by DeBesche¹, and since that time multiple allergens have been proposed as causing respiratory symptoms, and not only grain components but also components of their many contaminants (pollens, spores, enzymes, mites, ...).

Asthma caused by inhalation of diverse vegetable flours used by bakers (legume, soybean and vetch flours) has been described and studied in depth^{2,3}.

In the present case, the patient had been working since childhood in a family-operated bakery and had started evidencing symptoms three years prior to consultation; he clearly related these symptoms to exposure to dehydrated spinach powder. This material, because of its laxative and emollient properties and of its vitamin A, C and E,

iron, folate, magnesium and calcium contents, is used by some bakeries as a complement in the manufacture of whole grain bread. The material is used in dehydrated form, as cylinders some 3 cm in length, which are admixed to the flours and other additives.

Spinach, *Spinacea oleracea*, is an annual herbaceous plant of the *Chenopodiaceae* family, short in stature and with dark green leaves inserted into a short stalk. The leaves are adequate for human consumption while still young and tender. Its use was introduced in Spain by the Arabs, and the leaves are at present available during the whole year, and are used in fresh, frozen or also dehydrated forms.

Sensitisation to other green vegetables (green beans, potato, silver-beet) has been described in housewives who developed asthma after the inhalation of the cooking vapours⁴. Contact urticaria upon manipulation of these vegetables is common among these patients, and cross-reactivity studies between the various different vegetables have confirmed this phenomenon in skin tests and *in vitro* studies, yet without clinical relevance. These patients evidenced rhinitis and bronchial asthma due to grass pollen sensitisation and contact urticaria while manipulating the green vegetables, but tolerated the intake of those same vegetables.

The patient here reported had no urticarial symptoms when manipulating the dehydrated spinach, and he reported no problems when eating it, either raw or cooked. He also did not evidence sensitisation to grass pollens or to other pollens of the *Chenopodiaceae* family.

As for symptoms after spinach intake, there have been reports of the oral allergy syndrome⁵ and even of exercise-induced anaphylaxis, but in the latter case there was cross-reactivity to latex⁶, a finding also reported in relation to other *Chenopodiaceae*.

There is one report of extrinsic allergic alveolitis caused by the inhalation of spinach powder used as a foodstuff colouring. This patient developed severe disease with fever and dyspnoea, and with severe restrictive changes in the lung function tests. The diagnosis was confirmed by the demonstration of specific IgG₂ antibodies in the patient's serum⁷.

In the case here reported, hypersensitivity has been demonstrated to the dehydrated spinach he manipulates in the bakery in the course of its use as an enriching complement to whole grain flours, which is mixed to those flours during the manufacturing process.

An immediate, IgE-mediated mechanism has been

demonstrated *in vivo* through the positive skin tests with negative controls and through the specific nasal challenge test with the suspected allergen, which in our patient had positive results at a low allergen concentration.

The presence of specific IgE antibodies in the patient's serum has been demonstrated, and the SDS-PAGE procedure disclosed an IgE-binding protein band with a molecular mass of *ca.* 30 kDa upon incubation of the dehydrated spinach extract with the patient's serum.

The patient improved when using a face mask while at work, and at present he has no contact with the bakery area where the flour and the spinach powder are mixed. He thus remains symptom-free without any nasal or bronchial medication.

The patient has at all times tolerated the intake of spinach, both raw and cooked, and also of bread and other bakery products. No further sensitisation cases have been detected among the patient's work companion, despite the frequent use of this vegetable complement in flours as a result of the increasing demand for whole-grain bread.

It should therefore be underscored that sensitisation to other vegetable compounds used in the preparation of bread dough may be a cause of occupational asthma due to occult and/or infrequent antigens.

In summary, we have presented a case of rhinocon-

junctivitis and bronchial asthma caused by the manipulation of dehydrated spinach in the preparation of bread dough. An IgE-mediated mechanism has been shown to be responsible through the skin tests, the specific IgE quantitation and the demonstration of an IgE-binding protein with a *ca.* 30 kDa molecular mass in the SDS-PAGE immunoblotting studies.

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