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Sensitization to *Blomia kulagini* in a general population of a subtropical region of Spain (Canary Islands)

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The *Blomia* genus has been described as allergenic in man. The present study aimed to assess the prevalence of *B. kulagini* sensitization in a large population of allergic subjects without occupational exposure in a subtropical region (Canary Islands, Spain). Secondarily, a new standardized *B. kulagini* extract was evaluated. The study population comprised 207 patients. RAST for *B. kulagini* was positive in 76.2% of patients, and 47 of them were selected for the biologic standardization. When the prick test was performed with the nonstandardized extracts, results were positive in 76.6%, whereas when the test was repeated with the standardized extract, sensitivity rose to 95.7%. The conjunctival provocation test was positive in 78.3% of 46 evaluated patients. The bronchial provocation test was positive in 18 sensitized patients and negative in five controls. In conclusion, *B. kulagini* is an important cause of sensitization among the occupationally unexposed population of the studied area and should be included in allergy diagnostic tests. For reliable prick tests, the use of standardized extracts is mandatory.

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Key words: biologic standardization; *Blomia kulagini*; bronchial provocation test; conjunctival provocation test; house-dust mites; sensitization; skin test.

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Over the past years, some species of allergenic mites unrelated to the Pyroglyphidae or Acaridae family have acquired increasing relevance (1, 2). Among them, several genera of the Glycyphagidae family have been considered an important source of allergenic mites (2).

Three species of the *Blomia* genus (*B. kulagini*, *B. tropicalis*, and *B. freemani*) have been described as allergenic in man. Furthermore, they can be found in house-dust samples of various countries, mainly with tropical and subtropical climates (3–8). Although Spain has a mild, Mediterranean climate, the Canary Islands, a region of Spain, lie near the African coastline and benefit from a subtropical climate.

Various studies have demonstrated a positive skin test and/or presence of circulating IgE to *Blomia* spp. in sera from exposed subjects (3, 9, 10). However, the presence of *Blomia* sensitization in the occupationally unexposed population remains unknown, nor has its clinical significance been well established.

The present study aimed to assess the prevalence of *B. kulagini* sensitization in a large population of allergic subjects without occupational exposure in a subtropical area. Secondarily, a new standardized *B. kulagini* extract was evaluated.

Material and methods

Patients

The study population comprised the patients referred by their general practitioner to the allergy department of a general hospital for the first time. Inclusion criteria were clinical history of asthma and/or rhinitis compatible with mite sensitization and hypersensitivity to mites as demonstrated by positive response to prick test and/or specific IgE.

The area covered by the hospital (Tenerife, Canary Islands, Spain) comprises a total population of 643 000. The climate is subtropical, with an average temperature of 17.8°C in winter and 26°C in summer. The mean relative humidity is 63%, with an average rainfall of 233.4 l/m^2.

Specific IgE determination

RAST was performed with the following mite extracts: *B. kulagini*, *Dermatophagoides pteronyssinus*, *D. farinae*, *Euroglyphus maynei*, *Acarus siro*, *Tyrophagus putrescientiae*, *Lepidoglyphus destructor*, *Chortoglyphus arquatus*, and *Gohieria fusca*.

Solid-phase antigen was obtained by coupling the extract solution (at a concentration of 1 mg/ml) to a 6 mm-diameter, CNBr-activated paper disk, according to Ceska & Lundqvist (11). RAST was then carried out, by the method of Wide et al. (12), with commercial reagents (Phadezym RAST reagents, Pharmacia Diagnostics AB) and following the manufacturer's instructions. Values higher than 0.35 PRU/ml were considered positive.

Skin prick test

Commercially available skin prick tests (Ifidesa-Aristegui, Spain) were performed on all patients, according to the recommendation of the Subcommittee of Allergen Standardization of the European Academy of Allergology and Clinical Immunology (13). Skin pricks were performed on the anterior forearm with a standardized lancet (Dome Hollister, Leverkusen, Germany). Histamine dihydrochloride (10 mg/ml) and phenolated saline solution were used as positive and negative controls, respectively. Results were recorded after a 15-min exposure to the allergen by applying translucent tape over the wheals and transferring the impressions to a record sheet.

In addition to a standard battery (comprising pollens, molds, and epithelia), all the previously mentioned allergenic extracts were tested. Allergens were administered at a dose of 5.0 SPT/ml in the case of *D. pteronyssinus* and *D. farinae* standardized extracts (14), or at a dose of 5000 NE/ml in the case of the other nonstandardized extracts. The equivalent concentration of the commercially available *B. kulagini* nonstandardized extract was 0.5 mg/ml of freeze-dried crude extract.

Conjunctival provocation test

The conjunctival test was performed according to a previously described protocol (15). In brief, increasing concentrations (5, 50, 500, and 5000 NE/ml) of aqueous allergen extract were prepared, and one drop of the first allergen dilution was administered into the conjunctival sac. If no reaction was detected within 20 min, the following concentration was applied into the same eye. The criteria for a positive test were congestion of the conjunctival mucosa, itching and watering of the eye, and itching and runny nose. Negative control was carried out by administering one drop of diluent into the contralateral eye.

Bronchial provocation test

Bronchial provocation was performed according to the general guidelines for nonspecific bronchoconstricting tests of the European Society of Clinical Respiratory Physiology (16). Vital capacity, FEV_1, and FEV_{25-75} were recorded in a Datospir 2000 Admate DP-130 set (Sibel, SA) after inhalation through an Ultraneb-99 (De Vilbiss) of basal, diluent *B. kulagini* extracts, and terbutaline, respectively.

Patients inhaled 2 ml of each allergen concentration, increasing from 0.0001 up to a maximum of 1 histamine equivalent prick (HEP). Spirometric records were also performed at 4, 6, 8, and 24 h after terbutaline administration (or the last *B. kulagini* concentration in the case of negative tests). For detection of a possible late response, peak flow was also recorded 12 h later by a peak flowmeter. Challenges were considered positive when a 20% decrease in FEV_1 and/or a 35% decrease in FEV_{25-75} was registered. PC_{20}, expressed as HEP, indicates the concentration of *B. kulagini* extract which produces a FEV_1 decrease of 20%. The test was considered negative when 1 HEP did not produce a significant fall in either FEV_1 or FEV_{25-75}.

All tested patients remained at the hospital for 24 h. In order not to interfere with patients' treatment, *in vivo* tests were performed during asymptomatic phases of the disease.

Blomia kulagini *extract production for biologic standardization*

Mites for extract production were cultured on a finely ground medium consisting of yeast extract and a commercial mouse food, and were maintained at 25°C and 75% relative humidity. Cultures were stopped at the maximum exponential growth phase of their cycle by freezing and then maintained at –20°C until extraction. The whole cultures, containing mites and derivatives, were extracted by magnetic stirring (1000 rpm for 6 h at 4°C) in buffered saline solution 10% w/v, pH 7.5–8.5. Extracts were clarified by centrifugation at 5600 *g* for 30 min, filtered through a 0.45-μm pore

diameter membrane, and dialyzed by ultracentrifugation in a Pellicon System (Millipore, Milford, MA, USA) with a 5-kDa cutoff. The dialyzed extract was then sterilized by filtration through a 0.22-μm pore diameter membrane and freeze-dried.

For biologic standardization, 47 of the *B. kulagini* patients with positive prick test, positive RAST values, and compatible clinical findings were selected. Mean wheal areas produced by each allergen concentration tested (0.1, 0.5, 1, and 5 mg/ml) were plotted as a function of the allergen concentration in a log-log system, and linear regression was then performed. The inferred allergen concentration eliciting a wheal equal to the one produced by histamine (10 mg/ml) was defined as 1 HEP. A HEP unit was found to be equivalent to 2.7 mg/ml of allergen extract. Prick test was then repeated in those patients.

Statistical methods

This was a cross-sectional design prevalence study. Sample size was determined by the formula $n = p^*q^*(Z\alpha/i)^2$, resulting in $n = 0.5^*0.5^*(1.96/0.07)^2 = 196$ subjects to be studied. Test sensitivity was shown by the 95% confidence interval (CI).

Results

Patients

Of the 732 subjects evaluated during the recruitment period (November 1992 to April 1994), 207 (92 males and 115 females, aged 17.0±7.29 years, mean ± SD, range 5–44) were included in the study protocol, after giving informed consent. The allergic diagnosis was rhinitis or rhinoconjunctivitis in 89 subjects, asthma in 12, and both (rhinitis plus asthma) in 106. The remaining eligible patients were excluded for the following reasons: no signs of allergic disease ($n=152$), hypersensitivity to allergens other than mites ($n=101$), inappropriate age ($n=97$), previous immunotherapy ($n=75$), non-cooperation ($n=46$), previous residence in tropical areas ($n=27$), occupational involvement in cereal grain manipulation ($n=12$), and presence of concomitant disease ($n=12$). The number of included cases was consistent with the precalculated sample size of 196.

RAST and prick test

Table 1 shows the distribution of mite sensitization to the different species within the studied population. The prick test with the nonstandardized *B. kulagini* extract was positive in 80 patients (38.6%), whereas RAST was positive in 154 of the 202 patients tested (76.2%). Distribution by diagnosis of the *B. kulagini*-sensitized patients is shown in Fig. 1.

Conjunctival provocation test

Forty-six of the patients with positive results for RAST and prick test for *B. kulagini* were evaluated by the conjunctival provocation test. The test was positive in 36 (78.3%) of the tested patients, one suffering from asthma, 11 from rhinitis, and 23 from both. Among patients with negative result, one suffered from asthma, seven from rhinitis, and two from both.

Bronchial provocation test

The bronchial provocation test was performed in 18 asthmatic patients with RAST equal to or higher than class 3 and positive prick test to *B. kulagini*. As control group, five asthmatic patients with similar clinical features but negative RAST and prick tests to *B. kulagini* while positive to other *Dermatophagoides* species were evaluated.

Table 1. RAST and prick test responses to various mites. Prick test result for *B. kulagini* corresponded to nonstandardized extract

	RAST				Prick test			
	Positive		Negative		Positive		Negative	
	n	%	*n*	%	*n*	%	*n*	%
B. kulagini	154	76.2	48	23.8	80	38.6	127	61.4
D. pteronyssinus	192	94.1	12	5.9	203	98.1	4	1.9
D. farinae	197	96.6	7	3.4	193	93.2	14	6.8
E. maynei	187	90.3	16	7.7	74	35.7	133	64.3
A. siro	139	69.8	60	30.2	27	13.0	180	87.0
T. putrescientiae	78	37.7	123	59.4	78	37.7	129	62.3
L. destructor	146	70.5	53	25.6	61	29.5	146	70.5
C. arquatus	135	65.2	68	32.9	26	12.7	178	87.3
G. fusca	98	47.3	104	50.2	30	14.6	175	85.4

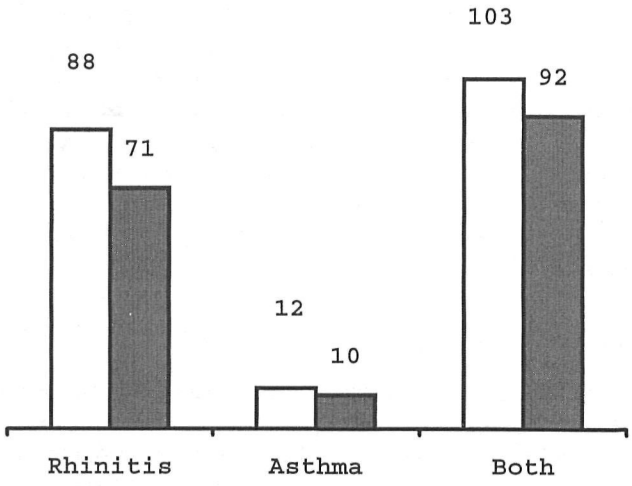


Fig. 1. Distribution by diagnosis of *B. kulagini*-sensitized patients (open bar = total patients; filled bar = *B. kulagini*-sensitized). Numbers over bars indicate number of patients.

The bronchial provocation test was positive in the 18 sensitized patients and negative in the five controls.

B. kulagini *biologic standardization*

Forty-seven patients sensitized to *B. kulagini*, with positive RAST values and clinical findings compatible with diagnoses, were selected for the biologic standardization. When the prick test was performed with a nonstandardized extract, results were positive in 36 of the 47 subjects, resulting in a sensitivity of 76.6% (95% CI: 61.6–87.2). When tests were repeated with the standardized extract, sensitivity rose to 95.7% (95% CI: 84.0–99.3). The mean extract concentration (freeze-dried crude extract) necessary to obtain a wheal equivalent to the one produced by 10 mg/ml histamine dihydrochloride was 2.7 mg/ml.

Discussion

Blomia is a common genus of house-dust mite, being predominant in tropical and subtropical areas, including those of Spain (3, 8). *Blomia* is also present in mild Mediterranean areas (5, 6). While most studies describe sensitization to *B. tropicalis*, very little has been published on the epidemiology of allergy to *B. kulagini*.

Noteworthy is the high prevalence of sensitization to *B. kulagini* found in the studied population, exceeded only by *Dermatophagoides* and *E. maynei*. This high prevalence agrees with data from other tropical areas, such as Brazil (10). However, the prevalence of 91% of an allergic population described in the latter study could not be compared with our findings, since no information on occupational exposure was given. A similar prevalence (80.5%) of sensitization to *B. tropicalis* was reported in Colombia (9), but again without information on the occupational exposure of the studied population. So far, only a few studies have demonstrated the presence of sensitization to *B. kulagini* in the Spanish Mediterranean area (5, 17) and the Canary Islands (18). Nevertheless, no systematic studies on storage-mite sensitization have covered *Blomia* spp. in this area.

In view of the high level of cross-reactivity described for the members of the *Pyroglyphidae* family (*Dermatophagoides* and *Euroglyphus*) (19) and the limited amount of cross-reaction with *B. tropicalis* (3), and considering that studied patients were carefully selected to avoid occupational exposure or sensitization abroad, it seems reasonable to consider *Blomia* spp. the most important species causing mite sensitization in this area, aside from the Pyroglyphidae. In consequence, *B. kulagini* must be included in the diagnostic armamentarium for mite sensitization, due to the importance of prevention measures and the future use of specific immunotherapy.

In relation to the clinical significance of this fact, several reports have illustrated the allergenicity of *Blomia* spp. as well as their capacity to produce significant clinical respiratory symptoms (3, 10). In the population evaluated, *B. kulagini* was present with similar frequency in patients affected by asthma or rhinitis or both. It is remarkable that almost 80% of the studied patients were positive to the conjunctival provocation test. However, more promising was the finding of a sensitivity and specificity of 100% in the bronchial provocation test. Nevertheless, more patients must be tested to confirm those results.

Although allergens of *Blomia* spp. are currently being studied exhaustively (20, 21), until now there have been no commercially available, biologically standardized *Blomia* extracts for diagnostic use that would yield reliable *in vivo* results. In this study, when patients were re-evaluated with the standardized extract, the diagnostic sensitivity of the prick test improved significantly. In addition, the biologic standardization methodology showed that the amount of allergenic mass necessary to perform the skin test should be five times greater than the nonstandardized dose. These data clearly demonstrate that the quality of extracts is crucial to obtain reliable results; therefore, this factor must be incorporated in a future diagnostic standard battery for this area.

In conclusion, *B. kulagini* is an important cause of sensitization among the occupationally unexposed population of the studied area and should be included in allergy diagnostic tests. For performance

of a reliable prick test, it is mandatory to use standardized extracts.

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