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ARAŞTIRMA RESEARCH ARTICLE

Nasal and bronchial challenges with *B. tropicalis* and *D. pteronyssinus* in children with asthma and/or rhinitis

Astım ve/veya rinitli çocuklarda *B. tropicalis* ve *D. pteronyssinus* ile nazal ve bronşiyal provokasyon*

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ABSTRACT

Objective: House dust mites were found to be the most prevalent inhalant allergens in our region. We aimed to investigate the sensitization rate to *Blomia tropicalis* and to evaluate the cross-reactivity between *Dermatophagoides pteronyssinus* and *B. tropicalis* in children with persistent allergic symptoms.

Materials and Methods: In order to determine the sensitization rate to these two mites, skin prick tests and specific IgE levels were used. Additionally, we performed nasal and bronchial challenges in a group of 38 patients who sensitized to both *D. pteronyssinus* and *B. tropicalis*.

Results: Skin prick tests and specific IgE levels with *D. pteronyssinus* and *B. tropicalis* were performed in 165 children with asthma and/or rhinitis having persistent allergic symptoms. Then, a total of 46 nasal and 30 bronchial challenges were conducted with both mite species. The sensitization rate to *B. tropicalis* was found 37.4% using prick tests and 42.4% using CAP results. Nasal challenges were performed

ÖZET

Giriş: Ev tozu akarları bölgemizde en sık rastlanan inhalan allerjenlerdir. Bu çalışmada, persistan allerjik semptomları olan çocuklarda, *Blomia tropicalis*'e duyarlılık ve *Dermatophagoides pteronyssinus* ile arasındaki çapraz reaksiyon oranını araştırmayı amaçladık.

Gereç ve Yöntem: Her iki akara olan duyarlılık, deri testi ve spesifik IgE düzeyleri ile değerlendirildi. Ayrıca, hem *B. tropicalis* hem de *D. pteronyssinus*'a duyarlı bulunan 38 hastaya her iki akar ile nazal ve bronşiyal provokasyonlar uygulandı.

Bulgular: Persistan semptomları olan, astım ve/veya rinitli 165 çocuğa, *D. pteronyssinus* ve *B. tropicalis* ile deri testleri yapıldı ve spesifik IgE düzeyleri ölçüldü. Daha sonra, her iki akar ile toplam 46 nazal ve 30 bronşiyal provokasyon yapıldı. *B. tropicalis*'e duyarlılık, deri testleri ile %37.4, CAP ile %42.4 oranında saptandı. Yirmi üç hastaya *D. pteronyssinus* ile nazal provokasyon uygulandı ve tümü pozitif bulundu. *B. tropicalis* ile nazal provokas-

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in 23 patients and all had positive results with *D. pteronyssinus*. 20 out of 23 (86.9%) patients had positive nasal challenges with *B. tropicalis*. Bronchial challenges were conducted in 15 patients and all of them had positive challenge with *D. pteronyssinus* except one. Twelve (80%) out of 15 patients had positive bronchial challenges with *B. tropicalis*.

Conclusion: We found that the sensitization rate to *B. tropicalis* is not as high as *D. pteronyssinus* in our patients with persistent allergic symptoms.

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Key words: Asthma, *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, nasal and bronchial challenges, rhinitis

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yonların ise 20 (%86.9)'si pozitif saptandı. Bronşiyal provokasyonlar ise 15 hastaya uygulandı; bunların biri hariç, tümü *D. pteronyssinus* ile pozitif iken, 12 (%80)'si *B. tropicalis* ile pozitif bulundu.

Sonuç: Persistan allerjik semptomları olan hastalarımızda *B. tropicalis*'e duyarlılığın, *D. pteronyssinus* kadar sık görülmediği söylenebilir.

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Anahtar kelimeler: Astım, *Blomia tropicalis, Dermatophagoides pteronyssinus*, nazal ve bronşiyal provokasyon, rinit

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INTRODUCTION

It has been known that dust mite allergens are the most important indoor allergens worldwide. They constitute a major allergenic component in house dust and play a significant role in the pathogenesis of asthma and allergic rhinitis[1-3]. Although up to several thousand species of dust mites have been described, only a limited number of them have been found as potent allergens. *Dermatophagoides* spp., in particular *D. pteronyssinus*, which belong to Pyroglyphidae family, are the main species that are highly allergenic[4]. *Blomia tropicalis* is the most important non-Pyroglyphid mite allergen in the tropics[5-7]. Although classified as a storage mite, it has been found in abundance in house dust of some subtropical and tropical regions of the world[7-10].

The high degree cross-reactivity between allergens of the *Dermatophagoides* spp. has been shown in different studies[11,12]. In regions where *B. tropicalis* is abundant, sensitization to this mite is as prevalent as *D. pteronyssinus*[13-15]. In the atopic population in Singapore, sensitization to both mites was as high as 90%[16]. The high rate of sensitization is not likely to be due to allergen cross-reactivity, as evidenced by in vitro inhibition studies[17].

Our region has climatic conditions that is highly conducive to mite growth like subtropical regions. In a previous study, we found that the sensitization rate to *D. pteronyssinus* and *D. farinea* were 65.7% and 60.4%, respectively, in 614 patients with respiratory allergy[18]. Recently, in a different study consisted of 336 allergic children followed up in our clinic, the sensitization rate for *D. farinea* and *D. pteronyssinus* was found to be 61.5% and 56.7%, respectively[19]. Therefore mites are the most important and ubiquitous indoor inhalant allergens in our region.

The objectives of this study were;

1. To investigate the prevalence of sensitization to *B. tropicalis* in allergic children with persistent symptoms using skin prick tests and specific IgE levels.

2. To conduct nasal and bronchial challenges with *B. tropicalis* and *D. pteronyssinus* in a group of children with asthma and/or rhinitis sensitized to mites in order to evaluate the allergenic cross-reactivity between these two mites.

MATERIALS and METHODS

Patients

The patient population consisted of 165 patients with asthma and/or rhinitis visiting the

Pediatric Allergy and Immunology Clinic in our University Hospital. Patients were diagnosed as persistent rhinitis and/or asthma according to ARIA or EAACI and GINA guidelines[20-22]. Subjects who had two or more symptoms (nasal obstruction, rhinorrhea, sneezing and ithchy nose) on more than four days in a week or more than four week during the past year were defined as having persistent rhinitis. The patients who had asthmatic daytime symptoms (cough, wheezing, dyspnea or chest thigtness) on at least three or more than three days of a week or at least more than three nights in a month were accepted as persistent asthma. All patients had a clinical history of allergic asthma and/or rhinitis at least one year of evolution and they had previously received treatment with antihistamines, inhaled steroids and/or beta-agonists.

In the first part of the study, all enrolled patients with persistent asthma and/or rhinitis were investigated for the prevalence of the sensitization rate of *B. tropicalis* by skin prick tests and specific IgE levels.

In the second part, 38 patients with positive skin tests or CAP results to *D. pteronyssinus* and *B. tropicalis* were enrolled to conduct bronchial or nasal challenge tests. Nasal challenges were performed in 23 patients, bronchial challenges in 15 patients. These 38 patients were challenged with both *D. pteronyssinus* and *B. tropicalis* extracts.

The study was approved by the ethics committee of our hospital and informed consent was obtained from all patients and their caregivers.

Prick Tests

Skin prick tests were performed according to EAACI recommendations with standardized extracts of *D. pteronyssinus* and *B. tropicalis*[23]. Histamine hydrogen chloride 10 mg/mL was used as the positive control and physiologic saline as the negative one. A mean wheal diameter greater than 3 mm was considered positive if no dermographism and/or positivity of the negative control was recorded.

CAP

Specific IgE to both mite species was determined by the CAP System (Pharmacia, Uppsala, Sweden).

Nasal challenge tests: Before the challenges, a nasal examination for obstruction, pulmonary function tests and physical examination for wheezing were performed. Patients were not challenged if their FEV_1 was less than 80% of predicted value or if they had complete obstruction of either nostril.

Active anterior rhinomanometry was performed according to the criteria of the Committee Report on Standardization of Rhinomanometry[24]. A Rhinospir 165 rhinomanometer (Sibelmed, Barcelona, Spain) programmed to perform nasal provocation test was used. Airflow and resistances were recorded in an X-Y mirror image.

The response was evaluated by measuring nasal resistance at 150 Pa with active rhinomanometry and by scoring the clinical symptoms. Total symptom scores represented the sum of the scores for: sneezing (0-2 sneezes: 0 points; 3-5: 1 point; > 5: 2 points); rhinorrhea (moderate: 1 point; severe: 2 points); tearing, itching (eyes, throat); 1 point; conjunctivitis, cough, urticaria and/or dyspnea: 2 points. Positive clinical nasal challenge was defined as > 3 points[25].

After spraying 0.2 mL of the diluent, increasing concentrations of allergen (2, 4 and 8 BU/mL, ALK, Horsholm, Denmark) were sprayed into the same nostril every 15 min until symptoms appeared and resistances doubled those induced by diluent.

FVC, FEV_1, PEF and MEF_{25-75} were recorded in a spirometry (ZAN 100 Spiromed, Germany) at 30 min intervals throughout 2 hours after nasal challenge test ended.

Forty six nasal challenge tests were performed in 23 patients (23 with *D. pteronyssinus* and 23 with *B. tropicalis*).

Bronchial challenge tests: Bronchial challenge tests were performed according to the general guidelines for standardization of bronchial challenge tests with allergens[26]. FVC, FEV_1, PEF and MEF_{25-75} were recorded in a spirometry (ZAN 100 Spiromed, Germany). All medications which could interfere with the results of the challenges were stopped prior the tests. A baseline spirometry was performed to confirm that the FEV_1 was above 80% of the predicted value and the patient's clinical condition was stable.

Bronchial challenge tests were performed with standardized allergen solutions (ALK, Horsholm, Denmark) and following standardized procedures. All allergen extracts belonged to the same batch. The solution is inhaled with continuous nebulization (PARI, Proneb Compressor Nebulizer, Midlothian, VA, USA) over 60 seconds during tidal breathing. After 15 minutes, FEV_1 value was obtained by spirometry and it was accepted as a reference value. Challenge continued with administering of the lowest allergen dose (0.001 BU/mL for each mite extract). After 15 minutes, new FEV_1 value obtained by spirometry and then the incresing allergen concentrations were administered as 0.01, 0.1 and 1 BU/mL. Spirometries were obtained after each increase in concentration. The test was stopped after a fall in FEV_1 of 20% or greater had occured, or the maximum concentration of 1 BU/mL was administered.

Bronchial challenge was first performed with *D. pteronyssinus* and the four weeks later bronchial challenge with *B. tropicalis* was conducted.

Thirty bronchial challege tests were performed in 15 patients (15 with *D. pteronyssinus* and 15 with *B. tropicalis*).

Statistics

All the analyses were performed using computer software (SPSS version 11.0; SPSS; Chicago, Illinois, USA). Descriptive statistics was performed.

RESULTS

Patients

Patient characteristics are seen in Table 1.

Skin Tests and CAP Results

In skin prick tests, 163 (98.8%) patients were positive to *D. pteronyssinus* and 55 (37.4%) were positive to *B. tropicalis*. The CAP test was positive for *D. pteronyssinus* in 164 (99.3%) patients and negative in one patient who was positive to *B. tropicalis*. Seventy (42.4%) patients had positive CAP results to *B. tropicalis*.

One hundred and sixty three patients had positive results to *D. pteronyssinus* by both skin prick test and CAP results, while one patient had only positive CAP result to *D. pteronyssinus*.

Fifty five patients had sensitization to *B. tropicalis* both by prick tests and specific IgE levels.

Nasal Challenges

All 23 patients had positive skin prick tests and CAP results to *D. pteronyssinus* and all of them had positive nasal challenges by *D. pteronyssinus*.

Twenty three patients were sensitive to *B. tropicalis* by using skin prick tests, while 16 of them had positive result to *B. tropicalis* by CAP only. Nasal challenges with *B. tropicalis* were found positive in 20 patients (Table 2).

Table 1. The characteristics of patients in the study group and provocation group (NC: Nasal challenge, BC: Bronchial challenge)

	Study group (n= 165)	Provocation group (23 patient NC 15 patient BC) (n= 38)
Age (min-max)	6-17	6-17
mean ± SD	10.7 ± 2.81	10.7 ± 2.81
Gender (male/female)	95/70	25/13
Diagnosis		
Rhinitis only	15 (9%)	3 (7.9%)
Asthma only	22 (13.3%)	6 (15.8%)
Rhinitis + asthma	128 (77%)	29 (76.3%)

Table 2. Results of the specific IgE levels (kU/L), skin prick tests and nasal challenges to *Dermatophagoides pteronyssinus* and *Blomia tropicalis*

Patient no	Prick Dp	CAP Dp	NC Dp	Prick Bt	CAP Bt	NC Bt
1	P	92	P	P	3.3	P
2	P	> 100	P	P	2.5	P
3	P	83	P	P	3.3	P
4	P	9.82	P	P	0	N
5	P	79.6	P	P	1.9	P
6	P	20	P	P	1.7	P
7	P	72.1	P	P	3.5	P
8	P	24.3	P	P	0.9	P
9	P	0.65	P	P	**0**	**P**
10	P	69	P	P	5.4	P
11	P	> 100	P	P	23.9	P
12	P	> 100	P	P	**0**	**P**
13	P	76.5	P	P	2.9	P
14	P	60.2	P	P	**0**	**P**
15	P	2.81	P	P	0	N
16	P	> 100	P	P	0.5	P
17	P	> 100	P	P	0.4	P
18	p	0.44	P	P	17.6	P
19	P	81	P	P	0	N
20	P	> 100	P	P	**0**	**P**
21	P	> 100	P	P	2.3	P
22	P	> 100	P	P	0.9	P
23	P	78.2	P	P	8.8	P

Dp: *D. pteronyssinus*, Bt: *B. tropicalis*, NC: Nasal challenge.

Eighteen out of 20 patients with a positive nasal challenge to *B. tropicalis* had both rhinitis and asthma, and there was neither concomitant wheezing/coughing nor a fall in FEV_1 of more than 20% within two hours following nasal challenge.

Two patients having both asthma and rhinitis had a decrease in FEV_1 (27% and 12%, respectively) at 1 h and 2 h postchallenge with *D. pteronyssinus*.

Bronchial Challenges

All 15 patients had positive result to skin prick and CAP tests for *D. pteronyssinus*, only one patient had negative CAP result and negative bronchial challenge to *D. pteronyssinus* (Table 3).

All of 15 patients were sensitive to *B. tropicalis* by prick tests, while 3 patients had negative result to *B. tropicalis* by using CAP.

Twelve patients had positive bronchial challenges for *B. tropicalis* (Table 3).

Five patients in the provocation group (four patient in nasal and one in bronchial challenge group) showed positive challenge results with *B. tropicalis* extracts although their Blomia CAP levels were negative.

The allergen concentrations which result in positive nasal and bronchial challenge were seen in Figure 1 and Figure 2.

Table 3. Results of the specific IgE levels (kU/L), skin prick tests and bronchial challenges to *Dermatophagoides pteronyssinus* and *Blomia tropicalis*

Patient no	Prick Dp	CAP Dp	BC Dp	Prick Bt	CAP Bt	BC Bt
24	P	> 100	P	P	15.3	P
25	P	> 100	P	P	0.43	N
26	P	> 100	P	P	1.7	P
27	P	61.2	P	P	0	N
28	P	> 100	P	P	2.3	P
29	P	48.6	P	P	0	N
30	P	0.44	P	P	0.41	P
31	P	> 100	P	P	7.6	P
32	P	53.5	P	P	**0**	**P**
33	P	94	P	P	106	P
34	P	> 100	P	P	17.6	P
35	P	> 100	P	P	18.6	P
36	P	0.28	N	P	1.1	P
37	P	> 100	P	P	19	P
38	P	22.1	P	P	1.6	P

Dp: *D. pteronyssinus*, Bt: *B. tropicalis*, NC: Nasal challenge.

DISCUSSION

We found that the prevalence of *B. tropicalis* sensitization in 165 children with persistent allergic symptoms was 37.4% using skin prick tests and 42.4% using CAP results. In order to evaluate allergenic cross-reactivity between *D. pteronyssinus* and *B. tropicalis*, we performed nasal challenges in 23 patients and bronchial challenges in 15 patients both by *D. pteronyssinus* and *B. tropicalis* extracts. Twenty (87%) out of 23 patients who were sensitized to *B. tropicalis* had a positive nasal challenge, while 12 out of 15 (80%) patients had a positive bronchial challenge.

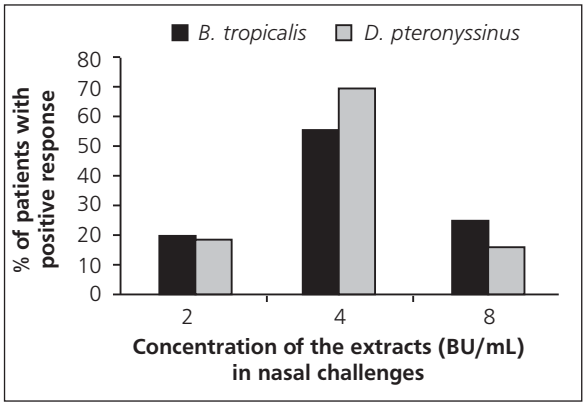

Figure 1. Percentage of patients responded positively to nasal challenge at a given allergen concentration of *B. tropicalis* and *D. pteronyssinus.*

The allergenicity of *B. tropicalis* has been demonstrated in subtropical and tropical regions of the world[7,13]. Sensitivity to *B. tropicalis* has been also described in many countries and is not limited just to tropical and subtropical areas[13-15,27,28]. Most of the studies that have investigated in vivo sensitization are based on prick tests[13-16]. It was reported as 71.9% in Indonesia, 96% in Singapore, 73.3% in Taipei 38.8% in Thailand 47% in Mexico City 93.7% in Sao Paolo and 77.8% in Caracas using skin prick tests[9,13,15,16,29,30]. In this study we found that the sensitization rate is 37.4% and 98.8% for *B. tropicalis* and *D. pteronyssinus*, respectively, using skin prick tests. Although *D. pteronyssinus* was the main source of the mite sensitization, we suggest that *B.*

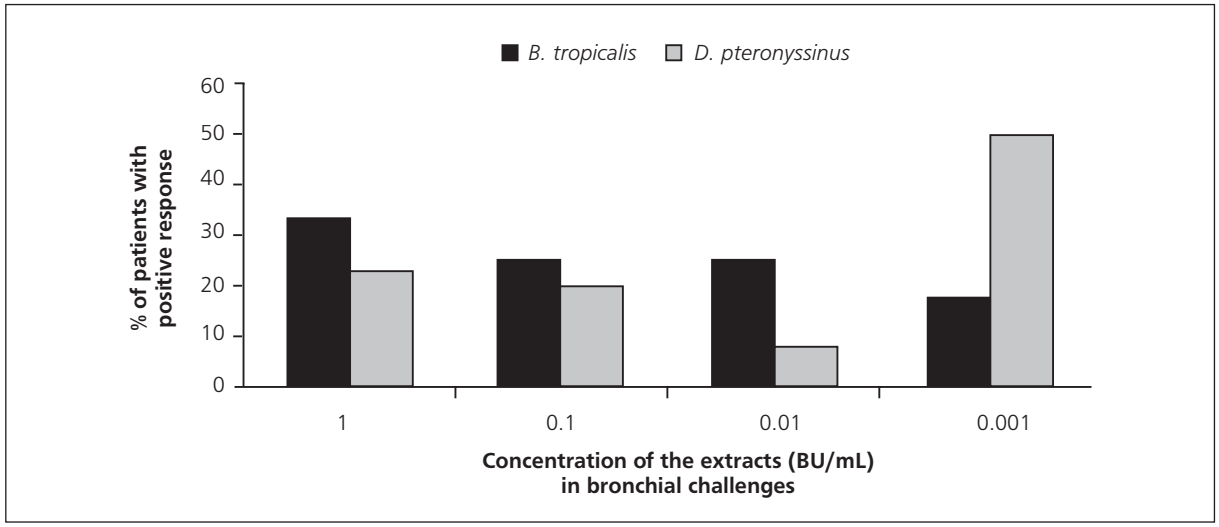

Figure 2. Percentage of patients responded positively to bronchial challenge at a given allergen concentration of *B. tropicalis* and *D. pteronyssinus*.

tropicalis may also be a significant mite species which may cause to persistent allergic symptoms in our patients.

In vitro crossreactivity studies between whole extracts of *B. tropicalis* and other mite species especially *D. pteronyssinus*, demonstrated that these two mites share common, as well as species-specific allergens. There are only few in vivo studies using inhalation test to investigate the clinical significance of sensitivity of *B. tropicalis* and its crossreactivity with *D. pteronyssinus*. Stanaland et al. demonstrated that 83% of *B. tropicalis* sensitive patients in Florida had a positive nasal challenge with *B. tropicalis* extract[31]. Therefore, they concluded that a positive skin prick test to *B. tropicalis* is a good indicator of possible allergic symptoms after inhalation of *B. tropicalis* allergens. In this study, we found that 87% and 80% of *B. tropicalis* skin prick test positive patients had positive nasal and bronchial challenges respectively, with *B. tropicalis* allergen extract. However, for the reason that the provocation group did not include adequate number of patients, we could not determined positive predictive values of CAP levels or skin prick tests for nasal and bronchial challenges.

Other studies performed in Brasil and Singapore have both demonstrated the allergenicity of *B. tropicalis* in vivo using nasal challenges[32,33]. In Brasil, a group of *D. pteronyssinus* and *B. tropicalis*-sensitive patients was evaluated; 90% of the patients had a positive nasal challenge to *D. pteronyssinu*s and 60% to *B. tropicalis*. In Singapore, provocation tests with *B. tropicalis* were performed to 20 adult patients with persistent allergic rhinitis; significant increases in subjective and objective nasal symptoms, together with significant increase of tryptase and LTC_4 concentrations in nasal secretion, were found in all patients. Garcia Robaina et al. demonstrated that conjunctival challenges to *B. tropicalis* were positive in 62.5% and bronchial challenges in 76.6% of the mite-sensitive patients[34]. Although we found a higher rate positivity in challenge tests of *B. tropicalis* sensitive patients, we suggest that there were some contradictions in patients 9, 12, 14, 16, and 17 whom nasal challenges were performed. These patients had a positive nasal challenge to *B. tropicalis* despite low levels of specific IgE and weakly positive skin tests[34]. Same contradiction was present also for patient 32 in bronchial challenge test; this patient had negative level of specific IgE and a

positive bronchial challenge to *B. tropicalis*. Similarly, six patients (patients 4, 15, 19, 25, 27, 29) in the provocation group showed negative challenge tests when skin prick tests were positive with *B. tropicalis*. In our opinion, these contradictions might be related with the standardization of the *B. tropicalis* allergen extract rather than the crossreactivity between *B. tropicalis* and *D. pteronyssinus*. Additionally, as seen in Table 2 and Table 3, specific IgE levels to *B. tropicalis* were generally very low in comparison to those of *D. pteronyssinus*. Therefore, there is need to perform some in vitro inhibition studies such as specific serum IgE detection by multiple blot antigen assay (MBAA) to differentiate crossreactivitiy between *B. tropicalis* and *D. pteronyssinus*.

We did not observed any decrease in pulmonary function tests of asthmatic children during the two hours following nasal challenge with *B. tropicalis*. However, it was reported that nasal provocation with *B. tropicalis* may also provoke asthmatic symptoms during the late-phase reaction in some asthmatics[33]. In our study, because of pulmonary function tests following nasal challenge were performed during the first two hours, probable late phase reactions to *B. tropicalis* might be overlooked.

To our knowledge this is the only study in our region which investigate the rate of *B. tropicalis* sensitization in allergic patients. Although the sensitization rate to *B. tropicalis* was found as 37.4%-42.4%, it was not as high as *D. pteronyssinus* in our patients with persistent allergic symptoms.

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