Original Articles

INHALANT ALLERGENS IN PATIENTS WITH ALLERGIC RHINITIS IN RIYADH

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تم اختيار مجموعة كواشف الاختبار الجلدي للمستأرجات بتحديدها عن طريق دراسة الأحياء الهوائية والمناعيات الكيميائية وذلك لتجربتها في مرضى سعوديين ولمعرفة المستأرجات الكامنة في الجو وفي عينات غبار المنازل في منطقة الرياض. أظهر ثلثا مجموعة المرضى الذين شخصت اصابتهم سريريًا بالتهاب الأنف الآلرجي ايجابية لواحدة أو أكثر من مستأرجات الاختبار الجلدي. شملت قائمة كواشف الاختبار الجلدي التي حددت جميع مرضى الحساسية نبات رجل الوز، عشب برمودا، الهرة، الصرصور وسوس الغبار.

A group of allergen skin-test reagents were selected for study in Saudi patients from aerobiological and immunochemical identification of potential allergens in the atmosphere and from dust samples of Saudi homes in the Riyadh region. Two thirds of a group of patients diagnosed clinically with allergic rhinitis were positive to one or more of the skin-test allergens. A minimal skin-test panel which identified all atopic patients included Chenopodiaceae, Bermuda grass, cat, cockroach, and dust mite.

Intensive analysis of allergies in Saudi patients is a recent study, and the issue is complicated by the rapidly changing nature of the Saudi environment with introduction of extensive imported flora: agricultural, domestic, and ornamental, which contribute an increasing load of potentially allergenic pollen and contaminating fungal spores. The selection of allergens for skin-testing patients in any region of the Kingdom has, to date, been based on educated guesswork as to which potential allergens might be present in the patients' environment. But a definitive, comprehensive range of skin-test allergens can only be determined after the various species of flora releasing pollen, fungi, insects, animal danders, and other allergens actually present have been established.

A three-city study of allergens in Saudi Arabia using aerobiological screening of pollen and fungal spores in the atmosphere, culture, and immunochemical analyses of domestic dust samples has, over the past 2 years, identified a catalogue of potentially allergenic material in the Saudi environment.[1-3] While these studies are not yet complete for Jeddah and Al-Khobar, the Riyadh data have been used to select the most commonly appearing allergens to skin-test a group of patients identified clinically with a diagnosis of allergic rhinitis. The strategy has been first to identify the most common potential allergens, and then to examine which of these are stimulating IgE-mediated sensitivity in patients to determine a screening battery of allergens for skin-testing Saudi patients.

Patients and Methods

Ninety-six patients with a clinical diagnosis of allergic rhinitis were identified at the Ear, Nose and Throat Department of King Abdulaziz Uni-

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TABLE 1. *Patient characteristics.*

	Allergic rhinitis	Control
Nationality		
Saudi	63(65.6%)	24(70.6%)
Yemeni	14(14.6%)	4(11.8%)
Egyptian	9(9.4%)	3(8.8%)
Sudanese	7(7.3%)	1(2.9%)
Jordanian	3(3.1%)	2(5.9%)
Total	96	34
Age(y)		
Mean	28.5	24.8
Range	9 – 52	17.40
Sex		
Male	52 (Saudi 31)	
Female	44 (Saudi 32)	

versity Hospital, Riyadh, and were referred for skin-testing. Where possible, consecutive patients were studied, and selection was restricted to nationals of Saudi Arabia or adjacent Middle Eastern countries. A group of 34 patients referred to the ENT clinic with diagnoses excluding rhinitis was skin-tested as a control group. Patient characteristics are summarized in Table 1.

Materials for skin-testing were selected as representing the most common regional allergens from preliminary data from the Riyadh aerobiological study[2] and the fungal allergen and dust study[1] and were supplied by ALK Laboratories, Copenhagen, Denmark, using standardized special purity (SQ) material where possible. A small number of samples were also purchased from Greer Laboratories, USA. As all material was imported, it was not possible, in many instances, to include the precise species growing in the Riyadh area, so representatives of related botanical families had to be included as substitutes.

A screening panel of 11 of the most common allergens was developed and used to test all 96 allergic rhinitis patients and the control group. Twenty-five of the patients found to have clear atopy with this initial screening panel were then recalled for further testing with an extended panel including an additional 21 allergens detected from the above studies. Details of both screening and expanded panel are presented in Table 2.

A standard skin-prick test was used. Droplets of test material were spaced on the forearms at 1- to 2-cm intervals. A positive control with histamine dihydrochloride (1 mg/mL) and a negative control

TABLE 2. *Skin-test allergens.*

	Species	Common name	Concentration
	Screening panel		
Fungi	*Alternaria alternata*		1:20 w/v
	Cladosporium herbarum		1:20
	Rhizopus nigricans		1:20
Pollen	Mixed grasses		
	Acacia		1:40
	Chenopodium album		1:100
Domestic	Cockroach		1:20
	Cat SQ		10 HEP*
	Dog SQ		10 HEP
	Dermatophagoides farinae SQ		10 HEP
	Additional allergens in full panel		
Fungi	*Phoma herbarum*		1:20
	Ulocladium chartarum		1:20
	Aspergillus fumigatus		1:20
	Aspergillus niger		1:20
Pollen	*Ambrosia artemisia*	Ragweed	1:100
	Rumex crispus	Dock	1:100
	Triticum sativum	Wheat	1:100
	Salix caprea	Willow	1:100
	Hordeum sativum	Barley	1:100
	Phragmites communis	Reed	1:100
	Brassica napus	Mustard	1:100
	Zea mays	Corn	1:100
	Uttica dioeca	Nettle	1:100
	Carpinus betula	Horbeam	1:100
	Cynodon dactylon	Bermuda grass	1:20
	Corylus avellana	Hazelnut	SQ 10 HEP
	Poa pratensis	Rapgrass	SQ 10 HEP
	Lolium perenne	Ryegrass	SQ 10 HEP
	Phleum pratensis	Timothy grass	SQ 10 HEP
Others	Horse epithelium		SQ 10 HEP
	Dust mite mix		SQ 10 HEP

*HEP indicates in vivo histamine-related standard.

were included for every patient. The skin was punctured through the droplet with a fine blood lancet to lift the upper layer of the dermis. Reactions were read at 10 to 15 minutes, and the mean wheal diameter was recorded. A positive reaction was determined as a minimum wheal diameter of 2.5 mm or more greater than the negative control.[4]

Results

Approximately two thirds of all patients diag-

TABLE 3. *Reactions to individual allergens of screening panel.*

Allergen	Total patients			Saudi patients		
	No. of positive reactions	% of all patients	% of skin-test-positive patients	No. of positive reactions	% of all patients	% of skin-test-positive patients
Chenopodia	31	32.3	54.4	22	34.9	53.6
Cat	28	29.2	49.1	19	30.2	46.7
Grasses	27	28.1	48.8	23	36.5	56.1
Cockroach	18	18.7	31.6	14	22.2	34.1
Dog	15	15.6	26.3	11	17.5	26.8
Dust mite	14	14.6	24.6	9	14.3	22.0
Plantain	9	9.4	15.8	5	7.9	12.2
Cladosporium	3	3.1	5.3	3	4.8	7.3
Acacia	3	3.1	5.3	3	4.8	7.3
Rhizopus	2	2.1	3.5	1	1.6	2.4
Alternaria	1	1.0	1.7	0		

nosed clinically with allergic rhinitis showed one or more positive skin reactions with the screening panel (Table 3). Sixty-three percent of the skin-test-positive patients reacted to two or more allergens. One quarter of the control patients showed a positive skin test.

Sixty-five percent of Saudis and half of the non-Saudis reacted to at least one allergen, and two thirds of these positive subjects reacted to two or more allergens. Positive wheal diameters ranged from 2.5 to 13 mm, with many smaller wheals being excluded by the definition of positivity. Only five of the 129 positive reactions in the allergic rhinitis patients were measured at the lowest 2.5-mm level larger than the negative control (3.9%), and in each instance, the patient also exhibited a wheal of 3 mm or more to another allergen.

Histamine-positive wheals averaged 5.83 ± 1.46 mm for the 57 skin-test-positive patients and 5.57 ± 1.32 mm for the 39 skin-test-negative rhinitis patients. The skin-test-negative control subjects showed a histamine wheal size averaging 4.68 ± 1.40 mm, a significantly smaller response than the rhinitis patients ($P < 0.001$).

The frequency of positive reactions to the individual screening panel antigens is presented in Table 3. *Chenopodium album*, fat hen (representative of the group which includes salt bushes), a panel of mixed grasses, and cat each induced positive reactions in over 25% of all patients tested and in half of the skin-test-reactive patients. For most allergens, the majority of patients showed a wheal size measuring 5 mm or less, but grasses and cat extract induced more than 65% of patients to react with wheals greater than 5-mm diameter.

No significant differences were found in the frequency of positive reactions between males and females. There was a trend for skin-test positivity to be more common in younger patients, with 71% of the 14 patients less than 20 years old reacting positively, 64% of the 39 patients 20 through 29 years old, and 50% of the 42 patients aged 30 and over.

Considering allergens as indoor (cat, dog, cockroach, and dust mite) or outdoor (others), 11 of the 57 skin-test subjects (19%) reacted to the inside battery alone, 27 patients (47%) responded to both inside and outside allergens, and 19 patients (33%) to outside allergens only. Thirty-eight patients (66.7%) reacted to inside allergens, more than half of these to two or more allergens.

To select a minimal panel of skin-test allergens which identified all atopic patients, the frequency of positive reactions to each allergen was compared with the others. Although many patients reacted to two or more allergens, only one is necessary to identify their atopic status. Table 4 lists in rank order the smallest panel which identified all atopic patients and the cumulative effect of adding to the panel.

The extended skin-test panel in the 25 selected atopic patients identified some clinically important additional allergens not included in the origi-

nal seven (Table 5). Bermuda grass, although represented in the screening grass mix, evoked positive reactions in 22 of the atopic subjects including seven previously negative or positive with wheals less than 2.5 mm above control with the grass mix screen. *Artemisia vulgaris*, *Phragmites communis*, *Zea mays*, and *Salix* were each positive in at least four of the atopics. *Lolium*, *Phleum*, *Poa*, and *Hordeum* are all grasses and were previously detected with grass mix. Individuals of the atopic group reacted to between two and 18 allergens (mean 5 to 7).

TABLE 4. *Rank order of skin-test reactivity.*

	No. of patients	No reaction to allergens	Cumulative positive No. (%)
Chenopodia	31	–	31(54.4)
Cat	28	15	46(80.7)
Grasses mix	27	8	54(94.7)
Cockroach	18	2	56(98.2)
Dust mite	15	1	57(100.0)

TABLE 5. *Positive skin reactions in atopic patients to allergens in expanded test panel.*

Allergen	Common name	No. positive(%)
Cynodon dactylon	Bermuda grass	22(88)
	Cat	14(56)
Chenopodium album	Fat hen	11(44)
Artemisia vulgaris	Mugwort	9(36)
Phragmites communis	Reed	8(32)
Periplaneta americana	American cockroach	7(28)
	Dog	7(28)
Acacia	Trees and shrubs	7(28)
Poa pratenses	Grass	6(24)
Dermatophagoides farina	Dust mite	5(20)
Cladosporium herbarum		5(20)
Phleum pratense	Grass	5(20)
Plantago lanceolata	Plantain	5(20)
Lolium perenne	Grass	4(16)
Zea mays	Maize	4(16)
Salix caprea	Willow	4(16)
Carpinus betula	Hornbeam	4(16)
Hordeum sativum	Grass	3(12)
Ambrosia artemisia	Ragweed	2(8)
Phoma herbarum		2(8)
Ulocladium chartarum		1(4)

Discussion

The study differs from other reports of skin-test results in Saudi patients[5] by selecting allergens for test on the basis of their proved presence in the Riyadh region using aerobiological and ELISA techniques.[1-3] Few patients have never left the region, thereby developing local sensitivities only, but a Riyadh test panel (and later appropriate panels for other regions) would at least present the best opportunity to identify allergens likely to precipitate clinical reactions in rhinitis and asthma within the region.

Three principal conclusions derive from this study. First, atopy among Saudi nationals is common and demands a formal study of incidence to compare with population in other countries. Second, a definitive pattern of sensitivities emerges which is influenced strongly by imported flora. Third, a preliminary allergen screen has been developed for use in Saudi nationals to identify atopy.

The determination of atopy requires a clinical picture of rhinitis, asthma, or eczema, together with a positive skin test and usually a positive family history. Sixty-five percent of Saudi patients diagnosed clinically as having allergic rhinitis showed positive reactions to at least one allergen. This allergen reaction frequency is comparable to values reported from other countries. Eriksson[6] reported 113 of 154 Swedish patients (73.4%) with seasonal rhinitis had positive skin tests. Freidhoff et al,[7] in 262 patients reporting allergies in Baltimore, identified 55% with positive skin reactions, while Tan and Teoh,[8] studying 138 patients with asthma in Singapore, found 69% gave positive skin-test reactions.

As the screening and enlarged test panel had to rely on skin-test material manufactured in Denmark and the United States, many test samples represented family members, but not identical species of flora growing in Saudi Arabia. Species of *Artemisia*, *Ambrosia*, *Rumex*, and *Salix* grow in the Kingdom, but no local species were available for testing. Cross-reactivity between allergens within botanical families is common,[9] but not universal, so the results obtained in this study are likely to represent an underestimate of the true-positive response rate. It is possible that a test panel selected specifically with species grow-

ing in the local area might give significantly higher responses.

Both local and imported flora were responsible for positive skin test reactions. The most common response was to grass mix which, on the expanded panel, appeared overwhelmingly to be Bermuda grass, which grows extensively in the Kingdom. While most grass species share many allergens, Bermuda grass has a much more limited allergenic overlap[10] and should be included for its own identity in any further test panel. Clearly, the influence of local irrigation and planting will influence the levels of pollen produced.

Fat hen and salt bush of the Chenopodiaceae family are representative of the group producing the highest pollen level in Riyadh.[2] Not surprisingly, this allergen group commonly provokes reactions in the local population. *Artemisia vulgaris* (mugwort) was included in the expanded panel, as it is a very potent allergen, and species of the genus *Artemisia* (e.g., *A. scoparia* and *A. monosperma*) are widespread in the desert outside Riyadh. The significant number of positive reactions to this in the atopic population (36%) means *Artemisia* should always be included in any local allergy test panel.

Fungal spores are common in Riyadh atmosphere,[1] but the proportion of patients reacting positively to the skin-test material was low. However, *Cladosporium*, *Alternaria*, *Phoma*, *Rhizopus*, and the rarely described *Ulocladium* have each stimulated reactions in some patients and so should be studied further in this environment.

Cat sensitivity was surprisingly common, with over half the atopic Saudis showing positive reactions. As cat-keeping is uncommon in Saudi families, this sensitivity was at first difficult to understand. The SQ material used is standardized with particular care to WHO standards. Contamination is most unlikely. But detailed questioning of positively reacting patients revealed the common presence of wild cats in their environments, in gardens, on stored carpets, and even in open areas of their houses. Clearly the potent allergenic nature of cat dander, with its propensity to cascade, and cat saliva provides a serious contribution to inhalant allergens in the Kingdom.

Immunologic analysis of allergens is a new procedure but a powerful tool in the search for potential allergens.[11] Preliminary information using an ELISA test to detect cockroach antigen revealed a very high level of this material in many household dust samples from the Riyadh region (R. Thorogood, MIBiol, personal communication). Cockroach is increasingly recognized as a very potent allergen,[12] so its presence demands inclusion in any skin-test panel. Eighteen of the 57 atopic patients reacted to American cockroach in the screening series. It is not yet clear if this figure might be much higher if a local species were used for skin-testing.

The house dust mite *Dermatophagoides* (of the species *farinae* or *pteronyssinus*) is ubiquitous in the United Kingdom, the United States, Scandinavia, and many other countries. It depends for its growth on a relative humidity about 50%, rarely the situation in Riyadh with its persistent dry climate of 20% humidity. Neither microscopic nor ELISA analysis has shown significant dust mite concentrations in Riyadh dust samples (R. Thorogood, MIBiol, personal communication). The relatively low dust mite positivity at 25% of the 57 skin-test-positive patients compares with figures as high as 80% for asthmatic children in an environment such as New Zealand (J.D. Wilson, FRACP,PhD, personal communication). The absence of house dust mites in Riyadh indicates that patients with positive reactions to the mite have acquired sensitivity in other centers, either in Saudi Arabia or other countries.

Sorensen et al[5] studied a group of 100 patients with perennial rhinitis in Saudi Arabia and showed Bermuda grass was the commonest allergen, reacting with 33% of all patients tested, while reed, cat, and dust mite were positive in about 15%. They did not include cockroach.

Although a large number of allergens can precipitate allergic rhinitis or bronchial asthma in susceptible patients, the great majority of allergic reactions arise from sensitivity to a very small number of allergens only. Pantin and Merritt[13] showed 95% of patients in the UK with IgE-mediated sensitivities reacted with one or more of a screening pool of only three allergens (grass, mite, and cat). Herbert et al[14] found 90% of atopic asthmatics could be detected with positive skin reactions to four allergens. While this screening group of allergens is insufficient to identify all the potentially triggering material for an individual

patient, it does serve as a valuable first step in indicating which patients have an atopic constitution and should be studied in depth. The present study identified a minimum battery of five allergens (Table 4), Chenopodiaceae, Bermuda grass, cat, cockroach, and dust mite, which detected all skin-test-positive patients and would serve as an atopy screen in the Riyadh region. From the expanded panel study, it is likely that *Artemisia* and *Phragmites* might detect some patients unresponsive to other antigens.

While the allergen panel for Riyadh is not complete until local species of potentially allergenic flora are tested, the study indicates that a screening test panel for inhalant allergens in the Riyadh region should include the pollens of *Artemisia*, Chenopodiaceae, Bermuda grass, *Acacia* species, plantain, *Phragmites communis* (reed); the fungi *Cladosporium*, *Rhizopus*, *Ulocladium*, and *Alternaria*; and the domestic allergens cat, cockroach, and dog. This panel might be extended on later investigation. Goat, camel, and sheep should be included where exposure to these species is likely, and rodent sensitivity is currently being investigated.

The authors are extending this work to both Jeddah and the Al-Khobar/Dhahran regions. In each area, the skin-test panel will be devised by studying information from the aerobiological and immunological analysis of the local environment. It is only by this means that an appropriate skin-test panel can be identified for particular regions.

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References

1. Al-Frayh AR, Hasnain SM, Wilson JD, Harfi H. Fungal allergens in the atmosphere of Riyadh: a preliminary communication. Ann Saudi Med 1988;8(4):248-51.
2. Al-Frayh AR, Reilly H, Harfi H, et al. A 12-month aerobiological survey of pollen in Riyadh. Ann Saudi Med (in press).
3. Hasnain SM, Al-Frayh AR, Harfi H, et al. Fungal allergens in the atmosphere of Riyadh: seasonal periodicities. Ann Saudi Med 1989;9(4):000-000.
4. Dreborg S, Belin L, Eriksson NE, et al. Results of biological standardization with standardized allergen preparations. Allergy 1987;42(2):109-16.
5. Sorensen H, Ashoor AA, Maglad S. Perennial rhinitis in Saudi Arabia: a prospective study. Ann Allergy 1986;56(1):70-80.
6. Eriksson NE. Allergy screening in asthma and allergic rhinitis: which allergens should be used? Allergy 1987;42(3):189-95.
7. Freidhoff LR, Meyers DA, Bias WB, et al. A genetic-epidemiologic study of human immune responsiveness to allergens in an industrial population: I. Epidemiology of reported allergy and skin test positivity. Am J Med Genet 1981;9(4):323-40.
8. Tan WC, Teoh PC. An analysis of skin prick test reactions in asthmatics in Singapore. Ann Allergy 1979;43(1):44-6.
9. Martin BG, Mansfield LE, Nelson HS. Cross-allergenicity among the grasses. Ann Allergy 1985;54(2):99-104.
10. Schumacher MJ, Grabowski J, Wagner CM. Anti-Bermuda grass RAST binding is minimally inhibited by pollen extracts from the other grasses. Ann Allergy 1985;55(4):584-7.
11. Lind P, Norman PJ, Newton M, et al. The prevalence of indoor allergens in the Baltimore area: house dust-mite and animal-dander antigens measured by immunochemical techniques. J Allergy Clin Immunol 1987;80:541-7.
12. Hulett AC, Dockhorn RJ. House dust mite (D. farinae) and cockroach allergy in a midwestern population. Ann Allergy 1979;42(3):160-5.
13. Pantin CF, Merrett TG. Allergy screening using a microcomputer. Br Med J 1982;285(6340):483-7.
14. Herbert FA, Weimer N, Salkie ML. RAST and skin test screening in the investigation of asthma. Ann Allergy 1982;49(6):311-4.