

AIRBORNE *ALTERNARIA* SPORES: POTENTIAL ALLERGIC SENSITIZERS IN SAUDI ARABIA

Syed M. Hasnain, PhD, FACAAl; Abdulrahman Al-Frayh, MD;
Muhammed O. Gad-el-Rab, MD; Sultan Al-Sedairy, PhD

Background: In order to investigate the role of airborne *Alternaria* spp. in the sensitization of individuals having respiratory allergy symptoms, particularly bronchial asthma, an aerobiologic and clinical (diagnostic) study was conducted at several centers in Saudi Arabia.

Materials and Methods: Airborne *Alternaria* spores were studied at four different centers in Riyadh, Jeddah and Al-Khobar, including three years at one site. Skin prick tests (SPT) were conducted on a total of 616 allergic individuals attending allergy clinics at six different hospitals in Abha, Qassim, Gizan, Jeddah, Hofuf and Makkah regions.

Results: *Alternaria* spores constituted between 1.9%-9.6% of the total fungal air spora, and the maximum concentration exceeded 5×10^2 spores per m^3 of air in Jeddah, followed by 4.9×10^2 spores per m^3 in Al-Khobar. Mean weekly and monthly concentration of *Alternaria* fluctuated, reaching up to 150 spores per m^3 and 60 spores per m^3 , respectively. Quantitative regional variations were also recorded. IgE-mediated SPT reactivities conducted on allergic patients resulted in an overall 21.6% positive reactions, showing mild, moderate and strong wheal and erythema.

Conclusion: The findings reveal that *Alternaria* spores are prevalent as a major component in the outdoor environment of the Kingdom, with peaks in April and October. The overall 21.6% positive SPT reactions indicate the sensitization level or cross-reactive sensitivity to *Alternaria* allergens. The study suggests that airborne *Alternaria* can be a potential allergic sensitizer in susceptible individuals and can be a risk factor in sensitized individuals with symptoms of bronchial asthma and allergic rhinitis in the Kingdom of Saudi Arabia. *Ann Saudi Med* 1998;18(6):497-501.

Key Words: *Alternaria* spores, skin prick tests, asthma, allergies.

Alternaria alternata allergens have long been considered to cause significant respiratory allergies in patients in the United States.¹ *A. alternata* spores were also implicated recently in serious cases of respiratory arrest² in children and young adults, and were temporarily associated with observed high levels of *Alternaria* spores in outdoor air.² Airborne incidence of *Alternaria* spores has been documented from various countries,³ and the antigenic properties of spores have been partially characterized.⁴⁻⁷ They have also been documented from the environment of Middle East countries,⁸ but mainly without volumetric data,^{9,10} and without their diagnostic implications.^{11,12} Sorensen et al.¹² reported about 7.7% *Alternaria* colonies, while data from Dammam¹³ with volumetric sampling

identified *Alternaria* spores between 1.9%-4%. In 1989, Hasnain et al.¹⁴ identified *Alternaria* as a major component

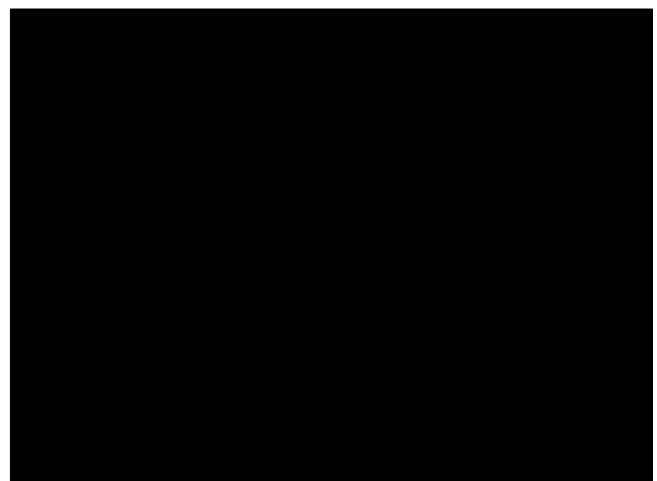


FIGURE 1. A photomicrograph of a conidium of *Alternaria* trapped from the air using volumetric sampler.

From the Department of Biological and Medical Research (Drs. Hasnain and Al-Sedairy), King Faisal Specialist Hospital and Research Centre, and the College of Medicine (Drs. Al-Frayh and Gad-el-Rab), King Saud University, Riyadh, Saudi Arabia.

Address reprint requests and correspondence to Dr. Hasnain: Department of Biological and Medical Research, MBC 03, King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia.

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TABLE 1. Catch percentage of airborne *Alternaria* spp. at various sites in Saudi Arabia.

Month	KFSH 1986-87	JEDDAH 1987-88	AKHB 1987-88	KKUH 1987-88	KKUH 1988-89	KKUH 1989-90
Jan	6.4%	1.8%	3.3%	5.0%	0.7%	3.8%
Feb	6.6%	2.3%	2.7%	6.6%	2.7%	0.8%
Mar	7.8%	1.2%	5.3%	3.7%	3.9%	1.8%
Apr	9.6%	1.0%	4.4%	6.5%	6.4%	4.4%
May	7.4%	0.8%	2.8%	6.7%	3.5%	2.2%
June	3.8%	1.8%	7.0%	4.0%	2.6%	3.0%
July	1.9%	1.5%	2.6%	4.4%	2.1%	2.1%
Aug	5.1%	2.8%	4.5%	3.7%	2.4%	1.5%
Sept	2.2%	1.7%	6.6%	1.5%	3.9%	2.4%
Oct	5.2%	2.3%	2.6%	1.9%	2.7%	4.0%
Nov	4.4%	1.0%	1.9%	1.6%	5.1%	3.9%
Dec	6.0%	2.4%	4.1%	2.5%	1.9%	2.6%
Mean	5.5%	1.7%	4.0%	4.0%	3.2%	2.7%
Range	1.9%- 9.6%	1.0%- 2.8%	1.9%- 6.6%	1.5%- 6.7%	0.7%- 6.4%	0.8%- 4.0%

TABLE 2. A summary of SPT-positive reactions in six different regions using *Alternaria* antigen.

Reaction	Age (yr)	Female/male	Total individuals	Total positive
Mild	1-12	9.6%/11.7%	67	
	13-18	3.2%/2.7%	18	
	>18	5.2%/3.5%	26	
	Blank	0.8%/1.4%	7	
	Total	18.9%/19.4%	118	19.16%
	Total F/M		47/71	
Moderate	1-12	0.8%/0.3%	3	
	13-18	-	-	
	>18	0/1.9%	7	
	Blank	0.4%/0	1	
	Total	1.2%/2.2%	11	1.79%
	Total F/M		3/8	
Strong	1-12	0/0.3%	1	
	13-18	-	-	
	>18	0.8%/0.3%	3	
	Blank	-	-	
	Total	0.8%/0.5%	4	0.65%
	Total F/M		2/2	
No reaction	Total	79.1%/77.9%	483	78.40%
	Total F/M		197/286	
	Total		616	100%
Total F/M		249/367		

F/M=female/male.

in the Riyadh outdoor environment, ranging from 1.9%-9% of the total airborne spores isolated.

The relationship of airborne *Alternaria* and allergic diseases, particularly bronchial asthma, has been established by many workers in this field.¹⁵⁻¹⁸ However, this has generally been done *in vivo*, by conducting skin prick test (SPT), and seldom by bronchial challenges or inhalation provocation tests. An *in vitro* test, such as the radioallergosorbent test (RAST), is often incorporated with the *in vivo* test.

The objective of this study was to investigate and accumulate comprehensive aerobiologic and clinical information on *Alternaria*, using continuous and

volumetric sampling and diagnostic SPT for IgE-mediated reactivities, in order to examine their role as a potent allergen and a potential sensitizer in patients with asthma and other allergic diseases in Saudi Arabia.

Materials and Methods

Spore Detection

Burkard Volumetric Spore Traps were operated at four different sites in three cities in Saudi Arabia (Riyadh, Jeddah and Al Khobar). Riyadh is the capital city, situated in the middle of the desert, in the Central Province. Jeddah is a coastal city by the Red Sea, and Al-Khobar is another comparatively new coastal city, in the Eastern Province of Saudi Arabia.

Sampling was conducted from 1986 through 1990. Air spora were trapped on melinex tapes, mounted and prepared for microscopic study. Slides were studied at 400x. One field of view at 400x was calculated to be 0.152053 mm². Ten such fields were each examined for an hour for the whole period. The numbers of *Alternaria* conidia were converted to concentrations per m³ of air.¹⁴

Site Selection

The following sites were selected for sampling of air in order to evaluate airborne incidence of *Alternaria* in Saudi Arabia: King Faisal Specialist Hospital and Research Centre (KFSH&RC) compound, Riyadh; King Khalid University Hospital (KKUH) compound, Riyadh; King Abdulaziz University compound, Jeddah (JEDD); King Faisal University Hospital compound, Al-Khobar (AKHB).

Allergic Patients

A total of 616 patients, comprising 249 females and 367 males from six different hospitals in Saudi Arabia, were included in this study. These included Asir Central Hospital in Abha, King Fahad Hospital in Qassim, Chest Diseases Hospital in Gizan, King Faisal University Hospital in Hofuf, King Abdulaziz University Hospital in Jeddah, and Noor Hospital, Makkah. All patients were attending allergy clinics in these areas and were suffering from various allergic diseases, including bronchial asthma. After obtaining their informed consent, those on antihistamines were instructed to stop the medication four days prior to the test. Routine SPT was conducted on the above patients, using *Alternaria* extract (ALK w/v 1:20).

The SPT results were examined and recorded as follows: weal size 0 = negative (saline control); weal size >2 mm = mild positive; weal size >3-5 mm = moderate positive; >5 mm = strong positive; 1% histamine phosphate was included as positive control.

Results

Percentages of airborne *Alternaria* spores (Figure 1) at four different sites, including three consecutive years at

one site (KKUH), are presented in Table 1. Mean weekly airborne concentrations per m³ of *Alternaria* spp. for KFSH&RC, JEDD and AKHB are presented in Figure 2. Mean weekly airborne concentration per m³ for 3 consecutive years (1987-90) at KKUH sites are presented in Figure 3. Mean monthly concentration of airborne *Alternaria* for the four sites are presented in Figure 4 (since KKUH was covered for 3 years, the findings are being presented as six different sites for the reason of comparison). Maximum hourly concentrations per m³ of *Alternaria* spores for all sites are presented in Figure 5.

The data presented in the above tables and figures show that *Alternaria* constituted up to 5.5% (yearly mean) of airborne fungal spores, with the highest concentration in April (9.6%) at the KFSH&RC site. The weekly mean concentration exceeded 140 per m³ of air, and the maximum concentration exceeded 500 per m³ at the Jeddah site. The AKHB site was close to 500 per m³ of air, while the KFSH&RC site exceeded 350 per m³ of air.

Results of SPT on 616 patients from several regions using *Alternaria* commercial antigen are presented in Table 2 (a summary of all tests), while the regional results are presented in Table 3.

Discussion

Approximately 20% of the human population is considered to be suffering from atopy, involving localized reactions to extrinsic allergens, such as pollens, animal fragments, fungal spores, house dust mites, etc. Contact between allergens and the cell-bound IgE in the bronchial tree, nasal mucosa and conjunctival tissues releases mediators of anaphylaxis to produce asthma and hay fever. Sensitivity is normally assessed by response to prick and intradermal challenges with antigen. The release of histamine and other mediators produces a wheal and erythema, which indicate the severity of the reaction as mild, moderate or strong. However, optimal use of laboratory allergy tests requires a high level of knowledge regarding allergens' composition, distribution, local importance and other properties which facilitate selection of adequate tests.¹

The current data on airborne *Alternaria* spores clearly demonstrate that they are widely distributed in the Saudi Arabian environment with quantitative regional variations. Maximum hourly concentration exceeds 500 spores per m³ (Figures 5). The value can be related to the suggested threshold concentration of 50 *Alternaria* spores per m³ (mean daily) for sensitization, and once sensitized, a lesser concentration can elicit the symptom or symptoms.

Fungal antigen preparation from commercial sources varies widely. The possible causes of variability are the following: 1) intrinsic variation between spores and mycelium in antigen content; 2) somatic mutations causing allergen content changes within single culture; 3) aging and culture variable changes; 4) interstrain variability; and

TABLE 3. SPT-positive reactions in allergic patients from different regions in Saudi Arabia using commercial *Alternaria* antigen.

Reaction	Female	Male	Grand total
Qassim			
Mild	4 (8%)	11 (12.5%)	15 (10.9%)
Moderate	0	3 (3.4%)	3 (2.2%)
Strong	0	0	0
No reaction	46 (92%)	74 (84.1%)	120 (86.9%)
Total	50 (100%)	88 (100%)	138 (100%)
Abha			
Mild	7 (10%)	14 (13.7%)	21 (12.2%)
Moderate	1 (1.4%)	4 (3.9%)	5 (2.9%)
Strong	2 (2.9%)	1 (1%)	3 (1.7%)
No reaction	60 (85.7%)	83 (81.4%)	143 (83.2%)
Total	70 (100%)	102 (100%)	172 (100%)
Gizan			
Mild	3 (100%)	3 (37.5%)	6 (54.5%)
Moderate	0	0	0
Strong	0	0	0
No reaction	0	5 (62.5%)	5 (45.5%)
Total	3 (100%)	8 (100%)	11 (100%)
Hofuf			
Mild	3 (6.8%)	7 (12.7%)	10 (10.1%)
Moderate	0	0	0
Strong	0	0	0
No reaction	41 (93.2%)	48 (87.3%)	89 (89.9%)
Total	44 (100%)	55 (100%)	99 (100%)
Jeddah			
Mild	1 (4.8%)	4 (11.8%)	5 (9.1%)
Moderate	1 (4.8%)	0	1 (1.8%)
Strong	0	0	0
No reaction	19 (90.4%)	30 (88.2%)	49 (89.1%)
Total	21 (100%)	34 (100%)	55 (100%)
Makkah			
Mild	29 (47.6%)	32 (40%)	61 (43.3%)
Moderate	1 (1.6%)	1 (1.3%)	2 (1.4%)
Strong	0	1 (1.3%)	1 (0.7%)
No reaction	31 (50.8%)	46 (57.5%)	77 (54.6)
Total	61 (100%)	80 (100%)	141 (100%)

5) interspecies variability.¹⁵ The sorting effect is the weak antigen causing a greater number of mild reactions than severe or strong reactions.

Alternaria alternata (also known as *A. tenuis*) is an extremely common species occurring in plants, soil, food, house dust and atmospheric samples, with optimal growth at 25°C–26°C.² It is also a complex species that exhibits significant interstrain differences in morphology, enzyme profile and allergen content when grown under various environmental conditions.²⁰ The complexity of the *A. alternata* allergen was studied by analyzing proteins, carbohydrates and allergenic activity of 10 different strains, by using RAST inhibition and crossed immunoelectrophoresis (CIE)/crossed radioimmuno-electrophoresis (CRIE). A total of 32 allergens were identified, including one major antigen (Ag-8), seven intermediate and six minor allergens.¹ Vijay also studied *A. alternata* and *A. solani* and reported that various strains of each species showed similarities but also showed considerable differences.²¹

As *in vivo* diagnostic tests, e.g., SPT, are usually considered to be more sensitive and economical than *in*

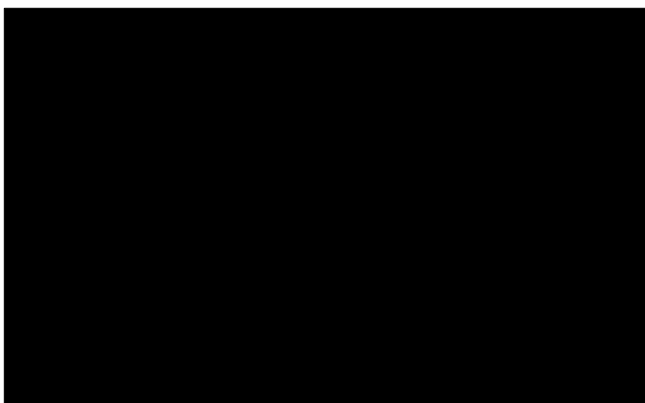


FIGURE 2. Weekly mean concentrations per m^3 of *Alternaria* spp. at three different sites.

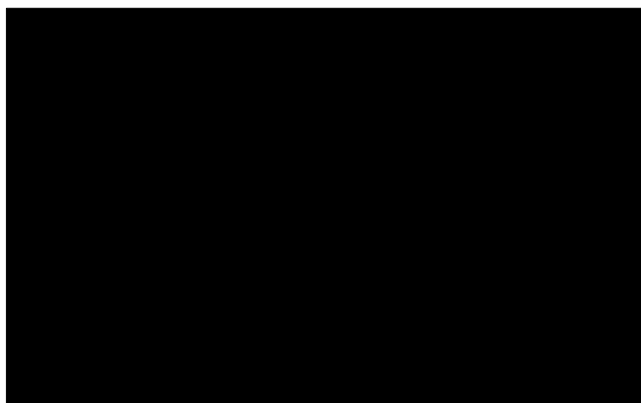


FIGURE 3. Weekly mean concentrations per m^3 of *Alternaria* spp. at KKUH site for three continuous years.

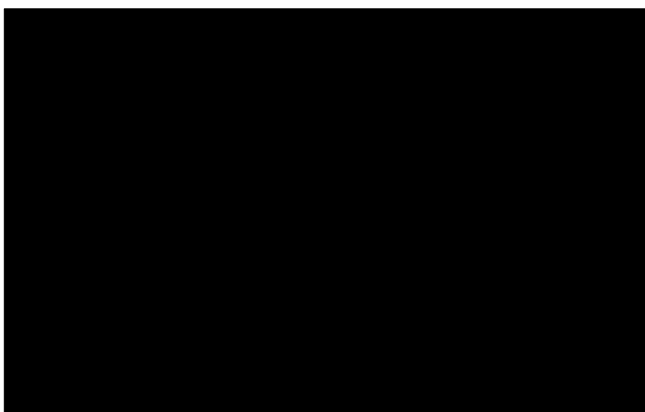


FIGURE 4. Monthly mean concentrations per m^3 of *Alternaria* spores at 6 different sites.

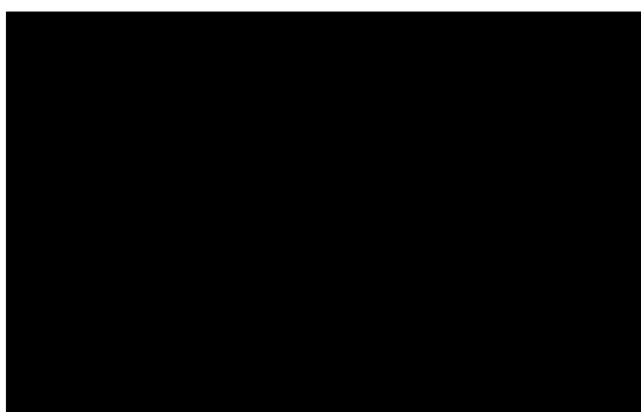


FIGURE 5. Maximum hourly concentrations per m^3 of *Alternaria* spp. at several locations in Saudi Arabia.

in vitro (RAST) tests, there appears to be a great variation in the relative frequency of positive skin reactivity to fungal extracts in patients with asthma and rhinitis in different countries. Positive results range from 3%-4% in Sweden to above 80% in some states in the USA.¹⁵

Skin prick testing with extracts of fungal species derived from homes of allergy clinic cases in Toronto, Canada, displayed up to 50% positive reactivity to *Alternaria alternata*, in comparison to only 4% indoor incidence in patients' homes. This indicates the possible exposure and subsequent sensitization to high fungal concentrations in outdoor environment, or in work-related environments.¹⁷ In a similar study of 2942 allergic cases with mainly bronchial asthma and rhinitis using partially purified *Alternaria* allergen extract, positive reactions varied from 5% to 20%, depending on the geographical and climatic characteristics.²² In Turkey, 130 children with respiratory allergies were submitted to scratch skin test and intradermal skin test. On scratch test, 18.6% of cases reacted to *Alternaria* antigen, while 31.5% of cases reacted to it in intradermal skin test.¹⁸

The SPT data presented in this paper support the above findings that IgE-mediated reactivity to *Alternaria* is very

common, and is the most reactive among all fungal reactions. It may also occur as a result of cross-reactivities with other *Alternaria* species or related genera, such as *Ulocladium*, which is also very common in Saudi Arabia.^{19,23} Hence, it is not inconceivable that there may be shared antigenic properties between the two genera, as with *Stemphylium*.⁵ However, the spores of *Stemphylium* are not so common in our environment.¹⁴ On the other hand, it is known that there are more species-specific antigens in fungi than the genus-specific antigen. These complexities and variations remain as yet unanswered, because only one species (*alternata*) of *Alternaria*, out of more than 45 species known to exist worldwide, has been studied in detail.^{24,25}

This study documents the presence of *Alternaria* spores at various centers of the country exceeding threshold concentrations for sensitization and acute attack of bronchial asthma in sensitized patients. Though no attempts were made to study and correlate the aerobiologic and diagnostic aspects at each center simultaneously at this stage, the SPT data obtained confirm that *Alternaria* spores are potential sensitizers in susceptible individuals showing allergic symptoms, particularly bronchial asthma

and allergic rhinitis. Therefore, those patients who are SPT-positive with *Alternaria* extract may be at risk for elicitation and/or exacerbation of their symptoms.

The mild IgE-mediated reaction (19.16%) (Table 2) is attributable to the poor quality of fungal extract, as these are prepared, unlike pollen extract, from culture and not from pure spores. Batch to batch variation in the potency of fungal extracts was recorded.¹ Hence, the possibility of both "false-positive" and "false-negative" reactions because of antigenic and diagnostic variabilities cannot be eliminated.

We, therefore, conclude that standardized or the highest available quality of *Alternaria* extract should be included in all diagnostic test profiles in Saudi Arabia in order to identify *Alternaria* sensitivity, and to obtain possible stronger reaction in sensitized patients. We recommend that, if possible, even the routine diagnostic test should extend beyond SPT to include intradermal test or RAST for better diagnostic accuracy.

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