

HUMAN SENSITIZATION TO *PROSOPIS JULIFLORA* ANTIGEN IN SAUDI ARABIA

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Background: Allergenicity to *Prosopis juliflora* pollen antigen has been reported from only a few countries, including the US, South Africa, India and Kuwait. In some parts of Saudi Arabia, species of *Prosopis* have been introduced by the millions as roadside ornamentation. There appear to be four flowering seasons during which pollen grains float in all directions. However, the role of *Prosopis* pollen as the sensitizing and/or triggering agent of allergic asthma and/or rhinitis in the Kingdom has never been evaluated.

Patients and Methods: A total of 473 allergic patients suffering from bronchial asthma in four different geographical regions (Abha, Qassim, Hofuf and Gizan), and attending allergy clinics and chest disease centers of university and Ministry of Health hospitals in the region were tested for immediate hypersensitivity reaction to *Prosopis juliflora* allergens. Airborne pollen grains at one center were also studied for one full year, using volumetric sampling techniques.

Results: A total of 76.1% patients in Qassim, 37.5% in Gizan, 29% in Abha and 11% in Hofuf reacted positively to *Prosopis* antigen. Multiple sensitivities to other pollen antigens were detected in all patients. The level of airborne *Prosopis* pollen detected in Gizan exceeded 90 grains m⁻³ of air.

Conclusion: In view of the documented evidence of *Prosopis*-involved allergenicity, the role of *Prosopis* pollen as a sensitizing factor in Saudi Arabia has been confirmed. However, the cause of elicitation of symptoms in many multiple sensitive patients, together with the question of cross-reactivities, needs thorough and detailed investigation. *In vitro* confirmation of all positive results is also required to incriminate *Prosopis* as one of the major allergens in parts of Saudi Arabia.

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Allergenicity to *Prosopis juliflora* pollen antigen has been reported from a few countries, including the United States,^{1,2,3} Kuwait,⁴ India^{5,6,7} and South Africa.⁸ Several species of the drought-resistant genus *Prosopis* have been introduced to Saudi Arabia as roadside ornamentation. Many roads and streets in several cities of the Kingdom have a large number of trees belonging to the genus *Prosopis*. *Prosopis* spp. are a native of South and Central America and India, and are closely related to *Acacia*, a known allergenic plant genus.

Out of the estimated 44 species of *Prosopis*, only nine are known to be present in the Kingdom. These are *Prosopis alba*, *P. chilensis*, *P. glandulosa*, *P. juliflora*

(also known as mesquite), *P. spicigera* (or *P. cineraria*), *P. tamarugo*, *P. velutina*, *P. farcta*, and *P. pallida*. Among these, *P. farcta* and *P. spicigera* are considered to have existed for a long time in the Arabian Peninsula.^{9,10}

P. juliflora is known to flower about four times a year in the region, and during the period large amounts of pollen debris are deposited underneath trees. These are easily distributed by moving vehicles as well as by human and animal activities. Individuals walking on the roadside are also likely to collect pollen on shoes and clothing and transfer them to indoor environments. Published data from different countries indicate that *Prosopis* pollen, which pollinates partly by insects, can become airborne,^{2,4,6} and can be trapped and identified on slides from air samplers. Airborne pollen of *Prosopis* can be inhaled through the nose and/or mouth as other pollen, resulting in sensitization of susceptible people and subsequent elicitation of symptoms of respiratory allergic diseases.^{7,8}

Because of the number of trees in Saudi Arabia, the role of *Prosopis* pollen as an airborne triggering agent for the elicitation of allergic symptoms has been a subject of

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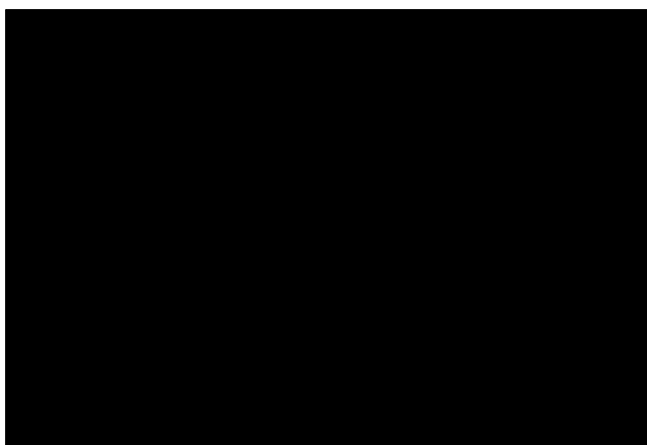


FIGURE 1A. A photomicrograph of a cluster of *Prosopis juliflora* pollen trapped in the environment of Saudi Arabia.

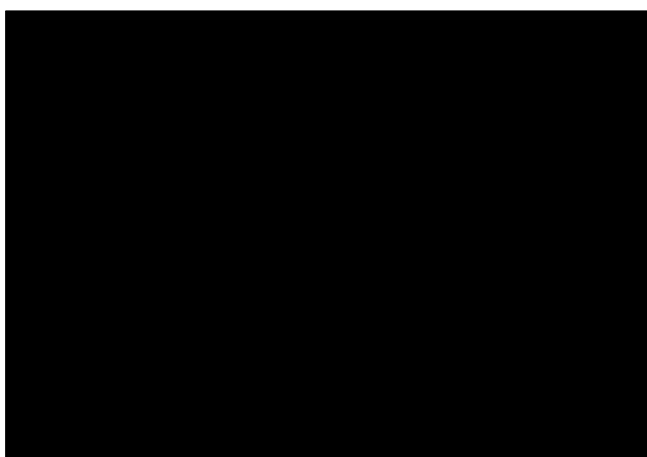


FIGURE 1B. Scanning electron micrograph of *Prosopis juliflora* (1800x magnification).

concern both at the Ministry of Health and the Ministry of Municipal and Rural Affairs. Apparently, no allergological and/or health-related study has been previously undertaken on *Prosopis* in the Kingdom. The objective of the current study was to estimate the sensitization level of individuals with allergic manifestations residing in the areas concentrated with *Prosopis*, as well as distant areas, and to determine the level of *Prosopis* pollen in the region.

Patients and Methods

Patients

A total of 473 allergic patients suffering from bronchial asthma in four different geographical regions were included in the study. These regions are situated within a radius of approximately 1000 km spanning agricultural, horticultural, coastal and mountainous regions. All the patients were attending allergy clinics and/or chest disease centers at universities and at the Ministry of Health hospitals in the regions.

Antigen

Commercial glycerinated extract of *Prosopis juliflora* (Meridian Biomedical, Round Rock, TX, USA, w/v 1:20) was employed in the study. All participating centers were provided with the same lot of extract. In addition to *Prosopis* antigen, a number of other pollen extracts were also tested on a lesser number of patients. The antigens included were *Atriplex polycarpa* (all scale), *Chenopodium album* (lamb's quarter), *Cynodon dactylon* (Bermuda grass), *Salsola tennifolia*, *Phoenix dactylefera* (date), *Olea europaea* (olive tree) (all Meridian Biomedical, w/v 1:20), *Phelum pratensis* (Timothy grass) (ALK, 10 Histamine Equivalent Potency (HEP), and *Rumex crispus* (yellow dock) (ALK, w/v 1:100).

Skin Prick Test

Skin prick test (SPT) was conducted by qualified staff under the supervision of a physician. Routine procedures were adopted, such as deletion of antihistamine or related medication prior to the testing. Normal saline as negative control and histamine as positive control were also employed. The SPT results were examined and recorded as follows: weal size 0 = negative (saline control); weal >2 mm = mild positive; weal size >3-5 mm = moderate positive; >5 mm = strong positive. One percent histamine phosphate was included as positive control.

Radioallergosorbent Test

In-vitro allergen-specific IgE assay using radioallergosorbent test (RAST) was conducted on 284 serum samples from asthmatic allergic patients referred from different regions to the investigators' clinic. RAST is a specialized and expensive test, and is only available in a few laboratories. The grading of RAST classes was done according to the following criteria:

Level of Antibody

Class 0 = <0.35 KUA/L: absent or undetectable

Class I = 0.5-0.7 KUA/L: low

Class II = 0.7-3.5 KUA/L: moderate

Class III = 3.5-17.5 KUA/L: high

Class IV = >17.5 KUA/L: very high

Pharmacia CAP-RAST (T20) was performed according to manufacturer's direction.

Pollen Detection

A Burkard Volumetric Seven-Day Recording Trap was operated on the roof of the Chest Diseases Hospital, Gizan, where SPT was conducted. Pollen grains were identified and analyzed as per details presented earlier.¹¹ Maximum pollen levels m^{-3} of air were collected from the data obtained. The choice of locations was based on convenience and available facilities under an ongoing project, where the above-mentioned sites were included for aeroallergens monitoring.

Results

Skin prick test results on a total of 420 allergic patients are presented in Tables 1, 2 and 3. Table 1 shows the SPT results by *Prosopis juliflora* extract in four different regions, with a high frequency (76.1%) in the mixed agricultural region (Qassim), where a higher number of *Prosopis* trees were observed. Another site, Gizan, showed 37.5% of positive SPT. The SPT positive in Abha was 29.1% and 11.1% in Hofuf. SPT are currently underway in Taif and Hail.

Table 2 shows the SPT results by various other pollen extracts as multiple reactions in these four different regions. In Qassim, being an agricultural region, SPT reactions by *Chenopodium album* (79%), *Salsola tennifolia* (78.2%), *Cynodon dactylon* (69.5%) and *Atriplex polycarpa* (71%) (n=66) were also frequent enough to be considered primary causes of sensitization or possible cross-reactivities with other pollen sensitization, such as *Prosopis* (76.1%). Adding all data together, we note that members of the Chenopodiaceae, *Prosopis* and *Cynodon dactylon* (Bermuda grass), are major outdoor factors causing sensitization in this agricultural region.

Table 3 presents mild, moderate and severe reactions in relation to various age groups of individuals, and indicates that severe reactions in both male (17.39%) and female (9.58%) were obtained in >18-year age group. Mild (F=8.38%, M=4.74%) and moderate (F=3.59%, M=5.14%) reactions were also higher in the >18-year age group of individuals. In total, severe reactions (18.8%) were higher than mild (14.5%) and moderate (8.6%) reactions.

Table 4 shows comparative regional SPT data from Abha, Qassim, Hofuf and Gizan, using *Prosopis juliflora* antigen.

Table 5 presents results of RAST on 284 serum samples from allergic patients. The data indicate that 32 patients (11.3%) were RAST-positive, with both high and very high values. Out of positive RAST for 32 patients, 11 were positive in Class II (moderate), while 15 were positive in Class III and IV (high to very high level).

Airborne *Prosopis* Pollen

For practical reasons, airborne *Prosopis* pollen (Figures 1A and B) were recorded for a full 12-month period, from Gizan only, from December 1995 to November 1996, as presented in Figure 2. The data presented are the maximum levels of airborne pollen recorded during that year.

Discussion

Despite the fact that international studies on *Prosopis*-induced allergenicity are very limited, the studies published¹⁻⁸ from a few countries strongly suggest the allergenic role of airborne *Prosopis* pollen. However, the

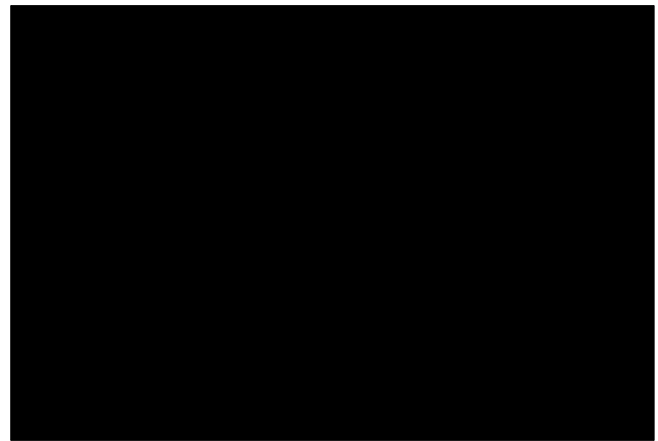


FIGURE 2. Maximum airborne concentration for *Prosopis* pollen in Gizan, Dec 1995-Nov 1996, using Burkard Volumetric Trap.

current study is the first detailed report on *Prosopis* allergenicity in Saudi Arabia. As such, it provides a basis for future biochemical and immunological investigations on *Prosopis*-induced asthma and allergies in the region.

The basic findings of this study revealed that: 1) A higher percentage (76.09%, n=138) of patients reacted to *Prosopis* antigen, using *in vivo* SPT diagnostic parameter; 2) there were marked regional variations in the results of the test; 3) *in vitro* RAST on 284 serum samples (n=284) from asthmatic patients showed 32 (11.9%) positive; 4) multiple SPT reactivities were recorded with eight other pollen allergens tested; 5) airborne pollen from *Prosopis juliflora* exhibited a peak in April and exceeded 90 grains m⁻³ of air at the Gizan site.

In vivo diagnostic results by SPT showing both a high degree of positive reactivities (a certain percentage might be false-positive) as shown in Table 1 (e.g., 76.09% in Qassim) and considerable regional variations (11.1% in Hofuf) are indicative of sensitization levels of patients in the region. This is also indicative of availability of allergen sources (*Prosopis* pollen) and their impact and/or ability to induce allergic reaction in and around the region. This finding is supported by the work of Novey et al.,² who trapped *Prosopis* pollen from a considerable distance from its source in California. By conducting diagnostic tests on 100 consecutive patients using 30 pollen antigens, he obtained 42% positive on scratch test and an additional 20% positive intradermal test to *Prosopis* extracts. He concluded that mesquite pollen is a potent allergen capable of evoking immediate hypersensitivity reactions in a susceptible population remote from the plant source. Lucas and Buckley³ also studied the prevalence of epicutaneous flare reactions to allergenic pollen including mesquite, and concluded that mesquite is the one which exhibited the most informative positive reaction.

In vitro specific IgE assays (RAST) conducted on 284 serum samples from patients referred from different regions resulted in 11.3% being RAST positive. Since

TABLE 1. Skin prick test results by *Prosopis juliflora* extract in four different regions.

	# of individuals	Normal or no reaction	# of pos. reactions (%)	Multiple reactivity
Abha (mountainous region)	172	122	50 (29.07)	++
Qassim (mixed agricultural region)	138	33	105 (76.09)	++
Hofuf (date trees agriculture)	99	88	11 (11.11)	++
Gizan (coastal region)	64	40	24 (37.50)	++

TABLE 2. Skin prick test results by various pollen extracts in four regions.

	Abha (n=156) (%)	Qassim (n=66) (%)	Hofuf (n=12) (%)	Gizan (n=11) (%)
<i>Atriplex polycarpa</i>	30.81	71.01	2.02	72.73
<i>Chenopodium album</i> (lamb's quarter)	32.56	78.99	5.05	81.82
<i>Cynodon dactylon</i> (Bermuda grass)	34.30	69.57	3.03	72.73
<i>Phoenix dactylefera</i> (date pollen)	23.84	52.90	19.19	81.82
<i>Olea europa</i> (olive)	21.51	39.13	7.07	81.82
<i>Pheleum pratensis</i> (Timothy grass)	17.44	45.65	3.03	72.73
<i>Rumex crispus</i> (yellow dock)	16.86	38.41	5.05	81.82
<i>Salsola tennifolia</i>	16.86	78.26	13.13	72.73

TABLE 3. Skin prick test reaction to *Prosopis* pollen in different age groups.

Reaction	Age	% female	% male	Total #
Mild	1-12 yr	5.39	4.74	21
	13-18 yr	1.80	3.16	11
	>18	8.38	4.74	26
	Unknown	0.60	0.79	3
	Total (n=61)	16.2	13.4	F:27, M:34 (14.5%)
Moderate	1-12 yr	1.80	1.19	6
	13-18 yr	1.80	0.79	5
	>18	3.59	5.14	19
	Unknown	2.40	0.79	6
	Total (n=36)	9.6	7.9	F:16, M:20 (8.6%)
Severe	1-12 yr	0.60	1.19	4
	13-18 yr	2.40	3.16	12
	>18	9.58	17.39	60
	Unknown	0.60	0.79	3
	Total (n=79)	13.2	22.5	F:22, M:57 (18.8%)
Normal	Total (n=244)			F:102, M:142 (58.1%)
Total (n=420)				F:167, M:253 (100%)

RAST is an expensive procedure and facilities are limited to certain institutions of the country, these patients did not only come from those regions where SPTs were conducted (Table 1), but they also came from other regions. However, at this stage, no attempt was made to separate the referred patients on a regional basis; instead, the result was compiled as an indicator of *in vitro* RAST positive by

Prosopis antigen in Saudi Arabian patients. Based on the different classes of RAST mentioned in the methods, results also indicated low to very high levels of antibody. The result is contrary to the findings of Novoy et al.,² where RAST with mesquite was negative unless concentrated extract was used in the allergosorbent-cellulose preparation.

Although multiple reactions were noted in almost all cases of SPT positive with *Prosopis* antigen, the similar multiple reactions with other pollen antigens (such as *Cynodon dactylon*) were also recorded by an earlier worker.² In a study of cross-reactivity among tree pollen,¹¹ which included *Prosopis* antigen, along with poplar (PO), birch (Bi), alder (Al), oak (Oa), beech, (Be), hornbeam (Ho), etc., the greatest cross-reactivities we recorded were among members of Fagales (Bi, Al, Ho, Oa, Be), and apparently no cross-reactivity was recorded with these and mesquite pollen. Therefore, it is not convincing that multiple reactivities by other pollen allergens presented in Table 2 may be cross-reactivities by *Prosopis* pollen or vice-versa. It is likely that within the other pollen group cross-reactivities may have taken place which are common in most grasses (but not in Bermuda grass) and some weeds. In general, allergen extract of only *Prosopis juliflora* is commercially available, and for this reason, information on even the most likely allergenic cross-reactivities within the species of *Prosopis* pollen appear to be missing from the literature.

In vivo and *in vitro* immunological response of *Prosopis juliflora* pollen allergen have also been measured in guinea pigs. Intercutaneous skin test showed an early wheal flare response and a late erythema redness sensitized with various concentrations (100, 50, 25, 5 and 1.5 mg/mL) of *Prosopis juliflora* pollen extract after administration of a challenging dose. A 50 mg/mL sensitizing dose of *Prosopis juliflora* pollen allergen gave optimum skin response as both early and late effects. The nature of immunochemical reactivity between pollen allergens and reagenic antibodies were further characterized by histamine-release test, gel diffusion test, radioallergosorbent test and passive cutaneous anaphylaxis test. The test confirmed allergenicity caused by *Prosopis juliflora* pollen allergens and showed the binding of allergens with reagenic antibody and its regulation in guinea pigs.⁷

In Kuwait, *Prosopis spicigera* (also known as mesquite, algarroba or hone locust) became the most common roadside plant in 1954. The species, known to be a native of North India, was imported from Abadan in 1951, and out of 30,000 plants introduced in Kuwait, 10,000 were planted in the town of Ahmadi. Two pollination periods of *Prosopis* in Kuwait were recognized in April and September, when hay fever in Ahmadi was recorded at its peak, suggesting that *Prosopis* pollen might be one of responsible allergens. Subsequently, an extract of the *Prosopis* pollen was prepared by Wright-Fleming

Institute in London under the supervision of Dr. A.W. Frankland and R.R. Davies and tested in patients in Ahmadi. Out of a total of 90 patients investigated, 82 showed strong positive reactions. However, despite the large number of *Prosopis* trees and high skin test reactivities, airborne *Prosopis* pollen was trapped only in small numbers on four occasions between August 1962 and March 1963: 10 grains/m³ on 10 September and 24 November and 5 grains/m³ on 28 November and 4 December.⁴ The current data for airborne *Prosopis* presented in Figure 2 for Gizan indicate even a higher concentration level (90 grains m⁻³), with peak in April. In India, a one-year sampling in different inhabited areas in 4 zones using volumetric samplers revealed *Prosopis* pollen as an important contributor, especially at lower heights.⁶

For the treatment of patients in Ahmadi, after confirmation of skin reactivity by extract of *Prosopis* spp., a supply of the vaccine was obtained and a sensitizing course was offered to those who had shown positive skin test reaction. Of the 42 patients who completed the course, 21 were available for interview three years later. Six had been completely relieved for 3 years or more, 4 improved to the extent of making the disorder bearable with the aid of antihistamines, while there had been no improvement in the other six. Thus 70% had derived some benefit from *Prosopis* desensitization.¹²

Some progress on the biochemical aspect of *Prosopis* allergen has been made, e.g., *Prosopis juliflora* pollen allergen extract has been fractionated by sephadex (G-100 gel filtration).¹³ Six different fractions were obtained, which were confirmed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.¹⁴ A fraction called E (MW 20,000 kd) consisted mainly of allergenic molecules.^{15,16} However, it appears that no other species of *Prosopis* have been studied in relation to antigenic properties or compared with *Prosopis juliflora*. Consequently, characterized and purified antigens from *Prosopis* spp., as per WHO reference, have not yet emerged.

The variable SPT data presented for the different parts of Saudi Arabia, with a considerable number of positive reactions obtained by *Prosopis juliflora* extract, indicate that individuals in these location are sensitized with specific IgE antibodies to *Prosopis juliflora*. The sensitizing effect may take place at any region where a higher level of these pollen may be found. But it is more likely that individuals living in densely populated *Prosopis* areas such as Qassim will be sensitized in that area. This is also evident from the data that out of 172 patients, 50 (29.07%) reacted in the mountainous region (Abha) compared to 105 patients (76.09%) out of 138 in the agricultural region (Qassim) known to have a large number of *Prosopis* trees. This highlights the fact that agricultural regions normally have a lot more to cause sensitization than *Prosopis*. This may be true, as almost all individuals also showed higher multiple reactions with pollen extracts in the Qassim region.

TABLE 4. Skin prick test reactions in patients from Abha, Qassim, Hofuf and Gizan regions, using *P. juliflora* antigen.

	Number female patients (%)	Number male patients (%)	Total (%)
Abha			
Mild	14 (10)	17 (16.6)	31 (18.02)
Moderate	1 (1.43)	3 (2.94)	4 (2.33)
Severe	5 (7.14)	10 (9.80)	15 (8.72)
Normal	50 (71.43)	72 (70.59)	122 (70.93)
Total	70 (100)	102 (100)	172 (100)
Qassim			
Mild	5 (10)	6 (6.82)	11 (7.97)
Moderate	13 (26)	17 (19.32)	30 (21.74)
Severe	17 (34)	47 (53.41)	64 (46.38)
Normal	15 (30)	18 (20.45)	33 (23.91)
Total	50 (100)	88 (100)	138 (100)
Hofuf			
Mild	6 (13.64)	4 (7.27)	10 (10.10)
Moderate	1 (2.27)	0	1 (1.01)
Severe	—	—	—
Normal	37 (84.09)	51 (92.73)	88 (88.89)
Total	44 (100)	55 (100)	99 (100)
Gizan			
Mild	2 (66.6)	7 (87.50)	9 (81.82)
Moderate	1 (33.33)	0 (0.005)	1 (9.09)
Severe	—	—	—
Normal	0	1 (12.50)	1 (9.09)
Total	3 (100)	8 (100)	11 (100)

TABLE 5. RAST in vitro results of *P. juliflora* (mesquite T20) on 284 serums from allergic patients from different parts of Saudi Arabia.

Pos. # of patients	RAST Class 0	RAST Class I	RAST Class II	RAST Class III	RAST Class IV
6	0	x	0	2	0
11	0	0	x	0	0
10	0	0	0	x	0
5	0	0	0	0	x
32	0	x	x	x	x

In view of the documented evidence of *Prosopis*-involved allergenicity, the role of *Prosopis* pollen as a sensitizing factor in Saudi Arabia is confirmed. However, the cause of elicitation of symptoms in many multiple sensitive patients, together with the question of cross-reactivities, needs thorough and detailed investigation. *In vitro* confirmation of all positive results on a regional basis is also required to incriminate *Prosopis* as one of the major allergens in parts of Saudi Arabia.

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