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a communication from India

A comprehensive investigation was done to evaluate the allergenic algae in the Delhi area. Results of 4,000 skin tests performed on 400 patients suffering from naso-bronchial allergy and 300 skin tests on 30 healthy persons with 10 common algae isolated from the Delhi atmosphere are presented and compared. Positive skin reactions (1+ to 3+) to algae extracts ranged from 25.7% with *Lyngbya major* extract to 1.7% with *Oscillatoria simplicissima* extract; in healthy non-allergic volunteers there were no positive skin reactions. The Prausnitz-Küstner (PK), bronchial provocation and conjunctival tests were negative in patients with negative skin reactions; in patients with positive skin reactions PK was positive in 70.9%, bronchial provocation in 50% and conjunctival in 48.5%. Levels of total IgE in patients with naso-bronchial allergy were higher, ranging from 1,225 international units per ml to 1,550 international units per ml, while in healthy volunteers the values were less than 885 international units per ml.

RESPIRATORY ALLERGY TO ALGAE: CLINICAL ASPECTS

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THE PREVALENCE OF ALLERGIC disorder in India is quite high.^{1,2,3,4} Although systematic, aerobiologic and clinicoimmunologic work has been done on the allergenic pollen, fungal spores and insects,^{5,6,7,8} the field of algae allergy has remained unexplored despite the climate of India, which is favorable for algae spores and fragments to become airborne. Very few studies have been reported in world literature of the role of algae in respiratory allergy.^{9,10} We have reported earlier from this laboratory on the aerobiological aspects^{11,12} and immunological aspects¹³ of this subject. This paper presents the clinical aspects of respiratory allergy to algae.

Materials and Methods

Preparation of Allergenic Extract:

After mass culturing of 10 forms of algae isolated from air, the pellicles obtained were harvested, dried in an incubator at 37°C and pulverized. Aqueous extracts

were then prepared in buffered saline according to the technique described by Sheldon et al.¹⁴ These were then standardized by weight/volume and their nitrogen and protein content were estimated by the Microkjeldahl method.²⁰ (See Table I).

Table I. Nitrogen mg/ml and Protein Nitrogen Contents of Aqueous Extracts (1:500 W/V) of 10 Common Airborne Algae.

S.No.	Algae extract	Nitrogen mg/ml	P.N.U.
1.	<i>Phormidium angustissimum</i>	0.427	17080
2.	<i>Nostoc commune</i>	0.270	10800
3.	<i>Oscillatoria simplicissima</i>	0.240	9600
4.	<i>Anabaena fertilissima</i>	0.206	8240
5.	<i>Westiellopsis prolifica</i>	0.178	7120
6.	<i>Chlorococcum humicola</i>	0.178	7120
7.	<i>Chlorella pyrenoidosa</i>	0.172	6880
8.	<i>Scytonema bohnneri</i>	0.140	5600
9.	<i>Lyngbya major</i>	0.140	5600
10.	<i>Anabaenopsis circularis</i>	0.079	3160

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Clinical Studies.

To determine which of the 10 algae extracts were important in clinical allergy, the following tests were performed on patients with naso-bronchial allergy; non-allergic healthy persons were used as controls.

1. *Intradermal Tests:* These were performed on 400 patients and 30 non-allergic controls with 0.01 ml of 1:500 weight/volume of the algae extract. A negative control test using buffered saline and a positive control using 1 microgram of histamine acid phosphate were also performed on each subject. The skin reactions were read at 20 minutes and graded according to the criteria of Shivpuri.⁶

2. *Prausnitz-Küstner Tests (P.K. Tests):* These were performed on the non-allergic relatives of the patients and gave negative skin reactions to the particular allergen. The standard technique²¹ using a dose of 0.1 ml of undiluted serum collected from the patient was employed. Care was taken to collect serum from patients showing a 1+ to 3+ reaction on direct intradermal testing as well as from those who gave negative intradermal reactions. These served as negative control sera.

3. *Bronchial Provocation Test (BPT):* These were performed on 30 subjects showing different intensities of intradermal skin reactions as well as on 10 patients who gave negative intradermal reactions and served as controls. This technique has previously been described by us.⁶ One modification is that instead of a closed circuit an open circuit design was employed.

4. *Conjunctival Tests:* These were performed on patients showing various grades of skin reactions as described above. Negative controls were also included. The technique employed has been previously described.⁶

5. *Estimation of Total IgE:* This was done in the sera of 15 patients by the Mancini technique with an IgE kit initially supplied by Meloy Laboratories, Virginia, U.S.A.

Results

1. *Intradermal Tests:* Results of 4,000 skin tests with 10 algae extracts performed on 400 patients suffering from bronchial asthma and/or allergic rhinitis are presented in Table II. It will be noted that none of the 30 healthy non-allergic controls showed a positive skin

Table II. Percentage of Patients with Respiratory Allergy Who Showed Type I Positive Reactions to Algae Extracts.

Total no. of patients tested	Patients showing 1+ to 3+ reactions to one or more algae extracts		Patients showing 2+ to 3+ reactions to one or more algae extracts		Healthy controls	
	No.	%	No.	%	No.	%
400	173	43.2	50	12.5	30	0.0

Note — There were no 4+ reactions.

Table III. Results of 4,000 Intradermal Tests Performed on 400 Patients of Respiratory Allergy with 10 Algae Extracts (1:500 W/V).

S.No.	Algae extract	Total tests done	1+ %	2+ %	3+ %	1+ to 3+ %	2+ to 3+ %
1.	<i>Lyngbya major</i>	400	19.5	5.5	0.75	25.7	6.25
2.	<i>Phormidium anguistissimum</i>	400	15.2	5.0	—	20.2	5.0
3.	<i>Anabaena fertilissima</i>	400	5.5	3.0	0.75	9.2	3.75
4.	<i>Scytonema bohnneri</i>	400	6.5	2.5	0.25	9.2	2.75
5.	<i>Chlorella pyranocidosa</i>	400	4.5	2.25	—	6.7	2.25
6.	<i>Westiellopsis prolifica</i>	400	8.5	1.25	—	10.0	1.5
7.	<i>Anabaenopsis circularis</i>	400	9.75	1.0	—	10.7	1.0
8.	<i>Oscillatoria simplicissima</i>	400	1.25	0.25	—	1.7	0.25
9.	<i>Nostoc commune</i>	400	3.5	—	—	3.5	0.0
10.	<i>Chlorococcum humicola</i>	400	2.0	—	—	2.0	0.0
	Total	4000	7.6	2.1	0.01	9.9	2.2

Total Positive

reaction whereas of the 400 patients tested 43.2% gave varying grades of positive reactions, ranging from 1+ to 3+. If the 1+ reactions are excluded, only 12.5% gave positive reactions ranging from 2+ to 3+. No. 4+ skin reaction was obtained.

Table III presents the results of intradermal tests with each algae extract in greater detail, showing not only the total positive reactions with each extract but also the grades of the positive reactions. *Lyngbya major* gave the maximum percentage of markedly positive reactions (6.25%).

2. *Prausnitz-Küstner Tests (P.K. Tests)*: All the 10 P.K. tests performed with the sera of patients who showed negative skin reactions were also negative. P.K. tests were positive in 16.6%, 85.0% and 100% of the sera from patients who showed 1+, 2+ and 3+ skin reactions respectively (See Table IV).

3. *Bronchial Provocation Tests*: Results of 30 bronchial provocation tests performed on the patients with bronchial asthma with various grades of skin reactions (negative to 3+) are presented and compared in Table V. It will be noted that bronchial response was negative in all the patients who had shown negative intradermal skin reactions. The positive bronchial response was seen in 63.6% and 75.3% of the patients who had shown 2+ and 3+ intradermal skin reactions, respectively. None of the patients with 1+ skin reaction gave a positive bronchial response.

4. *Conjunctival Tests*: A total of 45 conjunctival tests were performed. All the 10 conjunctival reactions were negative in the control group of 10 patients who had

shown negative intradermal skin tests to the respective antigen. However, where the intradermal test was positive the positive conjunctival reactions were seen in one of the six patients with 1+ skin reactions, in 12 of 24 cases with 2+ skin reactions and in three of five patients with 3+ skin reactions. (See Table VI.)

5. *Estimation of Total IgE Content in Sera of Patients*: Total IgE content was also estimated in 15

Table VI. Results of Conjunctival Tests Performed on Patients of Respiratory Allergy with Various Algae Extracts (1:50 W/V) as Compared with Intradermal Tests (1:500 W/V).

I/D test grades	Total tests done	Conjunctival test results		
		1+	2+	Total +ve No. (%)
Negative (controls)	10	-	-	0 (0.0)
1+	6	1	-	1 (16.6)
2+	24	12	1	13 (54.2)
3+	5	3	-	3 (60.0)
Total +ve (1+ to 3+)	35	16	1	17 (48.5%)

Table IV. Results of Prausnitz-Küstner (P.K.) Tests Performed on Non-allergic Volunteers with Various Algae Extracts (1:500 W/V) Compared with I/D Tests (1:500 W/V).

P.K. Test results				
I/D Test Grades	Total Tests done	1+	2+	Total No. (%)
Negative (controls)	10	-	-	0 (0.0)
1+	6	1	-	1 (16.6)
2+	20	11	6	17 (85.0)
3+	5	2	3	5 (100.0)
Total +ve (1+ to 3+)	31	14	9	23 (70.9)

Table V. Results of Bronchial Provocation Tests Performed on Asthmatics with Various Algae Extracts (1:50 W/V) as Compared with Intradermal Tests (1:500 W/V).

Results of bronchial provocation tests					
I/D Test Grades	Total tests done	Mild (1+)	Moderate (2+)	Severe (3+)	Total +ve No. (%)
Negative (controls)	10	-	-	-	0 (0)
1+	5	-	-	-	0 (0.0)
2+	11	-	5	2	7(63.6)
3+	4	-	1	2	3(75.3)
Total +ve (1+ to 3+)	20	-	6	4	10(50.0)

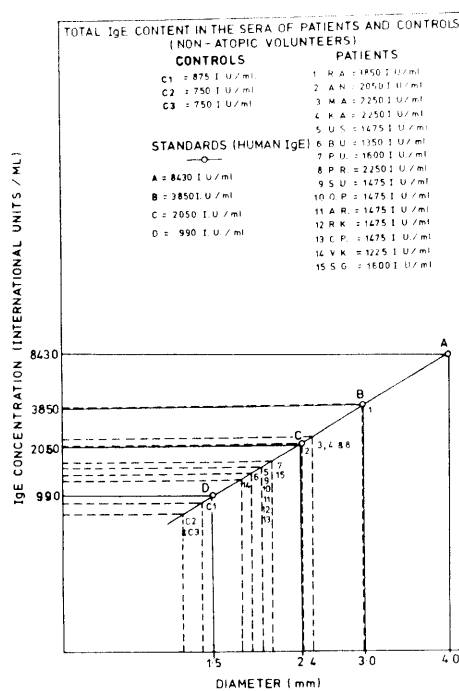


Figure 1.

Table VII. Relative Sensitivity of Different Diagnostic Tests in Patients Showing Positive Intradermal Reactions.

Diagnostic tests	Total skin tests Positive cases tested	% of positive reaction
P.K. test (1:500)	31	70.9
Bronchial provocation test (1:50)	20	50.0
Conjunctival test (1:50)	35	48.5

patients suffering from respiratory allergy. All showed high IgE titer ranging from 1225 international units/ml to 1550 I.U./ml while in the three healthy non-allergic volunteers IgE titer was less than 875 international units/ml (see Figure 1).

Discussion

The role of algae as inhalant allergens in Type I hypersensitivity is well substantiated by the findings presented in Table II. It will be noted from Table III that in allergic patients positive skin reactions (1+ to 3+) to algae extracts were quite common, ranging from 25.7% with *Lyngbya major* extract to 1.7% with *Oscillatoria Simplicissima* extract, while in healthy non-allergic volunteers there were no positive reactions, thus indicating that the positive skin reactions obtained in the patients were not due to the presence of any irritant but were likely evidence of an immunologic reaction, particularly in those with a reaction of 2+ or more.

From Table VII it is evident that when all the positive reactions of each diagnostic test are taken together, P.K. ranks first (70.9%), followed by bronchial provocation test (50.0%) and then by the conjunctival test (48.5%).

From the analysis of results of the conjunctival, P.K. and bronchial provocation tests it is clear that when high intensity positive reactions are taken alone, the sensitivity of different diagnostic tests are similar, although the degree and intensity of reactivity of human skin, bronchial tree and conjunctiva may differ in the same person even when he gives a positive response in each of the three tissues.

The percentage of correlation between negative bronchial responses in the patients with negative skin reactions supports Shivpuri's criteria for positivity and grading of skin reactions.

Higher levels of IgE in allergic patients compared to normals may be associated with the severity of the disease, if other causes of increase of IgE such as tropical eosinophilia, parasiticism, etc. have been excluded.

References

1. Indian Council of Medical Research, New Delhi: Morbidity survey of contributory health scheme beneficiaries, 1961.
2. Shivpuri DN: Studies in allergy and allergens in India. An introduction. *Aspects Allerg & Appl Immunol* 1: 71, 1968.
3. Viswanathan R, Mody RK, Prasad SS and Sinha SP: Bronchial asthma and chronic bronchitis. *J Ind Med Assoc* 45: 480, 1965.
4. Wig KL and Guleria JS: Bronchial asthma, its incidence, natural history and diagnosis. In *Respiratory Allergy - A Symposium*, 1961, pp. 9 and 10. New Delhi: Asia Publishing House, 1963.
5. Shivpuri DN, Viswanathan R and Dua KL: Studies in pollen allergy in Delhi area. Part II (survey of atmospheric pollens). *Ind J Med Res* 48: 21, 1960.
6. Shivpuri DN, Shali PL, Agarwal MK, Prakash D and Bhatnagar PL: Insect allergy in India. *Ann Allerg* 29: 588, 1971.
7. Shivpuri DN and Singh K: Studies in yet unknown allergenic pollens of Delhi State Metropolitan (clinical aspects). *Ind J Med Res* 59: 1411, 1971.
8. Bernstein H and Safferman RS: Sensitivity of skin and bronchial mucosa to green algae. *J Allerg* 38: 166, 1966.
9. Bernstein H and Safferman RS: Clinical sensitivity to green algae, demonstrated by nasal challenge and in vitro tests of immediate hypersensitivity. *J Allerg* 51: 22, 1973.
10. Heise HA: Symptoms of hay fever caused by algae. *J Allerg* 20: 383, 1949.
11. Luncford LM: Algae as an allergen. *J Kansas Med Soc* 69: 466, 1968.
12. McIlhenney TR, Bold HC, Brown RM and McGovern JP: Algae a cause of inhalant allergy in children. *Ann Allerg* 20: 739, 1962.
13. McGovern JP, Haywood LM and McIlhenney TR: Airborne algae and their allergenicity. II. Clinical and laboratory multiple correlation. Studies with four genera. *Ann Allerg* 24: 145, 1966.
14. Benaim-Pinto C: Airborne algae as a possible etiologic factor in respiratory allergy in Caracas, Venezuela. *J Allerg* 49: 356, 1972.
15. Mittal A, Agarwal MK, Goyal SK and Shivpuri DN: Preliminary studies on the allergenic algae of Delhi Metropolitan area - botanical aspects. *Aspects of Allergy & Appl Immunol* 6: 41, 1973.
16. Mittal A, Agarwal MK, Singh VP and Shivpuri DN: Studies on the prevalent algal forms of Delhi atmosphere. *Aspects Allerg & Appl Immunol* 7: 51, 1974.
17. Agarwal MK, Mittal A and Shivpuri DN: Investigations on the immunological role of airborne algae in Type I hypersensitivity. In *Advances in Chronic Obstructive Lung Disease*, proceedings at World Congress on Asthma, Bronchitis & Conditions Allied, (New Delhi, 1974) Viswanathan and Jaggi (Eds.) Pp. 743-757, 1977.
18. Sheldon JM, Lovel RG and Mathews KP: *A Manual of Clinical Allergy*. Philadelphia: W. B. Saunders, 1967.
19. Kabat EA and Mayer MM: *Experimental Immunochimistry*. Illinois: Charles C. Thomas, 1969.
20. Prausnitz C and Küstner H: Studies on hypersensitivity. *Zentralbl f Bakteriol* 86: 160, 1921.

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