

Etiologic significance of mosquito (*Anopheles stephensi*) in respiratory allergy in India 31285

Mahendra K Agarwal, PhD; Suman Chaudhry, PhD; Shashi Jhamb; Shailendra N Gaur, MD*; UPS Chauhan, PhD†; and Harish C Agarwal, PhD‡

The etiologic significance of Anopheles stephensi, a common mosquito species in Delhi, in respiratory allergic disorders was investigated. Intradermal (ID) tests with the mosquito whole body extract (WBE) were performed on 247 patients with bronchial asthma and/or rhinitis and 50 healthy nonallergic volunteers. Of these 247 patients, 118 (47.8%) had positive (1+ to 4+) reactions including 72 (29.1%) with markedly positive (2+ to 4+) ID reactions. We did not observe any 2+ to 4+ skin reactions in normal healthy volunteers, giving evidence for the specificity of markedly positive ID response. The skin test positivity was not influenced by various clinical characteristics (age at onset, duration and type of disease, and family history) of patients included in the study. The specificity of positive ID response was further supported by the results of mosquito RAST with the sera of 14 patients. Ten of the 12 sera (83%) from patients with positive ID response to mosquito WBE were RAST positive. Sera from two patients with negative ID reactions were RAST negative. The bronchial provocation tests (BPTs) were uniformly negative in all five healthy volunteers and two patients showing negative ID responses to mosquito WBE, while the three patients showing 2+ to 4+ responses gave positive BPTs. Our results suggest that mosquito-derived particles are important inhalant allergens in the etiology of IgE-mediated respiratory allergic disorders in India.

INTRODUCTION

Various insects including cockroach, May fly, Caddis fly, and "green nimitti" midge have been incriminated as sources of inhalant allergens.¹⁻⁸ Mosquito is a prevalent insect in India. During their life cycle, scales and other emanations from the body are disseminated into the air. Furthermore, the detritus produced after death and decay also becomes airborne. These mosquito emanations and detritus may serve as potent inhalant allergens and cause symptoms in patients with respiratory allergic disorders. No systematic study, however, has been reported so far on the role of mosquitoes as sources of inhalant aller-

gens. Hence, the present study was undertaken to investigate the role of *A. stephensi*, a common mosquito species in Delhi atmosphere, in the etiology of respiratory allergic disorders.

MATERIAL AND METHODS

Study Groups

Two hundred forty-seven consecutive patients (142 males and 105 females) aged 9 to 61 years (average 27 years) with bronchial asthma and/or rhinitis reporting to the Clinical Research Centre of V.P. Chest Institute, University of Delhi, India, were included in the present study. The patients were selected on the basis of their personal as well as family histories, physical examination, and various routine laboratory investigations. Each patient completed a questionnaire indicating the history of asthma and/or rhinitis, season and frequency of symptoms, age at onset of respiratory disease, duration of disease, and history of respiratory or any other allergic disorders in the first degree relatives.

All steroid-dependent patients,

those with associated cardiopulmonary diseases and those undergoing immunotherapy were excluded from the present study.

As controls 50 healthy nonallergic volunteers (32 males and 18 females) aged 17 to 61 years (average 28 years) were also studied. None of them had a personal or family history of any allergic disease.

Allergens

Mosquito whole body extract (WBE). Mosquitoes (*Anopheles stephensi*) were reared in our laboratory in sufficient numbers. The whole body extract from these mosquitoes was prepared by our modified method.⁹

Solid phase mosquito allergen. For preparing solid phase mosquito allergen discs, the method of Adolphson et al¹⁰ was followed. In brief, cyanogen bromide-activated cellulose discs (Whatman filter paper No. 50, 6mm, diameter) were allowed to react with mosquito WBE in borate buffered saline (pH 8.0) for 72 hours at 4 °C, on a rotator. Subsequently, the discs were thoroughly washed and noncoupled sites were blocked with 1 M etha-

From the Department of Respiratory Allergy and Applied Immunology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110 007, India.

* Clinical Research Centre, Vallabhbhai Patel Chest Institute.

† Department of Biochemistry, Institute of Nuclear Medicine and Allied Sciences, Delhi, India.

‡ Department of Zoology, University of Delhi, Delhi, India.

Received for publication December 11, 1990.

Accepted for publication April 20, 1991.

nomamine. Finally, the discs were washed and stored in RAST diluent at -20°C . Fifty milligrams mosquito WBE per 100 paper discs was used throughout the study based on the result of preliminary RAST experiments using varying amounts of mosquito WBE (10, 20, 50, and 100 mg/100 discs) ("Results" not shown).

Skin Tests

The allergenic significance of mosquitoes was evaluated by performing intradermal (ID) tests with mosquito WBE (1:500 wt/vol) on the patients with nasobronchial disorders. Phosphate buffered saline (PBS) and histamine acid phosphate (100 $\mu\text{g/mL}$) were also tested as a negative and a positive control, respectively. The tests were performed and graded according to the method and criteria of Shivpuri and Agarwal.¹¹ Markedly positive reactions (2+ to 4+) were confirmed by repeating the tests on the patients the same day. The tests were performed by the same trained technician and graded by one of the coauthors (MKA) throughout the course of study.

The specificity of ID reactions in patients was evaluated by performing similar tests on 50 healthy non-allergic volunteers.

Radioallergosorbent Test (RAST)

The disc RASTs were performed according to the procedure of Gleich and Yunginger¹² with a few modifications. In short, 50 μL of the test serum was incubated overnight with one allergen disc at 4°C . After washing the discs three times, 50 μL of ^{125}I -labeled anti-human IgE serum (Phadebas RAST isotope reagent; Pharmacia Diagnostics, Uppsala, Sweden) was added and incubated overnight at 4°C . The discs were washed thrice and counted for one minute in a gamma counter.

For evaluation of results, RAST binding (% counts bound) was calculated as the percentage of total counts added.

Determination of RAST Ratio

The counts bound with patient's sera in RAST experiments were expressed in terms of RAST ratio as per the following formula:

RAST ratio:

$$\frac{\% \text{ counts bound (CB) with patients serum}}{\% \text{ CB with normal human serum}}$$

A RAST was considered positive when the RAST ratio was 3 or more.

Bronchial Provocation Tests (BPTs)

Five asthmatics showing different grades of ID response (negative to 4+) to mosquito WBE and five healthy nonallergic volunteers were subjected to BPT with the homologous allergen extract according to the method of Shivpuri and Agarwal.¹¹

Statistical Analysis

A correlation coefficient between ID response (average diameters of wheal) and RAST ratios was calculated and significance tested by *t* test. The sensitivity and specificity of the two tests were evaluated in terms of Youden's Index.¹³

RESULTS

Skin Tests

The results of intradermal tests on 247 patients and 50 controls are presented in Table 1. Of the 247 patients, 118 (47.8%) had positive ID responses (1+ to 4+), 72 (29.1%) of them giving markedly positive (2+ to 4+) reactions. Only 6% of the 50 healthy nonallergic volunteers showed 1+ reactions. We did

not observe any markedly positive ID reaction in this group.

The distribution of the patients according to their clinical characteristics is included in Table 2 with the ID tests results in each group, separately.

Of the 247 patients, 42.1% were suffering from asthma, 13.4% from rhinitis, and 44.5% from both asthma and rhinitis. The relationship between clinical characteristics of patients and ID test results revealed that there was no significant difference in the ID positivity to mosquito WBE in the three groups. Similarly, duration of disease, age at onset, type of disease, and family history did not influence the skin test results.

RAST

The results of *A. stephensi* RAST using the sera of a limited number of 14 patients with different grades of skin reactions are presented in Table 3. Ten of the 12 sera from patients showing 1+ to 4+ ID responses were RAST positive. Both the sera from the patients with negative ID responses to the mosquito WBE were RAST negative.

The correlation between the RAST ratios and wheal diameters (ID response) was found to be significant ($r = .69$, $P < .01$). RAST results were found to be 100% specific when compared with ID response as all the patients showing positive RAST were ID positive (1+ to 4+) and all the ID negative cases gave negative RASTs. The RAST, however, was $83.3 \pm 10.8\%$ sensitive where 83% patients showing positive ID were RAST positive.

Table 1. Intradermal Test Responses with Mosquito Whole Body Extract in 247 Patients and 50 Healthy Volunteers

ID Response	Patients		Healthy Volunteers	
	No.	%	No.	%
1+	46	18.6	3	6.0
2+	60	24.3	0	0
3+	12	4.9	0	0
1+ to 4+	118	47.8	3	6.0
2+ to 4+	72	29.1	0	0

Table 2. Analysis of Skin Test Results with Mosquito WBE According to the Clinical Characteristics of the 247 Patients of Nasobronchial Disorders

Clinical Characteristics	No. of Patients	ID Positivity 1+ to 4+		Significance
		n	%	
Age of onset				
≤20 yr	139	68	48.9	NS†
>20 yr	108	51	47.2	
Duration of disease				
≤5 yr	133	57	42.9	NS
>5 yr	114	61	53.5	
Type of disease*				
Seasonal	67	27	40.3	NS
Perennial and nonseasonal	163	86	52.8	
Diagnosis				
Asthma	104	45	43.3	NS
Rhinitis	33	17	51.5	
Asthma + Rhinitis	110	56	50.9	
Family history of allergy				
Positive	113	58	51.3	NS
Negative	134	61	45.5	

* 17 patients had recent onset of the disease.

† NS = not significant, $P > .05$.

Table 3. RAST Results with the Sera of 14 Patients Showing Different Grades of Intradermal Response to Mosquito Allergen Extract

Serum	ID Response	% cpm	RAST Ratio (RAST +/-)*	Average RAST Ratio ± SD	% RAST Positivity
NHS†	—	0.49	1.0 (—)	1.0	0
Patients					
BSY	—	0.82	1.67 (—)	1.77 ± 0.13	0
RPC	—	0.91	1.86 (—)		
KK	1+	1.21	2.47 (—)	2.98 ± 0.72	50
GS	1+	1.71	3.49 (+)		
SA	2+	1.21	2.47 (—)		
OP	2+	1.73	3.53 (+)		
CP	2+	2.17	4.43 (+)		
HR	2+	2.95	6.02 (+)		
IS	2+	3.89	7.94 (+)	11.11 ± 9.3	88.9
A	2+	4.59	9.36 (+)		
DC	2+	6.93	14.14 (+)		
LB	2+	11.13	22.71 (+)		
A	2+	14.40	29.39 (+)		
R	3+	9.66	19.72 (+)	19.72	100
Total	1+ to 4+ 2+ to 4+				83.3 90

* — = negative and + = positive.

† NHS = normal human serum.

BPTs

The results of BPTs on five asthmatics and five controls are included in Table 4. The BPTs were uniformly negative in all the healthy

volunteers and two patients showing negative ID response to mosquito WBE, while all the three patients showing 2+ to 4+ ID responses had positive BPTs.

DISCUSSION

Insects and other arthropods are the most numerous animals in the world, the number of species described so far being 80% of all the animals. According to Sabrosky,¹⁴ 625,000 to 1,300,000 insect species are present throughout the world. These insects can be classified broadly as (1) stinging insects (2) biting insects (3) nonbiting and nonstinging insects. Mosquito is one of the biting insects and a few reports of anaphylaxis to mosquito bites are available.^{15,16} Even though IgE binding allergenic components in the mosquito WBEs (*Aedes* and *Culex* species) have also been demonstrated in a few preliminary studies,^{17,18} no systematic study on the role of mosquitoes as inhalant allergens in Type I respiratory allergic diseases has been reported so far.¹⁹

In the present study the allergenic significance of *Anopheles stephensi*, a common mosquito species in Delhi area, was evaluated by performing ID, BPT, and RAST experiments. Intradermal tests were performed with mosquito WBE in 1:500 wt/vol dilution as in an earlier study this strength of insect allergens was found suitable for ID tests.²⁰ Of the 247 patients, 48.7% showed 1+ to 4+ reactions while markedly positive responses (2+ to 4+) were obtained in 29.1% of the patients. Further analysis of ID results showed that the skin positivity to mosquito WBE was not related to various clinical characteristics such as age at onset, duration and type of disease, and family histories of the patients included in the study (Table 2).

The specificity of the positive ID reactions obtained in patients is supported by the fact that none of the healthy nonallergic volunteers showed a markedly positive ID reaction. Furthermore, even 1+ reactions were three and one-half times more frequent in patients than in controls (21% and 6%, respectively) (Table 1).

The specificity of skin reactions

Table 4. BPT Response to Mosquito WBE in Five Asthmatic Patients and Five Healthy Volunteers

Subjects	ID Response	BPT Response*	RAST Ratio
Healthy volunteers			
SJ	-	-	1.0
SB	-	-	ND†
PS	1+	-	ND
SJ	-	-	ND
RB	-	-	ND
Patients			
BSY	-	-	0.82
RPC	-	-	0.91
IS	2+	1+	7.94
DC	2+	2+	14.14
S	3+	2+	19.72

* BPT response¹¹

negative

1+

2+

Reduction in FEV₁

<15%

15% to 20%

21% to 50%

† ND = not done.

to mosquito WBE was further confirmed by mosquito RASTs. Of the sera from 12 patients eliciting a positive ID response (1+ to 4+), ten (83%) were RAST positive. The positivity of RAST increased to 90% in the patients showing markedly positive skin reactions (2+ to 4+). Further, the average RAST ratio increased with the increase in the grade of ID response ranging from 1.77 in patients with negative ID reactions to 19.7 in those with a 3+ response (Table 3). These results showed significant correlation ($r = .69$, $P < .01$) between ID response and RAST indicating that the skin tests graded according to the criteria followed in the present study are highly specific. The sera from another two patients who had negative ID responses to mosquito WBE also gave negative RASTs.

The reliability of ID positivity is also supported by the results of BPT performed on five asthmatics showing different grades of ID response (negative to 3+). All three patients who had positive ID responses and positive RASTs also had positive BPTs. Both the asthmatic patients with negative ID responses and negative RASTs gave negative inhalation challenge responses.

Furthermore, immunochemical quantitation studies revealed the presence of airborne mosquito allergens in Delhi atmosphere (unpublished results), confirming the exposure of our patients to these allergens.

In conclusion results of ID, RAST, and BPT suggest that mosquito-derived allergens are important causative agents in Type I respiratory allergic disorders in India. This is probably the first systematic study on the role of *Anopheles stephensi* as a source of inhalant allergens.

ACKNOWLEDGMENTS

The authors thank Mr. Dinesh Garg and Mrs. Manju Gupta for technical assistance.

REFERENCES

1. Bagenstose AH, Mathews KP, Homburger HA, Saaveard-Delgado AP. Inhalant allergy due to crickets. *J Allergy Clin Immunol* 1980; 65:71-4.
2. Figley KD. Asthma due to May fly. *Am J Med Sci* 1929;178:338.
3. Jamiesson HC. Housefly as a cause of nasal allergy. *J Allergy* 1938; 9:274.
4. Kang B, Vellody D, Homburger H, Yunginger JW. Cockroach cause of allergic asthma: its specificity and immunologic profile. *J Allergy Clin Immunol* 1979;63:80-6.
5. Kay B, Gad EI, Rab MO, Stewart J, Erwa HH. Widespread IgE-mediated hypersensitivity in Northern Sudan to the chironomid *Cladotanytarsus lewisi* ("green nimitti"). *Clin Exp Immunol* 1978;34:106-10.
6. Kino T, Oshima S. Allergy to insects in Japan. I The reaginic sensitivity to moth and butterfly in patients with bronchial asthma. *J Allergy Clin Immunol* 1977;61:10-6.
7. Kino T, Oshima S. Allergy to insects in Japan. II The reaginic sensitivity to silkworm moth in patients with bronchial asthma. *J Allergy Clin Immunol* 1979;64:131-6.
8. Parlato SJ. The sand fly (caddis fly) as an exciting cause of allergic coryza and asthma. II. Its relative frequency. *J Allergy* 1930;1:307.
9. Chaudhry S, Shashi Jhamb, Chauhan UPS, Gaur SN, Agarwal HC, Agarwal MK. Shared and specific allergenic and antigenic components in the two sexes of American cockroach-*Periplaneta americana*. *Clin Exp Allergy* 1990;20:59-65.
10. Adolphson CR, Yunginger JW, Gleich GJ. Standardisation of allergens. In: Rose NR, Friedman H. eds. Manual of clinical immunology. 2nd edition. Washington DC: American Society of Microbiology, 1976:778-88.
11. Shivpuri DN, Agarwal MK. Studies on the allergenic fungal spores of Delhi, India, metropolitan area-clinical aspects. *J Allergy* 1969;44: 204-13.
12. Gleich GJ, Yunginger JW. Standardization of allergens. In: Rose NR, Friedman H. eds. Manual of clinical immunology, 2nd edition. Washington DC: American Society of Microbiology, 1976:575-8.
13. Youden WJ. Index for rating diagnostic tests. *Cancer* 1950;3:32-5.
14. Sabrosky CW. Insects, year Book of agriculture, United States, Dept. of Agriculture Washington, U.S. Government Printing Office, 1952.
15. Frazier CA. Insect allergy, 2nd edition. St. Louis, Missouri: W.H. Green Inc, 1984:136-200.
16. Suzuki S, Negishi K, Tomizawa S,

- Shibasaki M, Kuroume T, Matsu-
mura T. A case of mosquito allergy.
Acta Allergol 1976;31:428-41.
17. Baldo BA, Panzani RC. Detection
of IgE antibodies to a wide range of
insect species in subjects with sus-
pected inhalant allergies to insects.
Int Arch Allergy Appl Immunol
1988;85:278-87.
18. Wu CH, Lan JL. Immunoblot
analysis of allergens in crude mos-
quito extracts. *Int Arch Allergy*
Appl Immunol 1989;90:271-3.
19. Kagen SL. Inhalant allergy to ar-
thropods. Insects, arachnids and
crustaceans. *Clin Rev Allergy*
1990;8:99-125.
20. Gaur SN, Kapoor M, Garg DC,
Agarwal MK. Etiologic significance
of insects in nasobronchial allergy.
Asp Allergy Appl Immunol 1985;18:
19-27.
- Request for reprints should be addressed to:*
Dr. M. K. Agarwal
Department of Allergy and Applied
Immunology,
Vallabhbhai Patel Chest Institute
University of Delhi
Delhi-110 007
India

EPIDEMIOLOGY OF ACQUIRED IMMUNODEFICIENCY SYNDROME IN WOMEN

The Centers for Disease Control reports there were 15,493 women with acquired immunodeficiency syndrome (AIDS) in the United States on December 31, 1990, 10% of the adults reported with AIDS. In 1985 only 538 women were reported to have AIDS, 6.6% of the adult cases reported that year. Only part of this increase was due to a change in AIDS case definition that took effect in 1988.

Cumulative incidence rates through 1990 were 67 per 100,000 women for blacks, 39 for Hispanics, and 5 for whites. Corresponding cumulative incidence rates for heterosexual men were 164 for blacks, 127 for Hispanics, and 14 for whites.

Mean age at the time of diagnoses of AIDS was 36 years for women; 85% were of reproductive age at the time of diagnosis. Areas with the highest reported cumulative incidence rates were Puerto Rico (71.3 per 100,000 women), New Jersey (62.9), New York (61.0), District of Columbia (59.8), Florida (34.7), Connecticut (24.4), and Maryland (21.0), but cases have been diagnosed in every state.

Fifty-one percent of women with AIDS were intravenous drug users, 21% were sexual partners of intravenous drug users, 9% were sexual partners of high-risk men who did not use intravenous drugs, and 9% had received transfusions, including treatment for hemophilia.

The median survival time from AIDS diagnosis to death was 9.8 months for women and 9.3 months for heterosexual men. The 3-year survival rate after diagnosis of AIDS was 20% for women and 19% for heterosexual men. The median time for progression from human immunodeficiency virus (HIV) infection to AIDS may be as long as 10 years.

The increasing incidence of AIDS among women indicates need for education and counseling of women about protection and treatment of drug addiction and sexually transmitted diseases that contribute to transmission of HIV.

—RMS

Ellerbrock TV, Bush TJ, Chamberland ME, et al. Epidemiology of women with AIDS in the United States, 1981 through 1990: a comparison with heterosexual men with AIDS. *JAMA* 1991;265:2971-5.