SKIN SENSITIVITY TO HISTOPLASMIN IN CALCUTTA AND ITS NEIGHBOURHOOD

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Summary

Skin sensitivity to histoplasmin was detected in 26 (9.4%) of 275 persons tested in Calcutta. Thirty-eight (13.8%) showed doubtful reaction. The positive reactors, all of whom were residents of the Gangetic plain, included 5 persons exposed to soil-source of *Histoplasma capsulatum* and a general duty assistant of the Medical Mycology laboratory, Calcutta School of Tropical Medicine. Among the doubtful reactors, there were 2 contacts of histoplasmosis patients and a sweeper of the Medical Mycology laboratory. Persons with pulmonary calcification were histoplasmin negative. *Histoplasma capsulatum* was not isolated from any of the positive reactors.

The endemicity of histoplasmosis in an area is determined by the occurrence of actual disease and cutaneous sensitivity to histoplasmin in the life-time residents and isolation of *Histoplasma capsulatum* (H. capsulatum) from the environment of the locality. Since the first study in Calcutta in 1954[1], several histoplasmin sensitivity surveys in the population in different parts of India including West Bengal have been carried out [2,3]. A number of case reports on histoplasmosis in the residents of West Bengal [4–13] prompted us to study the ecology and endemicity of the disease in this part of the country. This resulted in isolation of *H. capsulatum* from the soil of West Bengal[14] and this paper presents the results of a study of skin sensitivity to histoplasmin in 273 persons of this region.

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Received for publication on 22—2—1979

Materials and Methods

The subjects of this study, 4 to 70 years of age, were selected from the staff and patients of the Calcutta School of Tropical Medicine and the patients of Carmichael Hospital for Tropical Diseases and the Chest Clinic, Medical College, Calcutta. They were residents of Calcutta or the surrounding districts, e. g. 24 Parganas, Howrah and Hooghly, and other districts of West Bengal and comprised 154 males and 121 females. The subjects were grouped under 6 categories, as follows:

1. *Normal*: those who had no past or present pulmonary, oral, lymphoreticular or ocular diseases and no contact with known soil source of *H. capsulatum* or with histoplasmosis patients.

2. *Pulmonary*: those who had past or present evidence of pulmonary lesions, not responding to antitubercular treatment.

3. *Oral*: those who had ulcers in the oral cavity.

4. *Lymphoreticular :* those who had anaemia with hepatosplenomegaly and lymphadenopathy.

5. *Contact*: those who were exposed to known soil-source of *H. capsulatum* and were contacts of histoplasmosis patients.

6. *Miscellaneous*: Patients with ocular lesions, hospital staff and staff of the Medical Mycology laboratory.

The skin test was performed according to Parke-Davis & Co. as follows : 0. 01 ml of histoplasmin (Bio 498, Parke-Davis) was diluted with 1 ml of the buffered diluent supplied. After cleaning the flexor surface of the right forearm with 95% ethanol, 0.1 ml of the diluted material was injected intracutaneously with a sterile 1 ml tuberculin syringe (Microstat) and one half inch, 26 gauze, sharp needle (Luer). The reaction was read in natural light 24 and 48 hours after injection. An area of induration 5 x 5 mm or larger particularly after 48 hours, was taken as positive reaction, induration of 5 x 5 mm after 24 hours, which became less after 48 hours was regarded as doubtful reaction ; an induration of less than 5 x 5 mm or erythema only greater than 5 x 5 mm as doubtful reaction and no induration or erythema less than 5 x 5 mm as negative reaction. Control test was done with 0 .1 ml of the diluent in 5 cases only.

Tuberculin test was performed in positive reactors to histoplasmin belonging to the pulmonary and contact groups of the subjects. X-ray of the chest was performed in all positive reactors to histoplasmin and their sputum specimens were checked for acid-fast bacilli. Three consecutive samples of sputum from each person and scrapings in cases with oral lesions were investigated for *H. capsulatum* by direct microscopy and culture on slants of Sabouraud's dextrose agar with chloramphenicol (0. 05 mg /ml) and cycloheximide (0. 5 mg/ml) at 25° - 33° C. The culture tubes were held for 4 weeks.

Results

Of the 275 persons tested with histoplasmin, 26 (9. 4%) were positive reactors and 38 (13.8%) showed doubtful reaction. The area of induration in positive reactors varied from 5 x 5 mm to 25 x 30 mm (10 x 10 mm and more in 16 persons and 5 x 5 mm to 8 x 8 mm in 10) and persisted even upto 1 week or more in some cases. Control tests produced no erythema or induration.

The distribution of histoplasmin sensitivity in the subjects in relation to their categories, residences and sexes is shown in Table I. The results of investigations on the positive reactors are summarised in Table II.

TABLE 1

Distribution of skin sensitivity to histoplasmin in 275 persons belonging to different categories, areas and sexes.

Subjects	Number tested	Positive reactors	Doubtful reactors	Negative reactors
Categories :				
Normal	114	9	18	87
Pulmonary	84	8	7	69
Oral	34	3	6	25
Lymphoreticular	18	—	4	14
Contact	15	5i)	2ii)	8
Miscellaneous	10	1iii)	1iii)	8
Residents of:				
Calcutta	170	15	22	133
24 Parganas	39	2	6	31
Howrah	21	2	3	16
Hooghly	18	6	2	10
Other districts	27	1iv)	5	21
Sex :				
Male	154	17	23	114
Female	121	9	15	97

i) Exposed to soil-source of **Histoplasma capsulatum.**
ii) Contacts of histoplasmosis patients.
iii) Staff of medical mycology laboratory.
iv) From Nadia District.

TABLE 2

A summary of the results of investigations on 26 positive reactors to histoplasmin showing induration of 5 × 5 mm to 25 × 30 mm

Categories of subjects	Induration in mm	Chest X-ray	Tuberculin test	AFB in sputum	Sputum culture
Normal (9)*	10 × 10 mm or more (5)	Normal (9)	Not done (N.D.)	Not found (9) (N.F.)	No growth (9)
Pulmonary (8)	10 × 10 mm or more (4)	Bilateral, extensive infiltration (7)	Positive (7)	Positive (2)	Cryptococcus sp. (1) Nocardia sp. (1) No growth (6)
Oral (3)†	10 × 10 mm or more (2)	Normal (3)	N.D.	N.F. (3)	No growth (3)
Contact (5)‡	10 × 10 mm or more (4)	Normal (1)	Positive (1)	N.F. (1)	No growth (1)
Miscellaneous (1)	Above 10 × 10 mm (1)	Normal (1)	N.D.	N.F. (1)	No growth (1)

* Figures in parentheses indicate number of subjects
† Oral scrapings showed no growth on culture
‡ Only one was investigated; others did not co-operate

Persons with pulmonary calcification and ocular lesions and hospital staff including one in the medical mycology laboratory were histoplasmin negative. Among contacts of histoplasmosis patients was a girl of 19 years who was the sister of a young male patient with histoplasmosis[10]. This boy died in 1968. In 1969, *H. capsulatum* was isolated once in the sputum of the girl who had presented with irregular fever, cough, anaemia, hepatosplenomegaly and lymphadenopathy at that time. Her bone marrow was normal and free of fungus and splenic material did not reveal fungus. She was tuberculin positive and got well with antitubercular drugs. After 2 years, she had a recurrence of the earlier signs and symptoms with also ascites. Attempts to isolate *H capsulatum* from her sputum and bone-marrow failed this time. This girl was histoplasmin negative.

Histoplasma capsulatum was not isoalted from sputum or oral scrapings in any of the subjects.

Discussion

In the present study, induration in the positive reactors varied from 5 x 5mm to 25 x 30 mm. As has been pointed out earlier[3], so for there is no uniform or universal standard for dilution of histoplasmin and interpretation of positive reaction of histoplasmin test based on areas of induration. A 10 x 10 mm induration or higher, irrespective of the antigen used, may be acceptable as a positive reaction, but the lower limit of the induration for the same is a moot point. In this study, Bio 498 (Parke-Davis) histoplasmin was used and as suggested by the manufacturers, an induration of 5 x 5 mm or more was taken as a positive reaction; and hence the 9. 4% reported as positive reactors in this series. If 10 x 10 mm induration was to be taken as the index of positive reaction, the reactors would have been only 5.6%. Not withstanding the area of induration, however, the percentage of

positive reactors as compared to those observed by previous workers in West Bengal[2], is on the higher side in this study.

That 5 of the 7 persons exposed to the soil-source of *H. capsulatum* were positive reactors to histoplasmin, is significant, but the contacts of histoplasmosis patients, were histoplasmin negative. Even the girl from whose sputum *H. capsulatum* was isolated once in 1969, was histoplasmin negative.

In the pulmonary group, a positive reactor with active pulmonary lesion was tuberculin negative, and had no acid - fast bacilli in the sputum, *H. capsulatum* was however not isolated from his sputum. Two other positive reactors in the same group were tuberculin positive. One of them yielded growth of a *Cryptococcus* sp., (not *neoformans*) from sputum the other a *Nocardia* sp. (not resembling known pathogens). These organisms are not known to produce cross reaction with histoplasmin.

Those who showed doubtful reaction, comprised 13. 8% of the subjects. The significance of this doubtful reaction is yet to be determined. According to Edwards and Billings [2], a reaction less than 5 mm is a negative or a cross reaction. In this study, attempts were not made to rule out cross reaction by other fungal antigens. But all of the 64 persons in Calcutta[1] and 112 in Agra[15], who were tested with blastomycin and coccidioidin, were negative reactors excepting one person in Agra, who showed positive reaction to blastomycin.

Asgari and Conant[16] suggested that sensitivity to histoplasmin could be acquired by exposure to *Chrysosporium keratinophilum*. This keratinophilic fungus is commonly found in the soil of different parts of India[17-19], but its role in the development of sensitivity to histoplasmin in the population in this country is yet to be determined. On the other hand, more than 20 cases of histoplasmosis have appeared in the Indian literature and an endemic focus of the disease has been established by isolation of *H. capsulatum* from the soil of Gangetic plain in West Bengal, India[14]. In this connection, it is interesting to note that all the positive reactors in the present study were residents of the Gangetic plain. A true picture and the significance of histoplasmin sensitivity in the population of India can only be determined by more and extensive surveys with histoplasmin as well as other cross-reacting antigens and soil-studies.

Acknowledgement

This investigation was partly financed by the Indian Council of Medical Research. Mr. Barid Manna gifted 20 vials of histoplasmin. Parke-Davis (India) Ltd. and Upjohn Co., U. S. A. supplied chloramphenicol and cycloheximide respectively. Dr. B. B. Manna, Head, Department of Chest Diseases, Medical College, Calcutta provided all sorts of help regarding chest X-ray of the pulmonary group and tuberculin test. Dr. K. N. Dutta, Radiologist, Calcutta School of Tropical Medicine helped in chest X-ray of the remaining persons. Mr. A.K. Mukherjee typed the manuscript. The authors are indebted to all.

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Obituary

The members of I. A. D. V. L. Orissa State Branch condole the sad demise of Dr. Basudev Choudhury, one of the very active members of this branch on 8—4—80 and pray that his soul rest in peace.