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Epidemiology of chronic fungal rhinosinusitis in rural India

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Summary

A descriptive epidemiological study of fungal rhinosinusitis (FRS) was conducted in rural north India in the form of house-to-house survey of villages of two districts each of Punjab and Haryana provinces using a clinical case definition of chronic rhinosinusitis (CRS). The suspected cases were investigated further in the laboratory to confirm FRS. Air and environment were sampled in different seasons to find *Aspergillus* spore count. The prevalence of chronic FRS cases was at 0.11% of the population and *Aspergillus flavus* was the predominant (97.6%) agent of all types of chronic FRS. The chronic FRS patients were classified as allergic FRS 41 (56.1%), chronic granulomatous FRS 13 (17.8%), eosinophilic FRS 11 (15.0%), fungal ball 7 (9.5%) and chronic invasive FRS 1 (1.3%). *Aspergillus* spores were present in large numbers (~20%) in air with significantly higher counts of *A. flavus* during winter months in the wheat-thrashing areas of Punjab as compared to Haryana ($P = 0.0079$). The present study identified high prevalence (27.5% of CRS cases) of chronic FRS cases in rural north India and its possible association with wheat harvesting seasons.

Key words: *Aspergillus*, allergy, environment, epidemiology, fungus, sinusitis.

Introduction

Chronic rhinosinusitis (CRS) is an important chronic public health problem affecting the quality of life of more than 5% people.[1] In the US alone, 12.5% of the population is afflicted with CRS at least once during their lifetime.[2] The disease accounts for substantial health care expenditure and missed working days. Several factors like bacteria, viruses, anatomical abnormalities and fungi are claimed to cause CRS.[3] The role of fungi in CRS continues to be an important focus of research and extensive debate.[3,4] Clinically, CRS has been classified with or without nasal polyposis, or as allergic fungal rhinosinusitis (AFRS).[5] Fungal rhinosinusitis has been classified histopathologically into invasive and non-invasive disease depending on tissue invasion by fungi. The invasive disease is differentiated into acute invasive, chronic invasive and granulomatous types. The non-invasive disease is categorised into three different clinical forms: localised colonisation, fungal ball and eosinophil-related FRS including AFRS.[4,6,7] Other than acute invasive type, the rest may be clubbed under chronic FRS.[6]

Studies from tertiary care centres indicate a high prevalence of chronic FRS in India.[8–14] AFRS is the most common (56%–57%) form of CRS followed by chronic invasive granulomatous FRS (2%–17%), fungal ball/mycetoma (2%–4%), chronic invasive FRS (CIFRS; 1%–3%) in India.[10,15] The disease has been noted in ~70% young males in 10–39 years age group from rural India with *A. flavus* as the predominant causative agent.[8,9,11] Though the disease is presumed to be common in villages, no systematic epidemiological study has been conducted to determine its exact prevalence. The present study was undertaken to

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determine the prevalence, clinical pattern and determinants of FRS among patients with CRS in rural north India and to elucidate the impact of seasonal variation on the load of *Aspergillus* conidia in air.

Patients and methods

The study was under taken after obtaining the approval of the study proposal from Institute Ethics Committee, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Active surveillance

Our Institute is a referral teaching hospital in north India where patients from Haryana and Punjab provinces are referred for tertiary care. Analyses of our records revealed that majority of patients with FRS were referred from Patiala and Sangrur district of Punjab province. Hence, we conducted active surveillance in the villages of these two districts. For comparison, two districts (Ambala and Yamunanagar) were chosen purposively from the Haryana province, as we had good rapport with the local doctors and no patient with FRS had been referred from these areas. The help of the local Chief Medical Officer, medical practitioners, ENT specialists and elected head (Panchayat Pradhan) of the villages was also sought to conduct the active surveillance. Help of local temple (Gurudwara Prabandhak) committee was also taken to gain confidence of the villagers. Regular medical camps were organised to gain cooperation of the people. A house-to-house survey covering the entire population of the village was carried out to identify patients with CRS. The research team including doctors conducted the survey. A proforma (Data S2) with an operational definition of CRS was used for this survey. Active surveillance was performed in villages of Moonak block of Sangrur district and Pathran block of Patiala in Punjab province; villages of Sadhora PHC area of Yamunanagar and Narayangarh block of Ambala district in Haryana province.

Operative definition of CRS

A person with at least one major and one minor clinical symptom and sign for more than 12 weeks without complete relief in the intermittent period was diagnosed with CRS. The major symptoms and signs include facial pain/pressure, facial congestion/fullness, nasal obstruction/blockage, nasal discharge/purulence/postnasal drip, and/or hyposmia/anosmia; and the minor symptoms include headache, fever, fatigue, dental pain, cough, and/or ear pain/pressure/fullness.

Skin test survey

The villagers were addressed at a common place in each village to explain the study and to request participation in the skin test survey voluntarily. The skin test with *A. flavus* culture filtrate antigen was performed on patients fulfilling clinical definition of CRS and also age and sex-matched healthy controls from the same villages. The details of the patients and controls enrolled for active surveillance and skin tests in Punjab and Haryana are summarised in Fig. 1. The test was performed using *A. flavus* culture filtrate antigen. The antigen was prepared in our laboratory using standardised protocols, details of which are appended in the Data S1.[16] About 0.02 ml of the culture filtrate antigen (protein content 1 mg ml^{-1}) was injected intradermally. An equal volume of sterile phosphate-buffered saline (pH = 7.2) was injected intradermally in the other arm as control. Signs of immediate hypersensitivity including erythema and wheal formation were noted every 15 min till 60 min.

Evaluation of clinically diagnosed patients with CRS

During the door-to-door survey we identified the patients suffering from clinically suspected rhinosinusitis with the help of a questionnaire prepared by us.[5,6] Thereafter, all such patients were encouraged to undergo further investigation and treatment at our Institute. Transport was arranged to facilitate the process but no monetary incentive was provided. The patients were categorised into different types on the basis of histopathology, radiology, mycological and other investigations. The complete array of examination and investigations is detailed below:

Detailed history and clinical examination

The history included demographic data like age, sex, occupation, place of stay; and clinical data of presence of polyp, asthma, aspirin sensitivity, history of any nasal surgery and recurrences after previous surgery. Evidence of any underlying disease including diabetes, malignancy, HIV or any other immunodeficiency, and the treatment history including antibiotics, steroids and immunosuppressive drugs were also recorded. The findings observed during endoscopic examination or surgeries were noted.

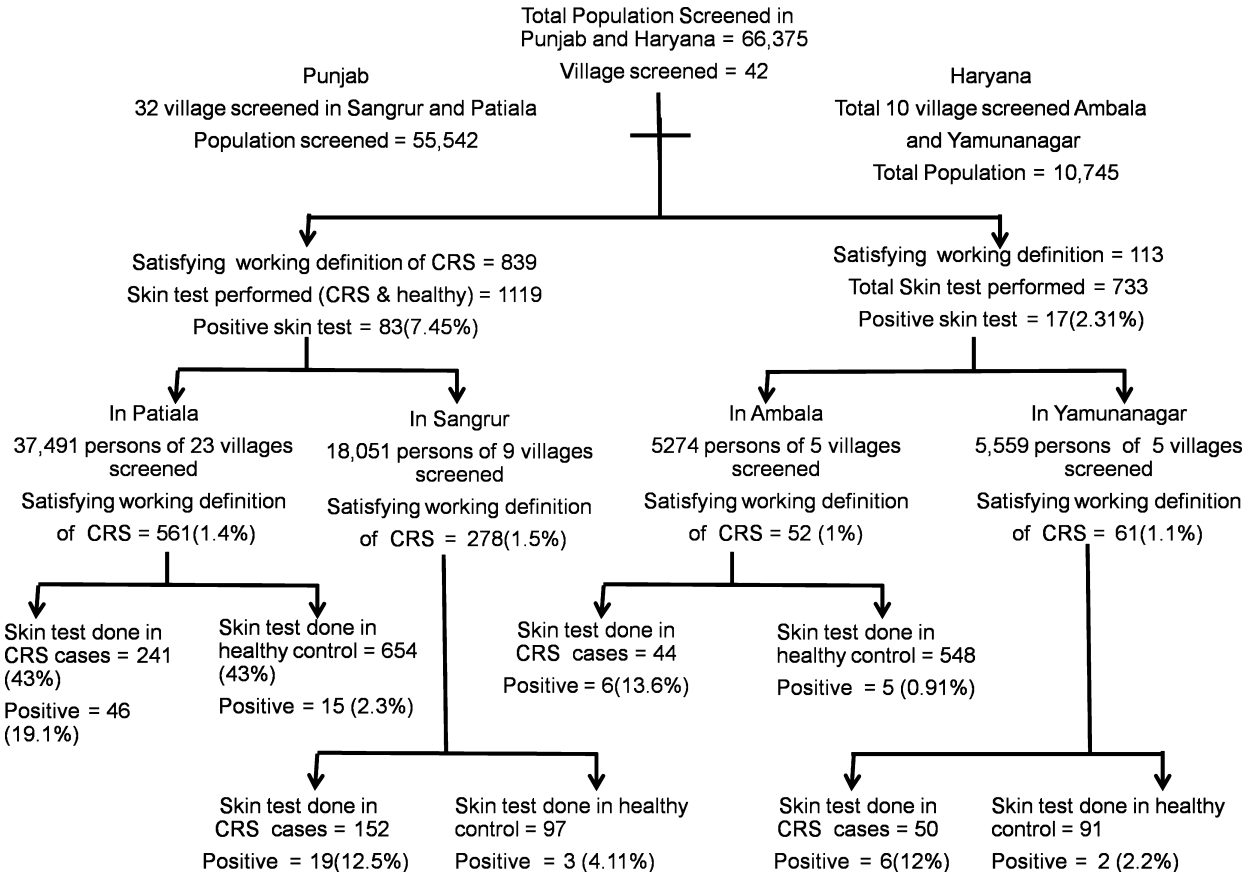

Figure 1 Schematic diagram showing the population of different study areas, skin test performed and prevalence of chronic rhinosinusitis.

Radiological investigation

Computed tomography scan of nose, paranasal sinus, orbit and frontal lobe area was performed for all clinically suspected patients to locate the site of lesion, sinus expansion, area of hyperattenuation, bone erosion, extension of disease into adjacent anatomical sites like orbit and intracranial region.

Mycological investigations

The investigation included direct microscopy using 10% potassium hydroxide wet mount and isolation of fungi from paranasal sinus biopsies. The fungal growth was identified on the basis of morphological features and conventional biotyping.

Histopathology examination

Histopathology examination was carried out on haematoxylin and eosin-stained slides from biopsies. Presence of allergic (eosinophilic) mucin, mucus plugs with eosinophils, Charcot-Leyden crystals was noted. Presence of fungi was specifically looked for using special stains like periodic acid Schiff and Gomori's methenamine silver (GMS) stain.

Other investigations

Absolute eosinophil blood count was recorded. A count of >500 cu mm^{-1} was considered as serum eosinophilia.

Categorisation of chronic FRS

Chronic FRS was classified into invasive and non-invasive diseases based on histopathological evidence of fungal tissue invasion.[6] The invasive diseases included granulomatous invasive FRS, and CIFRS. The non-invasive diseases included fungal ball and eosinophil-related FRS including AFRS. Granulomatous invasive FRS was defined as a granulomatous response with

considerable fibrosis and few fungal filaments on histopathology. Chronic invasive FRS had dense accumulation of hyphae, occasional presence of vascular invasion and mixed inflammatory reaction with involvement of local structures. A fungal ball was described as the presence of non-invasive accumulation of dense conglomeration of fungal hyphae in one sinus cavity, usually the maxillary sinus, or in multiple sinuses without any eosinophilic mucin. To diagnose AFRS, five diagnostic criteria were included: type I hypersensitivity, nasal polyposis, characteristic findings on CT scan, presence of fungi on direct microscopy or culture and mucin containing fungal elements without tissue invasion. The patients who had nasal polyposis, characteristic CT findings, detectable fungi in eosinophilic mucin, but had negative skin tests were classified under eosinophilic FRS (EFRS); and when fungi were also absent in the eosinophilic mucin, the patients were categorised as eosinophilic mucin rhinosinusitis (EMRS).

Determination of burden of *Aspergillus* conidia in air during different seasons

The air samples were collected from patients' houses, their neighbourhood and fields of seasonal crop of each village during four different seasons (summer, monsoon, autumn, winter). The number of conidia or spore present in the environmental air was determined by Air Petri Sampling System Mark II sampler (LA637: HiMedia Laboratories, Mumbai, India). The sampler could hold 90 mm petri plates containing Sabouraud dextrose agar and was operated for 5 min. The sampler had an air volume intake of 100 l min^{-1}. The plate was incubated at 25 °C and examined every day for 1 week. Three consecutive samples were collected from each area and colonies of *Aspergillus* species were calculated. The moulds and yeast were identified as described above. To correlate the colony forming units (CFUs) present on the agar plate with the most probable number of microorganisms per cubic metre of air sampled, the following formula was used.

$$P_r = N[1/N + 1/(N-1) + 1/(N-2) + \ldots + 1/(N - r + 1)]$$

where, P_r = most probable number of microorganisms in the volume of air sampled; N = number of holes on microflow sampling head; r = number of CFUs on the agar plates after incubation.

Statistical analysis

Skin test results from the two provinces were compared using unpaired *t* test. The mean spore counts, total *Aspergillus* spore counts and *A. flavus* spore counts between Punjab and Haryana provinces were compared using unpaired *t* test or Mann–Whitney test as appropriate. Seasonal variations in spore counts within each province were analysed using paired *t* test. Comparison of seasonal variation in spore count along with the variations between two provinces was carried out using repeated measures ANOVA. All statistical analyses were carried out on SPSS statistical package v17.0 (SPSS Inc., Chicago, IL).

Results

Active surveillance and skin test positivity

Active surveillance was conducted in villages of two districts of Punjab province, Patiala (Patran block) and Sangrur (Moonak block) and two districts of Haryana province, Yamunanagar (Sadhora block) and Ambala (Narayangarh block). The details of CRS cases and skin test results are shown in Fig. 1. A total of 66 375 individuals belonging to 42 villages of Punjab and Haryana provinces were screened; 37 491 individuals belonged to 23 villages in Patiala district, 18 051 to 9 villages of Sangrur district, 5274 from 5 villages of Ambala district and 5559 to 5 villages of Yamunanagar district (Fig. 1).

From the Patiala district 561 (1.5%) people satisfied the working definition of CRS and 241 (43%) of them voluntarily agreed for *A. flavus* culture filtrate antigen skin test. Skin test was also performed on 654 healthy controls; of which 41 were relatives of the CRS patients. Among CRS cases (241), skin test was positive (immediate hypersensitivity) in 46 (19.1%) individuals. In healthy controls, skin test was positive (immediate hypersensitivity) in 15 (2.3%) subjects. Among skin test positive healthy individuals, five (9.8%) were related and 10 (1.6%) unrelated individuals. Similar survey in Sangrur district enrolled 278 (1.5%) persons satisfying the working definition of CRS, and 152 (54.7%) of them agreed for aspergillin skin test. Among CRS patients, the skin test was positive (immediate hypersensitivity) in 19 (12.5%). In 72 healthy controls from the region, skin test was positive (immediate hypersensitivity) in 3 (4.1%) subjects (Fig. 1).

In Haryana province 10 833 individuals belonging to 10 villages were screened; of them 5274 belonged

Table 1 Statistical analysis of skin test positivity in Punjab and Haryana provinces.

	Total skin test done	Skin test positive *n* (%)	*P* value
Patiala	895	61 (6.82)	0.1247
Sangrur	224	22 (9.8)	
Ambala	592	11 (1.8)	0.01*
Yamunanagar	141	8 (5.6)	
Punjab (cases and healthy control)	1119	83 (7.4)	0.000009*
Haryana (cases and healthy control)	733	19 (2.6)	
All CRS positive cases	487	77 (15.8)	0.00000*
Healthy control	1365	25 (1.8)	
Punjab (CRS cases)	393	65 (16.6)	0.3676
Haryana (CRS cases)	94	12 (12.8)	

CRS, chronic rhinosinusitis.

*$P < 0.05$.

to five villages (under Narayangarh block) in Ambala district and 5559 belonged to five villages (under Sadhora block) in Yamunanagar district. In Narayangarh block of Ambala district 52 (1.0%) individuals met the working definition of CRS. Skin test was performed on 44 CRS cases and 548 healthy controls. Immediate hypersensitivity reaction was positive in six (13.6%) CRS patients and five (0.9%) healthy individuals. In Sadhora block of Yamunanagar 61 (1.1%) individuals satisfied the CRS working definition. Skin test was performed on 50 CRS cases and 91 healthy controls. Immediate hypersensitivity was positive in six (12.0%) CRS patients and two (2.2%) healthy individuals (Fig. 1).

Comparison of CRS cases and skin test positivity between Punjab and Haryana provinces

The statistical analysis of skin test surveillance is provided at Table 1. Of the 37 491 people screened in Punjab, 839 (2.2%; 95% CI: 2.1–2.4) satisfied working definition of CRS and 393 (46.8%) consented for *Aspergillus* skin test; 65 CRS patients (16.5%; 95% CI: 13.2–20.5) were skin test positive (Fig. 1). In Haryana province 10 745 people were screened for CRS, with 113 (1.1%; 95% CI: 0.9–1.3) satisfying the CRS working definition and 94 (83.2%) of those patients underwent skin test; 12 CRS patients (12.8%; 95% CI: 7.5–21.0) were positive for skin test (Fig. 1). The skin test positivity rate was significantly higher among the CRS patients as compared to healthy controls both in Punjab (16.5% vs. 2.5%; $P < 0.001$) and Haryana provinces (12.8% vs. 0.5%; $P < 0.001$). The overall burden of CRS patients was significantly higher in Punjab compared to Haryana (2.2% vs. 1.1%; $P < 0.001$) but the skin test positivity rate (16.5% vs. 12.8%; $P = 0.433$) among CRS patients showed no statistical difference across the two provinces (Table 1).

Fungal rhinosinusitis

We could evaluate 268 (28.5%) clinically suspected CRS patients who responded to our request for investigation at our Institute. Of them, 73 (27.2%) were diagnosed for FRS and 4 had EMRS. The FRS patients were classified as AFRS 41 (56.1%), chronic granulomatous FRS 13 (17.8%), EFRS 11 (15.0%), fungal ball 7 (9.5%), and CIFRS 1 (1.3%). The details of demography, clinical features, histopathology and microbiological findings of these 73 cases are provided in Table 2. Fungi could be isolated from 42 (57.5%) FRS cases and 97.6% of the isolates were *A. flavus*.

Environmental study

We measured the spore counts from different air samples in study regions. The total spore counts and *A. flavus* spore count obtained from selected villages of Punjab and Haryana provinces are shown in Tables S1 and S2 (Data S1). The total viable spore count was highest in Patiala district of Punjab at 123.3 CFU m^{-3} followed by Yamunanagar, Haryana at 118.2 CFU m^{-3} (Fig S1). *Aspergillus* accounted for ~20% of all viable spores in both provinces. Among the *Aspergillus* species isolated, *A. flavus* was dominant in all study areas, accounting for 85%–90% of all *Aspergillus* species in Punjab and 63%–80% in Haryana provinces (Fig S1).

During winter months the total spore count and *A. flavus* spore count in the air of villages of Punjab provinces were 138.14 and 28.76 CFU m^{-3} respectively. At the same season total spore count and *A. flavus* spore count in Haryana villages were 30.36 and 2.89 CFU m^{-3} respectively (Table 3, Fig. 2). Though spore count reduced during winter months compared to summer months in both Punjab and Haryana villages, the total spore count and *A. flavus* spore count in Punjab villages were significantly higher ($P = 0.0077$; $P = 0.0079$ respectively) compared to Haryana villages during winter months (Table 3, Fig. 2 and Fig. 3).

Among the 73 patients with FRS, 34 had the history of illness for <1 year duration. The case histories of these 34 patients were analysed to ascertain the season when their symptoms began. It emerged that in 15 (44.2%) patients the symptoms started in winters, nine (26.4%) in summers, and five (14.7%) each during monsoons and autumn seasons.

Table 2 Salient feature of FRS cases (n = 73).

	AFRS	EFRS	Fungal ball	CIGFRS	CIFRS	Total
Age-wise distribution (year)						
>10	2	0	0	0	0	2
10–20	5	1	1	2	0	9
20–30	18	3	0	6	1	28
30–40	11	5	2	4	0	22
40–50	4	0	0	1	0	5
50–60	0	1	2	0	0	3
>60	1	1	2	0	0	4
Occupation						
Student*	24	3	0	4	1	32
Housewife	6	3	2	2	0	13
Related with farming*	23	6	4	10	1	44
Major sign						
Nasal obstruction, nasal discharge	41	11	5	10	0	67
Proptosis	10	1	1	3	1	16
Diplopia	0	0	0	3	0	3
Headache	15	0	1	3	0	19
Insomnia	30	0	0	0	0	30
PND	10	1	1	0	0	12
Duration of illness (year)						
<1	19	2	4	8	1	34
1–5	16	3	3	4	0	26
5–10	6	3	0	1	0	10
10–20	0	3	0	0	0	3
Predisposing factor						
H/o allergy	14	3	0	2	0	19
Asthma	3	1	0	0	0	4
DNS	12	3	3	4	0	22
No predisposing factor	17	4	4	7	1	33
Mycological investigation						
Smear positive	36	7	5	6	0	54
Culture positive	30	6	0	6	0	42
Fungus isolated						
Aspergillus flavus	29	6	0	6	0	41
Alternaria alternata	1	0	0	0	0	1
Histopathology						
Allergic mucin	41	11	0	0	0	52
Charcot-leyden crystal	32	7	0	0	0	39
Invasion	0	0	0	13	1	14
Granuloma	0	0	0	13	0	13
Fungus	35	6	7	13	1	62
CT scan						
Intraorbital ext.	12	0	1	9	1	23
Intarcranial ext.	3	0	0	3	0	6
B/L heterogenous soft tissue density	32	7	3	8	1	51
U/L heterogenous soft tissue density	9	4	4	5	0	22
Endoscopy						
B/L polyp	32	7	3	8	1	51
U/L polyp	9	4	4	5	0	22
Allergic mucin	41	11	0	0	0	52

AFRS, allergic fungal rhinosinusitis; EFRS, eosinophilic FRS; GFRS, granulomatous FRS; CIFRS, chronic invasive FRS; PND, postnasal drip; DNS, deviated nasal septum.

*Some students were also doing farming activities hence both the groups contain overlapping population.

Table 3 Total spore, *Aspergillus* spore and *A. flavus* spore count (in CFU m^{-3}) of Haryana and Punjab provinces in different seasons.

	Total	*P* value	*Aspergillus*	*P* value	*A. flavus*	*P* value
Winter						
Punjab	138.14	0.0077	32.3	0.1376	28.76*	0.1626
Haryana	30.36		4.71		2.89*	
Summer						
Punjab	213.9	0.1365	10.78	0.0812	6.68	0.3022
Haryana	130.2		16.82		10.66	
Monsoon						
Punjab	73.8	0.3276	6.2	0.2519	3.1	0.1866
Haryana	192		12.32		6.9	
Autumn						
Punjab	95.15	0.8264	9	0.3528	6.9	0.9029
Haryana	102.16		13.2		32.5	

*The difference was significant when non-parametric analysis was performed by Mann–Whitney test ($P = 0.0079$).

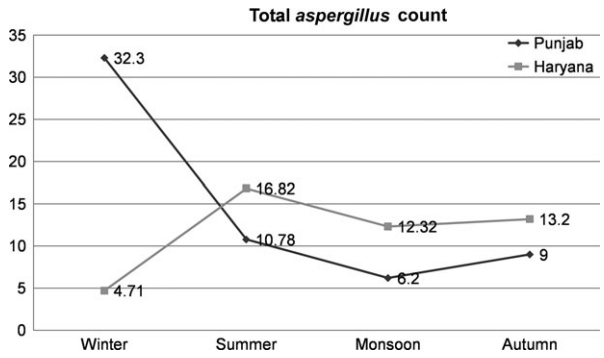

Figure 2 *Aspergillus* count (in CFU m^{-3}) in different seasons at Punjab and Haryana provinces.

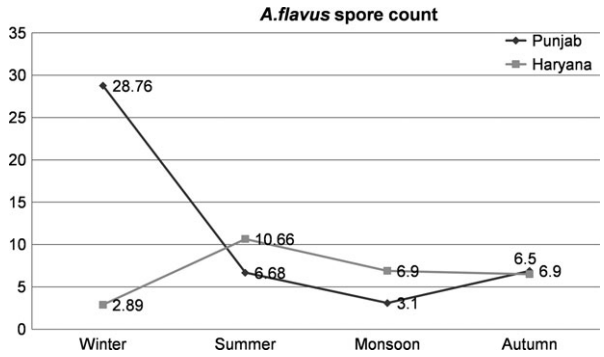

Figure 3 *Aspergillus flavus* count (in CFU m^{-3}) in different seasons at Punjab and Haryana provinces.

Discussion

The present study confirms CRS as a common health problem in north India affecting 1%–1.5% of rural population at any given time. According to the National Health Interview Survey from USA, CRS is a common disease affecting 12.5% of US population some time in their life.[1,2] The fungal aetiology of CRS is gaining importance in last two decades.[3–15] Majority (~70%) of our FRS cases were reported in young villagers of north India.[8,9,11] Though FRS has been recognised for many years in this area, the magnitude of the problem was never ascertained. The case series reported[8–14] are mainly from single centre without any population denominator data. The present study is maiden attempt to ascertain prevalence of FRS cases in rural India. However, the consensus definition of FRS is still elusive. It is more than a decade since Ponikau *et al.* [17] claimed fungus as a cause for all patients with CRS. However, many researchers debated their observation.[6] We categorised our patients according to the generally accepted classification.[6] Detection of fungus in nasal samples qualifies it as a case as fungus-related rhinosinusitis, though sensitivity of detection varies between conventional and molecular techniques.[17–20] Sensitive techniques for sample collection (nasal lavage) and detection of fungi (immunostaining) or its DNA offer higher sensitivity compared to conventional procedures.[17,18,20] Specific immunofluorescence stains using chitinase and anti-*Alternaria* antibodies are highly sensitive techniques for the detection of fungi. Guo *et al.* [21] recently described a modification of the GMS stain that could significantly increase the visualisation of fungi on histology. In the present study, we followed conventional direct microscopy of 10% potassium hydroxide and cacoflour white and histopathology for detection of fungi. Despite the use of conventional technique, FRS was diagnosed in 27.2% of CRS cases and 0.11% of the rural population. This implies that 1.1 persons per

1000 population suffer from FRS indicating a very high burden of FRS in north India especially when 0.83 billion people live in rural India.[22] The number of FRS cases per 1000 population may be even higher, as only 27.2% of CRS patients attended our hospital for confirmation of fungal aetiology. In our earlier observation, the FRS cases are not uniformly distributed across villages in north India.[9] The Patiala and Sangrur blocks of Punjab province are high prevalence zones for FRS. The present study confirmed the same findings. Similar population based studies across India would clarify the region-wise prevalence of FRS in this vast country.

Although much confusion surrounds the categorisation of eosinophil-related FRS, AFRS is a distinct entity and it is the most common type reported among Indian FRS patients. The present study confirms this with 56.1% of our patients suffering from AFRS. Among chronic invasive varieties, granulomatous invasive type has been described as an entity prevalent in tropical areas, like Sudan, Saudi Arabia, Pakistan and India.[23] In the present study, 93% of chronic invasive variety had granulomatous presentation. The reason for high endemicity of this category in our villages is unclear. *A. flavus* is the commonly isolated agent from these patients.[23] In the present study as well, *A. flavus* was the prevalent agent (97.6%) across all types of FRS. In AFRS cases, dematiaceous fungi especially *Alternaria* and *Cladosporium* spp. are the prevalent agent in the USA.[24] In contrast 96.8% of our isolates from AFRS patients were *A. flavus* and only one case was due to *Alternaria alternata*. High environmental contamination especially presence of *A. flavus* conidia in the air may be the reason for high *A. flavus* prevalence in Indian FRS patients.

The environmental air analysis for fungi revealed an abundance of *Aspergillus* spores (~20%) similar to earlier reports from different parts of India.[25–27] *A. flavus* was the predominant species among *Aspergillus* spp. isolated from air of all the regions studied. Both the total spore and *A. flavus* spore counts were significantly higher in winter months in the Punjab region as compared to the Haryana region.

The findings of the present study corroborate with the higher prevalence of FRS cases observed in our hospital records from Patiala and Sangrur blocks of Punjab province and an absence of cases from the blocks surveyed in the Haryana province. This higher prevalence of FRS in Punjab province appears to be linked to the higher spore counts observed in this study. Published data on cropping pattern of the two provinces revealed no major difference in the major crops (rice, wheat and jowar) and vegetables grown.[28] However, Haryana province is classified as arid region, whereas Punjab as semi arid region.[28]

In the present study we examined the influence of age, occupation and environmental exposure of fungal conidia in air with FRS development. The patients were young (70% within 20–39 years) and engaged in farming activity. More interestingly this study brought out the spike in *A. flavus* conidial burden in air during winter months, which coincides with the wheat harvesting period in north India. Punjab province is the largest wheat producer of the country. The *A. flavus* conidia count was much higher in wheat-thrashing area than other part of the villages (data not shown). Moreover, 34 of 73 patients who had <1 year of illness could reliably recollect the time of initiation of their symptoms; with 44.2% of these cases experiencing an onset in winters. Though the evidence is indirect, the relation of high count of *A. flavus* conidia in air and development of the disease is very significant. To confirm the association a comparison of fingerprints among clinical and environmental *A. flavus* isolates is required. Further, to evaluate the direct evidence of the effect of season and the relation of agricultural practices with development of FRS, a future longitudinal study is warranted. In conclusion, the present study determined 0.11% prevalence of FRS in rural India and highlighted the possible association of wheat harvesting season and acquisition of the disease.

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Conflict of interest

No conflict of interest of any author.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1. Total spore count, total *Aspergillus* count and *Aspergillus flavus* count in different districts of Punjab (Patiala and Sangrur) and Haryana (Ambala and Yamunanagar).

Table S1. Seasonal variation in spore count (in CFU m^{-3}) in villages of Haryana.

Table S2. Seasonal variation in spore count (in CFU m^{-3}) in villages of Punjab.

Data S1. Preparation of *Aspergillus flavus* antigen for aspergillin skin test.

Data S2. Proforma.