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# Presence of Fungal Organisms in Chronic Rhinosinusitis with Nasal Polyposis: A Clinico Pathological Study from Kerala

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## Abstract

**Background:** Fungi have been implicated in the etiopathogenesis of chronic rhinosinusitis (CRS) with nasal polyposis (NP). Our aim was to determine the presence of fungal organisms and to identify the fungal species as this would help in the management of the condition.

**Materials and Methods:** The study design was descriptive. Totally, 60 immunocompetent patients of CRS with bilateral and or unilateral NP comprised the study group. Fungal culture of nasal washings, direct microscopy, fungal culture, and histopathology of the specimen were carried out. Nasal washings of 16 healthy volunteers who served as control were cultured for fungus.

**Results:** Nasal washings of 48 patients (80%) were positive for fungus and the most common organism isolated was *Aspergillus fumigatus*. Direct microscopy was positive in 15 cases (25%). Specimen culture was positive in 35 cases (58.33%), the most common organism being *A. fumigatus*. The histopathology for fungus was positive in 19 cases (31.7%). The fungus was positive in the nasal washings of 15 volunteers (93.75%) and the most common organism isolated was *Aspergillus niger.*

**Conclusion:** The fungal prevalence in our study was determined by the histopathological result since the majority of the volunteers also had a positive fungal culture of the nasal washings. *Aspergillus* species was the most common isolate, which was found to be different when compared with the western literature. With a moderate prevalence rate in various studies, antifungal therapy may be considered in the medical management of CRS with NP.

**Key words:** *Aspergillus fumigatus, Aspergillus niger*, Nasal polyposis, Rhinosinusitis

## INTRODUCTION

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nose and paranasal sinuses which is present for at least 12 weeks, without complete resolution. The disease is characterized by the presence of symptoms such as nasal discharge or obstruction, facial pain, hyposmia; and with endoscopic features such as polyps, purulent discharge and mucosal edema.[1]

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Nasal polyposis (NP) is a chronic inflammatory disease of the mucous membrane of the nose and paranasal sinuses, presenting as smooth gelatinous, round or pear-shaped unilateral or bilateral pedunculated masses of inflamed mucosa prolapsing into the nose. The incidence of NP is between 1% and 4% of the population.[2] The main cause of polyp formation is not exactly understood, and the relationship between NP and chronic sinusitis is much debated.

Several factors have been implicated in the development of CRS. Ostial blockage by edema, inflammatory mucous and delayed recovery of mucociliary function, and mucous recirculation are some of the mechanisms that lead to a transition from an acute to chronic inflammatory process, while certain anatomical abnormalities may not be as much

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of a factor as believed earlier.[1] NP is considered as part of the spectrum of CRS.

Although NP is considered as a multifactorial disease with several different etiological factors, chronic persistent inflammation is the most significant factor in its formation.[3] Viral, bacterial, and fungal infection; and genetic factors have all been suggested as causes of inflammation in NP. Chronic inflammation leads to reactive hyperplasia of the mucous membrane, which can result in the formation of polyps.[4] Recently, fungal etiology has been proposed to underlie severe NP.[4] The presence of fungus in the nose and sinuses may be benign, or it may cause a spectrum of fungal diseases, which can range from noninvasive to invasive fulminant fungal diseases.[1]

Fungal specimen rates have been found to vary from 0% to 100% depending on the different techniques used for specimen collection and detection methods.[1] Ponikau *et al.*[5] have reported the presence of fungus in more than 90% among the controls, using his novel technique of collecting the nasal washings for fungal culture.

The purpose of this study was to determine the presence of fungal elements in the nasal washings and specimen of nasal polyps, the types of organisms and their role in the formation of polyposis in CRS.

### Objectives

1. To determine the presence of fungal organisms in CRS associated with NP by both culture and histopathology.
2. To identify the different types of fungal organisms in NP.

## MATERIALS AND METHODS

The study was conducted for a period of 2 years beginning from the first of January, 2013. A total of 60 patients with sinonasal polyposis who were treated surgically in the department of ENT at Government Medical College Alappuzha, Kerala during that period were included in the study.

The study design was descriptive.

### Eligibility Criteria

1. Cases of CRS with single or multiple, unilateral or bilateral NP from the age group of 15-65 years were included.
2. Totally, 20 volunteers with no symptoms of nasal or paranasal sinus disease, inhalant allergy, served as control group.
3. Patients and controls were instructed not to use any steroidal medication 1 month before the study to prevent any reduced eosinophilic inflammation.

### Exclusion Criteria

1. Patients with age below 15 years (to exclude congenital lesions) and above 65 years (to exclude tumor masses).
2. Immunocompromised patients.
3. Patients who had undergone surgery previously.
4. Patients who were on topical or systemic steroid for the past 1 month before the study period.

Ethical Committee clearance was obtained initially. The study included males and females. A total of 60 patients suffering from CRS with NP were enrolled. Totally, 16 healthy volunteers without any history of rhinosinusitis served as control. Informed consent was signed by all the patients and the volunteers for sharing in the scientific research. Specially designed proforma was used for data collection. All were subjected to the following:

- A full ENT examination including the face for any asymmetry or swelling in the sinus region and cheek. Nose and throat were examined to observe the effects of nasal obstruction. Eyes were examined by the ophthalmologist.
- Diagnostic nasal endoscopy to confirm the presence of the polyps and mucin.
- Computed tomography (CT) of the nose and paranasal sinuses with coronal and axial 5 mm thick cuts in both soft tissue and bone windows for hyperattenuation, calcification, bony erosion, and intracranial extension.

### Collection and Culture Techniques of Nasal Lavage

Specimens were collected using the nasal lavage method described by Ponikau in 1999.[5] This method allowed maximum collection of mucus and gave a better yield of fungus in the culture medium. Two puffs of oxymetazoline spray, 0.1% were sprayed into each nostril to produce vasoconstriction. The patient was asked to inspire deeply and hold it and then after 2 min, each nostril was flushed with 20 ml of sterile saline using a sterile curved blunt needle. The patient exhaled forcefully through the nose during the flushing. The return was collected in a sterile pan, put in a sterile centrifuge 50 ml tube and sent to the microbiology department in our college. This was processed and inoculated into the Sabouraud glucose agar media with chloramphenicol and cycloheximide. The dish was incubated at 30°C and 37°C, as dimorphic fungi will appear as mould at room temperature and as yeast at body temperature. The plates were examined at a 3 day interval for a period of 30 days for fungal growth. All the specimens provided by ENT Department with a clinical diagnosis of nasal polyp were included.

### Collection of Surgical Specimen and Histologic Examination

Endoscopic sinus surgery was tailored according to the need of the patient. Microdebriders were avoided, and suction devices were used discreetly. After surgery, samples were divided into two parts under sterile process in the operation room. Great care was taken to preserve the mucous over the inflamed tissue as fungus is generally found to colonize mucous. The specimen was placed in a nonstick sheet rather than on a towel to prevent absorption and loss of mucous.[5] One part was placed in formalin for histopathology, and the other in sterile normal saline and was sent to the microbiology department for the fungal culture of specimen with a complete history of the patient. Culture of the specimen was done as described earlier. Multiple serial sections of different specimens from each patient were stained with hematoxylin and eosin to identify the eosinophils in the mucous and gomori methenamine silver to identify the fungi.

Statistical analysis was performed with the use of SSPS 16.0 software, and Pearson's Chi-square test was used for comparing groups.

## RESULTS

Among the sixty patients of CRS with NP, 33 (55%) were male while 27(45%) were female, and their age ranged between 16 and 55 years. Maximum patients were between the age ranges of 46-55 (32%) years (Table 1).

The majority (44) belonged to the low socioeconomic group (73%). A total of 25 patients were manual laborers (41.7%), and 18 (30%) were housewives. Farmers, shopkeepers, and students formed the remaining group of 17 (28%) (Table 2).

A total of 36 patients (60%) are presented with bilateral nasal polyps, 20 with multiple unilateral polyposis (33.3%), and 4 with single unilateral polyp (6.7%). Proptosis was found only in 3 patients with multiple unilateral NP with corroborative CT scan findings of bony erosion of the infraorbital wall in 2 cases and of lamina papyracea in 1 case. In the majority of patients, more than one sinus was involved, with maxillary sinus being the most common.

Positive culture of the nasal washings was found in 48 patients (80%), among whom 25 (42%) were of bilateral multiple NP, 18 (30%) of unilateral multiple NP, and 5 (8%) of unilateral single polyp (Table 3). The most commonly isolated organism was *Aspergillus fumigatus* (58.3%) followed by *Aspergillus niger* (33.3%), *Candida* (4.2%), and *Fusareum* species (2.1%) (Table 4).

**Table 1: Age group distribution**

| Age in years | *n* | % age |
|---|---|---|
| 16-25 | 6 | 10 |
| 26-35 | 14 | 23 |
| 36-45 | 17 | 28 |
| 46-55 | 19 | 32 |
| 56-65 | 4 | 7 |

**Table 2: Occupation**

| Occupation | Frequency=*n* | Percentage |
|---|---|---|
| Manual laborer | 25 | 41.7 |
| Shopkeeper | 7 | 11.7 |
| Housewife | 18 | 30 |
| Farmer | 2 | 3.3 |
| Student | 5 | 8.3 |
| Nil | 3 | 5 |
| Total number | 60 | 100 |

Positive microscopy was found in 25%, 21.7% being from the multiple unilateral NP (Table 3). Direct microscopic examination of the specimens in the study showed branched septate hyphae ($n$ = 9) and beaded spherical spores ($n$ = 6) in 15 specimens (25%).

Allergic mucin was found in 12 (20%) consecutive surgical cases, among whom 11 patients were with multiple unilateral polyposis and 1 with bilateral multiple polyposis (Table 3). Mycological culture of the samples yielded pure growth of fungi in 35 cases (58.3%). The most commonly isolated fungi were *A. fumigatus* (45.7%) and *Niger* (22.9%) followed by *Aspergillus flavus* (11.4%), *Penicillium* (11.4%), *Candida albicans* (2.9%), *Fusareum* (2.9%), and *Alternaria* (2.9%) (Table 4).

The histopathological examination of these specimens revealed soft tissue invasion by fungal elements in 2 cases of multiple unilateral NP whose culture had yielded *A. flavus* in two cases and *Penicillium* in the other. Fungal elements like hyphae, conidiospores were found in 19 (31.7%) specimens.

Incidentally, the nasal washings of the volunteers yielded positive fungal culture in 15 cases (93.75%), *A. niger* was cultured in 14 (87.5%), *A. fumigatus* (6.25%) in one and one was culture negative (6.25%).

## DISCUSSION

CRS affects approximately 15% of the adult population. Several factors have been implicated in the development of CRS. Ostial blockage by edema, inflammatory mucous and delayed recovery of mucociliary function, and mucous recirculation are some of the mechanisms that

**Table 3: Clinical, endoscopic, CT, and culture results of the study group**

| Endoscopy findings | Total no of patient involved and % | Proptosis and facial swelling no of patients and % | CT scan bony erosion no of patients and % | Positive culture of nasal washing no of patients and % | Allergic mucin no of patients and % | Positive HPE no of patients and % | Positive HPE and tissue invasion no of patients and % | Positive microscopy no of patient and % | Positive culture of tissue specimen no of patients and % |
|---|---|---|---|---|---|---|---|---|---|
| Bilateral polyps | 31 (52) | 0 (0) | 0 (0) | 25 (42) | 1 (1.7) | 2 (3.3) | 0 (0) | 2 (3.3) | 11 (18.3) |
| Unilateral multiple polyps | 22 (37) | 3 (5) | 3 (5) | 18 (30) | 11 (18) | 12 (20) | 5 (8) | 13 (21.7) | 21 (35) |
| Unilateral single polyps | 7 (12) | 0 (0) | 0 (0) | 5 (8) | 0 (0) | 5 (8.3) | 1 (1.7) | 0 (0) | 3 (5) |
| Overall positivity | 100 | 5 | 5 | 80 | 20 | 31.67 | 9.7 | 25 | 58.3 |

CT: Computer tomography

**Table 4: Fungal organisms isolated in nasal washings and tissue specimens**

| Fungal organism isolated | No of culture positive nasal washings with % | No of culture positive specimen with % |
|---|---|---|
| *Aspergillus fumigatus* | 28 (58.3) | 16 (45.7) |
| *Aspergillus niger* | 16 (33.3) | 8 (22.9) |
| *Aspergillus flavus* | 0 (0) | 4 (11.4) |
| *Penicillium* | 0 (0) | 4 (11.4) |
| *Candida albicans* | 2 (4.2) | 1 (2.9) |
| *Fusareum* | 1 (2.1) | 1 (2.9) |
| *Alternaria* | 1 (2.1) | 1 (2.9) |
| Total number | 48 | 35 |

lead to a transition from an acute to chronic inflammatory process.[1]

NP affects 1-4% of the population.[2,4] Histologically NP is characterized by infiltration by inflammatory cells like eosinophils or neutrophils.[3] Frequently, nasal polyps are associated with asthma or aspirin sensitivity.[6] A genetic link has been demonstrated recently between HLA-A74 and NP but the current knowledge in this area remains very limited.[6]

In 1791, Reflaignaud[1] first reported fungal sinusitis.[5,6] The fungal spores in the environment on inhalation, settle down in the sinuses initiating a hypersensitivity reaction leading to the formation of polyps in certain individuals. At the same time, these spores get access to the nose or PNS of an individual already having nasal polyps. Chronic irritation of the narrow ostiomeatal complex, which is considered as the key area in development of polyps[2] causes mucosal edema, obstruction of natural ostia resulting in impeded mucociliary clearance, bacterial overgrowth, and release of chronic inflammatory mediators like cytokines which attract other inflammatory cells.[2] Polyps contain a high level of mast cells which release eosinophils. The inhaled fungal spores in the sinus mucous cause the eosinophils to be released into the lumen, which cluster around and attack the fungal elements, thereby leading to release of toxic mediators such as interleukins and ensuing secondary inflammation.[2,4]

There have been a number of reports of fungal infection in the immunocompetent people.[2] Polyps if left untreated, can become aggressive locally. They can cause bony erosion, extending intracranially, or orbit, causing proptosis, and visual impairment.[2] The presence of fungus in polyposis can lead to recurrence.[1]

Fungal rhinosinusitis (FRS) can range from benign localized fungal colonization to the extremely aggressive acute invasive FRS. Each of the clinicopathological variants of FRS is associated with unique climatic, geographical, and host-related risk factors, and different fungal organisms.[7]

FRS can affect both the immunocompetent and immunocompromised individuals. In immunocompetent people, it is usually indolent or chronic, whereas, in the latter, it is fulminant.[8] Once considered rare, the incidence of FRS has increased during the past two decades. Improved selection of patients based on clinical and radiological findings and newer methods of collection of the tissue samples have led to an increased evidence of fungal involvement in sinusitis.[9]

In the present study, the most common age group presenting with NP was 46-55 years with a slight male preponderance. All were immunocompetent. Manual laborers and housewives were the most commonly affected class.

In our study, the culture of the nasal washings was positive in 80% of cases, with *A. fumigatus* predominating (58.3%), followed by *A. niger* (33.3%). Incidentally, among the 16 volunteers, 15 individuals gave a positive fungal culture of the nasal washings, with *A. niger* in 14 cases and *A. fumigatus* in one case. It was noted that the presence of *A. fumigatus* in the culture of nasal washings among the patients with NP was statistically more significant when compared with that of the healthy, volunteers ($P < 0.04$).

Bony erosion was seen in 3 cases of multiple unilateral NP. Considering the number of bilateral multiple and unilateral single polyposis together, the bony erosion associated with multiple unilateral NP was statistically significant ($P < 0.05$).

About 18% of multiple unilateral polyposis had associated the presence of allergic mucin, which is considered as one of the diagnostic criteria for allergic fungal rhinosinusitis (AFRS).

Since, AFRS may not typically involve individuals with atopy, there have been suggestions to consider AFRS as eosinophilic fungal rhinosinusitis due to the predominance of eosinophils in the mucous,[3] which was similarly observed in the histopathology of the specimens in our study.

Positive HPE was seen in 31.67% of cases, with tissue invasion in 9.7% of cases, all of whom were of multiple unilateral NP, which was not statistically significant. Similarly, though *A. fumigatus* was the most commonly isolated fungal organism by the culture of the specimen, this was not found to be statistically significant.

In our study, an overall prevalence rate of 31.7% was noted histopathologically among the patients with clinical suspicion of FRS. Though fungal organisms were isolated by both cultures of nasal washings with a higher prevalence rate, the possibility of contamination could not be ruled out. Furthermore, nasal washings in volunteers had a high positivity rate of the fungal culture. Due to the presence of abundant fungal spores in warm, humid environment in Kerala, it is reasonable to assume that fungal colonization is present even in the normal nasal mucosa. Hence, histopathology seemed a more rational method to assess the association of fungal organisms in NP.

The prevalence rate by the study conducted by Amin and Kakru[10] at Srinagar was found to be 30%, and by Chakraborty *et al*.,[10] 42% at Chandigarh Venugopal *et al.*[11] in Tamil Nadu 45%, In a Malaysian study, the prevalence was found to be 26.7%,[9] Braun *et al.*[10] in Europe, found that 75.5% of specimens were positive fungal elements. Mayo clinical researchers diagnosed allergic fungal sinusitis in 93% of cases.[10]

Based on several studies in literature, fungal specimen rates have been found to vary from 0% to 100%.[1] Depending on the different specimen collection techniques and fungal detection techniques, and also influenced by geographical conditions both the prevalence rate and the type of organisms isolated have been found to vary from place to place.

In our study, the most common fungal organism isolated in fungal culture was *Aspergillus* species, with a predominance of *A. fumigatus* followed by *A. niger* and *A. flavus*. The prevalence of *Aspergillus* species was in accordance with the studies conducted by Lakshmanan *et al.*[9] in Tamil Nadu, Challa *et al.*[12] in Hyderabad, Deshmukh *et al.*[11] in Maharashtra and Garg *et al.*[13] in Delhi, being the various states of India, whereas, dematiaceous fungal organisms such as bipolaris and curvularia have been found to be prevalent in North America in these conditions.[9]

Among the *Aspergillus* species, *A. fumigatus* was the most frequently isolated organism followed by *A. niger* and *A. flavus* in our study, while *A. flavus* was most frequently isolated followed by *A. fumigatus* in fungal studies in Tamil Nadu, Srinagar, Maharashtra, Delhi. In Germany Vennewald *et al.* isolated *A. fumigatus* and in Malaysia, *A. niger* was most commonly isolated. This indicates that *Aspergillus* species has been found to vary from place to place.[10] It is interesting to note that *A. flavus* was isolated in tissue specimens with invasion histopathologically.

*Penicillium* species has been consistently found in FRS, 17.64% in Srinagar and Goh *et al.* isolated this in 14.3%.[10] In our study, *Penicillium* was isolated in 6.7% of cases.

Geographical variation has led to the isolation of different fungal species in different regions, as cited by various studies all over the world. In India, the most frequently reported genders are *Aspergillus* species, *Alternaria, Candida, Penicillium* and *Fusarium* in AFRS.[12] The fungal pattern isolated in Kerala was similar to other studies in India. Though no attempt was made to classify FRS clinically, presence of allergic mucin in 20% of the patients, predominantly in unilateral multiple NP, coupled with the fact that all the patients were immunocompetent, indicate that *Aspergillus* species is the most common fungus isolated in AFRS in India. On the contrary, dematiaceous fungal organisms are associated with AFRS in the western literature.[12] Therefore, the geographical pattern, climate and host factors play a significant role in the involvement of fungal organisms in CRS.

In the present study, FRS was more common among patients who lived in warm, humid environment. Unilateral multiple sinonasal polyposis was more implicated with fungal etiology than bilateral polyposis. The overall prevalence rate varies depending on the host factors, geographical conditions, and the methods of collection of specimen for fungal isolation.

The ubiquitous nature of fungal spores makes it difficult to determine their etiological role for fungal infection in CRS. Fungal spores are present in air, soil, and dust. The warm, moist environment of the upper respiratory tract is ideal for fungal colonization, and initiation of inflammation in susceptible individuals**.** Knowing the fungal flora and its prevalence helps in the diagnosis and management of FRS. It is believed that the presence of fungal organisms in sinonasal polyposis can lead to recurrence after treatment.[2] Considering the moderate, yet significant fungal prevalence rate in various studies in India, the use of antifungal drugs in the medical

management of CRS of selective cases may be considered to avoid serious complications associated with FRS.

## CONCLUSION

The mere presence of fungal organisms in association with NP is insufficient to implicate them as causative agents in CRS. Furthermore, the host resistance factors should be kept in mind as fungal infections are more aggressive in immunocompromised states. There is still a poor understanding of when fungi are present as pathogens or simply a part of the normal flora. Positive microscopy, histopathology, PCR assay may be considered more significant than culture alone as fungal spores are present everywhere in the environment. The different climatic pattern in India, when compared to the west, may account for the different pattern of organisms isolated in immunocompetent individuals as presented in the various studies conducted in India. As sinonasal polyposis recur frequently, further research is needed to determine the precise role of fungi in the pathogenesis of this condition and to the judicious use of specific antifungal drugs against FRS as this is associated with a prolonged morbidity among the sufferers.

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