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Microbial Study from Ethmoidal Mucosa in Patients Undergoing Functional Endoscopic Sinus Surgery

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Abstract **Original Research Article**

This study was conducted on 50 patients who underwent FESS under GA for Chronic Rhinosinusitis in a tertiary care hospital. The objectives were to determine the pattern of microbial flora in ethmoidal tissue in Chronic Rhinosinusitis and to know the antibiotic sensitivity of potential pathogen. The specimen was transported to microbiology lab in normal saline within 2 hours. Samples were inoculated into various media for aerobic, anaerobic and fungal growth. The study conducted showed Coagulase-negative Staphylococci to be the most common isolate, Staphylococcus aureus being second. Enterobacteriaceae were also frequently isolated. Klebsiella and Acinetobacter were infrequent isolates. Among fungi, Rhizopus and Aspergillus were seen. But no anaerobes were isolated.

Keywords: Aerobes, Chronic Rhinosinusitis, Coagulase-negative Staphylococci, Ethmoidal mucosa, Functional Endoscopic Sinus Surgery.

INTRODUCTION

Chronic Rhinosinusitis (CRS) is characterized by inflammation of nose and paranasal sinuses (PNS) of minimum 12 weeks duration. Patients with CRS may have intermittent acute flare ups and, in such cases, the disorder is called *'acute exacerbation of chronic rhinosinusitis'*. Although the term *sinusitis* is commonly used for any inflammation or infection of the sinuses, this term has largely been replaced by *rhinosinusitis* since the nose is almost always involved with the infection or inflammation of the sinuses at the same time [1].

Chronic rhinosinusitis is not a severe disease. However, it is important to realize that it is a common condition and causes considerable long term morbidity and hence is associated with poor quality of life. Many patients with chronic sinus disease receive multiple courses of antibiotics and undergo multiple procedures, with little or no improvement in their condition. Recently, there has been a tremendous advance in the management of rhinosinusitis, thanks to the knowledge of the microbes associated. Long-term results of medical and surgical therapies have resulted in cure rates varying between 29 and 80% [2].

The aetiology of chronic sinusitis is multifactorial, while bacterial infection is believed to be a major causative factor in the development of the disease. In the recent years there has also been a marked increase in the mycotic infections of the paranasal sinuses, especially in immunosuppressed patients. This has been attributed both to an increase in the survival of subjects at risk and to the improvements in diagnostic techniques enabling the identification of fungal sinusitis. Although bacteria, fungi and also, viruses, can be primary causes of sinus inflammation, they may also occur as secondary colonizers of the mucosa. The knowledge of normal flora can help in the assessment of significance of organisms isolated from the sinuses. But not many studies have been done on the normal flora of ethmoidal sinuses. Such studies guide us on choosing the most appropriate antibiotic to eliminate the infectious process, thus, helping in restoring the normal mucosa [1,2].

Maxillary sinus has always been considered as primary focus of disease, and they have been the focus of majority of the microbiological studies on chronic sinusitis due to its ease of access. The less accessible ethmoidal sinus was little studied until the development of rigid endoscopes which improved visualization of ethmoid sinus through the nasal cavity. Recent studies suggest that ethmoidal sinus play a central role in the

drainage and blockage of frontal, maxillary and sphenoid sinuses and hence form the key to aetiology and management of chronic sinusitis [2].

The diagnostic criteria for acute sinusitis is well established, but the definition of chronic sinusitis is controversial with respect to the importance of bacteria in the initiation and progression of the disease. The lack of progress in the management of CRS was due to the paucity of knowledge on microbiology and histopathology. In this prospective study, 50 patients with the diagnosis of CRS, who underwent FESS, were evaluated microbiologically by using biopsy specimens taken from the ethmoid sinus mucosa in an effort to identify the frequently associated organisms as well as their antibiotic sensitivity.

MATERIALS AND METHODS

Source of data

This is a prospective study conducted on 50 patients over a period of 18 months, who were diagnosed with CRS and underwent FESS. This study was conducted after receiving the approval of the Institutional Ethics Committee and an informed consent was obtained from all the patients or the patient's legal guardian.

Patients presenting with a history of chronic sinusitis of more than 12 weeks duration, which was supported by CT scan findings were included in the study. Specifically, the diagnosis was made based on the following criteria:

- Symptoms of nasal obstruction or purulent nasal discharge, discomfort or fullness over the sinuses, headache and/or disturbances in olfaction, lasting for more than 12 weeks with no response to medical therapy.
- History of recurrent sinusitis with more than 4 episodes in 1 year.
- Signs of inflamed nasal mucosa, purulent exudate in the middle meatus, nasal cavity or nasopharynx and/or Sinonasal polyposis.
- CT scan findings of thickening and/or opacification of the ethmoid sinus and/or a blurring of one or more paranasal sinuses and of the osteomeatal complex.

A detailed clinical examination was done in all the patients, belonging to the age group of 16 to 60 years, and 50 patients were selected depending on the above criteria. Among them 22 cases were females and 28 were males. Patients with acute infection and those who were diagnosed with conditions other than nasal polyp and chronic sinusitis, who were candidates for endoscopic sinus surgery, were excluded from the study. Those patients who were given local and systemic antibiotic therapy in the last 3 weeks before surgery were also excluded. None of the 50 patients selected were put on antimicrobials for 3 weeks prior to the sample collection. All the selected patients were subjected to the routine blood investigations.

Sample selection

Samples of ethmoidal mucosa were taken under GA from each patient during FESS under aseptic conditions. The endoscopes were sterilized in glutaraldehyde solution for 10 min and washed prior to use. In order to decrease the risk of contamination, biopsy specimens were taken from within the ethmoid sinus air cells upon entering the sinus. The removal of a mucosal fragment was done with care not to contaminate the sample with the nasal cavity mucosa. The fragments of ethmoidal mucosa collected for biopsy was immediately prepared for proper transportation. Strict asepsis was employed to avoid contamination. Samples taken were transported to microbiology lab in normal saline within 2 hours.

Samples were inoculated onto 5% sheep agar / chocolate agar for aerobic bacteria and incubated at 35 degree celsius, 5% CO2 for 24-48 hours. Samples were inoculated onto anaerobic blood agar; pre-reduced vitamin K1 enriched Brucella blood agar, blood agar with kanamycin and broth for anaerobic culture and incubated at 35 degree celsius in Gas-Pak anaerobic jars for 4-7 days. Samples were inoculated onto Sabouraud-Dextrose agar for fungi and left at room temperature for 20days.

All cultured bacteria were identified on the basis of standard microbiologic procedures, including morphological and Gram stain characteristics and oxidase reactions, as well as detection of catalase, coagulase and clumping factor for determination of Staph.aureus.

RESULTS

Of the 50 specimens collected and examined, in 36 cases (72%) an aerobic bacterial growth was obtained; 4 cases (8%) showed fungal growth; no growth was seen in 10 cases (20%). But no anaerobes were isolated. Among the aerobic bacteria isolated, Coagulase-negative Staphylococci was the most frequent isolate, grown from 16 cases (32%). Staphylococcus aureus was found in 12 cases (24%), being the second most common isolate. Klebsiella was found in 4 cases (8%); Pseudomonas in 1 case (2%); Acinetobacter in 3 cases (6%). The fungi isolated were Aspergillus and Rhizopus in 2 cases (4%) each. (Table 1)

Table-1: Distribution of the growth pattern

Isolates	Count	Percentage
Coagulase-negative Staphylococci	16	32 %
Staphylococcus aureus	12	24 %
Klebsiella	4	8 %
Acinetobacter	3	6 %
Pseudomonas	1	2 %
Aspergillus	2	4 %
Rhizopus	2	4 %
Anaerobes	0	0 %
No Growth	10	20 %
Total	**50**	**100 %**

Antibiotic sensitivity was tested for commonly used drugs. None of the organisms were found sensitive to Ampicillin, while macrolides and cephalosporins were found to be more sensitive against the common isolates.

DISCUSSION

CRS is an important public health problem, commonly seen both in children and adults, with an increasing incidence and prevalence across the globe, resulting in impressive cost of diagnosis and treatment. The present study was aimed at verifying the microorganisms present in cultures of mucosal fragments obtained from the ethmoid sinus of patients with CRS, not-responding to clinical treatment. This microbiological study is important to guide us towards choosing a specific antimicrobial, to augment the treatment of patients with CRS. The study showed predominance of aerobic microorganisms in the culture, similar to many of the previous studies done by various authors.

In contrast to maxillary sinusitis, the microbiology of the ethmoid sinusitis is not well established; and only a few reports have documented the organisms isolated. Moreover, no studies have examined the normal flora of the sinuses. Some studies found normal healthy maxillary antra to be sterile; however, other studies have shown conflicting results. Cultures for viruses were not done in most of the studies [2,3,4]. To compare our microbiology results with those in the literature, we selected those studies which used modern aerobic and anaerobic techniques.

The results of bacteriological studies are largely influenced by pre-analytic and analytical factors, including the collection and type of specimen, sampling site, transportation, and processing in the microbiology laboratory. One must also be careful in the interpretation of studies done with organisms from different anatomical locations or patients from different geographical locations and socioeconomic groups [2].

Our patients were selected after radiological confirmation of the disease. However, the severity of patients' symptoms does not always correlate well with the CT stage of the disease. Hence, all symptoms of sinusitis cannot be solely attributed to the objective findings obtained by radiological examination. The use of endoscopes expanded our knowledge about sinus infections by enabling the detailed examination of the intranasal cavity [5]. Taking specimens to determine the microbiology of each sinus with a lower probability of contamination has been possible by endoscopy.

The sample collection was done in an aseptic manner to avoid contamination. The results of the studies vary according to the population studied, the means of transportation used, time elapsed for processing the sample in the laboratory and the culture technique used. Therefore, we ensured rapid transport and plating of specimens and used culture media and conditions that facilitated detection of any microorganism; aerobes, anaerobes or fungi. Culture for virus was not done.

In our study, *Coagulase-negative Staphylococci (SNC)* was the most common isolate, accounting for 32% of all isolates. The role of *SNC* in the pathogenesis of CRS is still not clear. It is present in the flora of the nasal and sinus mucosa of healthy individuals, but it may play a role in the genesis of CRS, as this organism showed resistance to various antibiotics. The predominance of *SNC* was also described by Doyle and Woodham, and Aguiar Nigro J F *et al*. [2,6].

In most of the studies investigating the microbiology of CRS, *S.aureus* was present in 15% to 40% of sinuses. In our study, *S.aureus* is present in 24% of the results. In view of the chronicity of disease in our patients, the isolation of *S.aureus*, which has a propensity for causing chronic infections, is not surprising. In their examinations of ethmoid biopsy specimens, Doyle and Woodham described a predominance of *S.aureus* and *Enterobacteriaceae*, in addition to *SNC*, in patients with CRS and concluded that these bacteria played a possible role in the pathogenesis of CRS [2]. Even though Andrea Niederfuhr *et al*. also found a predominance of *SNC* in their study, they considered its pathogenic significance questionable as these pathogenic organisms occurred in almost the same numbers of CRS patients with Nasal

Polyp (52%) and control patients (48%) [7]. Busaba *et al*. also stated that rather than bacterial pathology, other factors play a major role in the pathogenesis of CRS [8].

Other aerobic bacteria found in our study were *Klebsiella* (8%), *Acinetobacter* (6%) and *Pseudomonas* (2%). No isolates of *S.pneumoniae* and *H.influenzae* were found in our study. The studies conducted by Doyle and Woodham, and Aguiar Nigro J F *et al*. showed relatively few isolates of *S.pneumoniae* and *H.influenzae* while both organisms were not found in the study done by S Jindal and M P Kamath [2,6,9]. Itzhak Brook, in his study demonstrated that *S.pneumoniae* and *H.influenzae* predominate in acute ethmoid sinusitis as with acute maxillary sinusitis, but not with CRS [3]. This difference can impact the choice of antibiotics.

Anaerobes were not isolated in our study of patients with CRS, in contrast to most other studies of patients with chronic and acute sinusitis. Relative to the total number of organisms isolated, Brook, and Frederick and Braude identified a much higher percentage of anaerobes than the other studies did [3,10]. Frederick and Braude studied chronically infected frontal, ethmoid, and maxillary sinuses and found anaerobes in pure culture in 23 of 83 specimens; they isolated 80 anaerobes in all. In those studies, they did not determine that all of these organisms were pathogens [10]. In contrast to the findings of Brook, and Frederick and Braude, Doyle and Woodham, Busaba *et al*. and S Jindal and M P Kamath, found anaerobes in none or only in a minority of biopsy specimens of ethmoid mucosa [3,10,2,8,9]. These differences could be explained by the difference in the sampling locations. In inflamed sinuses with blocked ostia, the growth conditions are favorable for anaerobic bacteria because of lower oxygen tension while the region of the anterior ethmoid sinus is assumed to be better aerated than a blocked sinus. We propose that the ethmoid sinus may have fewer anaerobes than the maxillary and frontal sinuses by virtue of the fact that it is less likely to be obstructed and is more likely to be exposed to inspired oxygen. With obstruction there is oxygen resorption, an increase in partial pressure of CO_2 (pCO_2), and transudation of fluid, which then becomes a growth medium for organisms trapped in the sinus, predisposing the patient to bacterial sinusitis. Clinically, this creates a purulent exudate with a low PO_2 that is a good medium to support anaerobes. In the presence of pus, ethmoid sinus mucosa also might grow anaerobes [2, 10, 11].

We also believe that one important factor for the absence of anaerobes in our study was the conservative pre-surgical medical treatment which was used. Such treatment may increase the drainage of purulent material and may sufficiently oxygenate the ethmoid sinuses to eliminate the anaerobes. It is also possible that technical factors or improper processing may account for the absence of fastidious anaerobic organisms in our study. The results of our study indicated that, in this select group of patients, anaerobes do not play a prominent role. Our results did not imply that anaerobes cannot be involved in other patients with chronic ethmoid sinus disease.

The mycological culture of the biopsy samples in our study yielded pure growth of fungi in 4 patients (8%). The isolated fungi were *Aspergillus* and *Rhizopus*, in 2 cases (4%) each. It is estimated that 8 - 10% of patients undergoing surgery for sinusitis or polyposis have fungal sinusitis; either non-invasive or invasive. Fungal involvement of paranasal sinuses requires an impairment of local or systemic host mechanism which facilitates the conversion of fungal organisms from saprophytic to pathogenic. Individuals with a history of recurrent bacterial sinusitis may develop thickening of the mucosa which leads to chronic sinusitis and the colonization of the airway with fungi secondary to frequent use of broad-spectrum antibiotics. The most common organism is *Aspergillus fumigatus* and affected individuals usually complain of long standing symptoms of allergic rhinitis or chronic bacterial sinusitis. Tissue biopsy, for culture and histologic identification of fungi is the gold standard diagnostic procedure. According to the studies conducted by P Kordbacheh *et al*. the mainstay of treatment is immediate surgical resection followed by aggressive medical therapy with topical steroids and subsequent debridement. The use of oral steroid and antifungal drugs is also recommended by some researchers [12].

In our study, in 10 cases (20%) growth of microorganisms couldn't be found. Literature review shows that the culture has been reported as sterile in 17 and 60% of cases. The absence of pathogenic microorganisms in 20% of the patients probably because of the obstruction of OMC and could have been the main reason for CRS. Thus, we believe that FESS is of high importance for these patients, because ventilation of the paranasal sinuses will allow for the re-establishment of the sinonasal physiology. This concept is in well agreement with the study of Busaba *et al*. [8].

Thus, CRS has to be considered a chronic inflammatory condition rather than an absolute microbial infection. The role of bacteria in the chronicity of inflammation is unknown. Ventilatory obstruction of the sinus ostium plays a key role in its pathogenesis. Acute infection destroys the normal ciliated epithelium impairing drainage from the sinus. Pooling and stagnation of secretions in the sinuses invites infection. Factors such as virulence of organism causing sinusitis, condition of sinus mucosa, decrease in mucociliary clearance, and immunity of host are all effective in the pathogenesis of sinusitis. Persistence of infection causes mucosal changes, such as loss of cilia, edema and polyp formation, thus continuing the vicious cycle.

The knowledge of the microbiology in these patients guides us towards choosing more appropriate antibiotics to lower the inflammatory-infectious process of the sinonasal mucosa. In the antimicrobial sensitivity test, different bacterial isolates showed resistance to various antibiotics and hence in each case, specific antibiotic therapy has to be considered. It is, however, recommended that the specimens should be routinely cultured for organisms so that appropriate antimicrobial therapy can be initiated. Our findings also suggest that if an empiric antimicrobial therapy is used to treat CRS, it should have activity against *S.aureus*.

This study has several important limitations that require discussion. The small sample size and patient population at a regional referral center may not be representative of community at large. Identification techniques like silicofluoride dye for direct fungi search and the PCR technique increase the sensitivity for fungi detection [13,14]. In our study we have not had access to such identification methods, which might explain why we found such a low positiveness for fungi. We attempted to follow a consistent and reproducible technique for harvesting specimens during FESS. We agree that it is impossible to design a bacteriologically pure study on sinusitis; this makes all studies subject to criticism, including our own.

SUMMARY AND CONCLUSION

In this cross-sectional study, we studied the microbiology of Chronic Rhinosinusitis. We found *SNC* to be the most frequent isolate, followed by *S.aureus*. Results also indicated that, in this select group of 50 patients, anaerobes do not play a prominent role in causing the disease. Our study supports the hypothesis that bacterial etiology is predominantly, but not the only cause of CRS. Fungal and allergic etiology was also found in few patients in the study. Hence, the pathogenesis of CRS is likely multifactorial with allergy, mucosal inflammation, the host's local immune system, viral infection, and fungus-based eosinophilic inflammation, all playing a role. Medical management becomes more effective if appropriate antibiotics are given in the right dose and for the right duration in these cases.

Abbreviations

SNC – Coagulase negative staphylococci
S. Aureus – Staphylococcus aureus
CRS – Chronic rhinosinusitis
OMC – Osteo meatal complex
FESS – Functional Endoscopic Sinus Surgery
GA – General Anaesthesia
PNS – Para Nasal Sinuses

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