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Research Article

MICROBIOLOGICAL PROFILE OF PATIENTS WITH CHRONIC SINUSITIS IN KASHMIR VALLEY

***Irfan ul shamas[1], Farooq Itoo[2] and Fayaz Wagay[2]**
[1]Department of Otolaryngology, Government Medical College Srinagar
[2]JK Health Department
**Author for Correspondence*

ABSTRACT

In contrast with the well established roles of microbes in the etiology of acute sinusitis, the exact roles of all of these microbes in the etiology of chronic sinusitis are uncertain. The objective of the study is to analyze micro-flora present in patients with chronic sinusitis in Kashmir valley. A cross sectional study was done to analyze the microorganisms of paranasal sinuses in patients having chronic sinusitis undergoing a functional endoscopic sinus surgery. Biopsy/Swabs were taken from the infected sinus of the patients during surgery and were sent for microbiological analysis within 4 hours of collection. *Staphylococcus aureus* was the most common isolate accounting for 43% of the patients followed by *Klebsiella* species in 9% and MRSA in 3%. Fungal organisms identified were *Aspergillus* and *Candida* spp. isolated from 9% of the patients. No anaerobes were isolated. The possibility of a fungal infection should always be considered in the differential diagnosis of difficult to treat diseases of the paranasal sinuses even in non endemic regions. Based on results we can vary the choice of antibiotics in chronic and acute rhino-sinusitis leading to a better management of the condition.

Keywords: *Chronic Sinusitis, FESS Surgery, Biopsy, Staphylococcus Aureus*

INTRODUCTION

Chronic sinusitis is an infection of sinuses lasting for more than three months. Despite of its prevalence the disease continues with poorly understood origin, pathogenesis and natural history. The etiology of chronic sinusitis continues to be the focus of much debate and research in the field of rhinology (Aral *et al.,* 2003).

With initial use of antibiotics and agents that decrease mucosal edema now surgical methods are employed in patients in whom medical treatment fails. Although diagnostic criteria for acute sinusitis are well established yet the definition of chronic sinusitis is controversial with respect to the importance of bacteria in the initiation and progression of disease. Chronic sinusitis has been considered to be chronic inflammatory condition rather than microbial infection. The role of bacteria in the pathogenesis of chronic sinusitis is currently being reassessed (Niederfuhr *et al.,* 2009).

The use of endoscopies has made it possible to determine the microbiology of each sinus with a lower probability of contamination (Busaba *et al.,* 2004). Persistence of infection causes mucosal changes such as loss of cilia, edema and polyp formation.

We feel that lack of progress is largely due to paucity of knowledge in microbiology and histopathology of chronic sinus disease available to us. This was the impetus of our study to evaluate the microbiology of chronic sinusitis in patient undergoing functional endoscopic sinus surgery.

MATERIALS AND METHODS

This is a cross-sectional study in the department of otorhinolaryngology at the Government Medical College Srinagar. The sample size is of 100 patients of all age groups and both males and females were considered in the study done from November 2012 to 2013.

Inclusion Criteria

(a) Patients with chronic inflammatory disease of sinuses undergoing functional endoscopic sinus surgery,

(b) Allergic rhinitis patients with chronic sinusitis not responding to medical treatment,

(c) Patients with chronic sinusitis with no response to medical treatment,

(d) Patients with recurrent sinusitis (>4 episodes/year) and chronic sinusitis not responding to medical treatment with complete opacification or mucosal thickening of >5 mm in one or two maxillary or ethmoidal sinuses in CT,

(e) Patients not on antibiotics at least one week before the surgery were included in the study.

Exclusion Criteria

The patients with acute sinusitis, malignancy of paranasal sinuses and patients on recent antibiotics were excluded from study.

During surgery, nasal cavity was disinfected with providing solution/chlorhexidine solution, swabs/biopsies were taken from the infected sinuses. Two biopsies/swabs were taken, one for aerobic and fungus, and another for anaerobic microorganisms. All biopsies/swabs were collected in a sterile container then inoculated into the culture media within 1–4 hours of collection.

For aerobic culture, biopsies/swabs were inoculated in Mac Conkey agar and Chocolate agar (Doyle and Woodham, 1991). The specimens were incubated at 35° C in a 5% carbon dioxide environment. The plates were evaluated daily for at least two days for any microbial growth. For anaerobic culture, biopsies/swabs were transported via thioglycolate broth and later inoculated in Schaedler agar (Busaba *et al.,* 2004). These were incubated anerobically at 35° C ()[11], and evaluated for any microbial growth daily for at least five days. Fungal analysis was done by KOH mount and culture on Sabouraud Chloroamphenicol agar (Paju *et al.,* 2003; Tang *et al.,* 2003). Data analysis was done by SPSS ver.11.5 and study was evaluated using the Chi-Square test.

RESULTS AND DISCUSSION

Table 1: Symptoms of the patients

Sypmtoms	No of patients	Percentage
Nasal discharge	54	54
Nasal obstruction	76	76
Headache	78	78
Disturbance of smell	21	21
Epistaxis	6	6
Sneezing	34	34
Sore throat	1	1
Hawking	1	1

Table 2: Result of microbiological analysis (Total 121 organisms)

Result	No. of positives	Percentage
Aerobic organisms (Both seen on smear and isolated)	62	51.24
Aerobic organism (Seen on smear but not isolated)	50	41.32
Anaerobic organisms	0	0
Fungus	9	7.4

Table 3: Aerobic organisms with their frequencies

Organisms	Frequency
Staphylococcus aureus	43
Klebsiella spp.	9
MRSA	3
Streptococcus	
Group A	1
Group B	1
Enterobacter spp.	1
Acinobacter spp.	1
Pseudomonas spp.	1
Botromycosis spp.	1
Citobacter spp.	1
Gram positive bacilli	11
Gram negative bacilli	23
Gram positive cocci	13
Gram neg coccobacilli	3
Total	112

During the period of study, 100 patients with chronic sinusitis entered into the study. The average age of the patients was 41 years (range 10–70 yrs). There were 60 males and 40 females in our study. The average duration of symptom was 20 months, ranged from less than 10 months to more than 30 months.

The most common symptom was headache in 78%. Other symptoms noted was nasal obstruction in 76%, nasal discharge in 54%, recurrent sneezing in 34%, disturbance of smell in 21%, and epistaxis in 6% (Table 1). History of allergy was present in 66% of patients who underwent surgery. On examination, 96% of patients had deviated nasal septum and 44% had congested nasal mucosa. On CT scan almost all patients had mucosal thickening with the DNS, and both CT scan and intra operative findings were similar.

On microbiological analysis of the biopsy/swab from infected sinuses, 51.24% were aerobic (both seen on smear and isolated); 41.32% were aerobic organism (seen only on smear not isolated) and 7.4% were fungi (Table 2). Of all the pathogens, in the aerobic group, Staphylococcus aureus was the most common organism isolated, seen in 48 out of 100 specimens (48%). The other organisms were Klebsiella spp. which was isolated in 9 out of 100 (9%) specimens and the rest being MRSA, 3 of 100 (3%) specimens. Streptococci which are again group A and group B hemolytic were isolated in one each of 100 specimens. Citrobacter spp., Botromycosis spp., Acinobacter spp., Enterobacter spp. and Pseudomonas spp. were seen in one each of 100 specimens. These organisms were seen on smear and isolated in the specimen (Table 3).

Others organisms which were seen on smear but not isolated were Gram negative bacilli, Gram negative coccobacilli, Gram positive bacilli and Gram positive cocci, all contributing for about 50 out of 100 specimens. These organisms were seen on smear along with other organisms but could not be isolated. No anaerobic organisms were isolated in our study.

The growth and isolation of fungus was 9 out of 100 specimens (9%). The most common fungus was Aspergillus Niger which was 4 out of 100 specimens (4%).Others being Candida albicans 3 of 100 (3%) and finally Candida krusei 2 of 100 (2%) (Table 4).

Table 4: Types of fungus and their frequencies

Type of Fungus	Frequency	Percentage
Aspergillus	4	44.4
Candida Albicans	3	30
Candida krusei	2	22.2
Total	9	100

Discussion

Bacterial etiology, pathophysiology and management of chronic rhinosinusitis have been one of the most controversial topics in otorhinolaryngology. As stated, the literature is sparse and difficult to interpret (Aral *et al.,* 2003). Many earlier studies dealing with the bacterial etiology of sinusitis have been compromised by technical problems, beginning from specimen collection to final step of identification of organisms, especially anaerobic bacteria. Taking into consideration problems faced by older studies, in our study we took biopsies directly from the infected sinuses rather than swabs from meatus or sinuses and also cleaning was done with poviodone (Busaba *et al.,* 2004) and chlorhexidine solutions reducing contamination of normal flora. Further the specimens were collected in sterile bottles with sterile saline and transporting was done within 4 hours to the lab.

To study the role of anaerobic bacteria in infectious process, prompt transport and processing of specimen are essential. The uses of selective media along with the non selective ones coupled with prolonged incubation period are prerequisites for success in isolation of anaerobic organisms ().[11] Results showing a difference from other studies are given in Table 5.

Table 5: Comparative analysis of microbial flora obtained in (Doyle and Woodham, 1991; Busaba *et al.*, 2004) and present study

Pathogens	Doyle and Woodham (1991) out of 94(%)	Busaba *et al.*, (2004) out of 179(%)	Present study out of 100(%)
1. Staphylococcus aureus	31(33)	33(18.4)	43(43.0)
2. MRSA	0(0.0)	0(0.0)	3(3.0)
3. Streptococci	5(5.3)	2(1.1)	2(2.0)
4. Pseudomonas	1(1.1)	9(5.0)	1(1.0)
5. Enterobacter spp.	3(3.2)	6(3.4)	1(1.0)
6. Klebsiella	2(2.1)	4(2.2)	9(9.0)
7. Actinomycetes	0(0.0)	0(0.0)	1(1.0)
8.Haemophilus influenzae	4(4.3)	8(4.5)	0(0.0)
9.Corynebacterium diphtheriae	2(2.1)	0(0.0)	0(0.0)
10.Citrobacter spp.	0(0.0)	0(0.0)	1(1.0)
11.Botromycosis	0(0.0)	0(0.0)	1(1.0)
12.Chlamydia rachomatis	0(0.0)	0(0.0)	0(0.0)
13. Fungus	1(1.1)	0(0.0)	9(9.0)
14. Anaerobes	0(0.0)	11(6.1)	0(0.0)

In most of studies investigating the microbiology of chronic sinustis, *S. aureus* was present in 15–40% of sinuses, in our study S. *aureus* was present in 43% (43 of 100). Our study showed Klebsiella spp. isolated in 9% of the patients was again significant as compared to the other study which showed only 2–4% of the patients. To our surprise MRSA was found in 3% of cases as compared to none in others. Our study also showed some organisms which were not isolated in any other study like Citobacter spp. which was in 1% of the patients, Actinomycetes spp. which was again in 1% of the patients and finally Botromycosis spp. which was one of the rare organisms found in the isolate, 1% of the patient. Our study also showed organisms which were seen on smear but not isolated which included Gram negative coccobacilli(3), Gram positive cocci(13), Gram positive bacilli(11) and Gram negative bacilli(23). This organism was either seen as an isolated organism in the specimen or in combination with other organisms like S. aureus, Klebsiella or fungus. The organism was only seen on staining with Gram stain but could not be isolated as they were few in number. The reason can be, that these patients had past infections which were getting treated by prior use of medications. Other reason may be technical factors, improper processing or non-infective/allergic pathology.

Bacterial pathogens in chronic rhinosinusitis are distinct from those found in acute rhinosinusitis, as is evident from our data. *Streptococcus pneumoniae* and *Haemophilus influenzae* are the predominant

pathogens in acute rhinosinusitis. However both organisms were not found in our study. This difference can impact the choice of antibiotics (Kingdom *et al.*, 2004).

Further comparison shows there is an absence of anaerobic organisms in contrast to other studies which may be because of technical factors which could have decreased the number of fastidious anaerobe isolated in our study. We noted that most anaerobes isolated by Brook (2005), and Fredrick were not particularly fastidious. If the percentage of no growth is used to assess the sensitivity of our technique to detect organisms in significant numbers, then our results with the lowest percentage of no growth specimens suggest that factors other than technical ones are likely to have contributed to the absence of anaerobes.

We believe that one important factor for the absence of anaerobes in our study was the conservative pre surgical medical treatment which was used. Such a treatment may increase the drainage of the purulent material and may sufficiently oxygenate the sinuses to eliminate the anaerobes. We found that fewer patients had pus on endoscopic examination of the middle meatus in the operating room than during the original endoscopic examination in the OPD.

We also studied the incidence of fungus in our patients of chronic sinusitis. And we found that 9% of the patients who had chronic sinusitis and who underwent surgery had fungal growth in the specimens.

The specimen collected was both sent for KoH mount and culture. The results were obtained both after 1 week of sending the specimen and also 2 weeks after receiving the first result. By following this protocol, it eliminates the chances of contamination of the specimen which is very common during the collection, transport, growth and culture of the specimen.

The final result was taken into consideration during the end of 3 weeks. That is if the results of fungal growth were positive during the end of 3 weeks that means the fungal presence is confirmed. Direct microscopic visualization of the fungus in the sinus biopsy samples suggests that fungus was indeed growing in the sinuses rather than existing as a dormant conidium. The Gram stain was useful in identifying the fungal elements even though the round to oval non budding cells was initially misinterpreted as yeast cells.

In our study we found 9% of the specimens/patients had a positive fungal growth, which is significant ($p < 0.005$) compared to the other studies, which showed 1–2% of the fungal growth. The most common fungus isolated was Aspergillus Niger 4 out of 9 specimens followed by candida albicans and candida krusei, 2 each of 9 specimens.

None of the above organisms belonged to invasive fungal sinusitis group and we also confirmed this fact by the CT scan finding, which was excluded by negative bone invading property. But this patient scan showed the other features of non invasive fungal sinusitis. Also intra operative this patient showed growth, which can easily be seen on the middle turbinate and middle meatus. Biopsy from some of the specimens showed typical characteristic of fungal sinusitis.

The increase in incidence can be explained by the fact that all the patients belonged from Southern India, near coastal area, that is a temperate zone with high humidity, where chances of fungus infection is very high. None of our patients, who were diagnosed to have fungal sinusitis had immuno compromised states like Diabetes, or HIV; neither any history of previous fungal infection. But all patients had history of allergy to dust or pollen, which may be again significant as allergic fungal sinusitis.

The study has several important limitations that require discussion. Patient population at a regional referral centre may not be a representative of the community at large. We attempted to follow a consistent and reproducible technique for harvesting specimens. However, in 50 of 121 organisms, it could not be isolated. This could be due to technical factors, improper processing, non – infective/allergic pathology or patients on past infections, now getting treated.

Conclusion

We found that *S. aureus*, MRSA, and *Klebsiella* were the most frequent organisms encountered and anaerobes do not play a prominent role. Our finding suggests that if empiric antimicrobial therapy is used to treat chronic sinusitis, it should have activity against *S. aureus* and *Klebsiella*.

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