INTRODUCTION

Nasal polyps are benign lesions commonly affecting up to 4% of the population [1]. Some theories considered polyps a consequence of conditions which cause chronic inflammation in the nose and nasal sinuses characterised by stromal oedema and cellular infiltration [2]. While many aspects of nasal polyps had been documented, the initiating cause remained unknown and might be different for different cases.

Bernstein et al 2005 [3] showed the influence of inflammation of nasal mucosa on the growth of nasal polyps. Bacterial and viral infections produced factors which damaged ciliated cells, causing dyskinesis and destruction of respiratory epithelium. Disturbed muco-ciliary clearance permitted bacteria to penetrate through mucosa facilitating their adhesion and colonisation leading to inflammation [4].

The microbiology of chronic maxillary sinusitis was found to be polymicrobial [7-11], but there was dearth of data on bacterial pathogenesis of nasal polyps involving the Indian population. The present study was aimed to identify the resident (control setup) and pathological (patients diagnosed with nasal polyps) bacterial flora; and establish empirical antibiotic policy to treat nasal polyp. Methods: This prospective study involving 105 patients with nasal polyp as “cases” and 105 healthy subjects as “controls” was conducted at Medical College, Kolkata to assess the involvement of bacteria in polyp formation. Post polypectomy core swabs were processed for isolation of aerobic and anaerobic bacteria and antibiotic sensitivity performed thereafter. Results: Polyp tissue core swabs from nasal polyp cases revealed 94.3% culture-positive specimens (p<0.05) with 99 aerobic and 129 anaerobic isolates. The predominant aerobes were Staphylococcus aureus (45.4%), Klebsiella (15.2%), Pseudomonas (15.2%), Coagulase-negative Staphylococcus (15.2%), and Moraxella (6.1%). Peptostreptococcus (16.35), Porphyromonas (16.3%), Eubacterium (13.9%), Fusobacterium (13.9%), B.fragilis (9.3%) and Peptococcus (9.3%) frequented the anaerobes. The normal nasal flora comprised CONS (56.4%) followed by S. aureus (35.0%) and Corynebacterium (8.6%). The anaerobes in the nasal mucosa included Peptostreptococcus (44%), Fusobacterium (20%), Peptococcus (16%) and Porphyromonas (12%) among others. Levofloxacin showed an impressive overall 94.9% activity with 37 out of 39 gram negative and 57 out of 60 gram positive isolates being sensitive to it. Conclusion: This study illustrated the presence of polymicrobial aerobic-anaerobic bacteria in patients with nasal polyps and considered bacterial infection as an etiological factor in the pathogenesis of nasal polyp.
weeks before surgery. 105 age-sex matched non atopic, healthy individuals without nasal polyp or any evidence of upper respiratory tract infection were taken as 'control'. Nasal polyps of fungal origin and neoplastic growth were excluded from the study.

Immediately after polyp excision, the polypoidal mass was dipped in povidone-iodine for 30 secs and then rinsed with sterile normal saline solution. It was then sectioned into two pieces under strict aseptic conditions. Core swabs of the polyp were taken with the help of a Sterile Nylon Flocked swab. For “control”, similar swabs were taken from the mucosa of middle meatal area of the nasal cavity.

The swabs were kept in a sterile test tube plugged with cotton wool for aerobic and in thioglycolate broth containing haemin and Vit K1 for anaerobic culture and transported to the microbiology laboratory without delay. The swabs were then inoculated onto MacConkey’s and 5% sheep blood agar plates for aerobic culture. The plates were incubated at 37°C and examined after 24, 48 and 72hrs. Samples were considered negative for bacterial colonisation if no growth was detected after 72 hrs of incubation. For growth of aerobes, the swabs were plated onto pre-reduced vitamin K1-enriched anaerobic blood agar plate and enriched thioglycolate broth [5]. The blood agar plates were incubated in anaerobic jars for 48 and 96hrs. Individual bacterial strains were identified and antibiotic sensitivity was performed by Kirby Bauer disc diffusion method. The study was carried out after clearance from the Institutional Ethics Committee.

RESULTS

In patients with nasal polyp, bacterial growth was present in 99 (94.3%) specimens. Aerobes were present in 84 (84.8%) and anaerobes were isolated from all 99 (100%) of the culture-positive specimens. Mixed aerobes and anaerobes were encountered in 84(84.8%) of the culture-positive specimens; while anaerobes alone were found in 15 out of 99 specimens (15.2%). 228 (99 aerobic and 129 anaerobic) individual bacterial isolates recovered. The predominant aerobes were S.aureus (45.4%), P.aeruginosa (15.2%), K.pneumoniae (15.2%), Coagulase-negative staphylococcus (CONS) (15.2%), and M.catarrhalis (6.1%). Anaerobes were predominated by Peptostreptococcus (16.3%), Porphyromonas (16.3%), Eubacterium (13.9%) and Fusobacterium (13.9%)(Table 1).

The nasal flora of healthy 'controls' was found to comprise of mixed aerobic and anaerobic bacteria. Aerobic bacterial presence was detected in the nasal cavities of all 105(100%) control specimens, while anaerobic growth accounted for 69 (65.7%) controls specimens. Out of 105 aerobic isolates, the nasal flora primarily included CONS (56.4%), S.aureus (35.0%) and Corynebacterium (8.6%). The 75 anaerobes isolated were predominated by Peptostreptococcus (44%), Fusobacterium (20%), Peptococcus (16%) and Porphyromonas (12%) (Table 2).

All aerobic and anaerobic components of the normal nasal flora were frequently recovered from core polyp swabs (with the exception of Corynebacterium). However, gram-negative aerobes were exclusively isolated from cases. There was no significant difference in anaerobes isolated in case and control groups apart from the isolation frequency being higher in cases (Eubacterium and B.fragilis were found only in cases).

Antibiotic sensitivity- K.pneumoniae showed decreased susceptibility (53.3%) towards third generation cephalosporin (Cefotaxime) and 100% susceptibility to Ceferoperazone-sulbactam and amino-glycosides (100%) but resistant to Amoxicillin-clavulanic acid. All strains of Paeruginosa were susceptible to Ceftazidime and Amikacin. Levofloxacain showed an impressive overall 94.9% efficacy with 37 out of 39 gram positive and 57 out of 60 gram positive isolates being sensitive to it. 66.7% of S.aureus and 60% of CONS were found to be resistant to cefoxitin (considered as methicillin resistant strains). Erythromycin was found short of complete gram positive coverage with 27 out of tested 60 gram positive isolates being resistant. Linezolid and vancomycin were effective against all gram positive isolates tested.

Table 1-Aerobic and anaerobic bacteria isolated from cases

<table>
<thead>
<tr>
<th>AEROBIC ISOLATES</th>
<th>NUMBER OF ISOLATES</th>
<th>FREQUENCY OF ISOLATION (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-positive organisms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus (Methicillin Resistant)</td>
<td>129</td>
<td>100</td>
</tr>
<tr>
<td>Staphylococcus aureus (Methicillin Sensitive)</td>
<td>129</td>
<td>100</td>
</tr>
<tr>
<td>Coagulase-negative staphylococcus</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td><strong>Gram-negative organisms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paeruginosa</td>
<td>15</td>
<td>15.1</td>
</tr>
<tr>
<td>M.catarrhalis</td>
<td>15</td>
<td>15.1</td>
</tr>
<tr>
<td>K.pneumoniae</td>
<td>15</td>
<td>15.1</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>3</td>
<td>3.0</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>100</td>
</tr>
</tbody>
</table>

*Out of 105 cases included in the study, 21 samples yielded no aerobic and 6 samples no anaerobic growth.
AEROBIC ISOLATES
Staphylococcus aureus (Methicillin Resistant) 9 8.6
(Methicillin Sensitive) 27 25.7
Coagulase-negative Staphylococcus (Methicillin Resistant) 12 11.4
(Methicillin Sensitive) 48 45.7
Corynebacterium spp. 9 8.6
Total 105 100
Peptostreptococcus 33 44
Peptococcus 12 16
Porphyromonas 9 12
Prevotella 3 4
Fusobacterium 15 20
Veillonella 3 4
Total 75 100

AEROBIC ISOLATES
Peptostreptococcus 33 44
Peptococcus 12 16
Porphyromonas 9 12
Prevotella 3 4
Fusobacterium 15 20
Veillonella 3 4
Total 75 100

*Out of 105 controls, all samples yielded aerobic growth; no anaerobic growth was detected in 36 control samples.

Table 3: Antibiotic sensitivity pattern of isolated bacteria

**Gram positive isolates**

<table>
<thead>
<tr>
<th>Antibiotic sensitivity (%)</th>
<th>AMC</th>
<th>PIT</th>
<th>CTX</th>
<th>CFS</th>
<th>CIP</th>
<th>LE</th>
<th>AMK</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>66.7</td>
<td>62.2</td>
<td>48.9</td>
<td>84.4</td>
<td>91.1</td>
<td>95.6</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>CONS</td>
<td>60</td>
<td>33.3</td>
<td>80</td>
<td>73.3</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

clear impact on infections in other anatomical locations and perhaps also in the sinuses. Staphylococcus aureus was considered the most potentially pathogenic bacteria in the nasal cavity. The isolated bacteria from control specimens had been consistent with the findings of previous studies [14, 15], thereby reinforcing the fact that the nasal mucosa was not sterile.

Most of the data regarding antibiotic therapy were based on the efficacy of macrolides, related to their anti-inflammatory properties rather than their antimicrobial characteristics [18]. Ichimura et al [19] noted that oxitromycin, 150 mg/day for 8 weeks, resulted in a diminution of polyps in 52% of patients with smaller polyps. When cetirizine was added, the success rate increased to 68%, although the difference was not statistically significant. This study revealed that Levofloxacin with its extensive gram positive sensitivity and appreciable gram negative coverage seemed the best suited antibiotic. Kim et al [20] recommended amoxicillin-clavulanate, cephalosporins and macrolides rather than penicillin as the first-line drug in chronic sinusitis with nasal polyps. Although antibiotic regimen was prescribed in CRS, they were found ineffective in the medical management of uncomplicated nasal polyp [21]. Ideally, medical therapy should include both, a broad-spectrum antibiotic with anaerobic coverage and a topical intra-nasal steroid to address the strong inflammatory component of this disease [22].

Bacteria isolated from normal nasal mucosa had also been concurrently encountered in cases of nasal polyps. But, the bacterial load and its rate of isolation were significantly higher in cases. Gram-negative rods were independently encountered in 37.14% (39 out of 105) cases of nasal polyps. Bacterial infection might be considered as an etiological factor in the pathogenesis of nasal polyp. These findings had been concordant with those of sinus aspirates in patients of CRS. Core swabs from polyp tissue were preferred to the common practice of using nasopharyngeal swabs in patients with CRS. Nasal cavity in healthy young men. Rhinology. 1986; 24:249-255.

CONCLUSION

This study illustrated the presence of polymicrobial aerobic-anaerobic bacteria in patients with nasal polyps with no variation in the bacteria isolated from sinus aspirates in patients with CRS. Bacteria as an etiological factor of nasal polyp were a plausible conclusion. The use of antibiotics with anaerobic coverage is recommended. Studies are warranted to elucidate the efficacy of the sensitive antibiotic to radically alter the course of disease progression in nasal polyp at an early stage.

REFERENCES