Original Research Article

Evidence of bacterial biofilms in chronic rhinosinusitis: factors affecting and the impact on prognosis

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ABSTRACT

Background: The aim of the study was to study the evidence of bacterial biofilms in chronic rhinosinusitis (CRS) and its effect on disease prognosis by correlating between symptom score, computed tomography (CT) score and endoscopy score.

Methods: 40 samples of bacteriologically positive CRS patients were studied and data analyzed using SNOT-20 questionnaire, nasal endoscopy scores and CT grading. Functional endoscopic sinus surgery was done in all CRS patients to clear blockage and ensures patency of osteomeatal complex. Postoperative evaluation continued at 2 weeks, 1 month and 3 months. A comparison made between preoperative and postoperative SNOT-20 and endoscopy scores of patients with and without biofilms.

Results: Patients with biofilm forming bacteria have significantly poor preoperative and postoperative SNOT-20 score and endoscopy score as compared to bio-film negative patients; however the quality of life improved in both the groups.

Conclusions: Biofilms play a potential role in postoperative prognosis of patients undergoing FESS. Further studies and research is required for assigning them as a definite proprietor for CRS.

Keywords: Bacterial biofilms, Chronic rhinosinusitis, SNOT-20

INTRODUCTION

Rhinosinusitis is defined as an inflammation of the mucosa of the nose and at least 12 weeks of inflammation is required for a diagnosis of CRS (chronic rhinosinusitis).1 CRS involves the disruption of the mucosa from allergens, infection, and immune system derangement. The mucosa of nasal airway and paranasal sinuses is accepted as a single unit and thus the term rhinosinusitis is commonly used.2

The common symptoms of chronic rhinosinusitis are nasal obstruction, headache, rhinorrhea and anosmia.

Chronic rhinosinusitis with nasal polyps is characterized by eosinophilia and tissue remodelling consisting of epithelial proliferation, goblet cell hyperplasia, basement membrane thickening, fibrosis and edema.3

Biofilms are structured, specialised communities of adherent micro-organisms encased in complex extracellular polymeric substances (EPS). The formation of biofilms is not restricted to bacteria, as fungal pathogens also form biofilms.4

Bacteria existing in a biofilm are surrounded by glycocalyx and grow in a coordinated fashion once a certain density is reached by induction signaling...
presence or absence and extent of nasal polyp, edema, discharge, crusting and scarring are the parameters graded in endoscopy scores.

Functional endoscopic sinus surgery (FESS) is a set of minimally invasive surgical techniques which allow direct visual examination and opening of the sinuses. FESS is a well-established strategy, for chronic rhinosinusitis which has not responded to medical management. The purpose of FESS is to re-establish ventilation and mucociliary clearance of sinuses. The principle of this technique is limited resection of inflammatory or anatomical defects that interfere with normal mucociliary clearance and result in local persistent inflammation.

The main aims and objectives in our study was to study the effect of biofilms on the outcome of endoscopic sinus surgeries in patients of chronic rhinosinusitis, factors affecting it and its impact on the quality of life. We also tried to find out the clinicopathological correlation in endoscopy, CT findings and symptom scores in these patients.

**METHODS**

**Design and setting of the study**

The proposed study was conducted in the Department of ENT and Microbiology, GTB Hospital and UCMS, Delhi during the period of November 2012 to January 2014.

**Sample size**

Forty patients of bacterial culture positive chronic rhinosinusitis were taken with 24 males and 16 females.

Patients with CRS were evaluated with history and routine ENT examination followed by evaluation with procedures like SNOT-20 questionnaire, nasal endoscopy with biopsy and CT paranasal sinuses.

CRS patients with bronchial asthma, pregnancy, impaired mucociliary functions, immunocompromised status were excluded from the study.

Relevant data was analyzed using SNOT-20 questionnaire and severity of symptoms was graded according to visual analogue scale (VAS) (0-5) where 0 stands for no response and 5 for maximum response.

Lund and Mackay CT scoring was used where each sinus was graded based upon opacification and osteomeatal complex based on occlusion. Total score could range from 0-24.

According to Lanza and Kennedy, findings on endoscopy were graded on the presence or absence and extent of nasal polyps, edema, discharge, crusting and scarring of each side.

Functional endoscopic sinus surgery (FESS) was done in all CRS patients to clear blockage and ensure patency of the osteomeatal complex. Samples of nasal polyps and tissue mucosa were collected in a brain heart infusion broth and sent to microbiology department. Isolates were initially identified by standard microbial techniques including Gram staining and were cultured for isolation of blood agar. Isolated strains were subjected to antimicrobial susceptibility testing by Kirby Beaur method as per CLSI guidelines.

Detection of bacterial biofilms was then performed by microrite plate method as described by Christensen et al.

Post-operative evaluation was done with SNOT-20 after 2 weeks, 1 month and 3 months.

A comparison was made between the preoperative and postoperative SNOT and nasal endoscopy scores of patients with positive biofilms (Group A) and with no biofilms (Group B).

**RESULTS**

Forty bacteriologically positive patients of chronic rhinosinusitis were studied. All patients underwent endoscopic sinus surgery and their sinonasal mucosal samples were sent for biofilm detection. The scores were then compared between the patients with positive biofilms and with negative biofilms in bacterial culture positive cases. Bacterial culture negative cases were discarded.

The age at the time of presentation in our study ranged from 10-50 years with a mean age of 25.35 years with male to female ratio almost being 3:2.

Maxillary sinus was involved in all the 40 cases with most of the cases involving more than 1 sinus (Figure 1).
Figure 1: Sinus opacification.

Following anatomical variations were found amongst the patients: Significant deviated nasal septum (DNS) was present in 14 cases, i.e. 35% cases; paradoxical middle turbinate was seen in 2 cases, i.e. 5% cases; concha bullosa was present in 6 cases i.e. 15% cases; Onodi cell was seen in 4 cases, i.e. 10% cases; and Heller cells was seen in 3 cases, i.e. 7.5% cases.

Mean preoperative symptom score of all the patients undergoing FESS were calculated. Grading of findings of diagnostic endoscopy was done and scores were added as a part of preoperative clinical work-up.

During follow up patients’ SNOT-20 scores and Endoscopy scores were taken at 2 weeks, 1 month and 3 months postoperatively. Comparison was then made between the scores of the patients having positive biofilms and patients without biofilms out of the patients showing positive bacterial growth.

Impact on quality of life of the patients was also assessed by asking the patients the number of days taken by them to rejoin their normal duties. The average number of days taken by patients to rejoin their normal daily activities was found to be 5.125±1.15 with a range of 3-7 days.

Figure 2: Bacterial culture results.

A total of 33 patients out of the 40 cases i.e. 82.5% were found to have biofilms. Most of the cultures i.e. 20 out of 40 revealed growth of bacteria S. aureus out of which 14 were methicillin resistant. *Coagulase negative S. aureus* was seen in 7 cultures.

*Pseudomonas* was found to have growth in 5 and *Citrobacter*, *E. coli*, *Acinetobacter baumanii*, *Staph epidermidis* and *Klebsiella* were found in 1 culture specimen each (Figure 2).

Patients with biofilm (Group A) have significantly worse (p=0.01) preoperative and postoperative score at 3 months (26.86±1.42 and 6.43±1.50) as compared to patients with biofilm (Group B) (24.45±1.11 and 5.82±0.3) (Table 1).

Also nasal endoscopy scores in patients with biofilms (Group A) were more preoperatively and at 3 months (8.97±1.6 and 2.52±1.2) as compared to patients without biofilm (Group B) (8.14±1.67 and 0.74±0.37, p=0.01) (Table 2).

### Table 1: Improvement in SNOT-20.

|                | Preop     | 2 weeks  | 1 month | 3 months |
|----------------|-----------|----------|---------|----------|
| **Group A**    | 26.86±1.42| 7.14±1.57| 4.57±0.59| 6.43±1.50|
| % improvement  | 71.71%    | 90.88%   | 78.53%  |
| **Group B**    | 24.45±1.11| 5.7±0.69 | 4±1.2   | 5.82±0.3 |
| % improvement  | 74.89%    | 97.70%   | 80.22%  |

### Table 2: Nasal endoscopy results after functional endoscopic sinus surgery (FESS).

|                | Preop     | 2 weeks  | 1 month | 3 months |
|----------------|-----------|----------|---------|----------|
| **Group A**    | 8.97±1.6  | 6.0±1.92 | 3.73±1.87| 2.52±1.2 |
| % improvement  | 33.78%    | 59.20%   | 73.25%  |
| **Group B**    | 8.14±1.67 | 4.29±2.62| 1.71±2.05| 0.74±0.37|
| % improvement  | 50.04%    | 82.19%   | 98.70%  |
**Recurrence**

Patients who had recurrence usually presented back 3-5 months after surgery. A total of 8 patients (i.e. 20% cases) showed recurrence or persistence of disease, i.e. persistence of disease in terms of ongoing symptoms or presence of polyoidal or inflamed mucosa. Patients showing recurrence had individual total SNOT-20 score above 8. Most of these patients also showed increased scoring on diagnostic nasal endoscopy.

In Group A i.e. group with biofilms recurrence was only in 7 out of 33 patients (i.e. 21.21%) whereas in group B i.e. group without biofilms recurrence was seen in 1 out of 7 patients (i.e. 14.2%).

After 3 months of initial postoperative period follow up, these patients having recurrence were put on medical treatment (oral steroids/antibiotics based on the sensitivity tests), minimum for a period of two weeks or till symptom free.

**DISCUSSION**

Biofilms represent an important influence on the pathophysiology of CRS. Bacterial biofilms are generally resistant to antimicrobial therapy at levels attainable by oral or intravenous administration.

Various studies demonstrate bacterial biofilms mediating the failure of treatment in such cases, particularly after surgery where there is probable failure to clear all biofilms present within the sinus cavities.

However, traditional culture based diagnostic microbiology is, in many cases, inadequate and there are conflicting reports outlining specific pathogens associated with CRS.

Nasal obstruction was commonest symptom and the need to blow nose was present in 100% patients. Post-nasal discharge and running nose were other common symptoms similar to study by Singhal and Psaltis.12

CRS also has impact on social and emotional life of patients as can be seen from the observation that patients had reduced concentration and reduced productivity in their routine work. Also few of them were sad, frustrated and embarrassed due to this disease.

All the 40 patients returned for follow up at 2 weeks and SNOT-20 score was evaluated. Average SNOT-20 score in biofilm group at 2 weeks was 7.14±1.57 with a range of 2-14 and in non-biofilm group it was 5.7±2.69 with a range of 1-11.

This improved to 4.57±0.59 with a range of 0-13 at 1 month in the biofilm group and to 4±1.2 with a range of 1-9 in the non-biofilm group. But at 3 month follow up there is worsening of symptoms in both the groups with the mean score being 6.43±0.50 with a range of 1-25 in biofilm group and in the non-biofilm group the score being 5.82±0.3 with a range of 1-23 (Figure 3). This worsening of symptoms at the end of 3 months could be a reflection of subjective increase of symptoms by patients or recurrence of disease in some of the cases. Recurrence was seen in 7 out of 33 patients in the biofilm group i.e. 21% recurrence while in the non-biofilm group recurrence was seen in 1 out of 7 patients i.e. 14% recurrence.

**Figure 3: Improvement in SNOT-20.**

**Figure 4: Nasal endoscopy results after functional endoscopic sinus surgery (FESS).**

The biofilm group patients experienced 71.71% improvement in SNOT-20 symptoms at 2 weeks increasing to 90.88% at 1 month and falling back to 78.53% at the end of 3 months.

In the non-biofilm group patients experienced 74.89% improvement in SNOT-20 symptoms at 2 weeks which improved to 97.7% improvement at 1 month but decreased to 80.22% at the end of 3 months (Figure 3).

In our study biofilm formation was noted mainly by *S. aureus* (MRSA and MSSA) in 20 patients, in 5 patients by *P. aeruginosa* and 7 by coagulase negative *Staphylococcus* and rest each by different bacteria. Out of 8 patients in which recurrence have been seen 7 cases are attributed to *S. aureus* only. It also signifies that biofilm...
formulation and its recalcitrance is dependent on individual bacteria and its capability to sustain environmental changes highlighted by a study done by Bendouah et al.\textsuperscript{13}

In our study it was observed that in both biofilm and non-biofilm group there was increase in percentage improvement of endoscopy score from 1 month to 3 month.

Percentage improvement of endoscopic score in Biofilm negative group (50.04%, 82.19%, 98.7%) was much more than the percentage improvement in Biofilm positive patients at 2 week, 1 month and 3 months (33.78%, 59.20% and 73.29%) (Figure 4).

SNOT 20 and endoscopy scores have correlation at 1 month and 3 months. It shows relation between objective and subjective outcomes. Hence the primary outcome in treatment of sinusitis should be based on both subjective as well as objective improvement.

In present study no patient underwent revision surgery for recurrences. These patients were put on medical treatment (oral steroids/antibiotics based on sensitivity tests) for a period of two weeks or more if needed which lead to improvement in patients.

Synechiae were present in three patients postoperatively which were corrected subsequently. Two patients developed periorbital edema that relieved spontaneously. Two patients had excessive postoperative bleed requiring nasal packing.

There was a significant correlation between biofilm formation and bacterial culture results, however, the difference of scores between biofilm positive and negative patients are not statistically significant among age, sex, disease severity, headache, decreased sense of smell, duration, running nose and nasal discharge with blood.

With the SNOT scores we evaluated subjective improvements of symptoms after surgery. However, SNOT scores are also influenced by patients cultural quality, awareness of disease, mental status, expectation of disease improvement and other affecting factors. Therefore objective assessment of endoscopy scores must be emphasized.

It is concluded that it is the presence of biofilms that is more important in pathogenesis of CRS than the mere positivity of bacterial culture. Bacterial biofilms play a potential role in postoperative prognosis of patients undergoing FESS. Further studies and research should assign them as a definite proprietor for CRS.

Bacterial biofilms represent one of many possible etiologies for the occurrence, persistence and recurrence of disease in chronic rhinosinusitis. Both the synergistic effect of multiple organisms and the efficacious treatment of biofilms are areas of extensive investigation which will lead to improvement in the case of chronic rhinosinusitis patients.

Further understanding of biofilm interactions and microbial organism behaviour will provide us with future treatment modalities for this disease.

**CONCLUSION**

It can be concluded that biofilms have a significant impact on patients of chronic rhinosinusitis and is associated with the bacterial culture. Patients with biofilms have worse objective findings and more recurrence as compared to patients without biofilms and patients without biofilms show more objective improvement after surgical intervention. Factors studied in our study like age, sex, duration, headache, decreased sense of smell, running nose and nasal discharge with blood have no effect on biofilm formation. Biofilms represented an important impact on prognosis of CRS patients.

Further understanding of biofilm interaction and microbial organism behaviour will provide us with future treatment modalities for this disease. Antibiotic therapy against bacteria is usually associated with relapse following cessation of treatment and may also have an adverse effect on normal commensal microflora. The impact of surgery on biofilms is beneficial. At present surgery combined with postoperative treatment (appropriate antibiotics and if required, steroids) is the most effective modality of controlling the majority of CRS infections.

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**Ethical approval:** The study was approved by the Institutional Ethics Committee

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