Original Research Article

Clinical and histopathological impact of biofilm in chronic rhinosinusitis with nasal polyps

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ABSTRACT

Background: Presence of biofilms in sinus mucosa of patients with chronic rhinosinusitis (CRS) remains controversial. Literature shows that biofilms may contribute to the recalcitrant nature of CRS and unfavourable outcome following surgery. This study was performed to evaluate the prevalence of biofilm and its clinical and histopathological impact in patients with chronic rhinosinusitis with nasal polyps (CRS\wNP)

Methods: 41 patients of CRS\wNP (study group) were included. SNOT-20 (sinonasal outcome test-20) score, radiological and endoscopic findings of these patients were evaluated preoperatively. Sinonasal polypoidal tissues removed during surgery were studied for the presence of biofilm and evaluated histopathologically. Postoperatively SNOT-20 score and endoscopic finding were recorded. 41 patients undergoing septoplasty for deviated nasal septum (control group) were also included in the study. Sinonasal mucosal samples of these patients were analysed for the presence of biofilm.

Results: 29 out of 41 (70.73%) samples in study group and 9 out of 41 (21.9%) samples in control group were positive for biofilm. We found a significant impact in preoperative SNOT-20 symptom scores in biofilm positive study group. But there is no significant impact in preoperative endoscopic scores, radiological scores and postoperative SNOT-20 scores and endoscopic scores in study group patients irrespective of biofilm status.

Conclusions: Prevalence of biofilm in patients with CRS\wNP was higher than normal population. Biofilms plays a major role in preoperative symptomatology. But biofilms have no endoscopic, radiological, and histopathological impact in CRS\wNP. It was concluded that apart from biofilms, host and other environmental factors plays a major role in the pathogenesis of CRS\wNP.

Keywords: Biofilm, Nasal polyps, Recalcitrant sinusitis, Rhinosinusitis

INTRODUCTION

Chronic rhinosinusitis (CRS), one of the most common diseases encountered by otorhinolaryngologists, endangers patients and places substantial socioeconomic burden with its high prevalence and chronic recalcitrant course.1 Patients with CRS report a deteriorating sense of general health and vitality when compared to general population. CRS represents a spectrum of inflammatory and infectious processes concurrently affecting the nose and paranasal sinuses and is characterized by a minimum of two symptoms. These include nasal congestion or nasal discharge, facial pain and a reduction in the sense of smell. The duration of the disease tends to exceed 12 weeks.2

CRS represents a heterogeneous group of diseases resulting from the multifaceted interaction between the host and the environment.3 Despite the fact that bacteria and fungi have been linked to the development of CRS, the nature of their interaction with the host remains largely unknown. It is not clear whether bacteria cause
infection, expose the host to superantigens causing an inflammatory response or are able to colonize due to pre-existing pathology of the sinus mucosa.\textsuperscript{4}

It is now believed that 99% of all bacteria exist in biofilms and only 1% live in a free-floating or planktonic state.\textsuperscript{5} The discovery of bacteria existing in biofilm form has led many researchers to revisit the pathogenesis of sinus diseases. Infection in the form of biofilm may have an important, if not central, role the recalcitrant inflammation for this increasingly common chronic disease. Now, CRS is thought to have an underlying biofilm etiology. In contrast to the planktonic infections, biofilms are highly capable of evoking sustained responses from host’s immune system.\textsuperscript{6,7}

Role of bacterial biofilm in otorhinolaryngologic infections was first evaluated by Post.\textsuperscript{3} Biofilms have been related to chronic tonsillitis, adenoiditis and device-related infections in voice prosthesis.\textsuperscript{9} These biofilms could explain why some patients improve while on antibiotics but relapse after completion of medications.\textsuperscript{10}

Chronic rhinosinusitis causes significant physical symptoms and negatively affects quality of life and can substantially impair daily function. The importance of outcome research in rhinosinusitis is clear considering that patient subjective description of symptoms and overall sense of wellbeing drive much of rhinosinusitis care. Therefore effective rhinosinusitis specific health quality of life instruments are needed. To help evaluate the effect of various treatments on patient status, Meltzer et al. have chosen to perform an independent evaluation of SNOT-20 (Sino-Nasal Outcome Test-20) which was developed by Piccirillo et al.\textsuperscript{11,12} The SNOT-20 asks the patient to rate the severity of their symptoms and social/emotional consequences of their rhinosinusitis. It is scored so that a higher SNOT-20 score indicates worse health related quality of life and functional status.

Computed tomography (CT) scan is mandatory in patients undergoing endoscopic sinus surgery. It gives information regarding regional bony anatomy and mucosa. It helps in diagnosis and provides the anatomic roadmap for the surgeon performing endoscopic sinus surgery. Lund and Mackay grading system is based on numeric score derived from CT scan. This grading system is used in quantification of inflammatory sinonasal disease before surgery.\textsuperscript{13}

Endoscopic grading of chronic rhinosinusitis was proposed by Lund and Kennedy. Parameters used for grading are presence or absence and extent of nasal polyp, nature of discharge, severity of edema, crusting and scarring in nasal cavity of patients with CRS.\textsuperscript{14}

Functional surgical treatment by endoscopic sinus surgery is presently most preferred treatment for CRS and is based on hypothesis that diseased sinonasal mucosa can get reverted if ventilation and drainage are improved, thus restoring mucociliary clearance.\textsuperscript{15} Functional endoscopic sinus surgery (FESS), like all minimally invasive surgery, is designed to combine an excellent outcome with minimal patient discomfort. The use of endoscope permits a better view of surgical field and hence lower rate of complications as compared to conventional surgery.

**METHODS**

The proposed study was conducted in the Department of Otorhinolaryngology and Head and Neck Surgery, Microbiology, and Pathology at University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi from November 2014 to April 2016.

Previous study revealed 70% biofilm positivity in chronic rhinosinusitis patients and 40% positivity in controls without chronic rhinosinusitis.\textsuperscript{22} Sample size of 41 in each group (41 cases of CRSwNP and 41 control patients) is sufficient to detect the difference(30%) between positivity rate with 80% power and 5% level of significance. Patients of chronic rhinosinusitis with nasal polyps were selected for this study using following criterion

**Inclusion criteria**

CRS with nasal polyp cases clinically diagnosed in adult age group (18-60 years), patients who are not under steroid or antibiotic medication in the week preceding surgery were included in the study.

**Exclusion criteria**

Cases with bronchial asthma, pregnant patients, immunocompromised patients were excluded from the study.

**Data collection**

**Before surgery**

Detailed history using Sinonasal Outcome Test (SNOT-20), CT scan findings were graded using Lund and Mackay CT scoring system, endoscopic findings were graded using Lund and Kennedy scoring system.

**Surgical procedure**

Functional endoscopic sinus surgery was carried out under general anaesthesia in patients of chronic rhinosinusitis with nasal polyp

**Lab procedure**

Samples of polyps and tissue mucosa were collected in a nutrient broth and sent to microbiology department for culture and sensitivity, and detection of biofilm producing capacity as per Christensen et al.\textsuperscript{16} Samples of polyps and tissue mucosa were sent to Pathology
Department for histopathological evaluation and cell differentiation.

Post-operative evaluation

SNOT-20 scores were recorded after 2 weeks, 1 month and 3 months following surgery. Postoperative diagnostic endoscopy was done after 2 weeks, 1 month and 3 months following surgery.

Control group

Patients undergoing nasal surgery without any infective etiology were selected for the study. Sinonasal tissue sample was taken in patients undergoing nasal surgery without infective etiology and analysed for the presence of bacterial biofilms by the same method as above.

Statistical analysis

Pearson’s Chi-Square test was used to compare the biofilm positivity rate between cases and controls. Mann-Whitney U test was used to compare the SNOT-20, endoscopic score and CT score in CRSwNP patients between those who were positive for biofilm and those who were negative for biofilm. Histopathological variables were also compared using Fisher’s Exact test between biofilm positive CRSwNP and biofilm negative CRSwNP.

RESULTS

Biofilm producing isolates in study group

Bacterial culture was positive in 31 out of 41 samples. Most of the cultures i.e., 26 out of 29 revealed growth of bacteria *S. aureus* out of which 12 were methicillin resistant. *Pseudomonas spp* were found in 2 samples. *E. coli, Klebsiella spp, and Coagulase negative Staphylococcus* were found in 1 culture specimen. 29 out of 31 isolated strains of bacteria had the biofilm forming capacity. Hence a total of 29 patients out of 41 cases i.e., 70.73% were found to have biofilms.

Biofilm producing isolates in control group

35 out of 41 samples were positive for bacterial culture. Most of the cultures i.e., 23 samples revealed growth of *S. aureus* out of which 13 were methicillin resistant. Coagulase negative Staphylococcus were seen in 3 cultures. *Pseudomonas species* were found in 4 samples. *Klebsiella species* were found in 2 and *Citrobacter, E. coli, Acinetobacter species* were found in 1 culture specimen. There were 6 samples that did not have any growth of bacteria. 4 out of 10 MSSA strains and 5 out of 13 MRSA strains had biofilm forming capacity. Thus, out of 41 patients 9 cases i.e., 21.9% were found to have biofilm and 32 cases i.e., 78% did not have biofilm.

SNOT-20 scores

SNOT 20 scores of study group patients were calculated pre and postoperatively. These scores are compared based on the biofilm status (Table 1).

The table shows that patients who were positive for biofilm had significant higher preoperative SNOT-20 score than those who were negative for biofilm. But there is no significant postoperative SNOT 20 scores difference between biofilm positive and negative patients.

Table 1: Mean snot-20 score.

<table>
<thead>
<tr>
<th></th>
<th>Before surgery</th>
<th>15 days</th>
<th>1 month</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biofilm +ve CRSwNP (n=29)</td>
<td>46.69±14.18</td>
<td>19.34±11.88</td>
<td>11.45±9.29</td>
<td>13.03±17.18</td>
</tr>
<tr>
<td>Biofilm -ve CRSwNP (n=12)</td>
<td>36.50±12.53</td>
<td>15.92±8.72</td>
<td>10.42±7.12</td>
<td>6.92±11.35</td>
</tr>
<tr>
<td>P value</td>
<td>0.036</td>
<td>0.359</td>
<td>0.954</td>
<td>0.353</td>
</tr>
</tbody>
</table>

Table 2: Mean preop CT score.

<table>
<thead>
<tr>
<th></th>
<th>Mean Preop CT score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biofilm +ve CRSwNP (n=29)</td>
<td>11.66±7.73</td>
</tr>
<tr>
<td>Biofilm -ve CRSwNP (n=12)</td>
<td>9.0±6.90</td>
</tr>
<tr>
<td>P value</td>
<td>0.204</td>
</tr>
</tbody>
</table>

Preoperative Lund and Kennedy endoscopy score

Preoperative and postoperative diagnostic nasal endoscopy was done. Scores of individual patients were documented. Mean scores are tabulated below (Table 3). We found that there is no significant difference in endoscopic scores based on biofilm status before and after surgery.

Histopathological evaluation

Nasal polyp samples of CRSwNP patients were sent for histopathological evaluation. Presence of edema, neutrophil, lymphocytes, plasma cells, eosinophil,
metaplasia, increased goblet cells, basement membrane thickening, increased glands, and fibrosis were studied. These parameters were evaluated and compared based on biofilm status (Table 4).

**Table 3: Comparison mean endoscopic score.**

<table>
<thead>
<tr>
<th>Biofilm</th>
<th>Before surgery</th>
<th>After surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 days</td>
</tr>
<tr>
<td>Biofilm +ve CRSwNP (n=29)</td>
<td>6.86±3.86</td>
<td>3.03±2.04</td>
</tr>
<tr>
<td>Biofilm –ve CRSwNP (n=12)</td>
<td>5.83±3.04</td>
<td>2.25±1.91</td>
</tr>
<tr>
<td>P value</td>
<td>0.454</td>
<td>0.242</td>
</tr>
</tbody>
</table>

**Table 4: Comparison of histopathological parameters between biofilm +ve CRSwNP and biofilm –ve CRSwNP.**

<table>
<thead>
<tr>
<th>Histopathological parameters</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edema</td>
<td>0.342</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>0.505</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.333</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0.897</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0.364</td>
</tr>
<tr>
<td>Metaplasia</td>
<td>0.577</td>
</tr>
<tr>
<td>Increased goblet cells</td>
<td>0.736</td>
</tr>
<tr>
<td>Basement membrane thickening</td>
<td>0.431</td>
</tr>
<tr>
<td>Increased glands</td>
<td>0.571</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0.786</td>
</tr>
</tbody>
</table>

The above Table shows that there is no significant difference in local inflammatory reaction in CRS patients with biofilm when compared with CRS patients without biofilm. No atypical cells were seen in the stroma in any case.

**DISCUSSION**

Chronic rhinosinusitis is a common inflammatory disease affecting millions of people each year in the world. It is estimated that the prevalence of CRSwNP is 2% to 5% in general population.17 Many patients with CRS without nasal polyp respond well to medical therapy and surgical treatment. But CRSwNP is recalcitrant to these therapies and relapse is increasing. CRSwNP represents a real challenge for otorhinolaryngologists, with many unresolved questions existing about the pathology of this disease.

Nowadays bacterial biofilms have been implicated in the chronic nature of CRS. Studies have shown that patients with biofilm have more persistent postoperative symptoms, ongoing inflammation, and infections. Zang et al reported that biofilms were found in nasal and sinus mucosa of CRS patients before and after FESS and they contribute to an unfavorable outcome after surgery.18

However, positive percentages of bacterial biofilm in CRS patients are varied among different researches. The discrepancy may be attributed to different detection methods, as well as the influence of impact factors of biofilm. There are multiple techniques to investigate the presence of biofilm in CRS. Some studies demonstrated prevalence from 25% to 70%.19 Fluorescent in situ hybridization/confocal laser scanning microscopy demonstrated a prevalence of about 77%.20 Indirect demonstration by in vitro formation was about 28.6% in a report, although a previous study reported 84%.21

Commonest symptom, patients presented to us was nasal obstruction and the need to blow nose which was present in 100% patients. The other common symptoms were thick nasal discharge, post nasal discharge and running nose. 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 most of them were sad, frustrated and embarrassed due to this disease.

In our study, we characterized the presence of biofilms in the nasal polypoidal tissues of 41 patients with chronic rhinosinusitis with nasal poly and in nasal mucosal tissues of 41 patients undergoing septoplasty. We found that 29 out of 41 (70.73%) samples were positive for biofilm in CRSwNP patients and 9 out of 41 (21.9%) samples of patients who underwent septoplasty.

Our study shows a strong association between the biofilm presence and CRSwNP (OR=8.593, p<0.001-Pearson Chi-Square test which is significant). In previous study Bezerra et al reported that biofilms were found in 24(72%) out of 33 CRSwNP patients and 13 (48.1%) out of 27 controls with p value of 0.051 which is not significant suggesting that there is no significant association between CRSwNP and biofilm.22

However, in other individual studies by Zernotti et al and Sun et al they found a strong association between biofilm presence and CRS.23,24 Similarly other study by Dlugaszewska et al revealed the presence of biofilm in 23 (76.7%) specimens of the 30 patients with CRS, and in 9 out of 20 (45%) of the septoplasty patients and they found a strong association between CRS and biofilm. Biofilm positivity in CRS patients and control group of our study is compared with previous studies.25 It is shown in Table 5. Most common organism that form biofilm in both case and control samples was *Staphylococcus aureus*. 41% of samples of CRSwNP and 21% of samples of control group were biofilm producing *Staphylococcus aureus*. Those *Staphylococcus aureus* with biofilm forming capacity found in control patients may in future lead to CRS. So these control patients need long term
follow up and further study. In a study conducted by Sachse et al they found that *Staphylococcus aureus* was the frequent bacterial isolates in CRS and was frequently seen in polymicrobial biofilms. So it is also believed to play a role in the pathogenesis of CRS by producing toxins and inducing specific immunity to superantigen.

Table 5: Comparison of preoperative mean SNOT-20 score between patients with and without biofilm during 15 days.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Biofilm in CRS patients</th>
<th>Biofilm in Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our study</td>
<td>32/41 (70.73%)</td>
<td>29/41 (70.73%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bezerra et al²</td>
<td>24/33 (72.7%)</td>
<td>13/27 (48.1%)</td>
<td>0.051</td>
</tr>
<tr>
<td>Zernotti et al²</td>
<td>9/12 (75%)</td>
<td>0/10 (0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sun et al²</td>
<td>13/19 (68.4%)</td>
<td>0/12 (0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Długaszewska et al²</td>
<td>23/30 (76%)</td>
<td>9/20 (45%)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Table 6: Comparison of mean preoperative Lund-Mackay CT score of our study with study by Han et al.

<table>
<thead>
<tr>
<th>Biofilm CRSwNP</th>
<th>Biofilm CRSwNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our study</td>
<td>11.65±7.73</td>
</tr>
<tr>
<td>Han et al</td>
<td>3.75±2.05</td>
</tr>
</tbody>
</table>

Table 7: Comparison of mean preoperative Lund-Kennedy endoscopy scores of our study with study by Han et al.

<table>
<thead>
<tr>
<th>Biofilm CRSwNP</th>
<th>Biofilm CRSwNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our study</td>
<td>4.25±1.44</td>
</tr>
<tr>
<td>Han et al</td>
<td>4.25±1.44</td>
</tr>
</tbody>
</table>

Postoperatively we evaluated the preoperative SNOT-20, CT score and endoscopy score based on biofilm status. We found a significant difference in preoperative SNOT-20 symptom score between CRSwNP patients with and without bacterial biofilm (46.69±14.18 vs. 36.5±12.53, p=0.036). No difference was found in preoperative endoscopy score (6.86±3.86 vs. 5.83±3.04, p=0.454) and preoperative CT score (11.66±7.73 vs. 9.0±6.9, p=0.204). Our preoperative SNOT-20 score, endoscopy score and CT score were compared with study by Han et al (Table 6, 7, 8). Similarly we compared the postoperative SNOT-20 score, CT score and endoscopy score within study group based on biofilm status. There was no significant difference in SNOT-20 score between patients with and without biofilm during 15 days (19.34±11.88 vs 15.92±8.72, p=0.359), 1 month (11.45±9.29 vs 10.42±7.12, p=0.954), and 3 month (13.03±17.18 vs 6.92±11.35, p=0.373) postoperative period. Similarly there was no significant difference in Lund-Kennedy endoscopic score between biofilm positive and negative patients during 15 days (3.03±2.04 vs 2.25±1.91, p=0.242), 1 month (2.21±2.0 vs 1.58±1.50, p=0.428), and 3 month (2.83±3.56 vs 1.42±1.67, p=0.397). Postoperative SNOT-20 and endoscopic score were in decreasing pattern on 15th day and 1st month postoperative visit. But these score were in increasing trend on 3rd month of postoperative visit.

Nasal polyp samples were studied for the presence of edema, increased neutrophil, increased eosinophil, increased plasma cells, increased lymphocytes, metaplasia, increased goblet cells, increased glands, basement membrane thickening, and fibrosis. Han et al in their study found the amount of total inflammatory infiltrates and number of goblet cells in the biofilm positive specimens were significantly higher than those in the biofilm negative samples in CRSwNP patients. Hekiert et al suggested that the presence of biofilms was related to a Th1 inflammatory response. Foreman et al provided evidence of a link between *Staphylococcus aureus* biofilms and specific Th2 response, independent of superantigen activities. They also observed Th2 responses with increased levels of eosinophilic cationic protein and interleukin-5 in CRSwNP who were positive for biofilm. However we did not observe a significant difference in local histopathological inflammatory reactions between biofilm +ve CRSwNP and biofilm –ve CRSwNP.

CONCLUSION

We concluded that prevalence of biofilm in patients with CRSwNP was significantly different from that in control. Previous researches show that bacterial biofilm have a significant clinical and pathological impact in CRS. But in our studies that biofilms have no clinical and histopathological impact in patients with CRSwNP apart from playing a significant role preoperative symptomatology. So host and other environmental factors may play major role in the histopathogenesis of chronic rhinosinusitis with nasal polyp.

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

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