Bacterial Biofilms in Chronic Rhinosinusitis and Their Implications for Clinical Management

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Abstract To study the microbiological profile in patients with chronic rhino-sinusitis. To correlate disease severity with the presence of biofilms and host risk factors. To assess outcome of Sinus Surgery 2 weeks post operatively in terms of presence of bacteria and their ability to form biofilm. Prospective study. 50 cases of chronic rhino-sinusitis requiring Functional Endoscopic Sinus Surgery admitted in SDM Hospital, Dharwad, Karnataka were studied using intra-operative mucosal samples for microbiological analysis. The organisms isolated were tested for biofilm forming ability using three in vitro tests. Severity of disease was assessed using SNOT 22 scoring system. Of 50 cases studied, 66% showed presence of chronic rhinosinusitis with polyposis and had higher SNOT scores compared to those without polyps. Bacterial isolates were obtained from only 17 samples. *Staphylococcus* species was isolated from 16 samples and *Klebsiella pneumoniae* from one. 11 *Staph* spp. isolates showed biofilm forming ability in vitro. Postoperative events in 3 cases yielded biofilm-forming *Staphylococcus*. *Staphylococcus* was the most dominant organism isolated and 11 isolates were biofilm formers. Thus the detection of biofilm forming organisms can be considered as a negative prognostic indicator and should forewarn the surgeon about the risk of recurrence.

Keywords Chronic rhinosinusitis · Bacterial biofilm · *Staphylococcus* · Nasal polyposis

Introduction

Despite extensive research efforts the pathophysiology chronic rhino-sinusitis (CRS) is not well understood. The contribution of anatomic, environmental and host immunological factors implicated in the pathogenesis are difficult to clearly separate and identify hence management is a challenge to the physician [1]. The role of bacteria as a primary or exacerbating factor in the etiology/pathogenesis of CRS remains still controversial. Nevertheless, there is increasing evidence implicating bacterial biofilms—in particular staphylococcal biofilms, in severe and recalcitrant forms of the disease [1]. The presence of biofilms within sinuses is also implicated in unfavorable outcome despite maximal medical therapy due to the inherent resistance of biofilms to antibiotics and this could also result in poor post operative mucosal outcomes requiring revision Functional Endoscopic Sinus Surgery (FESS). This calls for a pragmatic change in current management protocols with special attention to preventing the development of antibiotic resistance and development of new techniques that ensure clearance of biofilms.

Materials and Methods

The objectives of the study was fourfold (a) to study the microbiological profile of patients with CRS undergoing FESS (b) to study the ability of sinus associated organisms to form biofilms in vitro and to examine susceptibility of these organisms to antibiotics commonly used in clinical

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management of CRS (c) correlate the severity of the disease with the presence of biofilms and host risk factors and (d) study the outcome of surgical management (with adjuvant medical therapy) on CRS in terms of presence of bacteria and persistence of biofilm.

A total of 50 patients with CRS (case definition as per The European Position Paper on Rhinosinusitis and Nasal Polyps 2012) [1] with or without nasal polyposis admitted to the ENT ward in SDM Hospital, Dharwad were studied. Inclusion criteria were: (1) Patients with CRS who remained symptomatic after maximal medical therapy (2) Patients > 18 years of age. Cases excluded from the study were patients who did not fit the case definition; were < 18 years of age; patients with fungal sinusitis; and those with benign or malignant tumors of nose and paranasal sinus. Ethical clearance was obtained from the SDM Hospital Ethical Committee.

Patients who completed 2 or more courses of antibiotics or took antibiotics for 3–4 weeks, prescribed empirically or on the basis of culture along with topical steroids and nasal irrigation for a period of 3–6 weeks [2] were considered as having received maximal medical therapy. A detailed medical history and clinical examination followed by radiological examination of paranasal sinuses (CT PNS) along with Diagnostic Nasal Endoscopy (DNE) was obtained from all cases to confirm the diagnosis. Pre operative Sino Nasal Outcome Test (SNOT) 22 scores [3] were obtained to assess the severity of the patient's symptoms. All patients were empirically administered third generation cephalosporin—cefotaxime through intravenous route 12 h prior to the surgery as none of the patients had data on preoperative cultures. Patients with extensive nasal polyposis were additionally prescribed oral steroid, prednisolone 20 mg/day for 4 days prior to surgery [4]. The extent of surgery (FESS) was dictated by the extent and severity of the disease as noted by CT and on table. Intra-operatively, in addition to discharge from middle meatus, sinonasal mucosa from middle meatus region and unhealthy mucosa in any of the affected sinuses was collected aseptically using endoscopic guidance for bacteriological and mycological investigations.

During the initial phase of the study, repeated negative cultures were encountered despite aggressive mucosal pathology. This was attributed to the use of normal saline as transport medium and hence all further specimens were inoculated into brain–heart infusion broth (BHI, HiMedia, Mumbai) at the point of collection. The tubes were incubated at 37 °C for 24–48 h. All the BHI tubes were subcultured on Chocolate Agar and MacConkey Agar (Hi Media, Mumbai). Bacterial isolates obtained were identified using a battery of biochemical tests and studied for antimicrobial susceptibility using Kirby Bauer disc

diffusion method [5]. The biofilm forming ability of the isolates was tested by three methods, which included the tube method [6], Congo red staining [7] and micro titer plate method [8].

In the tube-based method, biofilm formation was indicated by the adherence of the slime producing strains along the inner wall of the tube. In the Congo red method, strong biofilm formers gave black colonies with a dry crystalline consistency while weak slime producers were pink. Tissue culture based technique was modified by extending the incubation to 24 h followed by staining and the results read at OD₅₇₀, values being an index of bacterial adherence to surface and biofilm formation.

Post-operatively, oral antibiotics were prescribed based on the antibiogram generated for the isolates. Patients with negative culture reports were also empirically prescribed a broad-spectrum oral antibiotic for 5 days together with an antihistaminic and topical decongestant. 2 weeks post surgery; the response was recorded using SNOT 22 scores [3] along with Diagnostic Nasal Endoscopy to evaluate mucosal healing or recurrence.

Results

Fifty patients included in the study comprised 31 males and 19 females in the age ranging 18–71 years. Phenotypic presentation showed, 33 patients (66%) having CRS with polyposis (CRSwNP) and 17 (34%) without polyposis (CRSSNP). 5 cases with CRSwNP had history of previous sinus surgery, now presenting recurrence.

Pre-and post-operative recording of SNOT 22 scores enabled patients to be categorized into 3 groups: Mild to moderate (score from 0 to 40); moderate to severe (scores 40–70) and severe to “as bad as it can be” (scores > 70). Pre-operative scores ranged from 87 to 25 with an average of 57.32. 14% of cases (7/50) had mild to moderate symptoms, 58% (29/50) moderate to severe and 28% (14/50) presented with very severe symptoms. Post-operative scores ranged from 8 to 2 with an average of 4.5. Patients with CRSwNP had higher SNOT 22 scores than those without (Fig. 1). Majority of the patients had scores < 70 in both phenotypes, with very severe symptoms observed in 30.3% in cases with nasal polyposis in contrast to only 23.5% cases of CRSSNP. Yet, there was no statistically significant correlation between the two phenotypes and the SNOT 22 scores.

16 patients (32%) gave positive history of allergic rhinitis and were on symptomatic treatment. CRSwNP was observed in all of them with 4 (25%) having undergone sinus surgery previously. However no statistically significant association was observed between allergic rhinitis and the CRS phenotypes.

Fig. 1 SNOT variation in CRS phenotypes

Microbiological Analysis

Culture positivity rate was 34% with 17 of 50 samples yielding isolates, 16 being *Staphylococcus* species and one being *Klebsiella pneumoniae*. Of the former, 11 were *S. aureus*, 4 being methicillin resistant (MRSA) and five were methicillin resistant coagulase negative *Staphylococcus* (MRCoNS) (Fig. 2). Of 17 culture positive cases, 5 (29.4%) had allergic rhinitis and majority (64.7%) of culture positive cases had CRSwNP. Among the culture positive cases 2 had history of previous surgery and one of these yielded *S. aureus* and the other MRSA.

Biofilm Production

Biofilm formation by any of the three methods used in the study was considered as positive. 11 of 16 isolates of *Staph* spp. showed the ability to form biofilms in vitro. 5 isolates

showed strong biofilm formation by tube method of which 3 were *Staph aureus*, one MRSA and one MRCoNS. 10 isolates showed ability to form biofilms by micro-titer plate method of which 5 were *Staph aureus*, 4 were MRCoNS and one MRSA. Only one isolate of *Staph aureus* showed positive biofilm formation by Congo red method. This isolate also showed strong biofilm formation by the other two techniques. Thus biofilm formation was observed in 68.8% of culture positive cases while 31.3% (5/16) isolates did not show biofilm formation.

Post-operative Observations

2 weeks post operatively, patency of sinus ostia and satisfactory mucosal healing was observed with no edema/active discharge in most cases (46/50). 2 patients, one with pre operative diagnosis of CRSsNP and another CRSwNP (pre-op SNOT score 32 and 45 respectively), developed post operative acute sinusitis with purulent discharge from sinus cavity (Table 1). One patient (CRSsNP) had negative culture from the specimen obtained intraoperatively while MRCoNS was isolated from the other (CRSwNP). Post operative cultures of these patients grew MRSA and MRCoNS respectively which responded well to oral antibiotics (selected based on sensitivity), prescribed for a period of 1 week along with nasal irrigation. The MRCoNS isolate showed strong biofilm forming ability by TCP method (Table 1). Another 2 patients with pre operative diagnosis of CRSwNP (pre op SNOT 22 score of 30 and 60 respectively), had mucosal edema in the region of the ethmoids and frontal recess as observed 2 weeks post operatively on nasal endoscopy. Since there was no evidence of acute inflammation (fever/pain/headache) or active discharge at the ostia post operative sampling of mucosa was not done. Intraoperative cultures of these cases showed MRCoNS and *Staph aureus* respectively and both the isolates showed biofilm forming ability by TCP method. The patients responded well to oral steroids (Prednisolone 20 mg/day) prescribed for a period of 1 week along with topical steroids, and nasal irrigation.

Fig. 2 Proportion of organisms isolated in this study

Discussion

Chronic rhino-sinusitis is a persistent symptomatic inflammation of the nasal and sinus mucosa and insights into the pathophysiology of this condition have largely expanded over the last 2 decades. However the exact etiology is still unknown and multiple host and environmental factors have been implicated. Although most patients respond to medical therapy, surgical intervention is required in a subset of patients. While the success rate of ESS is quite high [9], a significant proportion of patients

Table 1 Postoperative observations on four patients, who developed sinusitis or edema

Patient	2 weeks post-operative observations	Post op culture	CRS phenotype	Intra-operative culture	Biofilm forming ability
A	Developed sinusitis	MRCoNS	CRS _{WNP}	MRCoNS	Positive by TCP method
B	Developed sinusitis	MRSA	CRS _{SNP}	Negative	Not applicable
C	Mucosal edema	Not done	CRS _{WNP}	MRCoNS	Positive by TCP method
D	Mucosal edema	Not done	CRS _{WNP}	<i>Staph aureus</i>	Positive by TCP method

CRS_{WNP} chronic rhinosinusitis with nasal polyps, CRS_{SNP} chronic rhinosinusitis without nasal polyps, MRSA methicillin resistant *Staphylococcus aureus*, MRCoNS methicillin resistant coagulase negative *Staphylococcus*, TCP tissue culture plate

have recalcitrant disease. Early identification of patients at the risk of such recalcitrant disease after surgery might facilitate better management of the outcome. A number of both retrospective and prospective studies have demonstrated the association of bacterial biofilms with more severe disease pre-operatively and recalcitrant disease post-operatively [10, 11]. In support of the biofilm hypothesis, a number of the studies have noted their presence on the sinus mucosa by scanning electron microscopy. This is however an expensive technology, which is not easily available, and its routine use in clinical diagnostic laboratories is not feasible. Therefore, there is a need for an efficacious and cost effective alternative method to detect the presence of biofilms within sinuses.

This study included 50 cases of CRS categorized phenotypically as CRS_{WNP} 66% (33/50) and CRS_{SNP} 34% (17/50). Five cases with a history of previous sinus surgery presented with CRS_{WNP} on both occasions. 16 patients (32%) had positive history of allergic rhinitis and were diagnosed as CRS_{WNP}. Among them 4 (25%) patients had undergone previous sinus surgery 8–10 years ago for nasal polyposis. However, no statistically significant association was observed between allergic rhinitis and the CRS phenotypes in this study. This observation is in keeping with current literature findings where several investigators have discussed the role of allergy in pathogenesis of CRS, but there is no evidence for any cause and effect relationship [12].

The SNOT 22 scoring system was used as a CRS-specific Quality Of Life (QOL) measure. Pre-operatively, majority cases (58%) had moderate to severe symptoms, and 28% had very severe symptoms. An important observation here was that the post-operative scores averaged at 4.5 (maximum at 8 and minimum score of 2), which shows significant improvement in the quality of life. No statistically significant correlation was seen between the two phenotypes and the SNOT 22 scores, but this may be due to the small sample size of the study.

Current literature indicates that *Staphylococcus* is the most commonly isolated organisms from sinus cavities of CRS patients [13] and from frontal sinus stents [14]. In keeping with this, our study also found *Staphylococcus*

species to be the predominant species being isolated from 16 samples and *Klebsiella pneumoniae* from one (Fig. 2).

In this study higher culture positive rates were observed in cases of CRS_{WNP} (64.7%) in comparison to CRS_{SNP} (35.3%). *Staph aureus* was the most common isolate comprising 70.6% (12/17), followed by MRCoNS accounting for 29.4% (5/17). Though bacteriologic pathogenesis of CRS_{WNP} is unlikely [15], there are reports to suggest that staphylococcal toxins released from the biofilm can act as super-antigens and thus induce massive inflammatory response in CRS [16].

The low culture positivity observed here (34%) might be due to multiple reasons. The most likely being use of pre-operative antibiotics that kills the planktonic forms detected by conventional culture methods. Fastidious organisms such as *H. influenzae* are difficult to culture (considered gold standard). The importance of using appropriate transport media cannot be over-emphasized. The use of chocolate agar made from human blood may contain antibodies that inhibit bacterial growth hence sheep blood agar is recommended but is expensive and difficult to procure.

The antibiogram of the isolates in this study revealed that isolates of MR CoNS were resistant to all beta-lactams, cephalosporin, ciprofloxacin, beta lactam- beta lactamases combination and carbapenems, and *Staph aureus* isolates showed resistance to beta-lactams, cephalosporin and while MRSA showed resistance to cephalosporins and cotrimoxazole. This study supports the observation that methicillin resistant staphylococci maintain significant presence in CRS [17].

Biofilm Production

The ability of biofilm formation plays an essential role in the virulence of *Staphylococcus* spp. protecting the organism against host immune response and action of antibiotics. In the biofilm form, these organisms are notorious to cause persistent or recurrent infections particularly through indwelling devices. Formation of biofilms within sinuses is postulated to cause repeated infections, persistent mucosal inflammation leading to chronicity of the disease.

The hardy nature of biofilms makes their eradication near impossible and their symbiotic existence leads to the development of multi drug resistance.

11 isolates of *Staph* spp. showed the ability to form biofilms by the 3 in vitro methods used. In the tube method strong biofilm formation was noted in 5/11 isolates and 10/11 isolates showed ability to form biofilms by micro-titer plate method. By Congo red method, only one isolate of *Staph aureus* showed positive biofilm formation. Although Congo red agar method is fast and presents an advantage that the colonies remain viable in the medium for further analysis, results by this method appear to be inconsistent and hence is not a strongly recommended technique for biofilm production.⁸⁽²⁹⁾ These results are comparable to similar studies found in the literature [18]. Among the 11-biofilm formers, 10 were associated with moderate (6) to very severe (4) symptomatology and 9 were associated with nasal polyps.

Post Op Observations

Post operative SNOTT 22 scores showed a significant improvement in the patients symptomatology of all patients. As shown in Table 1, MRSA and MRCoNS was isolated at post-operative stage in 2 patients with discharge within the sinus. The isolation of MRSA 2 weeks postoperatively, in the patient with a negative intra op culture and relatively mild disease (as per the pre-op SNOT score), could be attributed to a nosocomial infection. The persistence of MRCoNS in the second patient in both intra operative and post op sample with the ability to form biofilm supports the ‘biofilm theory’ of chronicity of CRS wherein patients suffer with persistent mucosal inflammation leading to recalcitrant disease and poor therapeutic (medical and surgical) outcomes.

Another 2 patients with pre operative diagnosis of CRSwNP (pre op SNOT 22 score of 30 and 60 respectively), on nasal endoscopy had mucosal edema in the region of the ethmoids and frontal recess as observed 2 weeks post operatively. Intraoperative cultures of these cases showed MRCoNS and *Staph aureus* respectively and both the isolates showed biofilm forming ability by TCP method. This observation also supports the role of biofilms in the pathogenesis of CRS.

Conclusion

Although there was no statistically significant difference between CRS phenotype and SNOT score, the SNOT 22 scoring system was useful in the study of clinical outcomes. Pre-and post operative assessment of the patient’s symptomatology by means of any of the validated quality of life

assessment scoring systems helps the treating physician track the patient’s recovery and assess the treatment outcomes, both medical and surgical. In addition long-term follow up of patients will provide valuable information on the surgical outcomes and its impact on the quality of life of the patient. The role of bacterial biofilms in causing persistence and recalcitrance of CRS is being increasingly understood. Although overall culture positivity in samples studied was small (34%) CRSwNP had higher culture positivity rate (64.7%). *Staphylococcus* was the most dominant organism isolated and of the 16 *Staphylococcus* tested for the ability to form biofilms, 11 were positive. 2 weeks post operatively, in three of the four cases developing postoperative incidents, organisms capable of forming biofilm was detected. Thus the detection of biofilm forming organisms can be considered as a negative prognostic indicator and should forewarn the surgeon about the risk of recurrence.

Compliance with Ethical Standards

Conflict of interest Authors declare that they have no conflict of interest. No external funding was used for the study.

Ethical Approval This article does not contain any studies with animals performed by any of the authors.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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