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The relationship of biofilm *Staphylococcus aureus* with degree of severity and infection in patients of chronic rhinosinusitis

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ABSTRACT

Background: *Staphylococcus aureus* is the main pathogen infect chronic rhinosinusitis (CRS) and the biofilms play an essential role in the pathophysiology of infection and treatment of patients. However, research on clinical conditions is still limited. This study aimed to evaluate the relationship between *S. aureus* biofilms with severity and infection degree in CRS patients.

Methods: A post-test-only study was conducted at Dr Zaionel Abidin Hospital in Banda Aceh, Indonesia from January to April 2022. Thirty-five CRS patients, classified according to the disease severity and infection degree, were included and followed in the study. A mucosal swab on each patient was collected and biofilm formation analyzed quantitatively and qualitatively using a spectrophotometer and scanning electron microscope (SEM), respectively. The relationships between the bacterial growth, biofilm formation and mass of biofilms with the disease severity and degree of infection were assessed statistically.

Results: More than half of the patients (54.3%) were aged younger than 40 years, and 51.4% were male. Nasal congestion and rhinorrhoea were the most common reported obligate symptoms (78.2%) and 82.9% patients have additional symptom on facial pain. There was the relationship between bacterial growth and disease severity (p=0.046) and infection degree of CSR cases (p=0.031). Our data showed a significant association between biofilm formation and the disease severity (p=0.022) and infection degree of CSR patients (p=0.020). The mass of the biofilm was also associated significantly with disease severity (p=0.029) and degree of infection (p=0.018).

Conclusions: Our study shows that the formation and mass of biofilm associated with the disease severity and degree of infection in CSR. Biofilms may contribute to the clinical condition and also be considered during management of CRS patients.

Keywords: *Staphylococcus aureus*, chronic rhinosinusitis, biofilm.
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INTRODUCTION

Chronic rhinosinusitis (CRS) is the multifactorial inflammatory disease of the nasal and paranasal mucosae for more than 12 weeks, with a prevalence estimated to be 12.5% of the US population.[1,2] CRS is influenced by various factors such as allergens, toxins, microbes (fungal and bacteria), and immune system.[3] However the exact pathological mechanism of the disease remains unclear. Some studies have been carried out on the possible role of bacterial biofilms.[4,5]

Bacterial biofilms is one of many possible etiologies for the occurrence and persistence of inflammation in CRS.[4] Biofilm is a collection of external matrix-forming microorganisms that function as a barrier against bacteria from host defences and antibiotics.[6,7] In vitro study found that certain bacterial strains in the biofilm state can be 1000 times more resistant to antibiotics compared to the those that do not form the biofilms (known as planktonic stage).[8]

Multiple bacterial species are known to produce biofilms and *Staphylococcus aureus* biofilms are most commonly associated with recurrence CRS. A study on 39 patients undergoing sinus surgery functional endoscopy (BSEF) to detect biofilm-forming bacterial species found that as many as 30 of 39 patients were found to have a combination of different bacterial species, 30% was polymicrobial biofilms, and 70% was *S. aureus* biofilms.[9] Another study also evaluated bacterial biofilm production in rhinosinusitis with nasal polyposis (CRSwNP) patients and found that *S. aureus* was a more potent biofilm-producing bacterium than other bacterial species.[10]

To our knowledge, investigations of biofilms in clinical conditions are still limited. Therefore, we sought to assess the relationship between *S. aureus* biofilm with the disease severity and degree of infection in CRS patients. A better understanding of these relationships might offer important diagnostic and therapeutic for CRS.

METHODS

This study was conducted using a post-test-only control group design among CRS patients treated from January to April 2022 at the Otolaryngology, Head and Neck Surgery, polyclinic of Dr Zaionel Abiding Hospital in Banda Aceh, Indonesia. The patients' characteristics including age, gender, obligate symptoms

(nasal congestion and rhinorrhea) and additional symptoms (facial pain and smell disturbance) were collected and assessed. Inclusion criteria were CRS patients diagnosed based on European Paediatric Ophthalmological Society (EPOS) criteria who had not received antibiotic therapy and were more than 18 years old. CRS patients caused by non-*S. aureus* bacteria and diagnosed with fungal rhinosinusitis were excluded.

The dieses severity of CRS patients was assessed using the Lund-MacKay score using a CT-scan of paranasal sinuses (PNS). Each PNS (maxillary, anterior ethmoid, posterior ethmoid, sphenoid, and frontal) was scored between 0 to 2 (0: no abnormality, 1: partial opacification, and 2: total opacification) and the ostiomeatal complex was given a score of 0 for no obstruction and 2 for obstruction.[11] Lund Mackay score was categorized into low: score 1-3, moderate: score 4-10 and high: score >10.

The degree of infection was determined based on the sinus area covered by mucus fluid with a CT-scan of PNS and visualized with ImageJ software. The degree of infection was divided into low (<380.3 pixels/µm), moderate (380.3-919.9 pixels/µm), and high (>9191.9 pixels/µm).

Bacterial sampling was carried out using a sterile cotton swab on the meatus of the nasal cavity and stored at -25˚C for a maximum of 72 hours.[12] Bacteria samples were identified using the Gram stain and observed using a microscope at 100x magnification.[13]

Evaluation of bacteria biofilm formation and biofilm mass was conducted using a spectrophotometer and scanning electron microscope (SEM), respectively. Briefly, to assess the biofilm formation, bacteria were coated on 96 well plates, stained using 1.0% crystal violet, and measured by a spectrophotometer every 24 hours for 72 hours at 520 nm.[14] The absorbance was converted to 0.5 Mc Farland (0.5×10^8) CFU/mL and categorized into low (optical density (OD): 0.05–0.1), medium (OD: 0.2–3.9), and high (OD: 0.4 or more). The mass of the biofilm was assessed using SEM based on the electron ray approach and the data categorized into low (≤142 µm), medium (143–149 µm) and high (≥150 µm).[15]

Data analysis was carried out using the Pearson correlation or Chi-squared test with a 95% confidence level to determine the relationship between the biofilm formation (low, medium and high) and mass of biofilms (low, medium and high) with the disease severity (low, moderate and high) and degree of infection (low, moderate, and high).

RESULTS

In total, 35 CSR patients included in the study and the patient's characteristics are presented in Table 1. More than half of the patients (54.3%) were aged younger than 40 years, and 51.4% were male. Based on obligate symptoms, 33 patients (94.3%) had both nasal congestion and rhinorrhoea, and one patient (2.9%) each had congestion or rhinorrhea only. Facial pain was the most reported additional symptom (82.9). There were 3 (8.6%) patients reported both facial pain and smell disturbance and one patient reported no additional symptom (Table 1). Based on disease severity, patients were predominately had severe (high) (54.3%) followed by mild (low) (25.7%) and moderate (20.0%) degree. Similarly, based on infection degree, most of the patients had high infection degree followed by low and medium infection degree, 45.7%, 34.3% and 20.0%, respectively.

Table 2 presents the data of bacterial growth and its association with disease severity and degree of infection. There was 1 (2.8%) patient had very low bacterial growth, 28 (80.0%) patients had low bacterial growth, and there were 4 (11.4%) and 1 (2.8%) patient had moderate and high bacterial growth, respectively. Our data indicated there was the relationship between bacterial growth and disease severity degree of the CSR patients (p=0.046). In addition, bacterial growth also associated with infection degree of CSR cases (p=0.031) (Table 2).

Biofilm formation occurs in each patient with different levels (Table 3). Based on observations through a spectrophotometer, patients with low and moderate severity only produced low levels of biofilm. In contrast, patients with high severity showed biofilm formation at moderate (one patient) and high (one patient) levels, although more than 50% were at low levels. Statistical analysis showed the relationship between biofilm formation and the disease severity (p=0.022). In addition, there was a significant association between biofilm formation and infection degree of CSR patients (p=0.020) (Table 3).

The data of biofilm mass, assessed using SEM and was categorized into three groups (low, moderate, and high), from all CSR patients are presented in Table 4. Our data suggested that the mass of the biofilm was associated significantly with disease severity with p=0.029. The mass of the biofilm was also associated significantly with degree of infection (p=0.018).

DISCUSSION

CRS is an inflammatory disease involving the nasal mucosa and paranasal sinuses and its pathogenesis is related to infection, genetics, and environment.[16] *S. aureus* infection is associated with CRS, and it presence correlates with skin inflammation and disease severity.[17] Our present study reports an association between the production of biofilms by *S. aureus* with severity and infection degree in CRS patients. Our study found that the samples of the CRS patients were proven had *S. aureus* and mostly had low growth category (300-600 CFU/mL) post 72-hour incubation period. A previous study shown that CRS patients are highly susceptible to colonization by Gram-positive bacteria in particular *S. aureus*, *Staphylococcus epidermidis* and *Propionibacterium acnes*.[18] Recent studies related to CRS have focused on *S. aureus* because of its virulence capacity and it has a role in promoting nasal polyp formation and prolonged tissue inflammation. *S. aureus* infection is also a risk factor for the persistence and severity of chronic rhinosinusitis with nasal polyps.[17]

Our study results are consistent with the previous study that found that 52.8% of 59 patients were able to form biofilms with low category.[19] Our data indicate that biofilm formation and biofilm mass are significant correlation with disease severity and degree of infection. A study found that biofilms formed on mucosal tissue could increase the volume of purulent nasal discharge and preoperative Lund-Kennedy scores in patients with

chronic rhinosinusitis with nasal polyps.[20] Although antibiotic resistant can be caused by several mechanisms,[21] biofilms can block antibiotics 10-1000 times because of their ability as physical barriers and inducers of bacterial responses to environmental stress.[22,23] Bacterial biofilms have been associated with the development and persistence of CRS. Colonization of biofilms in the sinonasal mucosa has increased inflammation in CRS patients, with the most prominent colony was *S. aureus*.[22,23] In addition, the exoprotein ability of the *S. aureus* biofilm has a more effect as a mucosal barrier and it is more toxic than the exoprotein of planktonic clinical isolates, which causes an increase in the severity and infection of CRS patients.[24]

The study of biofilms can be used to guide the treatment in CRS patients. Patients with a history of more severe symptoms will be recommended to have treatment through surgery. Postoperative patient cannot produce biofilm due to changes in host cells and it will break the biofilm cycle.[25] In addition, biofilm removal also affects the performance of antibiotics. A previous study found that ultrasound therapeutic in CRS patients could improve the action of antibiotics.[26] Ultrasonography could change the biofilm into a planktonic form had lower resistance properties and therefore increase the ability of antibiotics to attack the bacteria that cause CRS.[26]

Table 1. Demographic characteristics, obligate and additional symptoms, dieses severity and degree of infection of CSR patients (n=35).

Characteristic	n (%)
Age	
< 40 years	19 (54.3)
> 40 years	16 (45.7)
Gender	
Male	18 (51.4)
Female	17 (48.6)
Obligate symptoms	
Nasal congestion	1 (2.9)
Rhinorea (anterior/posterior nasal drip)	1 (2.9)
Both	33 (94.3)
Neither	0 (0.0)
Additional symptoms	
Facial pain	29 (82.9)
Smell disturbance	2 (5.7)
Both	3 (8.6)
Neither	1 (2.9)
Disease severity	
Low	9 (25.7)
Moderate	7 (20.0)
High	19 (54.3)
Degree of infection	
Low	12 (34.3)
Moderate	7 (20.0)
High	16 (45.7)

CONCLUSION

Our results show that all CRS patients infected with Gram-positive bacteria *S. aureus*. Bacterial biofilms in the sinuses of CRS patients may be associated with disease severity and degree of infection. Biofilm seems to be a risk factor in severe symptoms and it could associate with antibiotic treatment failure in CRS patients. Further research is needed to

Table 2. Relationship between bacterial growth and degree of severity and infection.

Bacterial growth	Degree of severity n (%)			p-value	Degree of infection n (%)			p-value
	Low	Moderate	High		Low	Moderate	High	
Very Low	1 (100.0)	0 (0.0)	0 (0.0)	0.046	0 (0.0)	0 (0.0)	1 (100.0)	0.031
Low	8 (26.6)	6 (21.4)	14 (50.0)		9 (32.1)	5 (17.9)	14 (50.0)	
Moderate	0 (0.0)	1 (25.0)	3 (75.0)		2 (50.0)	1 (25.0)	1 (25.0)	
High	0 (0.0)	0 (0.0)	2 (100.0)		1 (50.0)	1 (50.0)	0 (0.0)	

Table 3. Relationship between forming and mass of biofilm with disease severity and degree of infection.

Variable		Degree of severity n (%)			p-value	Degree of infection n (%)			p-value
		Low	Moderate	High		Low	Moderate	High	
Biofilm formation	Low	9 (27.3)	7 (21.2)	17 (52.5)	0.022	16 (47.1)	7 (20.6)	10 (32.2)	0.020
	Moderate	0 (0.0)	0 (0.0)	1 (100.0)		0 (0.0)	0 (0.0)	1 (100.0)	
	High	0 (0.0)	0 (0.0)	1 (100.0)		0 (0.0)	0 (0.0)	1 (100.0)	
Biofilm Mass	Low	5 (31.2)	2 (12.5)	9 (56.3)	0.029	9 (56.3)	0 (0.0)	7 (43.8)	0.018
	Moderate	2 (20.0)	3 (30.0)	5 (50.0)		3 (30.0)	6 (60.0)	1 (10.0)	
	High	2 (22.2)	2 (22.2)	5 (55.6)		4 (44.4)	1 (11.1)	4 (44.4)	

identify the type of biofilm and the clinical factors that affect the biofilm in CRS patients.

ETHICS APPROVAL

This study was approved by the Research Ethics Committee of School of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia (040/EA/FK-RSUDZA/2022).

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DISCLOSURE OF CONFLICTS OF INTEREST

The authors declared no conflict of interest.

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AUTHORS CONTRIBUTION

The authors contributed to the research process, including preparation, conceptualization, data collection and analysis, drafting, and approval to publish.

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