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Prevalence of Bacterial Biofilms in Patients of Chronic Rhino Sinusitis (CRS) with or Without Nasal Polyposis

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Abstract **Original Research Article**

Background*:** Objective. There is growing evidence that bacterial biofilms may play a role in certain cases of recalcitrant CRS that do not respond to traditional medical and surgical therapies. ***Aim*:** The aim of this study was to determine the prevalence of biofilm-forming bacteria in clinical isolates of CRS patients with or without nasal polyposis. ***Methods: A Prospective observational study conducted in the Department of ENT & HNS GMC Srinagar and Department of Microbiology, from September 2018 to August 2020. 60 patients were enrolled in this study who reported to ENT & HNS OPD of Govt. Medical College Srinagar and diagnosed with CRS according to "clinical practice guidelines" 2015 criteria, and were willing to participate in the study. Samples were harvested in the OPD and/or intraoperatively for microscopic examination to determine biofilm presence. Statistical analysis was performed. For all statistical tests, P = .05 was considered significant. ***Results***: In this study on 60 patients Biofilms were present in 32 (53.3%) patients whereas in 28 (46.7%) patients Biofilms were absent (includes 14 culture positive and 14 culture negative patients). Biofilm formation was detected by three different phenotypic methods. Biofilm detection rate were highest by Tissue Culture Plate (TCP) method (56.3%) followed by Tube method (TM) as (46.9%) and Congo Red Agar (CRA) method as (43.8%). ***Conclusion*:** This study further elucidates the prevalence of bacterial Biofilms in sinonasal mucosa of CRS patients as Biofilms existed in 53% of the patients under study.

Keywords: Chronic Rhinosinusitis (CRS), biofilms, sinusitis, nasal polyps.

INTRODUCTION

A myriad of factors have been implicated in the underlying aetiology of chronic rhinosinusitis which includes anatomical, functional (impaired mucociliary clearance), allergic, infective, environmental and more recently microbial Biofilms [1, 2]. Bacteria can be found in two distinct phenotypes: planktonic, which consists of free-floating single cells, or biofilms which are a dormant adaptive mode that supports the viability of a bacterial community [3]. Biofilms are a three-dimensional communicating organization of bacterial cells surrounded by a protective extra-cellular water-based glycocalyx matrix. Pores within this organized structure are conduits for nutrient and waste exchange. Bacterial cells within these biofilms are more resistant to host defences such as immune system phagocytosis and can be up to 1000 times more resistant to antibiotics [4, 5]. Biofilm growth is associated with an increased level of mutations. Antibiotic resistance is likely enhanced by the ability of resident bacteria cells to share genetic material within the biofilm structure, thus facilitating the persistence of antibiotic-resistant genes [6, 7]. Diseases associated with biofilms are characterized by persistent infection with intermittent periods of acute exacerbations. Cells in the biofilm are in a dormant state allowing them to remain viable with minimal nutritional support [8]. There is growing evidence that bacterial biofilms may play a role in certain cases of recalcitrant CRS that do not respond to traditional medical and surgical therapies [9, 10]. The aim of this study was to determine the prevalence of biofilm-forming bacteria in clinical isolates of CRS patients with and without nasal polyposis.

METHODS

A Prospective observational study conducted in the Department of ENT & HNS GMC Srinagar and Department of Microbiology, from September 2018 to

August 2020. 60 patients were enrolled in this study who reported to ENT & HNS OPD of Govt. Medical College Srinagar and diagnosed with CRS according to "clinical practice guidelines" 2015 criteria, and were willing to participate in the study. In addition to above 60 patients 10 patients who were fungal culture positive were excluded from the study as per the exclusion criteria of this study. Basic demographic data including age, gender, and race were collected also. Information regarding medical therapy including systemic and topical steroids and nasal irrigations, as well as antimicrobial therapy, was acquired. Prior surgical therapy and comorbidities including asthma, aspirin intolerance, allergic rhinitis, diabetes, hypertension, gastroesophageal reflux, and cystic fibrosis were recorded. Samples were collected in the form of swabs of mucopurulent secretions from middle meatus, polyps and crusts from nasal cavity in sterile containers containing normal saline. Sample underwent microbiological specification and evaluation of biofilm-forming capacity.

Microbiological Examination

Samples were inoculated on 5% sheep blood agar, MacConkey and chocolate agar for bacterial culture and on sabouraud's dextrose agar (SDA) for fungal culture. Plates were incubated at 37^0C for 24 hours. Organisms were identified by standard microbiological procedures including various biochemical tests. Isolates were tested for in vitro production of biofilm by following three methods. i) Tissue culture plating (TCP) method. ii) Tube method (TM). iii) Congo red agar (CRA) method.

Classification of bacterial adherence by TCP method

Mean OD	Adherence	Biofilm formation
<0.12	Non	Non/Weak
0.12-0.24	Moderate	Moderate
>0.24	Strong	High

OD: Optical Density.

RESULTS

In this study, 60 patients were enrolled, 37 (61.7%) patients were males and 23 (38.3%) patients were females male: female ratio of 1.6:1.Twenty five (41.7%) patients were diagnosed as chronic rhinosinusitis without nasal polyposis (CRSsNP) and 35 (58.3%) patients were diagnosed as chronic rhinosinusitis with nasal polyposis (CRSwNP). All the three sample types i.e. polyp, crusts/mucosa and nasal secretion swab were collected from 35 (58.3%) patients followed by two samples i.e. mucosa / crusts and nasal secretion from 15 (25.0%) patients and one sample only i.e. swab of mucopurulent nasal secretion from 10 (16.7%) patients.

Distribution of patients on the basis of culture report (N=60)		
Bacterial culture of nasal samples	No of patients	positive
Positive	46	76.7%
Negative	14	23.3%
Total	60	100%

In this study in 46 (76.7%) patients bacteria culture of nasal samples were positive on routine culture whereas in 14 (23.3%) patients they were negative.

Distribution of presence / absence of Biofilms among study participants (N=60)		
Biofilm	No of patients	Percentage
Positive	32	53.3%
Negative	28	46.7%
Total	60	100%

In this study on 60 patients Biofilms were present in 32 (53.3%) patients whereas in 28 (46.7%) patients Biofilms were absent (includes 14 culture positive and 14 culture negative patients).

Method	**No of biofilm producers**	**Percentage**
Tissue culture plate method	18	56.3%
Tube Method	15	46.9%
Congo Med Agar method	14	43.8%

Note: Some bacteria were Biofilm positive by two or three methods so total is exceeding 100%

In this study Biofilm formation was detected by three different phenotypic methods. Biofilm detection rate were highest by Tissue Culture Plate (TCP) method (56.3%) followed by Tube method (TM) as (46.9%) and Congo Red Agar (CRA) method as (43.8%).

DISCUSSION

60 patients were enrolled in this study. Youngest patient was aged 18 years and eldest patient was aged 60 years. Mean age in years was 33.05 years with standard deviation of ±12.216. A similar study was conducted by Bezerra *et al*., (2009) [11] titled "Biofilm in Chronic Sinusitis with Nasal Polyposis" in which patients were in the range of 22-60 years with a mean age of patients as 37 years ±11 years. Another study by Tatar EC *et al*., (2012) [12] titled "Prevalence of Bacterial Biofilms and Their Response to Medical Treatment in Chronic Rhinosinusitis without Nasal Polyposis" observed mean age of patients as 38.1 years (range, 24-53 years). Age distribution and mean age in this study was more or less similar to above reported studies. In this study out of 46 culture positive patients 32 (69.6%) patients were Biofilm positive whereas 14 (30.4%) patients were biofilm negative. In a similar study by Sanderson AR, Leid JG and Hunsaker D (2006) [13] titled "Bacterial Biofilms on the Sinus Mucosa of Human Subjects with Chronic Rhinosinusitis. Bacterial biofilms were present on 14 (77.77%) of the 18 CRS specimens. In another study by Tatar E C *et al*., (2012) [14] titled, "Prevalence of Biofilms and Their Response to Medical Treatment in Chronic Rhinosinusitis without Polyps" determined that biofilms were present in 75% of CRS patients. In another study done by P Singh *et al*., (2015) [15] titled, "Bacterial biofilm on the sinus mucosa of healthy subjects and patients with chronic rhinosinusitis (with or without nasal polyposis)", 100 cases (50 control group and 50 disease group cases) were analysed for the presence of biofilm. Bacterial biofilm was present in 26 (52%) out of 50 cases in the disease group. In this study bacterial biofilm formation by three phenotypically different methods was 32(69.6%). In a study done by Bezerra TFP *et al*., (2011) [16] titled "Biofilms in Chronic Sinusitis with Nasal Polyps" Biofilms were identified in 24 (72.7%) of the 33 patients of CRSwNP. Jung JH *et al*., (2014) [17] in their study "Clinical Characteristics of Biofilms in Patients with Chronic Rhinosinusitis: A Prospective Case–Control Study'' found that Biofilms were present in 13 (50%) of the 26 patients in the CRS group, In another study done by Singhal D *et al*., (2011) [18] titled "The impact of biofilms on outcomes after endoscopic sinus surgery" Biofilm positive samples were 36 (70.5%) and Biofilm negative were15(29.4%). Biofilm formation in our study was more or less similar to the above mentioned studies.

CONCLUSION

This study further elucidates the prevalence of bacterial Biofilms in sinonasal mucosa of CRS patients as Biofilms existed in 53% of the patients under study.

Conflict of interest: Nil

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