Journal Of Medical Science And Clinical Research
IGM Publication   An Official Publication Of IGM Publication

Original Article

Role of Biofilms in the chronicity and resistance in patients of Chronic Rhinosinusitis: A Prospective study

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Abstract
The role of Biofilms in the disease process and chronicity of Rhinosinusitis has been evaluated and suggested by many researchers but its role in the resistance to antimicrobials and chronicity of the disease is still under evaluation. Hence taking intoconsideration the above fact a Prospective study was undertaken to evaluate the role of biofilms in the chronicity and resistance in patients of Chronic Rhinosinusitis.
Materials and Methods: *Patients were divided into two groups cases (as per the Rhinosinusitis Task Force Criteria of 1993 and its amendment in 2006) and controls. Nasal swabs were taken and were sent for culture and sensitivity. The specimens in which growth of organism was identified were further subjected to test their ability to form biofilms.*
Results: *Methicillin Resistant Staphylococcus aureus (MRSA) was found to be the most common organism isolated, A higher percentage of biofilm forming organisms were present amongst the cases as compared to controls rendering a p-value of 0.00001124, which is highly significant, the isolates from cases were sensitive to higher order antibiotics as compared to controls and also showed a higher degree of antimicrobial resistance.*
Conclusion: *Through this study we conclude that there is a definite role of Biofilms in chronicity and antimicrobial resistance amongst organisms which are the causative agents of Chronic Rhinosinusitis.*
Keywords: *biofilms, chronic rhinosinusitis, staphylococcus aureus, antimicrobial resistance.*

Introduction

Biofilm has been defined as "A complex aggregation of micro-organisms marked by the excretion of a protective and adhesive matrix. Biofilms are also often characterized by surface attachment, structural heterogeneity, genetic diversity, complex community interactions, and an extra-cellular matrix of polymeric substances" [1]. The biofilm hypothesis suggests that biofilms, in particular staphylococcal biofilms, can serve as etiologic agents that cause Chronic Rhinosinusitis[2]. It can be speculated that a defect in the

immune barrier might facilitate formation of biofilms, which would suggest a role in pathogenesis rather than etiology. In addition, it has been suggested that biofilms sequester staphylococci, which permits secretion of superantigens that trigger T Helper cell Type 2 skewing and eosinophilic polyposis.

Bio Films

The first description of bacteria and indeed biofilms was made by a Dutch lens maker, Anton Van Leeuwenhoek in 1683. Despite his observations of bacteria existing either as individual highly motile organisms or in seemingly stationary clusters, but it was not until the emergence of chronic diseases, that the concept of bacteria existing in biofilms was considered. Although it has taken more than two decades since the "re-discovery" of biofilms by Costerton et al in 1978[3].

The most recent definition put forward by Donlan and Costerton includes both the readily observable structural features of a biofilm as well as the specific physiological features of the organisms existing within these structures. They now define a biofilm as a microbially derived sessile community, characterized by cells that are irreversibly attached to a substratum or interface or to each other which are embedded in a matrix of self produced extracellular polymeric substances, and exhibit an altered phenotype in terms of growth rate and genotype [4].

Biofilm formation has been shown to occur by at least three mechanisms:

(1) The redistribution of attached cells by surface motility [5,6],

(2) The binary division of attached cells [7] and

(3) The recruitment of cells from the bulk fluid to the development of the biofilm [8].

The relative contribution of each mechanism will depend on the interplay between the organism and surface involved as well as environmental physical and chemical properties.

When attached, bacteria show a profound resistance, rendering biofilm cells 10-1000 fold less susceptible to various antimicrobial agents,

disinfectants and biocides than the same bacterium grown in planktonic cultures [9,10,11].

Hence taking this literature into account a study was undertaken to isolate various organisms causing Chronic Rhinosinusitis and their implication in formation of Biofilms.

Material and Method

The study was conducted in Department of E.N.T of MMIMSR, Mullana, Ambala. After a detailed clinical history and thorough clinical and radiological examination and taking into consideration the criteria laid down by the Rhinosinusitis Task force in 1996 and their modification in 2003, the patients were diagnosed as cases of chronic rhinosinusitis. A proper written inform consent in the regional language was sought from the patient for his acceptance towards consideration in the study and also for further workup and investigations. The patients were divided into two groups: Cases and Controls.

Inclusion criteria

All patients of Chronic Rhinosinusitis
1) Age between 10 -50:
2) Criteria laid down by Rhinosinusitis Task Force in 1996 and its modification in 2003

Controls were selected from the patients who are having other nasal pathologies other than Rhinosinusitis.

Exclusion criteria

1) Pregnant women
2) Pre-existing medical condition leading to an immune-compromised state.

This is a hospital-based randomized case control study that will be conducted in the Department of E.N.T in a rural tertiary care centre of northern India, after proper Institutional ethical approval. A total of 30 cases and 30 controls were considered. After their complete history and examination and seeking a written informed consent in the regional language the nasal swab of the patient, from the nasal cavity and paranasal sinuses (from patients who underwent Sinus surgery) was collected.

The sample was sent for the evaluation of the growth of pathogens present and their ability to form biofilms by performing the following tests:

1. Tissue culture plate method

I. Organisms isolated from fresh agar plates were inoculated on brain heart infusion both (BHI) with 2% sucrose incubated for 24 hrs at 37^0C.

II. Adherent bacterial cells usually form biofilm on all side wells and were uniformly stained with crystal violet. Optical Density (OD) of stained adherent bacteria was determined with a micro ELISA auto reader at wavelength of 570 nm (OD 570nm)

III. These OD values was considered as index of bacteria adhering to surface and forming biofilms.

2. Modified Congo red agar method

I. Bacteria were inoculated on specially prepared solid medium – Brain heart infusion agar (BHA) supplemented with glucose and Congo red.

II. Black coloured colonies with dry crystalline consistency were interpreted as positive biofilm producing strains.

III. Red coloured colonies were interpreted as negative for biofilm produced.

3. Tube adherence method

I. Bacteria were inoculated on the Brain heart infusion broth with 2% sucrose in a glass tube and incubated for 24 hours at370c.

II. Biofilm formation will be considered positive when visible film was present on the walls and bottom of the tube.

Results

Microbiological Spectrum

In our study the most common organism isolated from both cases and controls was Methicillin Resistant Staphylococcus aureus (MRSA) but he prevalence of it was higher amongst cases compared to controls. Other organisms included S. epidermidis, Mehicillin Sensitive Staphylococcus aureus, and H. influenza.
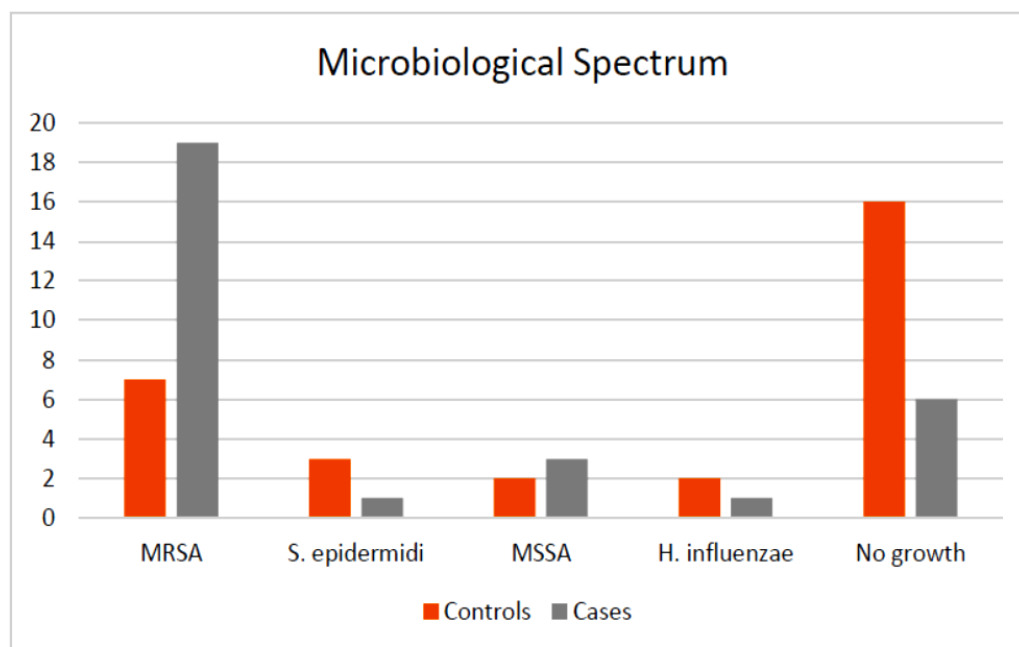

Figure 1: Microbiological Spectrum

Tendency to form Biofilm

Organisms isolated were considered for laboratory testing to test for their ability to form biofilms and the results obtained are shown in the figure:
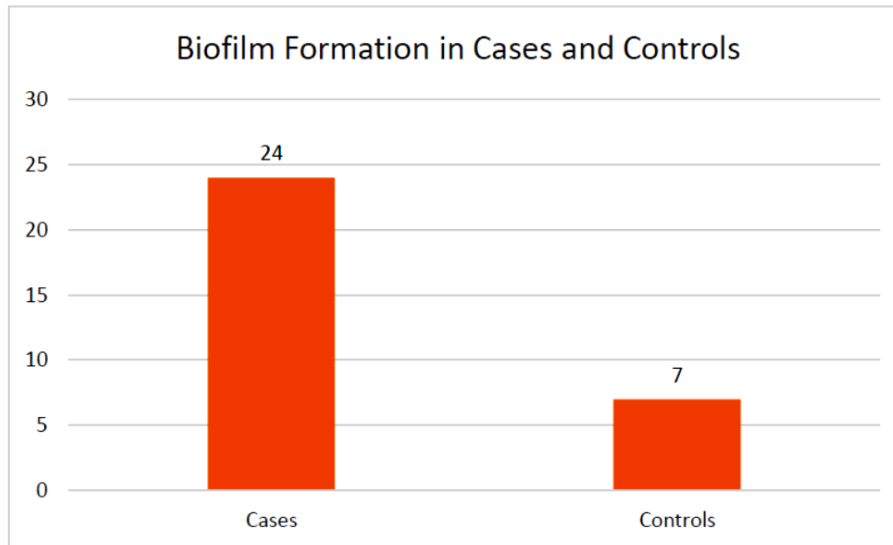

Figure 2: Tendency to form Biofilms

A higher percentage of biofilm forming organisms were present amongst the cases as compared to controls rendering a p-value of 0.00001124, which is highly significant.

Antibiotic Sensitivity

The nasal discharge from the cases and controls was also subjected to culture and sensitivity which yielded the following:
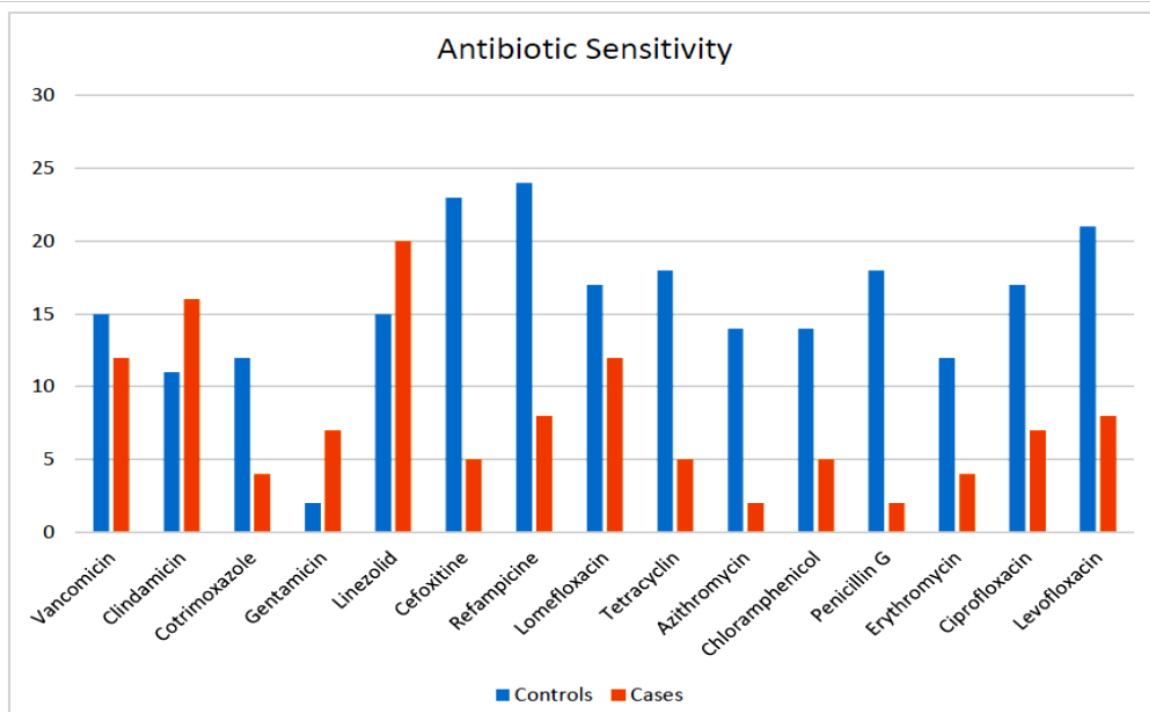

Figure 3: Antibiotic sensitivity amongst cases and controls.

It can be seen that the isolates from cases were sensitive to higher order antibiotics as compared to controls and also showed a higher degree of antimicrobial resistance.

Discussion

A higher percentage of biofilm forming organisms were present amongst the cases as compared to controls rendering a p-value of 0.00001124, which is highly significant.

The nasal discharge from the cases and controls was also subjected to culture and sensitivity which yielded that the isolates from cases were sensitive to higher order antibiotics as compared to controls and also showed a higher degree of antimicrobial resistance.

Antimicrobial resistance was initially postulated to be mediated by a single generalizable mechanism but recent studies suggest that it more likely to be a multi-factorial process and that the mechanism may vary among different organisms. The main hypotheses have been summarised below.

(a) Delayed antibiotic penetration of biofilms. The presence of the exopolysaccharide matrix of biofilms has long been held to have a role in limiting the penetration of antimicrobials to deep within biofilms. It was hypothesised that the matrix did this by either physically influencing the rate of transport of the antimicrobial agent or by deactivating it on its passage through the matrix. Recent in vitro studies have disproven this hypothesis for the majority of antimicrobials by documenting unimpaired antimicrobial penetration of the biofilm [12-18]. Three exceptions must be noted however involving aminoglycosides, beta-lactams and some glycopeptide antibiotics.

There is some evidence suggesting that electrostatic binding of positively charged aminoglycosides to the negatively charged polymers of the biofilm matrix, may retard the penetration of these antimicrobial agents and allow bacteria the necessary time to implement adaptive stress responses [19-22].

Additionally some biofilms such as those produced by Klebsiella pneumoniae, accumulate beta-lactamase in the biofilm matrix as a result of secretion or cell lysis and can subsequently deactivate beta-lactam antibiotics in the surface layers more rapidly than they diffuse into the biofilm [12, 20,23,24].

Finally it has been noted that slime associated with certain strains of S. epidermidis has been shown to physically complex with and antagonise specific glycopeptide antibiotics [25-27].

(b) Altered Microenvironment and Reduced Growth Rate. It is now well established that within biofilms, micro-gradients occur in the concentration of key metabolites and products [28]. These chemical gradients have been shown to directly alter antibiotic potency. Tack and Sabath showed that oxygen availability alone, modulated the action of aminoglycosides, with bacteria in anaerobic environments more resistant to these antibiotics than those in aerobic ones [29]. Similarly, gradients in pH have also been shown to impact negatively on antibiotic efficacy [30, 31]. Additionally, in areas of nutrient depletion, studies using fluorescent probes and reporter genes, have demonstrated that bacterial cells also significantly reduce their growth and metabolic rate [32-34]. As almost all antimicrobial agents are more effective in killing rapidly growing cells, this slow growth undoubtedly also contributes to biofilm resistance to antimicrobial killing [35].

(c) Altered Genetic expression. DNA microarray and proteomic studies have demonstrated differences in gene expression and protein profiles of biofilm and planktonic bacteria. It has been postulated that increased expression of biofilm-specific resistance genes, such as those coding for multidrug efflux (MDR) pumps or periplasmic glucans may also contribute to antimicrobial resistance [36,37]. The additional finding by a recent study that genetic disruption of expression of MDR pumps in Pseudomonal biofilms, also affected their biofilm attachment, suggests that antibiotic resistance may be under the same regulatory or genetic control as other biofilm associated traits [38].

Conclusion

Through this study we conclude that there is a definite role of Biofilms in chronicity and antimicrobial resistance amongst organisms which are the causative agents of Chronic Rhinosinusitis.

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