ORIGINAL ARTICLE

Does bacterial Biofilms have a role in the development of human chronic rhinosinusitis?

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Abstract

Background: The presence of bacterial biofilms may explain the recalcitrant nature of some forms of chronic rhinosinusitis (CRS) despite proper medication and good aeration of the sinuses after functional endoscopic sinus surgery (FESS).

Objectives: The aim of this study was to assess the existence of bacterial biofilms on the sinus mucosa surfaces of patients with recalcitrant chronic sinusitis.

Patients and methods: Fourteen patients with CRS scheduled for FESS or patients with persistent rhinosinusitis after FESS were enrolled in the study. Cultures were obtained from the sinus secretions and mucosal biopsies were harvested from the bulla ethmoidalis for *scanning electron microscopy (SEM).*

Results: Nine patients showed picture of chronic rhino-sinusitis with pathological thick adherent mucociliary blanket, five specimens showed bacilli and four specimens showed cocci.

Conclusion: Bacterial biofilms can be considered to have a major role in the failure of medical and/or surgical treatment of CRS resulting in its recalcitrant nature.

Key words: Biofilms, chronic rhinosinusitis, FESS, mucociliary blanket.

Introduction

Chronic rhinosinusitis (CRS) is one of the most common diseases in the Gulf area especially the central area due to the very hot, dry dusty weather and the continuous use of air conditions. CRS is defined as the presence of continuous symptoms and signs for more than 12 weeks with the identification of signs of inflammation on anterior rhinoscopy, endoscopy and imaging (1). There are many potential etiologies for CRS including allergy, bacteria, fungi, superantigens and biofilms (2). Although medical and surgical strategies for CRS have been greatly refined during the last 2 decades, many patients continue to suffer.

Bacterial biofilms are three-dimensional aggregates of bacteria that recently have been shown to play a major role in many chronic infections. There is growing evidence that bacterial biofilms may play a role in some forms of recalcitrant CRS that persists despite the surgically properly opened sinus cavities and culture-directed antibiotic therapy. New directions in therapy aimed at biofilms may provide some success in treatment for patients with CRS (3). Bacterial biofilms are communities of microorganisms in a matrix attached to a moist surface; they are formed when free-floating bacteria bind to a surface. These communities are composed of distinct microcolonies in towers extending from the substratum. Towers are penetrated and separated by channels supplying water and nutrients by diffusion (2, 3). Bacteria in nature exist in two states, free-floating planktonic bacteria and matrix-enclosed bacteria (biofilms) attached to a surface. Planktonic bacteria are genotipically and phenotipically different from the bacterial biofilms. Bacterial biofilms undergo phenotypic changes before they are released in the host as free-floating bacteria (4, 5, 6). The bacterial biofilms growth in humans serves as a nidus of infection periodically shedding planktonic cells that are responsible for the patients' systemic response with the symptoms and signs of infection (7, 8). In animals biofilms produce a very little systemic response in the

form of minimal cellular and humoral immune response; these have been demonstrated by identifying the cytokines and chemokines released by leukocytes exposed to the biofilms. Bacteria prefer to exist in the biofilm forms, which are responsible for more than 65% of all hospital infections. Biofilms are also up to 1,000 times more resistant to traditional antibiotics than their corresponding planktonic forms (8, 9, 10).

The aim of this study was to determine the presence of specific bacterial biofilms on the sinus mucosa of patients with recalcitrant CRS.

Methods

This prospective observational study was done in Saudi German Hospital and King Abdul Aziz University Hospital following institutional review board approval and receipt of patient consent. The study included 14 patients, five of them were undergoing FESS for CRS, three patients were undergoing revision FESS and six were undergoing office based debridement. At the start of the procedure a swab was obtained from the patients' sinus secretions for cultures. Ethmoid sinus mucosal specimens were obtained using a through-cutting forceps (Hermann CEH 146) to minimize mucosal injury. Specimens were fixed in 4% paraformaldehyde for at least 24 hours, dehydration of the specimens followed by incubation with increasing concentrations of ethanol to a final concentration of 100% (absolute). Specimens were then dried in CO2 to critical-point, mounted on stubs, and supper-coated with gold–palladium to a thickness of 12 nm. Finally mucosal specimens were examined with an XL 20 scanning electron microscope SEM (Philips Electronics, The Netherlands), representative images were obtained at a variety of magnifications.

Patient information data collected included symptoms, duration of illness, and presence of allergy symptoms, treatment history and diagnostic results radioallergosorbent test (RAST), Immunoglobulin E (IgE) and CT scan results.

Results

Fourteen patients were included in the study chosen in the ENT clinic suffering of CRS of different durations, irrespective to age or sex. Eight patients underwent previous FESS in the same center or different centers. The analysis of patients' symptoms, allergic response and radiological findings are shown in table (1).

Fourteen ethmoid sinus mucosa specimens were harvested and examined as mentioned before using SEM, all specimens showed signs of chronic infection including inflammatory cells infiltration and areas of ciliary loss. Nine specimens (64%) showed near-total coverage of the ciliary surface by a coat that is thicker and more adherent than that associated with the mucociliary blanket in healthy mucosa (figure 1).

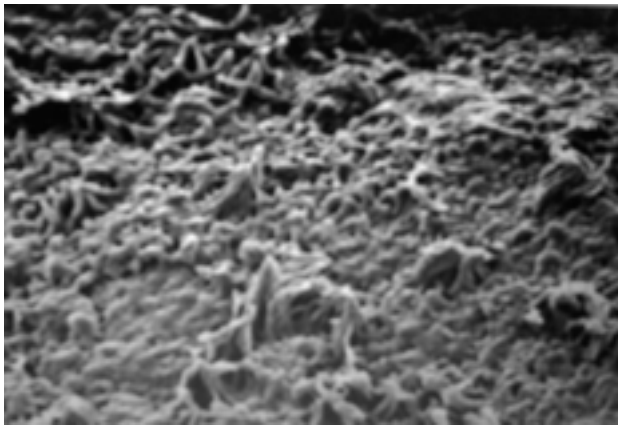

Figure 1: SEM image showing thick adherent coat to the mucociliary blanket with areas of ciliary loss.

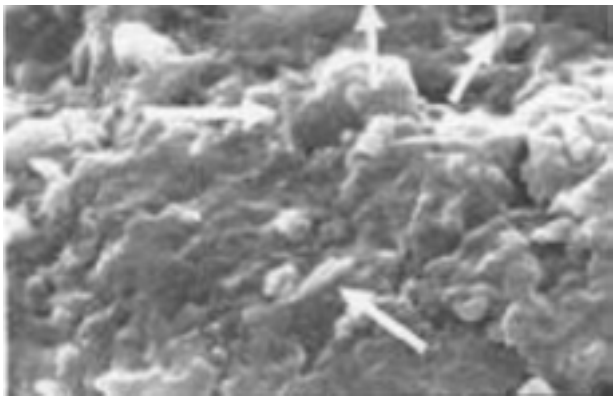

Figure 2: High power SEM image of the tower like structures containing many rod shaped elements (white arrows) P. aeruginosa.

Five (36% of total) of the nine specimens showed areas with three dimensional structures within which rod-shaped structures resembling bacteria, this was seen in specimens number 2,3,6,10and13 as shown in (figure 2). Specimens 4, 9, 11 and 14 (29%) showed three dimensional tower-like structures containing spherical elements resembling cocci as shown in (figure 3).

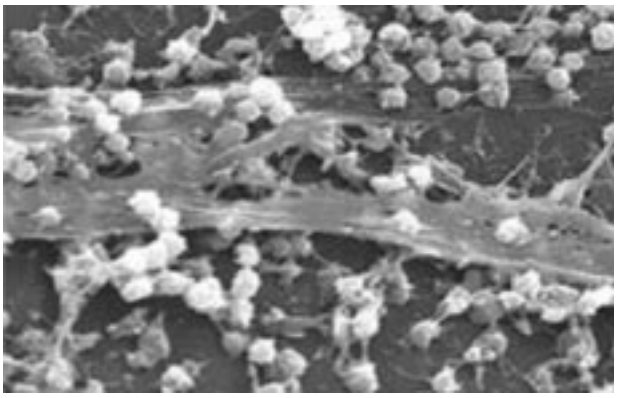

Figure 3: High power SEM image of the tower like spherical structures (Staphylococcus aureus)

Patient No.	Symptoms	History of Surgery	Allergy		CT scan	Biofilms	Culture
			RAST	IgE			
1	Facial pain, PND, stuffy nose	-ve	-ve	26	Pansinusitis	-ve	-ve
2	PND and stuffy nose	+ve	-ve	34	Maxillary + ethmoids	+ve, bacilli	-ve
3	PND, stuffy nose	+ve	-ve	51	Bilateral maxillary	+ve, bacilli	-ve
4	PND and stuffy nose	+ve	-ve	35	Maxillary + ethmoids	+ve, cocci	-ve
5	Facial pain, PND, stuffy nose	-ve	+ve	782	Pansinusitis	-ve	-ve
6	PND, facial pain	+ve	+ve	1246	Bilateral maxillary	+ve, bacilli	H. influenza
7	PND and stuffy nose	-ve	-ve	26	Maxillary + ethmoids	-ve	-ve
8	PND and stuffy nose	-ve	-ve	56	Maxillary + ethmoids + frontal	-ve	-ve
9	Facial pain, PND, stuffy nose	+ve	-ve	23	Pansinusitis	+ve, cocci	-ve
10	Facial pain, PND, stuffy nose	+ve	-ve	43	Pansinusitis	+ve, bacilli	P. aeruginosa
11	Facial pain, PND, stuffy nose	+ve	+ve	680	Pansinusitis	+ve, cocci	-ve
12	PND and stuffy nose	-ve	-ve	24	Ethmoiditis + frontal	-ve	-ve
13	PND, stuffy nose	+ve	-ve	37	Bilateral maxillary	+ve, bacilli	-ve
14	Facial pain, PND, stuffy nose	+ve	+ve	445	Pansinusitis	+ve, cocci	-ve

Table (1): Clinical data and results of study patients

PND=Post nasal discharge, RAST= radioallergosorbent test, IgE= immunoglobulin E.

Discussion

Biofilms [matrix-enclosed microbial accretions that adhere to biological or non-biological surfaces] represent a significant and incompletely understood mode of growth for bacteria. Recent advances show that biofilms are structurally complex, dynamic systems with attributes of both primordial multicellular organisms and multifaceted ecosystems (12). Biofilm formation represents a protected mode of growth that allows cells to survive in hostile environments and also disperse to colonize new niches (6). Bacteriologic studies of otitis media with effusion (OME) using highly sensitive techniques of molecular biology such as the polymerase chain reaction have demonstrated that traditional culturing methods are inadequate to detect many viable bacteria present in OME. The presence of pathogens attached to the middle-ear mucosa as a bacterial biofilm, rather than as free-floating organisms in a middle-ear effusion, has previously been suggested to explain these observations (2, 3). Although medical and surgical strategies for CRS have been greatly refined during the last 2 decades, many patients continue to suffer. There is growing evidence that bacterial biofilms may play a role in some forms of recalcitrant chronic sinusitis that persists despite surgically opened sinus cavities and what seems to be appropriate and culture-directed antibiotic therapy (11,12). The current study showed the presence of bacterial biofilms in nine of the examined specimens, and only two bacterial growths in sinus secretions cultures. The +ve specimens showed the tower-shaped formations comprised of aggregates of matrix-encased

bacteria that is considered as a morphologic hallmark of biofilms. Different forms of biofilms were detected, towers containing rods that could be bacilli as haemophilus influenzae or P. aeruginosa and towers containing spheres that might have been Staphylococcus aureus. It was planned to do fluorescent in situ hybridization (FISH) as it the only test that can identify specific bacteria creating the biofilm matrix. This test was not done as it was very expensive (466$) for a single sample. A possible limitation of the standard SEM preparation is that artifacts may appear as biofilm on SEM because of dehydration and protein cross-linking. We need to evaluate whether SEM is the best technique available for identification of biofilms or whether there are other modalities for identifying the biofilm as well as the bacteria in the biofilm.

As mentioned in literature, biofilms cannot be cultured, this study showed positive growth only in two cases only (14%); this could be contamination of the swabs with nasal commensals and not from the sinuses proper. Most of the positive biofilm specimens were harvested from cases of persistent CRS after FESS undergoing office debridement or revision FESS. This is explained by the high virulence of the offending organism, high resistance and irresponsiveness to commonly used antibiotics or even surgical clearance, ending in residual CRS. Detection of these biofilms on the surface epithelium of human sinonasal mucosa highlights the nature of recalcitrant CRS. Further studies and research work should be focused on the means of eradication of bacterial biofilms either by dissolving its matrix preventing it from colonization or by blocking the channels penetrating the towers to prevent diffusion of water and nutrients to the bacteria ending in its death.

Bacterial biofilms can be considered to have a major role in the failure of medical and/or surgical treatment of CRS resulting in its recalcitrant nature.

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