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Bacterial biofilms in chronic rhinosinusitis and their relationship with inflammation severity

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Abstract

Aims: To identify the presence of bacterial biofilms on mucosal specimens from chronic rhinosinusitis (CRS) patients, and evaluate their relationship with severity of CRS.

Methods: A prospective study of biofilms presence on 24 CRS patients compared with 12 controls was designed. The presence of biofilms was determined by scanning electron microscopy (SEM), and associations with the preoperative Lund–MacKay CT scores, Johansson endoscopic scores, and the history of ESS were assessed.

Results: Biofilms were found in 13/24 CRS patients (54.2%) but in only 1/12 controls (8.3%; $P < 0.01$). CRS patients with and without biofilms had similar preoperative Lund–MacKay CT and Johansson endoscopic scores ($P > 0.05$). Patients with revision ESS showed a tendency of higher biofilms incidence (5/7, 71.4%) than those undergoing their first procedure (8/17, 47.1%), but did not reach a significant difference ($P > 0.05$).

Conclusions: The higher incidence of biofilms in CRS patients suggests a role in the pathogenesis of CRS, but no correlation with severity of CRS.

Keywords: Bacterial biofilms; Chronic rhinosinusitis; Lund–MacKay CT scores; Johansson endoscopic scores; History of endoscopic sinus surgery

1. Introduction

Bacterial biofilms are highly organized structures composed of bacterial communities encased within a protective extracellular matrix, which are resistant to both antibiotic treatment and host defense systems [1]. Biofilms are considered a common and important cause of persistent infections. The National Institutes of Health has estimated that at least 65% of all bacterial infections in humans are related to biofilms [2]. In the field of otolaryngology, biofilms have been documented on otitis media with effusion [3], cholesteatoma and tonsillitis [4,5], rhinosinusitis and adenoids removed from children with chronic rhinosinusitis (CRS) [6], and they have also been isolated on some prosthetic devices, such as tracheotomy and tympanostomy tubes, front recess stents, and cochlear implants [7].

Current research suggests that biofilms may contribute greatly to the recalcitrant nature of CRS. There have been several reports regarding the presence of biofilms in surgical specimens obtained from patients with CRS. In 2005, Ramadan examined five specimens from five CRS patients, all of which were positive for biofilms [8]. Using confocal scanning laser microscopy, Psaltis found biofilms formation in 44% of 38 CRS patients [9]. A recent prospective study in over 150 consecutive CRS patients identified a positive rate of almost 30% for bacteria with biofilm-forming capacity, as assessed by an in vitro biofilm-formation assay [10].

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Although the association between biofilms and the recalcitrant nature of CRS has been gradually accepted by most researchers, the precise role of biofilms in the pathogenesis of CRS remains unclear. Additionally, the pathogenesis of CRS may differ between different populations and areas.

There are only a few reports regarding the pathogenesis of CRS with biofilms in China, and even fewer concerning the clinical relevance of CRS with biofilms. The primary aim of this study was to determine the presence of biofilms on CRS patients in Chinese community and its correlations with Lund–Mackay CT scores, Johansson endoscopic scores, and history of endoscopic sinus surgery (ESS).

2. Materials and methods

2.1. Study design and population

This was a prospective study performed to determine the presence of bacterial biofilms on the sinus mucosa of 24 CRS patients undergoing ESS compared with 12 controls without CRS. All patients were from the Otolaryngological Department of the 1st Affiliated Hospital of Zhejiang University and Zhejiang Provincial Tongde Hospital during the period July 2008 to September 2010. All subjects gave informed consent prior to enrollment in the study.

All CRS patients fulfilled the diagnostic criteria for CRS according to clinical practice guidelines [11]. Clinical data, including demographic information, presence of bronchial asthma, macrolide therapy or steroid therapy before surgery, preoperative Lund–MacKay computed tomography scores, Johansson endoscopic scores and history of ESS, were recorded. Specimens were obtained intraoperatively from diseased sinuses, including the uncinate process, anterior ethmoid sinuses, posterior ethmoid sinuses or maxillary sinus, as evidenced by the preoperative CT scan. Multiple specimens were obtained per subject (47 specimens in all from 24 CRS patients), each of them varied from 3.0 mm × 3.0 mm to 6.0 mm × 6.0 mm. Specimens were washed thoroughly in saline, and then placed immediately into 2% glutaraldehyde for scanning electron microscopy (SEM) specimen preparation.

The control group consisted of 12 patients without CRS (1 patient with nasopharyngeal carcinoma, 8 patients scheduled for nasal bone-plasty due to nasal trauma, 3 patients undergoing septoplasty). Specimens were obtained from the uncinate process or the anterior ethmoid bulla at the time of surgery.

2.2. Scanning electronic microscopy

All specimens were prepared for SEM using the following techniques. Tissue was initially fixed for 2 h in 2.5% glutaraldehyde in phosphate-buffered saline (PBS, pH 7.4) at 4 °C. Two rinses of 15 min each were then carried out using PBS. Next, the specimens were fixed with 1% osmium tetroxide for 1 h. They were then dehydrated through a graded ethanol series as follows: 50% for 15 min, 70% for 15 min, 80% for 15 min, 90% for 15 min, and 100% twice for 15 min each time. The tissue was immersed in 100% acetone for 15 min and washed in 100% isoamyl acetate for 15 min, followed by critical point drying. Finally, specimens were mounted on metal stubs and subsequently sputter-coated with gold preparation for imaging.

Observations were carried out in our SEM laboratory using a Stereoscan 260 microscope (Leica, Wetzlar, Germany) at an acceleration voltage of 15 kV. Structures characterized by water channels, 3D structure, and matrix-embedded spherical or elliptical bodies were identified as evidence of biofilms. It is differed from viscous mucous, the latter is a flat blanket, under the blanket, sometimes you may see the comparative orderly cilia, above the mucous blanket, sometimes you can find irregular foreign granule. The entire area of each specimen was scanned for the presence of biofilms structures. Images were taken at various angles to effectively display the specimens and to minimize errors and artifacts. The investigators examining the samples were blinded to the disease status of the patients.

2.3. Statistical analysis

The results of this study were analyzed using the SPSS software (ver. 13.0; SPSS Inc., Chicago, IL). Continuous data were analyzed using Student's *t*-test, and dichotomous data were analyzed using Fisher's exact test. In all analyses, P values <0.05 were deemed to indicate statistical significance.

3. Results

3.1. Patient demographics and clinical data

Twenty-four CRS patients and 12 controls were included in the study. The CRS group consisted of 16 males and 8 females with a mean age of 41.88 ± 12.58 years (range 18–65 years), while the control group consisted of 8 males and 4 females with a mean age of 30.2 ± 12.6 years (range 17–56 years). Table 1 lists the presence of bronchial asthma, macrolide therapy or steroid therapy before surgery, preoperative Lund–MacKay CT scores, Johansson endoscopic scores, and the history of ESS for each CRS patient.

3.2. SEM findings

Specimens from the 24 CRS patients and 12 controls were examined for the presence of bacterial biofilms by SEM. Using strict SEM morphological criteria as well as the characteristic features of biofilms, all 24 CRS patients