ORIGINAL ARTICLE

Association of Adenoid Surface Biofilm Formation and Chronic Otitis Media With Effusion

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Objectives: To investigate bacterial biofilm formation on the adenoid surface of pediatric patients undergoing adenoidectomy and to reveal whether this formation is associated with chronic otitis media with effusion (COME).

Design: Prospective, single-blinded study with scanning electron microscopy.

Setting: Dışkapı Yıldırım Beyazıt Research and Educational Hospital, Ankara, Turkey.

Patients: Thirty-four children between 4 and 15 years of age (mean age, 7.76 years) who had undergone adenoidectomy from June 1 through December 31, 2007, were divided into 2 groups. The first group (17 children) had undergone adenoidectomy alone, whereas the second group (17 children) had undergone adenoidectomy combined with ventilation tube insertion for COME.

Main Outcome Measures: Scanning electron microscopy was performed to observe biofilm formation on the surface of adenoid tissues. The biofilm formation was graded according to extension. The roles of adenoid size, age, sex, and duration of COME symptoms were evaluated.

Results: Biofilm formation was detected on all samples. Adenoids removed from patients with COME had higher-grade biofilm formation than the other group ($P = .001$). No correlation was found between adenoid size and biofilm formation.

Conclusion: In pediatric patients, adenoid surface biofilm formation may be associated with COME etiopathogenesis.

Arch Otolaryngol Head Neck Surg. 2010;136(6):550-555

CHRONIC OTITIS MEDIA WITH effusion (COME) is the most common chronic ear disorder in children. COME is defined as the persistence of middle ear fluid beyond 12 weeks and has multiple causes, including environmental and host factors.[1,2] Medical treatment is the first choice of management, but it does not have a standard. It usually includes eradication of upper respiratory tract infections, control of allergy, and environmental precautions. When the effusion does not respond to medical treatment, ventilation tube insertion and/or adenoidectomy is required. Both are safe and commonly applied surgical procedures, but each surgical intervention has associated costs, morbidity, and failure.[1-3]

The increasing resistance to antibiotics in patients with chronic sinonasal infections has attracted an interest in biofilm formation. Biofilm is the thin layer of bacteria encased in a hydrated matrix of polysaccharide and protein, which adheres to implanted medical devices or surface tissues. Reversible attachment of motile and nonmotile bacteria to the surfaces initiates the biofilm formation. Then with elaboration of a glycocalyx by the bacteria, adhesion becomes irreversible. Growth continues by the division of sessile bacteria and the recruitment of other bacteria from the environment, which constitutes a biofilm of glycocalyx-enclosed microcolonies.[4] This process was first described within dental plaque in the 17th century by Antonj van Leeuwenhoek. However, its clinical importance was not fully appreciated until the late 20th century.[5]

The role of biofilms in the etiopathogenesis of chronic rhinosinusitis was demonstrated in several studies.[6-9] Biofilms were shown on the surface of several devices, such as endotracheal tubes, tracheostomy tubes, voice prostheses, tympanostomy tubes, and even cochlear implants.[10] Biofilm formation was also detected on adenoid and tonsil surfaces, particularly in children with recurrent infections.[11-13] The correlation between COME and biofilm formation was also studied with respect to resistance to medical treatment and the need for ventilation tube application.[14,15] The aim of this

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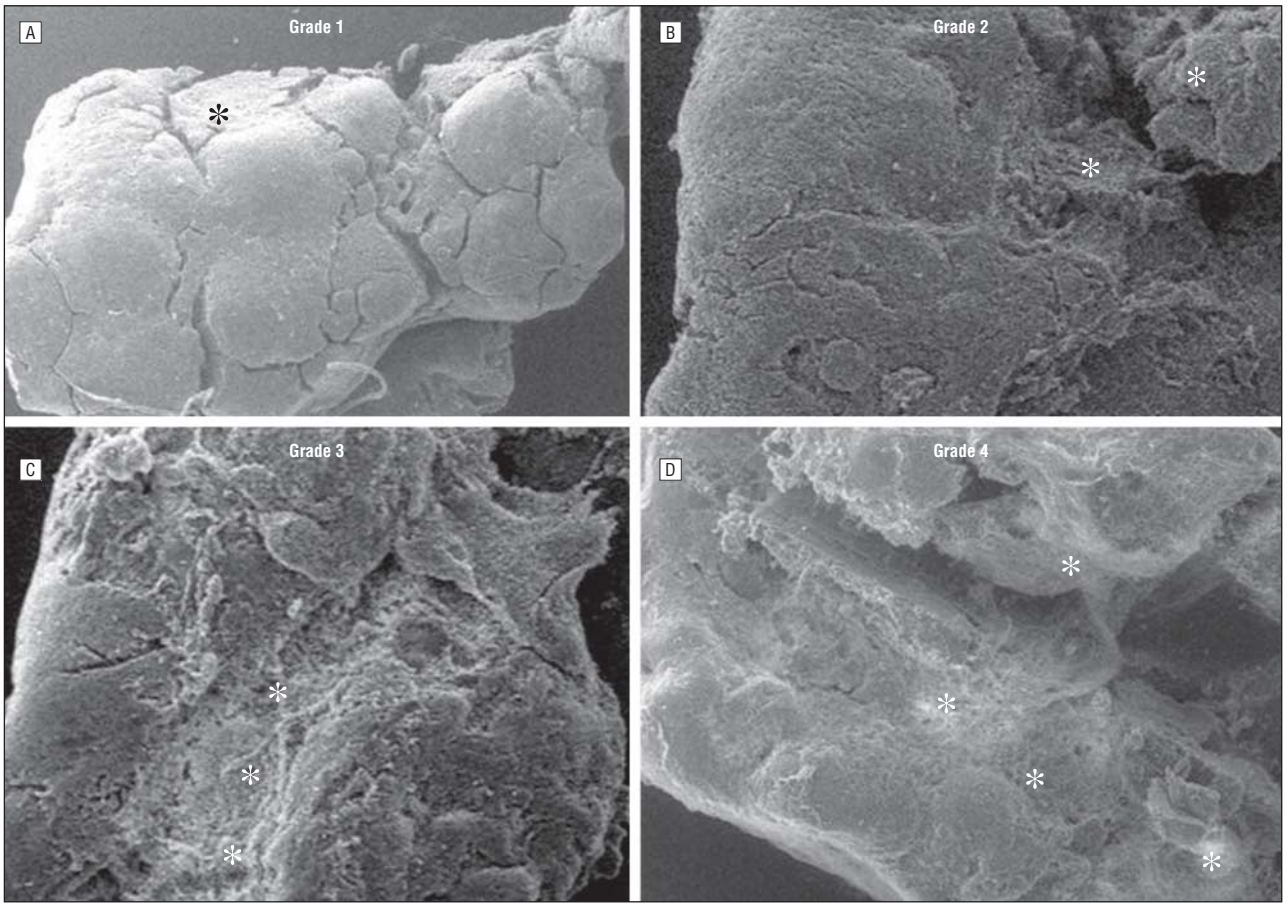

Figure 1. Different grades of biofilm formation are seen with low magnification (original magnification approximately ×100). A, Grade 1; B, grade 2; C, grade 3; and D, grade 4. Asterisks indicate biofilm areas on the adenoid surface.

study was to investigate whether there was a relationship between adenoid surface biofilm formation and COME by using scanning electron microscopy (SEM).

METHODS

We planned a prospective study with 34 children who underwent adenoidectomy in the Dışkapı Yıldırım Beyazıt Research and Educational Hospital from June 1 through December 31, 2007; the local ethics committee approved the study. The patients were preoperatively evaluated by nasal endoscopy or lateral x-ray examination (in noncooperative children) for adenoid hypertrophy. Patients with healthy ears and nasal obstruction with or without apnea were included in the first group. Patients with COME and adenoid hypertrophy irrespective of nasal symptoms were included in the second group. The exclusion criteria were the presence of a previous adenoid operation and/or tube insertion. The size of the adenoid was reassessed with a flexible fiberoptic endoscope (2.4 mm; Karl Storz GmbH & Co KG, Tuttlingen, Germany) with the patient under general anesthesia at the beginning of the operation to avoid misevaluation because of the child's noncooperation. Then the adenoids were graded arbitrarily according to the degree of choanal obstruction, with less than 50%, 50% through 75%, and greater than 75% designated as levels 1, 2, and 3, respectively. Adenoidectomies were performed with adenotomes, and the specimens were obtained from 2 different parts of the nasopharyngeal surface of adenoid tissues with a knife.

The surface layer of the adenoid tissues was examined for biofilm formation by using SEM, by the same researcher (I.T.) in a single-blinded manner, at the Electron Microscopy Laboratory, Department of Anatomy, Medical School, Hacettepe University. The fresh specimens were immediately fixed in 2.5% glutaraldehyde for 24 hours, washed in phosphate buffer (pH 7.4), postfixed in 1% osmium tetroxide in phosphate buffer (pH 7.4), and dehydrated in increasing concentrations of alcohol. After dehydration, the specimens underwent the critical drying point process and were mounted on metal stubs with double-sided adhesive tape. Then the samples were sputtered with a 15-nm-thick layer of gold in a sputter apparatus (Bio-Rad Laboratories, Hercules, California). The images were taken with the JEOL SEM ASID-10 (JEOL Ltd, Tokyo, Japan) and the LEO 4.3 HVP SEM (Carl Zeiss AG, Oberkochen, Germany) electron microscopes.

To examine the areas of interest on the surface of an adenoid tissue specimen, we took sample SEM images within a voltage range of 5 to 80 kV and within a magnification range of 50 to 5000 times. We determined the biofilms as the areas where multilayered remnants of tissue and microorganisms exist. With respect to the average biofilm extension, grade 1 (**Figure 1**A), grade 2 (Figure 1B and **Figure 2**), grade 3 (Figure 1C and **Figure 3**), and grade 4 (Figure 1D and **Figure 4**) biofilm formations were determined when less than 25%, 25% to 49%, 50% through 75% and more than 75% of sample surfaces were involved, respectively.

In each group, biofilm formation was analyzed with respect to age, sex, and duration of clinical symptoms. Then the groups were compared for the severity of biofilm formation in terms of grades.

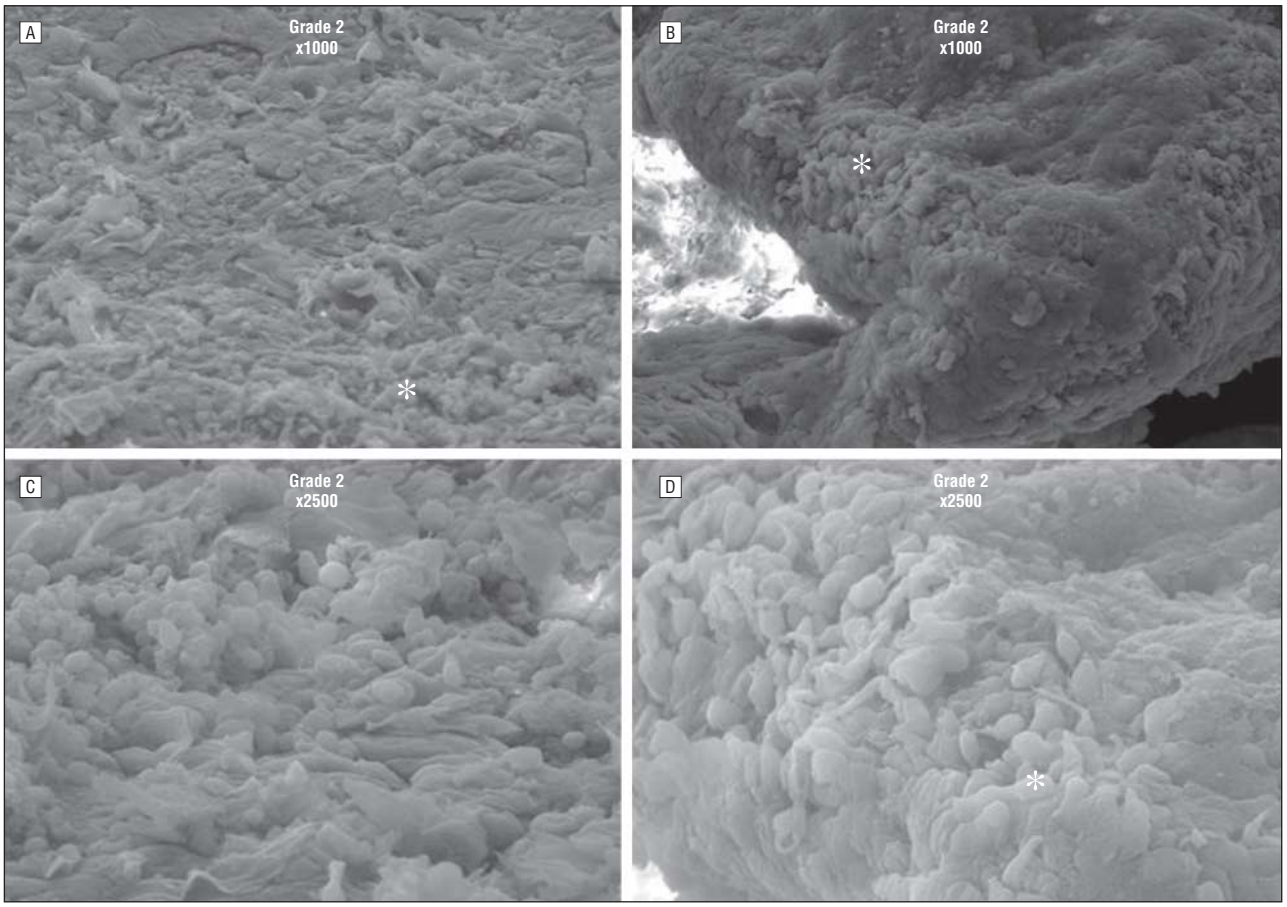

Figure 2. Grade 2 biofilm formations from 2 different samples. A and B, Sample 1 (×1000 magnification); C and D, sample 2 (×2500 magnification). Asterisks indicate biofilm areas on the adenoid surface.

Statistical tests were conducted using SPSS statistical software, version 10.0 (SPSS Inc, Chicago, Illinois). The nonparametric Mann-Whitney test was used in the comparison of biofilm formation and adenoid size between groups. The role of sex was also evaluated with the Mann-Whitney test. A Pearson correlation coefficient was used to analyze the effect of age and duration of symptoms on biofilm formation and adenoid size. $P < .05$ was accepted as significant.

RESULTS

There were 20 male and 14 female patients in the study; their ages ranged from 4 to 15 years (mean, 7.76 years). The duration of the symptoms was 1 to 10 years (mean, 2.85 years). The demographic characteristics of the 2 groups are given in the **Table**.

BIOFILM FORMATION

The biofilm formation was detected in all samples in the range of grades 1 to 4. Adenoid samples removed from the children with COME (group 2) had higher-grade biofilm formation than the group who were operated on for adenoid obstruction (group 1). The difference between the 2 groups was statistically significant (Mann-Whitney test, $z = -3.355$, $P = .001$) (Table).

Biofilm formation did not change with respect to sex (Mann-Whitney test, $P = .34$). There was no relationship of biofilm formation with either age (Pearson correlation coefficient, $r = 0.045$, $P = .55$) or duration of symptoms (Pearson correlation coefficient, $r = 0.045$, $P = .80$) in all patients. When the same analysis was performed for each group, there was an increase of biofilm formation with age in group 2 (Pearson correlation coefficient, $r = 0.610$, $P = .001$).

ADENOID TISSUE SIZE

The adenoids were graded according to size; 2 samples were determined as level 1, 15 samples as level 2, and 17 samples as level 3. No statistically significant impact of the adenoid size on biofilm formation was found ($P = .33$). Also, adenoid size did not change significantly between the groups (Mann-Whitney test, $P = .21$). No level 1 adenoid was seen in the first group because this group was operated on for obstructive symptoms.

No significant correlation of adenoid size was seen with sex (Mann-Whitney test, $P = .23$), age (Pearson correlation coefficient, $r = 0.118$, $P = .51$), and duration of symptoms (Pearson correlation coefficient, $r = 0.276$, $P = .11$). These analyses were performed in all patients and in each group separately.

COMMENT

In recent years, the role of biofilms has gained popularity as a component in the explanation of chronicity of

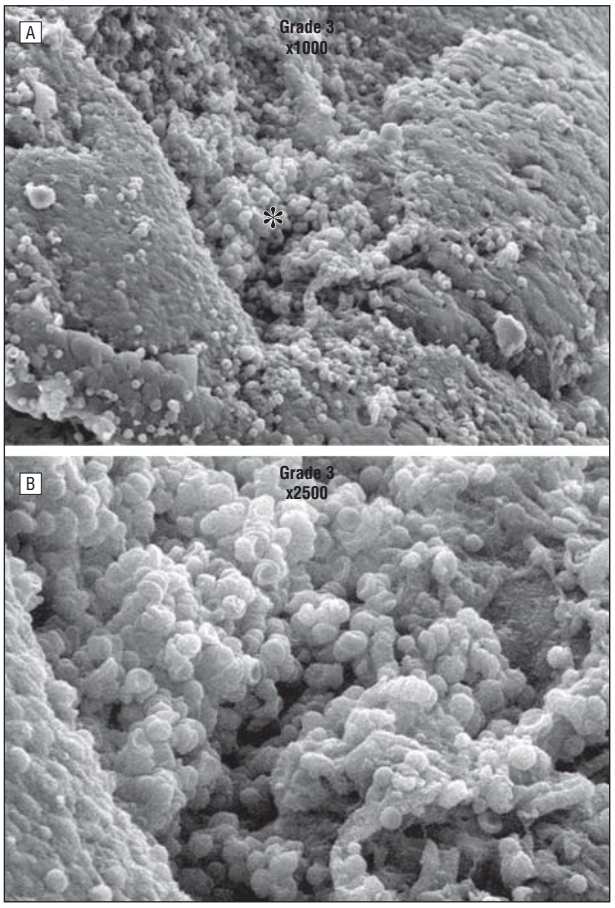

Figure 3. Grade 3 biofilm formation. A, Sample (×1000 magnification); B, close-up of sample (×2500 magnification). Asterisk indicates biofilm area on the adenoid surface.

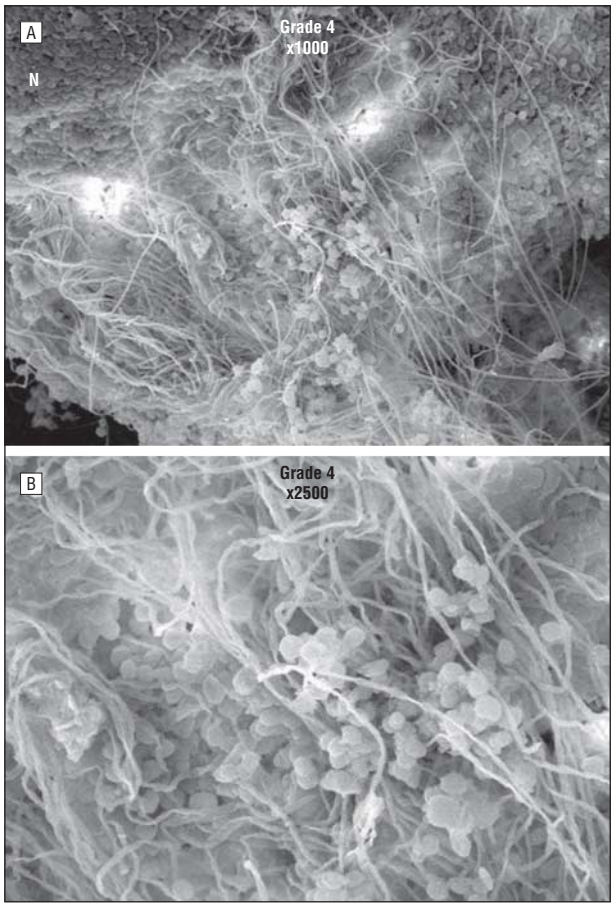

Figure 4. Biofilm formation graded as 4 can be seen on these images. Hypha formation, which is rarely observed according to type of the microorganism, is also seen. A, Sample (×1000 magnification; B, close-up of the sample (×2500 magnification). N indicates normal surface.

Table. Demographic Characteristics of the Study Children, Duration of Symptoms, and Distribution of the Patients According to Adenoid Tissue Size and Biofilm Grades[a]

Characteristic	Group 1 (n=17)	Group 2 (n=17)	*P* Value[b]
Sex, No.			
Male	8	12	.30
Female	9	5	
Age, mean (SD) [range], y	8.05 (2.83) [4.00-15.00]	7.47 (2.62) [4.00-13.00]	.55
Symptom duration, mean (SD) [range], y	3.06 (2.19) [1.00-10.00]	2.65 (1.41) [1.00-6.00]	.75
Adenoid tissue size, patients, No. (%)			
Level 1 (<50)	0	2 (12)	.21
Level 2 (50-75)	7 (41)	8 (47)	
Level 3 (>75)	10 (59)	7 (41)	
Biofilm formation, samples, No. (%)			
Grade 1 (<25)	7 (41)	0	.001
Grade 2 (25-49)	7 (41)	6 (35)	
Grade 3 (50-75)	3 (18)	9 (53)	
Grade 4 (>75)	0	2 (12)	

[a] Group 1 had adenoid hypertrophy; group 2 had adenoid hypertrophy and chronic otitis media with effusion.
[b] Fisher exact test was used for sex, and Mann-Whitney test was used for adenoid tissue size, biofilm formation, age, and symptom duration.

infections and resistance to antibiotic chemotherapy. The persistence of bacteria in a biofilm happens with mechanisms other than efflux pumps, modifying enzymes, and target mutations. There are 3 main hypotheses to explain this phenomenon. The first hypothesis is the possibility of slow or incomplete penetration of the antibiotics into the biofilm. The second hypothesis depends on the altered chemical microenvironment within the biofilm. A third and still speculative hypothesis is that a subpopulation of microorganisms

in a biofilm forms a unique and highly protected phenotypic state—a cell differentiation similar to spore formation.[16] With these possible mechanisms, biofilms may play an important role in the formation of many chronic or recurrent otorhinolaryngologic diseases, such as otitis media, sinusitis, cholesteatoma, tonsillitis, adenoiditis, and device infections.

Tonsils removed for recurrent infections and/or obstructive enlargement had been studied to identify a possible mechanism to explain chronicity by using light microscopy and transmission electron microscopy. It was stated that there was strong anatomical evidence of bacterial biofilms in chronically diseased tonsils.[11] In another study, the presence of biofilms on tonsils was shown using confocal scanning laser microscopy with double fluorescent staining, and 17 of 24 tonsils (71%) removed for chronic or recurrent tonsillitis contained biofilms.[12]

In recent literature, articles have supported the hypothesis that COME pathogenesis may also be biofilm related. Presence of bacterial biofilms was shown in experimental otitis media induced in chinchillas by transbulbar injection of nontypable *Haemophilus influenzae* and in tympanostomy tubes removed from children with otorrhea.[14] Mucosal biofilms were also demonstrated in 46 of 50 (92%) middle ear mucosal specimens of patients undergoing ventilation tube placement for COME by using confocal scanning laser microscopy, fluorescent in situ hybridization, and immunostaining methods, although their control group had no biofilm.[15] In our study, the role of adenoid surface biofilm formation was investigated in children with COME. The patients undergoing adenoidectomy were divided into 2 groups; group 1 had healthy ears and group 2 had COME and ventilation tube insertion. Adenoids were examined for biofilm formation by SEM and were graded for quantification purpose according to Tatar et al.[17] Our hypothesis was that coexistence of biofilms with adenoid hypertrophy might be a factor in COME origin. The findings supported this theory, and the adenoid samples from children with middle ear effusion had higher-grade biofilm formation than those without effusion.

The standard surgical treatment of COME, which is resistant to medical management, is ventilation tube insertion and/or adenoidectomy.[18-20] The critical question is whether adenoidectomy is effective through removal of mechanical obstruction of eustachian tube or removal of reservoir of chronic infection. It is our policy to remove the adenoids if the pediatric patients (<15 years old) with COME undergo an operation to have ventilation tubes inserted. If the effusion persists more than 3 months despite medical management, the child is a candidate for surgery in our practice. In patients with healthy ears and adenoid hypertrophy, history of apnea is the main indication for adenoidectomy and frequent upper respiratory tract infections. Our findings support the role of adenoidectomy in COME to eradicate the focus of chronic bacterial infection, even if it is not obstructive.

In a study[21] comparing biofilm formation on adenoid tissues removed from patients with obstructive sleep apnea and those with chronic rhinosinusitis, it was stated that biofilms in the nasopharynx of patients with chronic rhinosinusitis might act as a chronic reservoir for bacteria. Mucosal biofilms were demonstrated on the adenoid tissues removed from the children with COME in 54% of the patients.[22] Our findings supported these results, and it was found that there was an association between adenoidal biofilms and COME. However, there was no correlation between adenoid size and biofilm formation. Grade 4 biofilm formation was not detected in patients with only adenoid hypertrophy and, similarly, grade 1 biofilm formation was not detected in patients with COME.

In our study, it was determined that adenoid tissues of children with COME contained denser surface biofilms compared with those without COME. These findings support the hypothesis that there may be an association between adenoidal biofilm formation and COME. Adenoids in COME may act as a reservoir of chronic infection rather than causing mechanical eustachian obstruction. However, with our findings it is not possible to speculate that biofilms spread into the middle ear through the eustachian tube. Our results support the role of adenoidectomy in the management of COME resistant to medical therapy to eradicate the reservoir of chronic infection. Further studies may reveal whether adenoidectomy and/or prolonged antibiotic course can eradicate biofilm formation or whether the biofilms also coexist in the middle ear in COME.

Submitted for Publication: December 8, 2008; final revision received November 13, 2009; accepted January 6, 2010.

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Financial Disclosure: None reported.

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