ADENOID VEGETATIONS – RESERVOIR OF BACTERIA FOR CHRONIC OTITIS MEDIA WITH EFFUSION AND CHRONIC RHINOSINUSITIS

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Abstract
Introduction: Otitis media and rhinosinusitis are commonly encountered illnesses in pediatric population. Literature reports have documented the association between the occurrence of these two conditions and even their almost identical microbiological findings. Until recently, the key factor in the association of these two conditions was considered to be the hypertrophic adenoid tissue, but within the past few years there have been evidences in the literature about the presence of bacterial biofilms on the adenoids suggesting biofilms to be also responsible for both conditions, chronic otitis media with effusion and chronic rhinosinusitis.

Aim: The aim of this study was to make a microbiological analysis of the adenoid tissue specimens taken from patients with chronic otitis media with effusion and chronic or recurrent rhinosinusitis and to determine their potential for biofilms formation.

Methods: After the surgical intervention, adenoidectomy, microbiological evaluation and analysis of the adenoid tissue specimens taken from 20 patients were made. Having in mind the disease history, chronic otitis media with effusion was diagnosed in all 20 patients and chronic rhinosinusitis in 9 patients.

Results: The results obtained from the microbiological analyses showed many potentially pathogenic bacteria in the adenoids that were almost identical with the most common organisms incorporated in the etiopathogenesis of both conditions, in chronic otitis media with effusion and in chronic rhinosinusitis. In 7 (35%) patients Haemophylus influenzae was isolated, in 6 (30%) Streptococcus pneumoniae, in 4 (20%) Moraxella catarrhalis, in 2 (10%) patients Staphylococcus aureus and in 1 (5%) patient Staphylococcus pyogenes – group A was isolated. One bacterium was isolated from all adenoid vegetations, except in one case when two bacteria (Haemophylus influenzae and Staphylococcus aureus) were concurrently isolated.

Conclusion: Our results have shown that the key role in adenoid vegetations in chronic otitis media with effusion and chronic rhinosinusitis is not only the mechanism of rhinopharyngeal obstruction, but also the presence of bacterial strains with a large potential for formation of biofilms adhered to their surface, especially in cases with symptoms of chronic otitis media with effusion and chronic rhinosinusitis that were resistant to antibiotic therapy.

Key words: adenoid vegetations, bacterial reservoir, chronic otitis media with effusion, chronic rhinosinusitis

Introduction
Otitis media with effusion and rhinosinusitis are commonly encountered illnesses in pediatric population. According to the literature 43%–47% of the children with diagnosed otitis media with effusion have concurrent sinusitis
[1, 2], and 23% of the patients with chronic sinusitis have otitis media with effusion [3]. A large percentage of the children (78%) with otitis media with effusion, who did not respond to the effect of the anti-inflammatory therapy, had concurrent rhinosinusitis. In children with a combination of otitis media and sinusitis microbiological findings from the middle ear, the effusions were identical with the sinus aspirates [4].

The failure of the antibiotic treatment in the eradication of suspicious organisms has motivated microbiologists to hypothesize the presence of a bacterial community organized and attached to the organic and inorganic surfaces, the so-called biofilm [5, 6]. A biofilm is a colony of one or several bacterial strains embedded in a polymer matrix of their own, which is composed of nucleic acids, polysaccharides and proteins. In comparison with the planktonic form, the biofilm matrix protects the microorganisms, provides better survival and protects them from the effect of the macrophages, antibiotics, fluctuations in temperature and pH. The successful survival of bacteria by biofilm formation is a subject of investigation of many authors [7–9], in order to define the role of the biofilm in the etiopathogenetic mechanism of the antibiotic resistance as well as in the chronic course of the upper respiratory tract inflammatory processes. The latest publications from the Center for Disease Control and Prevention have reported that 65% of the human bacterial infections involve biofilms [10].

The aim of this study was to determine the most common microorganisms in the tissue specimens of adenoid vegetations in patients with chronic otitis media with effusion (COME) and chronic rhinosinusitis (CRS) resistant to anti-inflammatory treatment, as a result of which a surgical intervention i.e. adenoid-deadenoidectomy was performed.

Material and methods

A total of 20 adenoid tissue specimens were examined. They were taken from children (12 boys and 8 girls of an average age of six years) hospitalized at the University Ear, Nose and Throat Clinic. The patients’ history revealed that all children had recurrent or chronic inflammation of the middle ear, and 9 of these children had concurrent chronic rhinosinusitis. Adenoidectomy was performed in 15 children and adenotonsillectomy in 5 children.

Each specimen was rinsed and put in a sterile bottle with physiologic solution and sent for microbiological examination and analysis at the Institute of Microbiology and Parasitology, Medical Faculty in Skopje. All specimens were analyzed with standard microbiological techniques. The specimens were inoculated on four solid and one liquid media. Each specimen was inoculated on the following media for bacteria isolation: blood agar (for isolation of aerobic bacteria), Schaedler agar (for isolation of anaerobic bacteria), chocolate agar (for isolation of Haemophilus), liquid medium – glucose broth (for faster bacterial growth) and Sabouraud agar for fungi isolation. All media were from the manufacturer Oxoid, UK. From each specimen a direct microscopic specimen was made and it was stained by Gram staining method showing the eventually present leukocytes and bacteria. Susceptibility to different groups of antibiotics was examined in all isolated bacteria by application of the standard disc diffusion method. The following antibiotic discs were tested: from the penicillin group – penicillin, ampicillin, cloxacillin, amoxicillin-clavulanic acid, vancomycin; cephalosporins – cefadroxil, cefpodoxime, ceftriaxone, cefotaxime; aminoglycosides – amikacin; lincosamines – clindamycin; macrolides – erythromycin; quinolone – ciprofloxacin and cotrimoxazole. All discs were from the manufacturer Oxoid, UK in concentrations corresponding to the CLSI (Clinical and Laboratory Standard Institute) standards. The results from the disc diffusion method were also interpreted according to the CLSI standards.

The statistical analysis was made with the statistical program SPSS for Windows, 17.0. The data are presented as absolute or relative numbers. For comparison of the isolates found in children with COME and with COME and CRS Fischer’s exact, two-tailed test was used. The values of p < 0.05 were considered to be statistically significant.

Results

The microbiological results were positive in all 20 adenoid tissue specimens. In the lar-
gest percentage (35%) of specimens *Haemophilus influenzae* was isolated, followed by *Streptococcus pneumoniae* (30%), *Moraxella catarrhalis* (20%), *Staphylococcus aureus* (10%) and *Streptococcus pyogenes* – group A (5%).

The statistical analysis of the presence of different types of bacteria showed no significant difference of *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Moraxella catarrhalis* and *Staphylococcus aureus* between the group of children with COME and children with both COME and CRS.

*Haemophilus influenzae* and *Streptococcus pneumoniae* were more frequently isolated from adenoids of children with COME and CRS while *Streptococcus pyogenes*, *Moraxella catarrhalis* and *Staphylococcus aureus* were more frequently isolated from adenoids of children with COME (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Number of isolates-COME (n = 20)</th>
<th>Number (%) of isolates-COME+CRS (n = 9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. influenzae</em></td>
<td>8 (40%)</td>
<td>4 (44.5%)</td>
<td>p = 1.0</td>
</tr>
<tr>
<td><em>St. pneumoniae</em></td>
<td>6 (30%)</td>
<td>4 (44.5%)</td>
<td>p = 0.67</td>
</tr>
<tr>
<td><em>St. pyogenes</em></td>
<td>1 (5%)</td>
<td>0</td>
<td>p = 1.0</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>4 (20%)</td>
<td>1 (11%)</td>
<td>p = 1.0</td>
</tr>
<tr>
<td><em>St. Aureus</em></td>
<td>2 (10%)</td>
<td>0</td>
<td>p = 1.0</td>
</tr>
</tbody>
</table>

P (Fisher exact, two tailed)

The results obtained for the susceptibility of microorganisms to certain antibiotics showed that *Haemophilus influenzae*, *Streptococcus pyogenes* and *Staphylococcus aureus* strains were susceptible to all tested antibiotics, except to cotrimoxazole. Mild susceptibility and resistance to certain antibiotics was found for *Streptococcus pneumoniae* and *Moraxella catarrhalis* isolates (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Pathogenic bacteria</th>
<th>Antibiotics – (S,LR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria/number of patients</td>
<td>Penicillin</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>4-R</td>
</tr>
<tr>
<td><em>St. pyogenes</em></td>
<td>1-S</td>
</tr>
<tr>
<td><em>St. Pneumoniae</em></td>
<td>6-1</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>7-S</td>
</tr>
<tr>
<td><em>St. aureus</em></td>
<td>2-S</td>
</tr>
</tbody>
</table>

Legend: S-susceptible; I-moderately susceptible; R-resistant

**Discussion**

Hypertrophic adenoid vegetations support the development of COME and COME causing mechanical obstruction of choanae and Eustachian tube, which results in secretion sta-

sis in nasal fossa and middle ear [11]. In addition to the effect of obstruction, recent investigations have emphasized that adenoids might act as a reservoir of pathogenic bacteria in both conditions, in COME and COM [12]. The re-
duction in the number of COME episodes fol-
lowing adenoidectomy in children > 3 years,
individually of the adenoid’s size, suggests
that the mechanical obstruction in the upper
respiratory tract is not the unique risk factor for
recurrent COME [13]. The debate about this
bacterial reservoir and its role in the etiology
of these two clinical entities is still open. The
results presented in one study including a large
number of children showed that COME and
CRS were found in 11% of the total number of
children who did not have adenoids, and the
percentage was significantly increased up to
50% in children with adenoid vegetations [14].

Recently a group of authors introduced a
fluorescent in situ hybridization (FISH), and
immunostaining combined with confocal laser
scanning microscope to prove that bacterial
strains in adenoid and tonsil tissue specimens
were identical with the pathogens commonly
isolated in patients with chronic infections
[15]. Other authors have demonstrated that the
percentage of Haemophilus influenzae de-
creases with the increasing age [16].

In the study of Galli et al. [17] biofilms
were identified in 100% of samples, and Haem-
ophilus influenzae was the most commonly
isolated bacteria in adenoid tissue.

One study has shown that Haemophilus
influenzae has a large propensity of in vitro
biofilm formation. This observation might be
associated with the physiologic state that dif-
fers from that of the bacteria growing in a plank-
tonic form, which might explain the unres-
ponsiveness to antibiotic treatment [18]. As an
opportunistic pathogenic bacterium Haemoph-
philus influenzae coexists with other organisms
in the nasopharynx. Its transmission into path-
gen happens most probably due to variations in
the protection system such as alterations in
mucocilliary transport and Eustachian tube dys-
function [19].

In this study there was no statistically
significant difference in the incidence of Haem-
ophilus influenzae, Streptococcus pneumoniae,
Streptococcus pyogenes and Staphylococcus
aureus, M. catarrhalis and St. aureus between
children with COME and children with both
COME and CRS suggesting interference of
these two conditions with adenoids from
which these potentially pathogenic bacteria
were isolated.

Our results are similar with other presen-
ted in a large number of studies indicating that
adenoidectomy is effective in the treatment of
COME and CRS, having in mind that adenoids
act as a reservoir of pathogenic bacteria and
play an important role in the pathogenesis of
these two conditions [13, 20–22].

The analyses about the susceptibility of
microorganisms to certain antibiotics have
shown that Haemophilus influenzae, Strepto-
coccus pyogenes, Moraxella catarrhalis and
Staphylococcus aureus were susceptible to all
tested antibiotics, except to cotrimoxazole to
which Haemophilus influenzae and Strepto-
coccus pyogenes were resistant. Mild suscep-
tibility and resistance to certain antibiotics was
observed in Streptococcus pneumoniae and
Moraxella catarrhalis isolates. This result sug-
gests the need for treatment of these conditions
in line with the microbiological finding and
antiogram.

Conclusion
The treatment of COME and CRS in the
pediatric population is still an issue under dis-
cussion among the specialists in otorhinolary-
gology and microbiology.

In this study the microbiological findings
obtained from the adenoid tissue specimens of
patients with COME and CRS have indicated
that isolated bacteria might form a biofilm,
which on the other hand might be a reservoir
of pathogens for COME and CRS. This has a sub-
stantial diagnostic-therapeutic importance.

Based on the results obtained we assume
adenoidectomy, independently on the size of
the adenoid vegetation, to be justifiable in chil-
dren with COME and CRS.

The results of in vitro tests of bacterial
susceptibility to certain antibiotics have poin-
ted out the need of a greater collaboration be-
 tween the otorhinolaryngologists and microbio-
logists in order to suggest the most adequate
antibiotic agent in treatment of chronic rhinosi-
nitis and otitis media.

To prevent the ability of bacteria to form
a biofilm as well as to prevent biofilm forma-
tion it is necessary to develop a biofilm-related
method. All methods for detection of biofilms are not available for routine work, except in highly specialized research centers. There are still no methods for routine detection of biofilms and hence the effective intervention depends on the quality of the clinical diagnosis.

REFERENCES


Резиме

АДЕНОИДИ ВЕГЕТАЦИИ – РЕЗЕРВОАР НА БАКТЕРИИ ЗА ХРОНИЧЕН ОТИТИС МЕДИЈА СО ЕФУЗИЈА И ХРОНИЧЕН РИНОСИНУСИТС

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Вовед: Риносинуситис и отитис медија се заболувања кои многу често се сретнуваат во педијатриската популација. Во литературата јасно е потврдена асоцијацијата помеѓу овие две состојби и дури е потврдено совпаѓање на инв-
ните микробиолошки наоди. До неодамна се сметаше дека клучен фактор што ги поврзува овие две состојби е хипертрофичното аденоидно ткиво, но од пред неколку години литературата опишува присуство на бактерски биофилм на аденоидите, што сугерира дека клучен фактор што ги поврзува овие две состојби е хипертрофичното аденоидно ткиво, но од пред неколку години литературата опишува присуство на бактериски биофилм на аденоидите, што сугерира дека биофилмот, истакнат, е одговорен за двете состојби за хроничен отитис медија со ефузија и за хроничен риносинуситис.

Цел: Оваа студија имаше цел да се утврди и за се направи микробиолошка анализ на аденоидното ткиво кај пациенти со отитис медија и хроничен/или рекурентен риносинуситис и да се одреди нивниот потенцијал за формирање на биофилм.

Методи: По спроведена хируршка интервенција, аденоидектомија, беше направена микробиолошка евалуација и анализа на аденоидното ткиво кај 20 пациенти. Според податоците од историјата на болеста, кај сите 20 пациенти беше дијагностициран хроничен отитис медија со ефузија, а кај деветима од вкупниот број пациенти беше верифициран хроничен риносинуситис.

Резултати: Резултатите од микробиолошката анализа покажаа дека аденоидните содржат многу потенцијално патогени бактерии, кои се поклопуваат со најчестите организации инкорпорирани во етнопатогенезата на двете состојби риносинуситис и отитис медија со ефузија. Кај седумина (35%) пациенти беше изолиран *Haemophylus influenzae*, кај шестмина (30%) беше изолиран *Streptococcus pneumoniae*, а потоа следувала *Moraxella catarrhalis* изолирана од четворица (20%) пациенти, *Staphylococcus aureus* кај двадесета пациенти (10%) беше изолиран и *Streptococcus pyogenes* кај еден пациент (5%). Од сите аденоидни вегетации беше изолирана една бактерија, освен во еден случај кога беше исто времено изолирана двете бактерии: *Haemophylus influenzae* и *Staphylococcus aureus*.

Заклучок: Резултатите од овој труд покажуваат дека клучната улога на аденоидните вегетации кај хроничните риносинуситис и отитис медија со ефузија не е само механизмот на ринофарингеална опструкција, туку и присуството на бактериски соеви со голем потенцијал за формирање биофилм на нивната површина, особено кај случаи со симптоми на хроничен риносинуситис и хроничен отитис медија со ефузија, кои беше отпорни на антибиотска терапија.

Ключни зборови: аденоидни вегетации, бактериски резервоар, хроничен отитис медија со ефузија, хроничен риносинуситис