Assessment of the Streptococcus milleri group on Adenoid Tissues

Jerin K. Joseph, BS, Patrick J. Antonelli, MD, William O. Collins, MD, Rodrigo C. Silva, MD, and Carolyn O. Dirain, PhD

Department of Otolaryngology, College of Medicine
University of Florida, Gainesville, FL

ABSTRACT

Objectives: To determine if the Streptococcus milleri group (SMG) constitutes a greater microbial burden in adenoid tissues from patients with recurrent acute otitis media (RAOM) or chronic otitis media with effusion (COME) compared to patients with obstructive sleep apnea (OSA). Study Design: Controlled, ex vivo. Methods: Human adenoids were obtained from children undergoing adenoidectomy for RAOM (n=7), COME (n=5), or OSA (n=16). Specimens were processed for total deoxyribonucleic acid (DNA) isolation. All specimens were analyzed by real-time polymerase chain reaction for the quantification of the S. milleri group and total bacterial load.

Results: All adenoid specimens had evidence of microbes with levels that were not different between the three groups (p=0.20). DNA levels of S. anginosus and S. intermedius/S. constellatus were not different in adenoids from RAOM patients as compared to those with OSA. In contrast, adenoids from patients with COME displayed higher DNA levels of S. anginosus (p=0.005) and S. intermedius/S. constellatus (p=0.02) compared to those with RAOM or OSA.

Conclusions: The microbial load of the S. milleri group appears to be higher in patients with COME. The role of the S. milleri group in the pathogenesis of COME warrants further exploration.

INTRODUCTION

Inadequately treated RAOM or COME is thought to lead to chronic supplicative otitis media.1 The pathogens commonly involved in RAOM and COME are Streptococcus pneumoniae and Haemophilus influenzae,2 whereas the pathogens commonly associated with chronic supplicative otitis media include Pseudomonas aeruginosa and Staphylococcus aureus.3 The presence of chronic pathogens in the middle ear has long been linked to the clinical disease. Human adenoid tissues are thought as a likely reservoir for bacteria4 and the nasopharynx has been shown in animal models to be a route for otitis media infection with chronic pathogens.5,6 However, it remains unclear what leads to middle ear infection with chronic pathogens.

The Streptococcus milleri group is a subgroup of viridans streptococci that includes S. anginosus, S. intermedius, and S. constellatus.7 The S. milleri group is commonly found in the upper aerodigestive tract and has implicated in the exacerbation of a variety of serious infections.8 Biofilms that harbor S. milleri bacteria are thought to be involved in many infectious diseases, such as the exacerbation of cystic fibrosis (CF). Therefore, we hypothesized that the S. milleri group might be involved in the transition from RAOM and COME to chronic supplicative otitis media.

Adenoid tissue is readily available after an adenoidectomy, which is commonly performed for patients with RAOM, COME and obstructive sleep apnea (OSA), allowing for comparison of children at risk of developing chronic supplicative otitis media against those that are not. The purpose of this study was to determine whether the S. milleri group constitutes a greater microbial burden in adenoid tissues from patients with RAOM or COME compared to patients with OSA.

RESULTS

A total of 28 samples were obtained from children undergoing adenoidectomy for RAOM (n=7), COME (n=5), or OSA (n=16). All adenoid specimens had evidence of microbes with levels that were not different between the three groups (p=0.20; data not shown). The threshold cycle (Ct) of 16S was used to normalize the Ct for S. anginosus and S. intermedius/S. constellatus. When samples were analyzed for S. anginosus, we found that the 16S-normalized Ct for S. anginosus was significantly lower (p=0.005) in the adenoids from patients with COME compared to adenoids from OSA or RAOM patients, indicating higher DNA levels of S. anginosus in COME adenoids (Figure 1).

Figure 1. S. anginosus in adenoids measured by real-time PCR. S. anginosus threshold cycle (Ct) values were normalized by total bacterial load (16S Ct). A lower Ct indicates higher amount of target DNA in the sample. Adenoids from COME patients have higher DNA levels of S. anginosus compared to adenoids from OSA and RAOM patients (p<0.005). Error bars represent standard error.

CONCLUSIONS

The microbial load of the S. milleri group appears to be higher in patients with COME. The role of the S. milleri group in the pathogenesis of COME warrants further exploration.

REFERENCES

8. Antonelli PJ, et al. S. anginosus is associated with chronic supplicative otitis media: A lower Ct indicates higher amount of target DNA in the sample. Adenoids from COME patients have higher DNA levels of S. anginosus compared to adenoids from OSA and RAOM patients (p<0.005). Error bars represent standard error.

Figure 2. S. intermedius/S. constellatus in adenoids measured by real-time PCR. Threshold cycle (Ct) values were normalized by total bacterial load (16S Ct). A lower Ct indicates higher amount of target DNA in the sample. Adenoids from COME patients have higher DNA levels of S. intermedius/S. constellatus compared to adenoids from OSA and RAOM patients (p<0.02). Error bars represent standard error.

Figure 3. Scanning electron micrographs of adenoids. Eukaryotic cells (3.5 to 5μM) predominate but cocci-shaped microbes (about 0.5 to 1μM, indicated by orange arrows) appear to be present in adenoids from all three groups.