A new horizon in the treatment of biofilm-associated tonsillitis

Z. Ciftci¹., O.N. Develioglu.¹, <u>S. Arbak S.²</u>, T. Ozdoganoglu .¹, E. Gultekin.³

1. Taksim Training and Research Hospital, Department of Otorhinolaryngology Taksim Istanbul Turkey

2. Acıbadem University, Medical Faculty, Department of Histology and Embryology, Maltepe Istanbul Turkey

3. Namık Kemal University, Medical Faculty, Department of Otorhinolaryngology, Tekirdag Turkey

arbaks@yahoo.com

Key words: tonsillitis, biofilm, scanning electron microscopy

Infections of the palatine tonsils are ranking first among the diseases affecting both the children and adults. [1, 2]. Tonsillitis is a challenging medical condition both for the patient and physician due to its possible early and late complications and high rate of treatment failure. The causative agents of the acute bacterial tonsillitis still could not be totally eradicated. The mechanisms of this bacterial persistence have been thoroughly investigated and several theories have been proposed to explain the reason of this treatment failure.(4).Among these theories, the most recent and probably the most intriguing theory is the biofilm theory. According to this theory, the underlying pathology of chronic tonsillitis is the presence of biofilm bound to the surface of the palatine tonsils. [3].

In our study we used scanning electron microscopy (SEM) to show the behavior of the biofilm on the tonsillectomy specimens against various surface cleaning methods. Our aim was to assess the effect of rinsing and brushing on the biofilm layer of the palatine tonsils.

Patients suffering from chronic tonsillitis who attended to Taksim Training and Research Hospital were enrolled in this study. The decision to perform a tonsillectomy was based on the Paradise criteria [4]. Randomly selected sixteen male and nine female patients were enrolled in this study. The ages of the patients ranged between 4 and 42. In order to create a homogeneous sample group, we have excluded the patients with a history of acute tonsillitis or antibiotic use within the last month. The tonsillectomy specimens taken from each patient were divided into four portions using a scalpel no 15 under sterile conditions.

Each of four portions has undergone four distinct procedures. Tissue specimens from the first group were processed for routine scanning electron microscopy. The specimens in the second group were rinsed thoroughly for 10 seconds in a saline containing sterile container. The third group has also undergone the same procedure as the second group but before that, a soft brush was used to brush the surface of the tonsils for a period of 30 seconds. The specimens in the fourth group were brushed using a hard brush before the rinsing procedure. After these procedures, all the tissue specimens from 2nd, 3rd and 4th groups were immediately transferred for scanning electron microscopy. For scanning electron microscopy, tissue samples were prefixed for 2 hours in 2 % phosphate - buffered glutaraldehyde solution. (0.1 M, ph 7.2) and post - fixed for 1 hour in 1 % phosphate - buffered osmium tetroxide solution and passed from increasing alcohol and amyl acetate series. After drying the tissue samples with Bio - Rad ''Critical Point Dryer'' and gold coating with a Bio-Rad Sputter Coater (SC 502)(Bio-Rad, United States). Tissue samples were examined under a JEOL 5200 JSM scanning electron microscope (JEOL Tokyo, Japan).

The examination of the first group of tonsils which were neither brushed nor rinsed revealed a thick layer of biofilm on the mucosal surface. The biofilm layer could be seen on the surface of 20 specimens (80 %). Erythrocytes and fibrin residues were seen to accompany the biofilm layer. This group served as the control group of the study The second group of tonsils which were only rinsed showed a more homogenous surface appearance devoid of any blood cells or fibrin residues but the integrity of the biofilm layer was still not breached. Biofilm layer could be readily seen in 21 specimens (84%). However, despite rinsing, a few erythrocytes and fibrin residues still could be seen in 10 % of the samples. It was impossible to visualize any structural components associated with surface epithelium Scanning electron microscopical investigation of the third group which were rinsed following gently brushing by a soft teeth brush revealed a decreased biofilm layer. However, in 20 % of the samples, the continuous biofilm layer could still be observed and the tonsil epithelium could not be seen. The fourth group of tonsils was almost devoid of a biofilm layer except for scattered small biofilm fragments. 100 % of the samples showed complete defragmentation of the biofilm layer.

The presence of biofilm was suggested as the underlying mechanism of chronic tonsillitis and this hypothesis was supported with microscopic evidence of biofilm in the crypts of tonsils crypts using transmission electron microscopy. It is interesting to note that, only rinsing or rinsing and mildly brushing could not remove the biofilm layer on the tonsillectomy specimens. Using a harder brush resulted in disintegration and removal of biofilm layer from the tonsil surface. These findings may alter our approach in the treatment of chronic tonsillitis, a biofilm associated infection. As a result, a tendency to focus on local treatment modalities may be expected. However, further studies need to be performed to assess the effects of brushing on the tonsils in vivo.

Our findings may lead to revolutionary changes in the treatment of chronic tonsillitis and give rise to new treatment modalities focusing on local treatment of biofilm associated infections.

- 1. S. Wagner, et al., Clin Padiatr. 205 (1993) p14
- 2. H.G. Birnbaum, et al., Chest. 122 (2002) p 603
- 3. R. A. Chole RA et al., Arch Otolaryngol Head Neck Surg. 129 (2003) p 634
- 4. J.L. Paradise et al., N EnglJ Med. 310 (1984) p 674