



Relationship between gradation density of biofilm bacteria with tonsillar hypertrophy on patients with chronic tonsillitis

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Abstract

Background: Tonsillar hypertrophy, which is an indicator of tonsillectomy, is less accurate in making indications, so more specific indicators are needed. In chronic tonsillitis, biofilm bacteria are found which can cause antibiotic resistance. The presence of biofilm bacteria in chronic tonsillitis causes antibiotic drugs to not work properly, which is indicated to be the cause of delayed recovery in chronic tonsillitis. **Purpose:** Knowing the relationship between the gradation of biofilm bacterial density and tonsillar hypertrophy in patients with chronic tonsillitis. **Method:** This study was an observational analytic study with a cross-sectional study design. All patients with chronic tonsillitis who undergo tonsillectomy were selected by consecutive sampling. Gradations of tonsillar hypertrophy and bacterial biofilm were examined by scanning electron microscopy (SEM). **Result:** The results of biofilm density gradation from 26 samples obtained grade 0 results of 1 (3.85%) patient with T2 tonsils, class 1 as many as 1 (3.85%) patient with T2 tonsils. Class 2 were 2 (7.69%) patients with T2 tonsils and 3 (11.54%) patients with T3 tonsils. Class 3 were 1 (3.85%) patient with T2 tonsils, 7 (26.92%) patients with T3 tonsils and 1 (3.85%) patient with T4 tonsils. Class 4 (biofilms > 76%) as many as 2 (7.69%) patients with T3 tonsils and 8 (30.76%) patients with T4 tonsils. **Conclusion:** There is a relationship between the gradation of biofilm bacterial density and tonsillar hypertrophy in patients with chronic tonsillitis. The higher the gradation density of biofilm bacteria, the higher the gradation of tonsillar hypertrophy.

Keywords: chronic tonsillitis, tonsillar hypertrophy, density gradation of biofilm bacterial, scanning electron microscopy

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INTRODUCTION

Tonsillar hypertrophy has been used as one of the main indicators of tonsillectomy, but some clinical experience has shown that subsequent evaluation of tonsils has diminished with conservative therapy. This fact informs that tonsillar hypertrophy is less accurate in determining indications of tonsillectomy, therefore more specific indicators are needed (Brodsky, 2001. Bista, et al. 2005). Chronicity from tonsillitis can occur due to sufferers often experience Acute Respiratory Infections (ARI) or acute tonsillitis that is not treated adequately.

Electronic medical record (EMR) data on Outpatient Unit of the ear nose, throat, head surgery (ENT-TH) Dr. Soetomo regional public hospital Surabaya based on the international classification of diseases-10 (ICD-10) in 2013-2015, states that there were 141 new chronic tonsillitis patients, only 80 patients or 56.7% who underwent tonsillectomy surgery in the same year period. This data shows that tonsillectomy has not yet become a therapeutic choice for sufferers. One feature

of chronic tonsillitis is enlargement or hypertrophy of the tonsils (Brodsky, 2001).

Recent research shows that there are deposits of biofilm bacteria in infected tonsillar tissue. Biofilm itself consists of microbial cells and extracellular polymeric substance (EPS), which generally have a size of 5-500 μm (Agrippina, Widiyanti, & Yusuf, 2017. Kriswandini, et al. 2019). These biofilm bacteria presence indicates the basis of the chronic pathophysiology mechanism of tonsillitis (Ciftci, et al. 2014. Chole, & Faddis, (2003). The presence of biofilm bacteria plays an important role in the pathophysiology of chronic tonsillitis (Ciftci, et al. 2014. Chole, & Faddis, 2003. Alasil, et al. 2013). Biofilm bacteria can be 1000 times more resistant to antibiotics compared to free-living bacteria. Biofilm bacteria play a major role in chronic tonsillitis which is considered as

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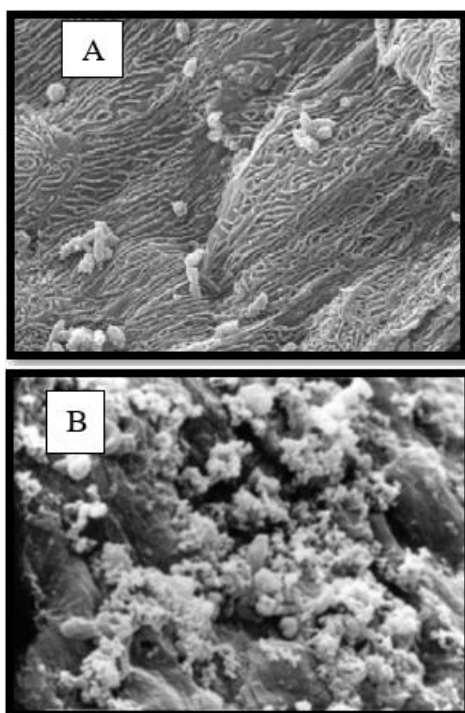


Fig. 1. SEM Examination; A. Negative Biofilm; B. Positive Biofilm

one of the most common pathologies found in children. Although the use of antibiotics is quite extensive, the recurrence of tonsillitis is still common (Alasil, et al. 2013; Sami, & Marin, 2017). The growth of biofilm bacteria will cause chronic infections which are characterized by inflammation and persistent tissue damage. The chronic infection persists will still be present despite antibiotic therapy had already done (Alasil, et al. 2013). Based on this, it is necessary to know the relationship between the presence of biofilm bacteria and tonsillar hypertrophy. This study aims to determine the relationship between the density of biofilm bacteria with tonsillar hypertrophy in patients with chronic tonsillitis.

METHODS

This research is an observational analytic study with a cross-sectional approach with a sample of 26 cases. Biofilm bacteria from tonsillar tissue were seen and validated using scanning electron microscopy (SEM) with a magnification of 2500 times. Tonsil tissue examined was in the form of fresh tissue (immediately after surgery) or a maximum of 72 hours after the surgery process with 2% glutaraldehyde fixation solution media at 4°C. On the SEM examination, it is determined whether there are bacterial biofilms or not. Biofilm is negative (-) if there are no biofilm bacteria and biofilm is positive (+) if there are bacterial biofilms as in **Fig. 1**.

Examination of biofilm bacterial density gradation at 150 times magnification is divided into several classes, namely class 0 if there is no biofilm, class 1 if there is

Table 1. Interpretation of correlation coefficients

Coefficients Interval	Relationship Level
0,00-0,199	Very Low
0,20-0,399	Low
0,40-0,599	Medium
0,60-0,799	Strong
0,80-1,000	Very Strong

Table 2. Assessment of Tonsil Hypertrophy Gradation Results

Tonsil Hypertrophy gradation	Amount	Percentage (%)
T1	0	0,00
T2	5	19,23
T3	12	46,15
T4	9	34,62
Total	26	100,00

<25% surface covered by biofilm, class 2 if there is 26-50% of the surface area covered by biofilm, class 3 if there is 51-75% of the surface is covered by biofilm, and grade 4 if 76-100% of the surface is covered by biofilm. Tonsillar hypertrophy gradations are classified based on previous studies, T0 if the tonsils are located in the tonsillar fossa, T1 if the proportion of tonsils to the oropharynx is <25%, T2 if the proportion of tonsils to the oropharynx is 25-50%, T3 when the proportion of tonsils to the oropharynx is 50-75%, and T4 if the proportion of tonsils to the oropharynx is > 75% (1).

Data presented and displayed in the form of distribution and frequency of hypertrophy gradations of patients with chronic tonsillitis and the results of the detection of bacterial biofilms. The type of statistic used is the Spearman test with $p < 0.05$. The strength of the relationship is determined based on the correlation coefficient which can be seen in **Table 1**.

RESULT

In this study, the youngest age was 4 years and the oldest age was 24 years. The average age is 11.96 years. Age distribution of patients is 6-10 years old, as many as 10 (38.46%) patients. Male sex distribution recorded at 16 (61.54%) patients and 10 (38.46%) patients for the female. The ratio of men to women is 1.6: 1. The main complaint is the throat pain as many as 9 (34.62%) sufferers, followed by the throat blocking which was recorded at 7 (26.92%) sufferers, snoring 4 (15.38%) sufferers, nasal 3 (11.54%) sufferers, difficulty swallowing (dysphagia) 2 (7.69%) sufferers, and bad breath 1 (3.85%) sufferers.

The examination of tonsillar hypertrophy gradation is done before tonsillectomy. **Table 2** shows the results of the examination of tonsillar hypertrophy gradations in patients with chronic tonsillitis and are categorized into T1, T2, T3, and T4. The results of gradual assessment of tonsillar hypertrophy obtained T2 results in 5 (19.23%) patients, T3 in 12 (46.15%) patients, T4 in 9 (34.62%) patients, no patients were included in the T1 category. The results of bacterial biofilms examination from tonsillectomy surgery tissue using SEM shown in **Table**

Table 3. Biofilm Bacteria Assessment Results

Biofilm Bacteria	Amount	Percentage (%)
Positif (+)	25	96,15
Negatif (-)	1	3,85
Total	26	100,00

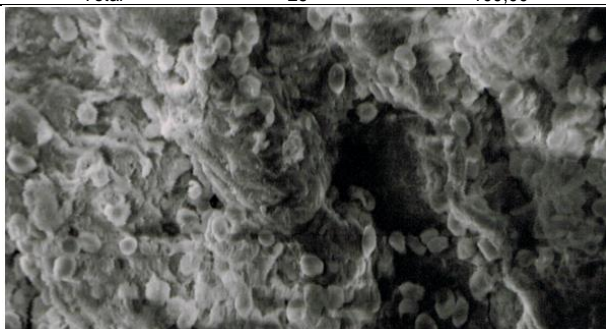


Fig. 2. The results of the examination of infected palatine tonsillar tissue using SEM with magnification 1000 times, obtained positive biofilm bacterial results. (1) Biofilm bacteria, which appear in the form of coccus which are piled and surrounded by EPS matrix; (2) EPS matrix indicates the presence of biofilms

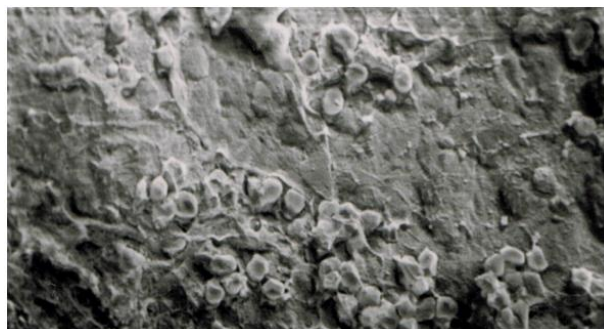


Fig. 3. The examination result of infected palatine tonsillar tissue using SEM with 1000 times magnification, negative biofilm bacteria results were obtained. (1) Coccus-shaped bacteria without biofilms; (2) Palatine tonsillar tissue

3. Results of examination of 26 tonsillectomy surgery tissues using SEM obtained positive biofilm bacteria (+) in 25 (96.15%) tissues and negative biofilm bacteria (-) in 1 (3, 85%) tissue.

Examination results revealed that the biofilm bacteria were positive if the bacteria appeared to be concentrated in the interface area (usually between solid and liquid media) and surrounded by the EPS matrix.

Biofilms are characterized by a three-dimensional complex of bacteria enclosed in the self-producing extracellular matrix of polysaccharides, nucleic acids, proteins, and extracellular DNA (**Fig. 2**). The examination results stated that biofilm bacteria were negative if there was no EPS matrix obtained (**Fig. 3**).

The examination result of biofilm density gradations from tonsillectomy surgery tissue using SEM shown in **Table 4**. The results of 26 tonsillectomy surgery tissues using SEM obtained grade 0 biofilm density gradation in 1 (3.85%) tissue, class 1 in 1 (3.85%) tissue, class 2 in 5 (19.23%) tissues, class 3 in 9 (34.61%) tissues, and class 4 in 10 (38.46%) tissues (**Fig. 4**). The analysis

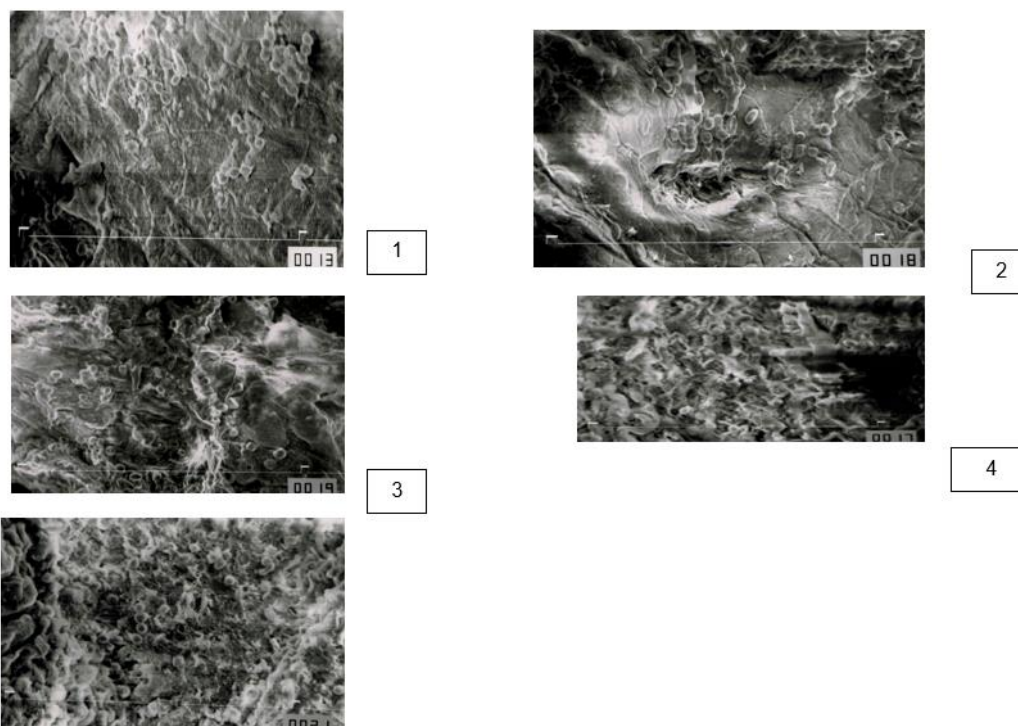


Fig. 4. Examination result of biofilm density gradation using SEM with a magnification of 150 times. (1) Class 0 does not have biofilms; (2) Class 1 recorded at <25% of the surface is covered with biofilm; (3) Class 2 recorded at 26-50% of the surface covered by biofilm; (4) Class 3 at 51-75% of the surface is covered by biofilm; (4) Class 4 at 76-100% of the surface is covered by biofilm

Table 4. The Density of Biofilm Gradation Assessment Results

Biofilm Gradation	Amount	Percentage (%)
Class 0	1	3,85
Class 1	1	3,85
Class 2	5	19,23
Class 3	9	34,61
Class 4	10	38,46
Total	26	100,00

Table 5. The Analysis Result of The Relationship of Biofilm Bacterial Density Gradation with Tonsillar hypertrophy

Tonsillar Hypertrophy Gradation	Biofilm Density Gradation					Total
	0	1	2	3	4	
T2	1 20,0%	1 20,0%	2 40,0%	1 20,0%	0 0,0%	5 100%
T3	0 0,0%	0 0,0%	3 25,0%	7 58,3%	2 16,7%	12 100%
T4	0 0,0%	0 0,0%	0 0,0%	1 11,1%	8 88,9%	9 100%
Total	1 3,8%	1 3,8%	5 19,2%	9 34,6%	10 38,5%	26 100%

results of the relationship of biofilm bacteria from tonsillectomy surgery tissue using SEM and tonsillar hypertrophy gradations on 26 patients with chronic tonsillitis shown in **Table 5**.

The results of statistical analysis with the Spearman test obtained a significance value of 0,000 <0.05 and a correlation coefficient of 0.795. Based on these results, there is a strong positive relationship between biofilm bacterial density gradation with tonsillar hypertrophy in patients with chronic tonsillitis ($p < 0.05$). The higher the gradation density of biofilm bacteria, the higher the gradation of tonsillar hypertrophy (**Fig. 5**).

DISCUSSION

Significant differences of biofilm bacterial density gradation between recurrent tonsillitis and the control group indicate that biofilms can be inside the tonsils without causing obvious symptoms so that greater biofilm development is needed to cause symptoms of an infection (Homenta, 2016). Previous studies using light microscopy and transmission electron microscope

(TEM) to examine 19 tonsils, tissues were taken through tonsillectomy to overcome previous recurrent infections or alleviate airway obstruction due to hypertrophy. Amorphous polysaccharide matrices and biofilm bacteria were found in tonsillar crypts on 11 of the 15 infected tonsils and bacteria found in smaller groups of 3 hypertrophic tonsils (Chole, & Faddis, (2003).

In a more recent study, a confocal laser scanning microscope (CLSM) with multiple fluorescence staining was used to examine 24 tonsils taken from children with previous chronic or recurrent tonsillitis and obtained bacterial biofilms in 17 of 24 specimens (70.8%). Biofilm bacteria are shown in most patients with chronic acute respiratory infections that fail to be treated aggressively. Streptococcus pyogenes infection that fails to be treated with antibiotics can be associated with biofilm formation (Bakaletz, 2012). Other studies that support the results of this study, found that biofilm bacteria act as a reservoir in causing persistent infections that cause enlargement or hypertrophy of the tonsils (Alasil, et al. 2013). Symptoms that indicate biofilm bacteria are hoarseness, hypertrophy of the tonsils and adenoids, obstructive sleep apnea, and cervical adenopathy (Diaz, et al. 2011).

The results of this study are also supported by other studies on different organs which show that the high density of biofilm bacteria (grades 3 and 4) is significantly proportional to the severity of symptoms and sufferers or patients of chronic rhinosinusitis (Atay, et al. 2013). Other studies that support the results of this study indicate that there are bacterial biofilms in pediatric patients with chronic exacerbation of chronic tonsillitis. Tonsillar hypertrophy gradations are important indicators of the presence of biofilm bacteria, but they have not been able to answer whether biofilm bacteria are a causative factor or a consequence of chronic exacerbation chronic tonsillitis (acute exacerbation tonsillitis. Torretta, et al. 2013).

Biofilm bacteria found in all tonsillar hypertrophy confirms that tonsillar hypertrophy is one of the

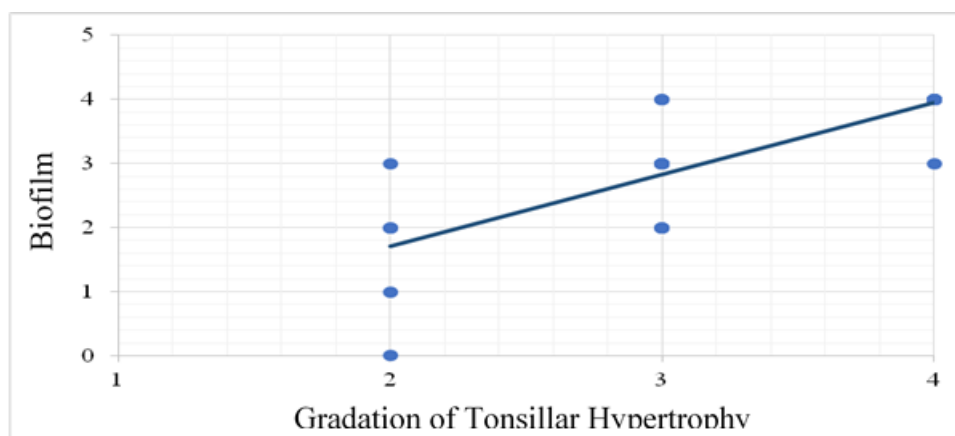


Fig. 5. The curve of the relationship between biofilm bacterial density gradation with tonsillar hypertrophy in patients with chronic tonsillitis

important symptoms associated with the presence of biofilm bacteria. In tonsillar hypertrophy, there is an increase in the number of lymphatic follicles related to the presence of bacterial biofilm infection. Biofilm bacteria have too large structures to be swallowed by host macrophages, consequently, the presence of biofilm bacteria in the tonsils will disrupt the normal functioning of the tonsil lymphatic tissue, causing chronic or recurrent infections (Alasil, et al. 2013). The density of biofilm bacteria in the SEM examination material can be determined based on the presence of clusters and towers embedded in the EPS matrix with a diameter of 0.5-2 μm attached to the surface. Grading is made based on the maximum visible field with a magnification of 75-150x which is equivalent to 12.25 mm^2 area (Atay, et al. 2013).

Based on the data in this study, tonsillar hypertrophy in the T3 and T4 categories in patients with chronic

tonsillitis strengthens the indication for tonsillectomy. This is because the biofilm bacterial density gradation at the size of the tonsils is relatively high, can be a cause of failure of medical therapy. While the T2 category or smaller still does not have a strong indication of tonsillectomy because the biofilm bacterial density is still relatively low.

CONCLUSION

There is a strong positive association or relationship between the gradation of biofilm bacterial density and tonsillar hypertrophy in patients with chronic tonsillitis. The higher the gradation of biofilm bacterial density, the higher the gradation of tonsillar hypertrophy. High bacterial density in grades 3 and 4 is also directly proportional to the severity of tonsillitis.

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