BIOFILM BACTERIA PLAYS A ROLE IN CSOM PATHOGENESIS AND HAS SIGNIFICANT CORRELATION WITH UNSAFE TYPE CSOM

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ABSTRAK

Otitis media supuratif kronik (OMSK) dapat menyebabkan morbiditas dan mortalitas sehingga sampai sekarang masih menjadi masalah kesehatan utama di seluruh dunia. Angka kejadian OMSK di Indonesia (1994-1996) diperkirakan sekitar 8,36 juta penderita dan prevalensi OMSK secara umum sekitar 3,8% (Helmi 2005). Penelitian ini bertujuan membuktikan adanya bakteri biofilm pada penderita OMSK tipe aman dan tipe bahaya yang dilakukan operasi mastoidectomy. Rancangan penelitian ini adalah analitik observasional dengan pendekatan cross sectional. Penelitian dilakukan di Ruang Teratai, IBP RSUD Dr. Soetomo, dan UPT Mikroskopi Elektron FK Unair Surabaya, mulai November 2013 hingga Juni 2014. Sampel berupa jaringan patologis yang diambil secara consecutive sampling dan dilakukan pemeriksaan bakteri biofilm dengan SEM. Hasil SEM dikategorikan bakteri biofilm positif dan negatif. Bakteri biofilm dinyatakan positif bila tampak gambaran tiga dimensi bakteri dengan bentuk dan ukuran yang jelas dan bergerombol, adanya bentukan bahan amorf yang terdiri dari bahan glycocalyx mengelilingi bakteri, dan adanya perlekatan permukaan. Bakteri biofilm dinyatakan negatif bila tidak didapatkan bentukan gerombolan bakteri, glycocalyx dan perlekatan permukaan. Penelitian ini dilakukan pada 33 sampel penderita OMSK yang dibagi OMSK tipe bahaya (17 penderita) dan OMSK tipe aman (16 penderita). Bakteri biofilm positif didapatkan pada 12 penderita tipe bahaya (75%) dan 6 penderita tipe aman (35,35%). Bakteri biofilm negatif didapatkan pada 4 penderita tipe bahaya (25%) dan 11 penderita tipe aman (67,9%). Uji regresi logistik didapatkan nilai p = 0,027, menunjukkan bakteri biofilm memiliki hubungan bermakna dengan OMSK tipe bahaya (p < 0,05). Simpulan, bakteri biofilm berperan pada patogenesis OMSK dan bakteri biofilm berhubungan bermakna dengan OMSK tipe bahaya. ***(FMI 2015;51:208-213)***

Kata kunci: *otitis media supuratif kronik, bakteri biofilm, tipe aman, tipe bahaya,*

ABSTRACT

Chronic suppurative otitis media (CSOM) may cause severe morbidity and mortality and remains a major health problem worldwide. The incidence of CSOM in Indonesia (1994-1996) is estimated at about 8.36 million people and CSOM general prevalence is 3.8% (Helmi 2005). This study aims to prove the existence of bacterial biofilm in patients with safe type and hazard type CSOM from mastoidectomy. The design was observational analytic with cross sectional approach. This study was conducted at the Teratai Wards, IBP Dr. Soetomo Hospital, and Electron Microscopy Unit, Faculty of Medicine, Airlangga University Surabaya, from November 2013 to June 2014. Samples of pathological tissues were taken by consecutive sampling and bacterial biofilms examination was done by SEM. SEM results categorized the biofilm bacteria as positive or negative. Biofilm bacteria tested positive when it shows three-dimensional representation of bacteria with clear shapes and sizes and clusters, the formation of amorphous material consisting of glycocalyx material surrounding the bacteria, and surface attachment. Biofilm bacteria was regarded as negative when there is no bacterial cluster, glycocalyx and surface attachment. This study was performed on 33 CSOM patients. Samples were divided into unsafe type CSOM (17 patients) and safe type CSOM (16 patients). Positive biofilm bacteria was found in 12 patients with unsafe type (75%) and 6 patients with safe type (35.35%). Negative biofilm bacteria was found in 4 patients with the unsafe type (25%) and 11 patients with safe type (67.9%). Logistic regression analysis revealed p value = 0.027, indicating the biofilm bacteria have a significant correlation with unsafe type CSOM (p <0.05). In conclusion, biofilm bacteria plays a role in CSOM pathogenesis of biofilm bacteria has significant correlation with unsafe type CSOM. ***(FMI 2015;51:208-213)***

Keywords: *chronic suppurative otitis media, biofilm bacteria, safe type, unsafe type*

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INTRODUCTION

CSOM frequently causes morbidity and mortality so that remains a major health problem worldwide (WHO 2004, Helmi 2005). The incidence of CSOM in Indonesia (1994-1996) was estimated at about 8.36 million people and CSOM general prevalence was 3.8% (Helmi 2005). Research at Dr. Soetomo Hospital in 2007-2008 obtained 61 CSOM cases subjected to mastoidectomy operations. The results of the evaluation at 2 months postoperatively showed 53% of the cases remained wet and 47% dry (Pramesthi & Ahadiah

2011). These recurring, even persisting, otorrhoeal problems havce driven the experts to conduct further research.

CSOM etiology is multifactorial. Bacteria infection is a major factor in inflammation that lasts longer on CSOM. Bacteria that cause CSOM type of *Pseudomonas aeruginosa* and *S. aureus* has the ability to secrete polysaccharides exopolymer which will form the glycocalyx matrix so that the bacteria will have a form of biofilm (Hall-Stoodley et al 2004). Bacterial biofilms have some defensive ability of an unfavorable environment that are resistant to antibiotics. Bacterial biofilms on CSOM can provide signals that result in increased turnover of epithelial cells and keratin production. This triggers the formation of cholesteatoma, resulting in complicated treatment (Drenkard 2003, Hall-Stoodley et al 2004, Morris & Hagr 2005).

Research using electron microscopy has been shown to indicate the presence of biofilms on medical devices such as tubal implants timpanostomy and chronic infection in the field of otorhinolaryngology and head and neck surgery, including CSOM. Studies on middle ear tissues in CSOM with cholesteatoma also found biofilms bacteria (Kumar & Prasad 2006, Saunders et al 2011, Lampikoski et al 2012). Until now, direct detection of bacterial biofilms in CSOM by Scanning Electron Microscopy (SEM) has never been done in Surabaya and Indonesia. This study sought to demonstrate correlation betwen the presence of biofilm bacteria and CSOM. The influence of the presence of biofilm bacteria can answer occurrence of CSOM resistance against systemic and topical antibiotics. Management with surgery for debridement and cleaning up patho-logic tissues is an effective standard treatment.

MATERIALS AND METHODS

The design of this study was cross sectional observational analytic study to determine correlation between biofilm bacteria and ith CSOM. The study population was CSOM patients who visited to Otorhinolaryngology Clinic, Dr. Soetomo Hospital, and underwent mastoidectomy in IBP Dr. Soetomo Hospital. The study samples were 33 tissues from patients with pathological tissue of safe type and hazard type CSOM patients while undergoing mastoidectomy. Inclusion criteria were pathological tissue of CSOM patient during surgery from all types of mastodectomy and the patient was willing to participate in the study by signing a statement of willingness (informed consent). Exclus-ion criteria were pathologically inadequate and damaged tissue unreadable with an electron microscope and the patient refused to follow the research.

Tools for collecting specimens (material examination) during mastoidectomy were curette and forceps. Examined tissues were put into fixative and sent in the cooler box. Biofilm bacteria were examined with scanning electron microscopy (SEM) brand JEOL, JSM-T100 Scanning Microscope. The specimen drier was Critical Point Drying (CPD) and tool coating was done with with Vacuum Evaporator. Materials needed were a solution of 2% glutaraldehyde fixation, phosphate buffer, osmic acid, various concentrations of alcohol, amyl acetate as a preservative and pure gold for coating. The study also requires data collection sheets and letters of approval to follow the study.

Patients who undergo surgery CSOM given an explanation of the purpose and procedures of the study and then signed the informed consent. Mastoidectomy specimen collection during the operation can be granulation tissue or cholesteatoma then inserted into the fixative and stored in the cooler. Specimens were sent to the Electron Microscopy Unit for detection of bacterial biofilms by SEM. Data obtained were displayed in the form of the distribution and frequency of CSOM patients before and during mastoidectomy and results. of biofilm bacteria detection. Inferential analysis was done to determine the existence of a significant relationship between biofilm bacteria and safe and unsafe types CSOM. Obtained nominal scale data were compiled, processed, analyzed and presented. The type of statistical analysis used was logistic regression.

RESULTS

The study was conducted between November 2013 and June 2014 to CSOM patients who underwent mastoidectomy at IBP, Dr Soetomo Hospital, Surabaya. Sample consisted of 33 surgical tissues from safe and unsafe types CSOM. The tissues were examined at Airlangga University Electron Microscopy Unit to obtain data on the existence of bacterial biofilm. All samples were analyzed descriptively to obtain basic data. Analysis was conducted to find correlation between biofilm bacteria and CSOM. Baseline data recorded in the study included age, gender, CSOM type, mastoidectomy type, and obtained tissue types. Age distribution is shown in Table 1.

In this study, the youngest age was 8 years old and the oldest 61 years old. Mean age was 25.78 years (SD = 13.90). CSOM patients were mostly in age group 11-20 years as many as 15 (45.45%). The following basic data is about sex distribution that can be seen in Table 2.

Table 1. Age distribution

Age (years)	Total	%
≤ 10	2	6.06
11 – 20	15	45.45
21 – 30	9	27.27
31 – 40	2	6.06
> 40	5	15.16
Total	33	100.00

Table 2. Sex distribution

Sex	Total	%
Male	17	51.50
Female	16	48.50
Total	35	100.00

Sex distribution showed male: female ratio was 1.1 : 1. CSOM types of the samples in this study are presented in Table 3 which shows patients with unsafe type CSOM comprised as many as 16 patients (48.48%) and safe type CSOM of 17 patients (51.51%).

Table 3. CSOM types

CSOM types	Total	%
Unsafe	16	48.48
Safe	17	51.52
Total	35	100.00

Table 4. Mastoidectomy and tissue types

Mastoidectomy surgery	Total	%
Types of surgery		
Canal wall up	12	36.36
Canal wall down	21	63.64
Types of tissue		
Granulation	17	51.52
Cholesteatoma	6	18.18
Granulation & cholesteatoma	10	30.30

Canal wall down surgery type was performed on 21 patients (63.64%) and canal wall up surgery was performed on 12 patients (36.36%). Granulation tissue was found in 17 patients (51.52%) and total tissue containing cholesteatoma was found in 16 patients (48.48%).

Table 5. Results of bacteria biofilms examination with SEM (n = 33)

Biofilm bacteria	Total	%
(+) positive	18	54.54
(-) negative	15	45.46

Positive (+) bacterial biofilm from surgical tissue examined was totally 18 tissues (54.54%) and negative (-) biofilm tissues were as many as 15 (45.46%). Table 6 shows correlation between biofilm bacteria and CSOM types. The analysis was conducted on the correlation. Logistic regression analysis was conducted to determine correlation significance between biofilm bacteria and CSOM type.

Table 6. Correlation between CSOM type and biofilm bacteria.

CSOM types	Biofilm bacteria		Total
	(+)	(-)	
Unsafe	12 (75.0%)	4 (25.0%)	16 (100%)
Safe	6 (35.3%)	11 (64.7%)	17 (100%)
Total	18 (54.5%)	15 (45.5%)	33 (100%)

Test results showed that in unsafe type CSOM 12 (75%) was positive (+) with biofilm bacteria and 4 (25%) negative (-) bacteria biofilm. Safe type CSOM showed 6 (35.35%) with positive (+) biofilm bacteria and 11 (67.9%) negative (-) biofilm bacteria. The statistical test of this study can be seen in Table 7.

Table 7. Logistic regression correlation between biofilm bacteria and cholesteatoma in CSOM

Variables	β	p	Risk Ratio
Unsafe type CSOM	1.705	0.027*	5.55
Safe type CSOM	(comparison)		

Logistic regression results had p = 0.027. As it was significant if p <0.05, it shows that there was significant correlation between bacterial biofilm with unsafe type CSOM. The risk ratio was found to be 5.55, indicating that unsafe type CSOM has a risk of bacterial biofilm formation as much as 5.55.

DISCUSSION

Characteristics of patient age in Table 1 indicate the highest number was in age group 11-20 years as many as 15 individuals (45.45%). They were from school-age group. The results are consistent with a sight and hearing health survey in 1994-1996 in seven provinces in Indonesia which showed prevalence of CSOM was a 3.1% where the age of patient population with middle ear infections was mostly 7-18 years, belonging to school-age group (Helmi 2005). The same results were obtained in studies in H. Adam Malik Hospital, Medan, in 2000-2001 that in 40 patients with CSOM there were 12 (33.33%) patients aged 12-20 years as the highest percentage (Nursiah 2003).

Age characteristics in this study were according to WHO data in which CSOM prevalence based on surveys in several countries found many patients in

school-aged children. The incidence of middle ear infections was mainly found in the first 6 years with a peak at the age of 2 years and decline along the children's age. Until now, the time of transition from OMA be CSOM is still controversial. However, the prevalence of CSOM is high in school age and young adults. CSOM incident with cholesteatoma in the United States was found in children aged 10-19 years as many as 9.2 cases in 100,000 population. Similarly, in Israel CSOM incident with cholesteatoma was in children aged 1-15 years as many as 39 cases per 100,000 population (WHO 2004, Meyer et al 2006).

Sex distribution in Table 2 shows the ratio of men to women is almost the same at 1.1: 1. These results are similar to previous studies by Wahyudiasih et al (2011) in Dr. Saiful Anwar Hospital, Malang, in 2005-2010 which reported that from 45 patients with mastoidectomy for CSOM 55.6% were male and 44.4% were female. A study in Makasar by Faridah et al (2010) also found no significant difference between both sexes with otitis media.

The distribution of the sexes in this study differs from previous studies in Otorhinolaryngology Clinic, Dr. Soetomo Hospital. From all new patients with unsafe CSOM in Surabaya between 2000-2002, 225 underwent radical mastoidectomy, with males more than females by a ratio of 2:1 (Harmadji 2006). Likewise, studies in Medan 2011-2012 and in Pakistan reported that CSOM patients were predominantly males than females (Baiq et al 2011, Dewi & Zahara 2013). This may be due to the number of samples in this study which is less than that of the above-mentioned studies.

Table 3 shows the types of CSOM in this study, which were based on the existence of cholesteatomas, are divided into two types: safe type and unsafe type CSOM. Safe type CSOM did not show cholesteatoma, while unsafe type CSOM did. The number of both types was nearly as many. Cholesteatoma is an abnormal accumulation of keratin and squamous epithelium in tympanic cavity, mastoid or petrous apex and has a destructive nature.

The type of surgery performed on the sample of this research was canal wall down, performed in 21 patients (63.64%) and the canal wall up in 12 patients (36.36%). Canal wall down mastoidectomy tears down MAE posterior walls separating the tympanic cavity with mastoid cavity. This type of surgery may clean cholesteatoma and other pathological tissues to maximally so that the ear becomes dry and prevent complications (WHO 2004).

Canal wall up mastoidectomy is highly dependent on the surgical technique and often leads to relapse because access to the middle ear is limited. Canal wall down mastoidectomy is easier to do and have wide access to the middle ear to clear existing cholesteatoma. After canal wall down mastoidectomy the patients less often require control or revision surgery and it has relatively low recurrence rate. Selection of the surgical procedure depends on published studies, the patients' factors, and the ability of the operator (WHO 2004).

Table 4 shows the type of tissue obtained during the mastoidectomy. Granulation tissue was found in 17 patients (51.52%). Granulation tissue will replace some of the mucosa due to persisting infection. Granulation consists of vascular tissue with blood vessel formation, histiocytes and fibroblasts. When histiocytes number becomes higher, the fibrosis becomes thicker, the mucosa become thinner until the entire mucosa is replaced by granulation tissue (Telian & Schmalbach 2003).

Total tissue containing cholesteatomas in this study was found in 16 patients (48.48%). Cholesteatoma associated with chronic otitis media is from the aquisital type. Cholesteatoma an expansive lesion of the temporal bone coated with stratified squamous epithelium containing keratin desquamation. Cholesteatoma erodes bones affected by the effects of suppression by the buildup of keratin debris or due to osteoclast enzyme mediating activity. Resorption of trabecular bone can cause destruction of the mastoid, erosion of ossicles, labyrinth fistula, N. VII exposure , as well as the duramater and lateral sinus (Telian & Schmalbach 2003, Helmi 2005, Meyer et al 2006).

Biofilm bacteria examination results

The whole samples in this study were pathological tissues form mastoidectomy in CSOM patients enough for biofilm bacteria examination process. The tissues were processed before being examined for the presence of biofilm bacteria. Detection of bacterial biofilms in this study was conducted directly using electron microscopy. This microscope can do enlargement objects up to 2 million times, which uses electrostatic and electromagnetic control lighting and display images as well as have the ability to object magnification and resolution are finer than the light microscope.

This study used SEM as it s able to observe the details of the surface of cells or other microscopic structure and displaying the observation of objects in three dimensions without thinning. SEM facility in Faculty of Medicine, Airlangga University, has the brand of JEOL, JSM-T100 Scanning Microscope. Tissues obtained

during surgery were put into fixative and delivered in coolers to the Electron Microscopy Unit. The preparation started from fixation with 2% glutaral-dehyde, phosphate buffer, osmic acid, various concent-rations of alcohol, amyl acetate as a preservative, and pure gold as coating.

Table 5 shows the results of positive biofilm bacteria from surgical tissues examined as many as 18 tissues (54.54%) and negative biofilm of 15 tissues (45.46%). This shows that positive bacterial biofilm was in higher number compared to the negative tissues from the CSOM patients. In this study, tissues with positive biofilm were found with cocci bacteria clustered and surrounded the formation of amorphous material in the form of glycocalyx. In tissues with negative biofilm, the formation of cocci bacteria was found but not clustered or sessile-shaped and glycocalyx formation was also not found.

This study was in line with that of Pinar et al (2008) that in the middle ear tissue of CSOM patients with timpanomastoid surgery, biofilms were found in 9 (56.2%) of the 16 samples with positive cultures. A study by Lee et al (2009) proved the same thing where the biofilm bacteria was found in 6 of 10 (60%) of the tissue from CSOM patients with middle ear surgery. In control group, biofilm bacteria was found only in 1 of 10 (10%) tissues. A study by Akyildiz et al (2013) showing all tissues from CSOM patient had biofilms (19 patients), while the control group, comprising patients performed cochlear implant (9 patients), did not have the biofilm. This showed statistically significant differences on the presence of bacterial biofilm in CSOM group and control group.

Biofilm bacteria correlation with cholesteatoma in CSOM

Table 6 shows the results of analysis of bacterial biofilm correlation with CSOM. Bacterial biofilm were found in 12 of 16 patients with unsafe type CSOM (75%) and 6 of 17 patients safe type CSOM (35.35%). The results of logistic regression test revealed p value of 0.027. This shows that there is correlation between biofilm bacteria and cholesteatoma in CSOM. Relative risk was found to be 5.5 which indicates cholesteatoma has a risk value of biofilm bacterial formation as much as 5.5.

This study supports the results of a previos study by Saunders et al (2011) 3 of 5 specimens obtained cholesteatoma showed biofilm (60%) and 1 of 7 CSOM without cholesteatoma specimens were positive for biofilms (14%). A study by Lampikoski et al (2012) obtained bacterial biofilm in 14 (82%) of 17 CSOM patients with cholesteatoma and 5 (42%) tissues had positive bacterial biofilm from 12 tissues of CSOM patients without cholesteatoma, while the control group gained 1 (9%) of 11 patients with cochlear implants. A study by Kaya et al (2013) showed 7 out of 10 CSOM patients had biofilm (70%), 6 of the 11 groups of non-suppurative chronic otitis media showed biofilm (54.5%). Cholesteatoma group showed biofilm in 8 of 13 patients (61.5%).

The results of those studies indicate that there is correlation between CSOM of cholesteatoma group and non- cholesteatoma group with bacterial biofilm on the mastoid mucosa. The study also showed higher incidence of bacterial biofilm on CSOM with cholesteatoma compared CSOM without cholesteatoma. The difference between the two CSOM groups in the presence of biofilm was statistically significant (Saunders et al 2011, Lampikoski et al 2012).

The results support the theory that biofilm bacteria plays an important role in CSOM. Biofilm bacteria obtained more in groups with unsafe type CSOM than in safe type CSOM with relative risk as much as 5.5. Thus, this study may explain the aetiopathogenesis of recurrence and antibiotic resistance in patients with unsafe type CSOM. Recurrence in unsafe type CSOM results from endotoxins produced by bacterial biofilm and the attachment of sessile bacteria to the epithelial cells. This causes interference signal regulation to increased epithelial cell turnover and keratin production so that makes infection more difficult to stop (Morris & Hagr 2005). Biofilm resistance against antibiotics was suspected through several mechanisms, such as failed penetration due to limited transport of antibiotics. Decreased levels of bacterial growth occur due to differences in oxygen and nutrients concentration, causing physiological gradation preventing antibiotics action to stop bacterial growth. Other mechanism of biofilm resistance to antibiotics is a new phenotypic variation and the presence of efflux pumps (Drenkard 2003).

The results of this study showed that the safe type CSOM may also harbor bacterial biofilm. Although few in number, it needs to be noticed by the otorhino-laryngologists. Further research can be conducted to determine the type of bacteria that form biofilms on CSOM. In addition, it is necessary to carry out studies on the development of new therapies that have the target to stop the growth of bacteria in the biofilm CSOM.

CONCLUSION

Biofilm bacteria are present in CSOM and these bacteria have significant correlation with unsafe type CSOM.

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