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Correlation of Matrix Metalloproteinase-9 (MMP-9) expression and bone destruction in Chronic Suppurative Otitis Media (CSOM) patients with cholesteatoma at Adam Malik General Hospital Medan - Indonesia

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ABSTRACT

Introduction: Cholesteatoma is a cyst which coated by stratified squamous epithelium containing keratin epithelial desquamation. The recurrence and bone destruction arerelevant features in cholesteatoma pathophysiology, which makes cholesteatoma tend to be dangerous and difficult to treat. Recent studies have shown that variations in cellular systems of matrix metalloproteinase-9 (MMP-9) production contribute to the pathophysiology of cholesteatoma, especially in of bone erosion.

Objective: The aim of this study is to determine the correlation between MMP-9 with bone destruction in CSOM with cholesteatoma patients.

Materials and Methods: This study was conducted by using cross-sectional design to the 40 samples of cholesteatomas patients at Otorhinolaryngology Department of Adam Malik General Hospital in May 2016. The level of MMP-9 expression was assessed by immunohistochemical staining of cholesteatoma tissue. The immunoreactivity score was obtained by calculating the broad scores with the intensity score in the assessment. Then, the correlation test was also carried out between MMP-9 expression and bone destruction statistically.

Results: This study found that most of participants were male gender patients (23 patients; 67.5%), 6-24 years old interval (23 patients; 67.5%), having intratemporal complication (35 patients; 87.5%); moderate level bone destruction (19 patients; 47.5%), and MMP-9 overexpression (34 patients; 85%). In addition, there was a significant correlation between MMP-9 expression and bone destruction (P = 0.000) in this study.

Conclusion: There was a significant correlation between MMP-9 expression with bone destruction in this study.

Keywords: CSOM with cholesteatoma, MMP-9, Immunohistochemistry, Bone Destruction

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INTRODUCTION

Chronic Suppurative Otitis Media (CSOM) is a common disease in the Ear, Nose, and Throat (ENT). This disease is commonly found in patients with low socioeconomic.[1] CSOM is a result of acute otitis media episode characterized by persistent fluid secretion from the middle ear through tympanic perforation where this lead to hearing loss.[2] CSOM with or without cholesteatoma is a major worldwide health problem and a burden particularly in developing countries.[2,3]

Cholesteatoma is a non-neoplastic lesion of keratin tissue as one of CSOM complications. The origin of cholesteatoma early formation is still unknown, but it slowly developed and had a high destructive progression which resultsin damage against the surrounding of dense and soft tissue. Cholesteatomas may involve the osicles, blood vessels, facial nerves, and even invade the inner and intracranial ear. Cholesteatomas can also cause hearing loss, tinnitus, vertigo, loss of balance, and other severe complications such as meningitis, sigmoid sinus thrombosis, facial paralysis, and brain abscess. The balance between MMP and TIMP is critical in determining the integrity of the extracellular matrix. MMP is a zinc and calcium-dependent synthesized endopeptidase by various cell types such as fibroblasts, keratinocytes, macrophages, and endothelial cells. Increased levels of MMP-9, MMP-2, MMP-1, MMP-8 and MMP-13 in cholesteatoma have been reported. MMP-9 (92 kDa) can be specifically seen in areas with inflammatory infiltration.[7] However, recent studies have shown that variations in cellular systems of matrix metalloproteinases (MMPs) production and specific inhibitors of Tissue Inhibitors of Metalloproteinases (TIMPs) contribute to the pathophysiology of cholesteatomas especially in the process of bone erosion.

Recently, there is no literature specifically discusses the MMP-9 in CSOM with cholesteatoma patient in Indonesia, so that aim of the study is to know the relationship of MMP-9 expression with the degree of bone destruction in CSOM with cholesteatoma patients in Adam Malik General Hospital Medan.

METHOD

It was a cross-sectional analytic study which conducted at Otorhinolaryngology Department of Adam Malik General Hospital in May 2016. The study population is all CSOM patients who also have diagnosed with cholesteatoma based on history, ear examination, as well as X-ray/CT-Scan of patient's mastoid were treated in the Otology Division of ENT Department at Medical Faculty of Universitas Sumatera Utara /Adam Malik General Hospital Medan has undergone tympanomastoidectomy and made paraffin block preparations. The inclusion criteria of this study are the preparation of paraffin block of cholesteatoma patients, taken from the action of a good tympanomastoidectomy operation, as well as could be performed thein immunohistochemical examination. The sample size was 40 subjects according to formula being used.

The cholesteatoma examination is carried out through the examination of cholesteatoma tissue. The examination of MMP-9 expression was performed by immunohistochemical staining with a positive or overexpression outcome of a brown colored pulp in the cytoplasm of the cholesteatoma epithelium. The MMP-9 immunoreactivity assessments were assessed by summing the results of a broad score with an intensity score to obtain animmunoreactive MMP-9 score. The broad score consists with: 1) 0 = if no brown cytoplasm is found; 2) 1 = if a brown cytoplasm is found <10% the number of cells; 3) 2 = if a brown cytoplasm around 10-50% of the cell count; and 4) 3 = if a brown cytoplasm is present>50% of the cell count. The intensity score is calculated with a score of 1 = weak; 2 = medium and 3 = strong.

The immunoreactive score was obtained by summing the broad scores with the intensity score with the assessment: Not overexpression = immunoreactive score 0-3; Overexpression = immunoreactive score 4-6. Bone destruction examination is measured from CT-Scan Temporal and during surgery with the following classification: 1) Mild = erosion of scutum and osicles; 2) Moderate = destruction of the tegmen and all osicles; and 3) Severe = destruction of all osicles, labyrinth bone, facial canal and external ear canal.

Data regarding with sex, age, and complications were obtained from the patient's medical record at Adam Malik General Hospital Medan. The analysis will be performed on the data collected. Univariate analysis is done by distributing data in the form of tables and drawings. The data collected were processed and analyzed using SPSS program.

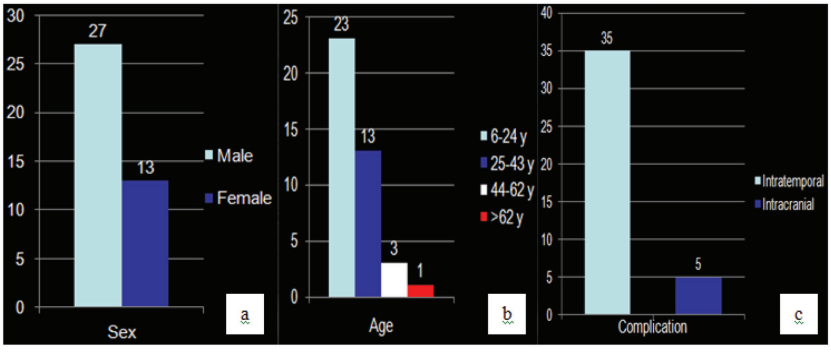

Figure 1 Characteristic of CSOM with cholesteatoma; (A) Sex; (B) Age; (C) Complications

RESULTS

This study found that 40 samples of CSOM with cholesteatoma were males (27 patients; 67.5%) and females (13 patients; 32.5%).According to the age group, 6-24 years old group was dominant (23 patients; 57.5%), followed by 25-34 years old group (13 patients; 32.5%); 44-62 years old group (3 patients; 7.5%), and >62 years old group (1 patient; 2.5%) (Figure 1).

Thirty-five CSOM with cholesteatomapatients in this study experienced intratemporal complications (87.5%), while intracranial complications around 5 samples (12.5%). According to the degree of bone destruction, the moderate form was predominance (19 patients; 47.5%), followed by severe form (18 patients; 45%), as well as the mild form (3 samples; 7.5%). In addition, the expression of MMP-9 in patients CSOM with cholesteatoma was overexpression in 34 samples (85.0%), while 6 samples left were non-expression (15.0%) (Figure 2).

Figure 3 shows the MMP-9 expression pattern based on the degree of bone destruction. Regarding with the statistical calculations, it can be concluded that MMP-9 is overexpressed; bone destruction has been at a moderate and severe form (47.5% and 45%, respectively). While in mild destruction, overexpression of MMP-9 was not found. The statistical tests showed a statistically significant relationship between MMP-9 expression and bone destruction (p=0,000).

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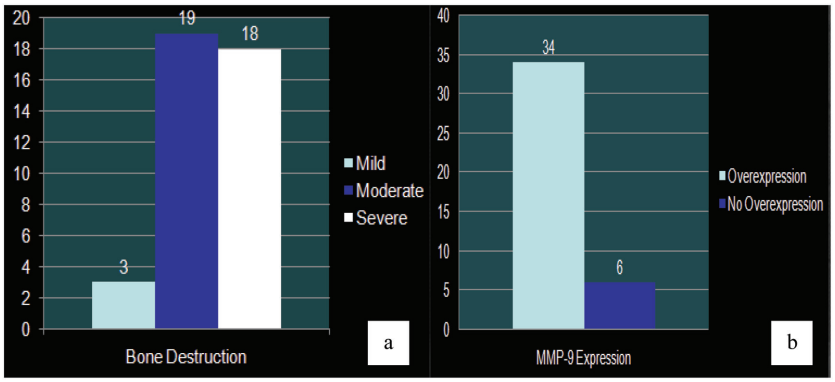

Figure 2 Characteristics of CSOM with cholesteatoma patients; (A) Based on the degree of bone destruction; (B) MMP-9 Expression

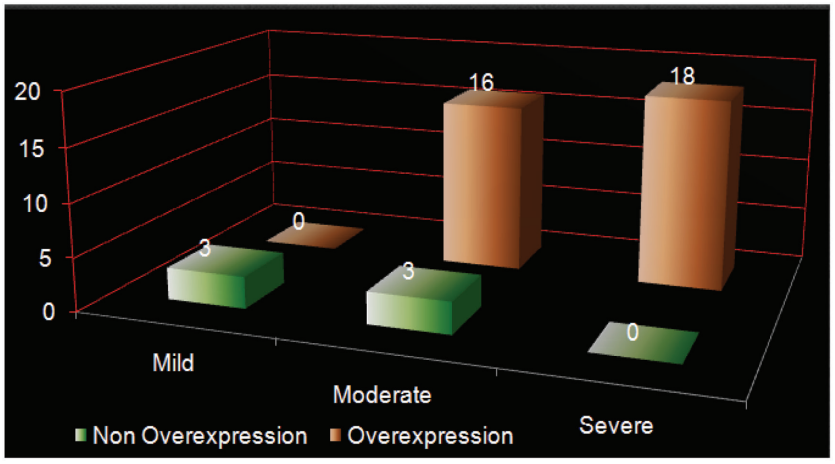

Figure 3 Relationship of MMP-9 expression with bone destruction

DISCUSSION

CSOM is a common entity in ENT. CSOM with cholesteatoma formerly known as aticoantral type, usually appear with marginal perforation with the formation of cholesteatoma and also considered as the cause of various complications.[9]

The distribution of CSOM with cholesteatoma patients according to gender group was dominant with males around 27 patients (67.5%) and follower by females around 13 patients (32.5%). This result is similar to the study conducted by Aquino[10] in which from 1146 CSOM with cholesteatoma, 66% males and 33.4% were female. In addition, Chole & Nason[11] also mentioned that men were more dominantly suffering from CSOM, but there is no study to prove the relationship between CSOM and gender. The distribution of CSOM with cholesteatoma according to ageis most prevalent in the 6-24 years age group around 23 samples (57.5%), followed by 25-43 years age group around 13 sample (32.5%). Some studies also found similar results, such as a study carried out by Aquino[10] whose the patients >16 years around 63.70%, and <16 years as much as 16.30%. Yousuf[12] gained the most prevalent of the age group in CSOM with cholesteatoma patients is 11-20 years group as much as 54%, followed by the age group 0-10 years around 20%. In another study, Kumar[13] obtained the most age group of patients CSOM with cholesteatoma was 25-35 years group around 40%, followed by the age group of 15-25 years as many as 35 (35%). Then, the study conducted by Islam[14] found that the most prevalent of the age group in CSOM with cholesteatoma patients is 11-20 years group around 50%, while Srivastava[15] got in two different age group, such as the 11-20 years and 21-30 years group as much as 25 patients (22.7%).

Adult patients are more common than children due to likely to tolerate the disease until they seek further treatment, it is also more likely to affect patients with low economic classes. The causes of late diagnosis are due to the fact that the patients don't realize for mild autologic symptoms until the pain, headache, and bleeding as well as the delay of the general practitioner refers to the patient who is already at the cholesteatoma stage.[10]

From the data of complication caused by cholesteatoma, the most complication of CSOM patients in this study was intratemporal complication around 35 samples (87.5%), and intracranial complication found 5 people (12,5%) as seen in Table 2. A study conducted by Yousuf[6] found the same results where the highest complications rate from 100 peoples with cholesteatoma is intratemporal complications by 25%, followed by intracranial complications around 6%. Baig[9] also found that intratemporal complications were the most complication compared to intracranial complications.

Cholesteatomas first appear from Prussakspace and begin to spread through the posteroanterior epitympanic, as well as the posterior mesotympanic. Chronic infection and hyperproliferative induction in each layer of cholesteatoma have implications for both internal and external idiopathic potential responses in the form of release of various cytokines by inflammatory cells.[5] In this case, it can be concluded that the complications occurred due to the invasive nature of cholesteatoma which giving bone destruction accompanied by infection due to bacterial accumulation contained in matrix cholesteatoma. Bacteria biofilm can found both in CSOM and middle ear cholesteatomas. A cholesteatoma keratin layer is an ideal place for the growth and development of bacterial biofilms. The presence of biofilm in cholesteatoma responds to thechronic inflammatory formation, proliferation and bone resorption.[16] The epidemiological and microbiological characteristics of the various microorganisms found in CSOM provide knowledge of the pathogenesis and pathophysiology regarding with their complications.

The highest degree of bone destruction was found in moderate samples of 19 samples (47.5%), followed by a severe form of 18 samples (45%), and mild form around 3 samples (7.5%). A study conducted by Baig[9] found that bone destruction, particularly in hearing-loss sequences, was the most common complication of CSOM with cholesteatoma. Similarly, Memon[1] concluded that the osicles destruction occurred in all CSOM with cholesteatoma patients studied. Cholesteatoma consists of a matrix which composed by squamous keratin epithelium and perimatrix. The perimetrix also composedof loose connective tissue with the content of collagen fibers, fibrocytes, and inflammatory cells. Some authors describe perimatrix as a peripheral part of cholesteatoma. Bone destruction occurs due to the pressure effect causing remodeling of bone structure and enzyme activity on perimatrix which leads to activation of osteoclasts.[5]

Many enzymeshavebeen investigated with regard to bone damage in CSOM with cholesteatoma. In inflammatory areas, collagenases may damage surrounding collagen molecules resulting in more severe damage to the collagen structure.[1] The most pathological conditions caused by cholesteatoma are the result of osteoclast-mediated bone destruction. Cytokines, nitric oxide, neurotransmitters and growth factors are associated with chronic inflammation as well as bone destruction due to cholesteatoma.[19]

The pattern of MMP-9 expression in CSOM with cholesteatoma was overexpression for 34 samples (85%), while non-overexpression only in 6 samples (15%). Olszewska[20] in his study found that there was an increaseof MMP-9 expression in cholesteatoma as well as inCSOM with cholesteatoma patients serum. Similar findings were obtained by Juha'sz[21] where an increase of MMP-9 expression in cholesteatoma compared to normal ear skin was the most common in the moderate positive category. Jesionek[22] in a study with a zymographic method in Poland of 14 patients who undergone mastoidectomy found an increase of MMP-9 expression almost three times higher than normal ear skin in cholesteatoma. Another study conducted by Schmidt[23] also found the similar results in which there wasa significant increase in MMP-9 expression of 37 cholesteatoma samples through zymographic examination. Similar results also obtained Schonermark[24] through his research on 16 samples of cholesteatoma, where there was an increase of MMP-9 expression compared to the ear canal skin and tympanic membrane.

Many studies have found elevated MMP-9 levels in various pathological conditions. A study investigated by Usmanova[25] found an elevated of MMP-9 levels in serum patients with atherosclerosis. Another study conducted by Nukarinen[26] also found an elevated of MMP-9 levels in acute pancreatitis patients. Those finding due to MMP is an enzyme synthesized by various cells such as fibroblasts, keratinocytes, macrophages and endothelial cells that are activated by proteolysis.

In normal conditions, MMP activity is strictly controlled, since an increase in MMP may interfere with the extracellular matrix. In cholesteatoma, several studies have revealed that the regulatory imbalance of MMP, leading to an increase in MMP expression resulting in damage to the extracellular matrix of bone.[8] The increase in MMP expression in the matrix of cholesteatoma are unexpressed in the tympanic membrane and normal middle ear mucosa. The expression of this enzyme contributes to the potential of resorption and triggers proliferation.[22] In Table 3 showed that the pattern of MMP-9 expression in relationship with the degree of bone destruction. The statistical results found that the increased expression of MMP-9 also leads to an increase of the bone destruction. This is evidenced by the result of MMP-9 overexpression in moderate and severe bone destruction of cholesteatoma. However, there is no destruction in mild degrees cholesteatoma. So it can be concluded that the increased expression of MMP-9 in cholesteatoma will be more severe in bone destruction. Hamed[17] argued that increased MMP-9 expression has been widely evidenced by numerous research and examination methods such as Enzym-linked immunosorbent assay (ELISA), zymography, immunohistochemistry, and gene expression through Polymerase Chain Reaction (PCR) examination.

Juhasz[21] found an increase in MMP-9 and tenascin expression associated with the aggressiveness of cholesteatoma. The results of this study also suggest that increased expression of MMP-9 is strongly associated with the destructive nature of bone occurred. Besides the increase in MMP-9 as well as to suppress apoptosis, which supports the growth of cholesteatoma and its accompanying destructive nature. Jesionek[22] also concluded that the increased levels of MMP-9 also plays a major role in the molecular mechanism of the invasive nature of cholesteatoma in the middle ear bone destruction.

A study conducted by Zhu, Xie & Wang[27] also entitled expression of MMP in cholesteatoma and cancer, through the immunohistochemistry examination of 36 samples of cholesteatoma, found a strong association between cholesteatoma with MMP-2 and MMP-9. It can be concluded that the interference regulation of MMP and inhibitor

responsible for the destruction Bone in cholesteatoma and ear tumors. The Schonermark[24] study also found that there was a strong association between MMP-9 expression of bone destruction activity due to cholesteatoma.

Bone destruction and recurrence are relevant features in the pathophysiology of cholesteatoma, resulting in a hazardous and difficult to treat condition.[7] In 1969, Abramson reported the potential of collagenolytic of cholesteatoma for the first time. This study provides an important clue to the biochemical theory of bone destruction processes in the CSOM. Since then, many authors have focused on the molecular mechanisms of temporal bone osteolysis during CSOM that jeopardize the integrity of the middle and deep ear structures and surrounding tissue.

The mechanism of bone destruction by cholesteatoma has not been fully explained. Until now, there are several mechanisms deemed to be associated with this condition including pressure necrosis, perimatrix granuloma inflammation, chronic osteomyelitis and osteoclast and osteocyte involvement stimulated by various local factors produced by inflammatory cells. Thus, histochemical studies have demonstrated the fact that in the development of inflammatory processes in CSOM as well as cholesteatoma, local activation of collagenase, phosphatase, proteases, and pH changes play an important role in the mechanism of bone destruction.[28]

Collagenase, which is one of the subgroups of metalloproteinase, is present in cholesteatoma perimatrixas the primary factor responsible for the osteolysis process.[28] In this study, the statistical test obtained p=0,000; this indicates that there is a significant relationship between MMP-9 expression with degree of bone destruction in CSOM with cholesteatoma and so the research hypothesis is accepted.

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