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Association between Matrix Metalloproteinase-2 with Bone Destruction Degree in Patients with Atticoantral Chronic Suppurative Otitis Media at Haji Adam Malik Central General Hospital, Medan, Indonesia

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ABSTRACT

Chronis suppurative otitis media (CSOM) with cholesteatoma may disturb the balance between bone formation and resorption. The destruction caused by cholesteatoma growth can reach the surrounding areas due to dermal enzyme and acid production. Matrix metalloproteinase-2 (MMP-2) is a zinc proteolytic enzyme and calcium endopeptidase that degrades extracellular matrix. This cross-sectional analytical study aimed to determine the association between MMP-2 expressions and bone destruction degrees due to cholesteatoma in patients with atticoantral CSOM at Haji Adam Malik Central General Hospital, Medan Indonesia. Cholesteatoma was taken from 40 cases of CSOM and then made into paraffin blocks. MMP-2 overexpression was more dominant in paraffin blocks taken from patients with moderate degree bone destruction (47.5%). This study concluded that MMP-2 was significantly associated with the degree of bone destruction (p=0.002).

Keywords: Chronic Suppurative Otitis Media, Cholesteatoma, Matrix Metalloproteinase, Bone Destruction Degree.
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INTRODUCTION

Chronic suppurative otitis media (CSOM) accompanied by cholesteatoma may disturb the balance between bone formation and resorption. This disease is associated with damage to the structure of the middle and inner ear and may cause conductive and sensorineural deafness, facial nerve palsy, vestibular function disturbances or intracranial complications. Damage due to cholesteatoma can destruct surrounding bones and soft tissues.[1]

Temporal bone proteolytic erosion is a key event in cholesteatoma progression. Several studies have focused on the molecular mechanism of temporal bone osteolysis during chronic middle ear inflammation. Temporal bone osteolysis can destruct the integrity of the middle, inner and surrounding ear structures. Matrix metalloproteinase (MMP), a new member of the proteolytic enzyme family, functions in matrix and bone homeostasis and inflammation in several osteolytic diseases including osteoarthritis and periodotitis.[2]

Matrix metalloproteinases are zinc and calcium-dependent endopeptidases that are synthesized by several cell types including fibroblasts keratinocytes macrophages and endothelial cell activation by proteolytic cleavage. Proteolytic activities are controlled by their respective precursors during activation and inhibition by endogenous inhibitors alpha macroglobulins and tissue inhibitors of metalloproteinases (TIMPs). The balance between MMP and TIMP is important in maintaining extracellular integrity.[3]

Metalloproteinase 2 (MMP-2) or Gelatinase A or 72 kDa collagenase is a nucleus induced protein and is associated with several characteristics such as tumor progression. Tumor progression is a process that comprises of growth invasion, metastasis and angiogenesis (blood vessel growth).[4]

Specific MMP isoenzymes in cholesteatoma (MMP-2, MMP-3, and MMP-9) were first introduced by Schonemark in 1996 by immunohistochemical testing. Since then immunohistochemistry has been extensively used to analyze and compare enzyme level changes in cholesteatoma and normal tissues. Increased levels of MMP-9, MMP-2, MMP-1, MMP-8 and MMP-13 have been reported. Matrix metalloproteinase-2, 3 and 9 immune labeling have been observed in basal and suprabasal epithelial layers of cholesteatoma.[3] Morales et al

Table 1 Distribution of respondents (atticoantral CSOM) based on age and sex

Characteristics	Total (n = 40)	%
Age (years)		
< 20	20	50
20-40	13	32.5
41-60	6	15
> 60	1	2.5
Total	40	100
Sex		
Male	27	67.5
Female	13	32.5
Total	40	100

Table 2 Distribution of CSOM patients with atticoantral CSOM based on clinical symptoms

Clinical Symptoms	Total (n = 40)	%
Watery ears	40	100
Headaches		
Yes	6	15
No	34	85
Dizziness		
Yes	6	15
No	34	85
Haering disturbances	40	100
Posterior ear swelling		
Yes	11	27.5
No	29	72.5
Posterior ear hole		
Yes	7	17.5
No	33	82.5
Deviated mouth		
Yes	6	15
No	34	85

Table 3 Distribution of atticoantral CSOM based on bone destruction degree and MMP-2 expressions

Characteristics	Total (n = 40)	%
Bone destruction degree		
Mild	3	7.5
Moderate	19	47.5
Severe	18	45
MMP-2 expression		
Overexpression (score 4-9)	36	90
No overexpression (score 0-3)	4	10

(2007) in his study on cholesteatoma reported high MMP-2 expressions in invasive cholesteatoma.[3]

This study aimed to determine the association between MMP-2 expression and degree of bone destruction in patients with atticoantral CSOM.

MATERIALS AND METHODS

This cross-sectional analytical study took place at the Department of ear, nose, and throat, Faculty of Medicine, University of Sumatera Utara/ Haji Adam Malik Central General Hospital and retrieved cholesteatoma from 40 patients with chronic suppurative otitis media and made into paraffin blocks. Immunohistochemical testing was then performed on these blocks at the Anatomical Pathology Installation at Haji Adam Malik Central General Hospital, Medan. Cholesteatoma that could be immunohistochemically examined, which were taken from patients with atticoantral CSOM through mastoidectomy, were included in this study. Cholesteatoma that could not be immunohistochemically examined were excluded from this study, the criteria of which were determined by the Anatomical Pathology installation.

Expressions of MMP-2 was obtained by multiplying extent scores and intensity scores. The results were immunoreactive scores which divided into two, no overexpression of MMP-2 (score 0-3) and overexpression of MMP-2 (score 4-9). The degrees of bone destruction due to cholesteatoma was based on Kuczkowski et al (2011) and classified as follows: mild degree (scutum and ossicle erosion) moderate degree (destruction of the segment and entire ossicle), severe degree (destruction of the entire ossicle, labyrinth bones, facial canal, and outer ear canal). Destruction degree was obtained by temporal CT scan.

RESULTS

Table 1 showed that from 40 subjects with atticoantral CSOM, 27 were males (67.5%).Table 2 reports the distribution of atticoantral CSOM based on clinical symptoms and showed that watery ears and hearing disturbances were complained by all samples followed by posterior ear swelling (11 sample/9.48%), posterior ear holes (7 samples/6.03%), deviated mouth (6 samples/5.17%), headaches (6 samples/5.17%), and dizziness (6 samples/5.17%).

Based on table 3, the majority of samples had overexpression of MMP-2, with a total of 36 samples (90 %), and least dominant in the no overexpression category (10%). Based on the degree of

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Table 4 Proportion of MMP-2 expression associated with bone destruction degree of respondents

	Bone Destruction Degree						Total		
	1st degree		2nd Degree		3rd degree				
MMP-2	n	%	n	%	n	%	n	%	*p*
No overexpression	2	5	2	0	0	0	4	10	0.002
Overexpression	1	2.5	17	42.5	18	45	36	90	
Total	3	7.5	19	42.5	18	50	40	100	

bone destruction, 19 samples (47.5%) had a moderate degree of destruction, with only three samples (7.5%) had a mild degree of bone destruction.

DISCUSSION

Chronic suppurative otitis media (CSOM) also known as chronic inflammation of the middle ear may cause extracranial and intracranial complications, causing increased morbidity rates.[5] Reports from WHO has stated that prevalence rates of CSOM worldwide were 65-330 million people with 94% of whom were found in developing countries. From this amount, mortality rates reach 60% (39-200 million cases) with < 2 million develop disabilities. In Indonesia, prevalence rates of CSOM reach 3.6%.[6]

Chronic suppurative otitis media with cholesteatoma is an atticoantral of infection and requires surgical treatment. The complications that may occur include intratemporal complications such as mastoiditis, facial nerve palsy, labyrinthitis, and intracranial complications with fatal consequences and death.[7]

In atticoantral CSOM cellular debris accumulation occurs and keratinocytes are invaded by cells from the immune system, including Langerhans cells, T-cells, and macrophages. This process is stimulated by unbalanced epithelial proliferation, keratinocyte differentiation and maturation, and prolonged apoptosis that eventually results in the formation of cholesteatoma. Cellular migration is replaced by hyperplasia in an inflammatory condition. Inflammation that promotes epithelial proliferation is associated with increased lytic enzyme and cytokine expressions, that would stimulate osteoclast differentiation and maturation. Epithelial proliferation may also react to the bone matrix, and expose it to the osteoclast. This would cause extracellular matrix bone degradation resulting in erosion or bone destruction that may cause complications such as atticoantral CSOM.[8]

This study was performed on 40 samples of atticoantral CSOM patients. The distribution of atticoantral CSOM proportion based on sex shows that this disease was more prevalent in men than in women (67.5% and 32.5%, respectively). Chole and Nason (2009) also stated that in several studies, males were more dominantly diagnosed with CSOM. Shresta et al. showed that CSOM with cholesteatoma was 1.96 times more frequent in men than in women.[9] Yarisman et al. (2017) also reported that most subjects were men (57.5%).[10] Malirmasele et al. (2014) showed that 51.9% of CSOM patients were men.[11] Whereas Desbassarie et al. (2015) reported that 55.7% of the CSOM patients included were men.[12]

Concerning age, this study showed that 55% of respondents were aged < 20 years old, whereas only 2.5% were aged > 60 years old. This finding concurs with results by Viswanatha and Naseeruddin (2014) that reported 48.1% of CSOM patients were aged < 20 years old5 whereas Yarisma et al. (2017) found that 47.5% of CSOM patients were also aged < 20 years old.10 In this study, CSOM was more frequent in newborns and children as CSOM starts with acute otitis media in children, which associated with a shorter and more flat eustachian tube and lower immunological function, consequently making the middle ear more prone to infection.[11]

In this study, the symptoms of CSOM ranges from watery ears, hearing disturbances, swelling behind the ears, deviated mouth, headaches, and dizziness. All 40 respondents experienced watery ears and hearing disturbances. Swelling behind the ear was reported in 11 respondents, hole behind the ear by seven respondents, and complaints of a deviated mouth, dizziness, and headaches by six respondents for each. Yarisma et al. (2017) found that all samples experienced watery ears whereas hearing disturbances was experienced by 36.04% of subjects.10 Siregar et al. (2013) in a study at Haji Adam Malik Central General Hospital showed that 73 patients complained watery ears (61.3%).[13] Desbassarie et al. (2015) in his study showed that CSOM patients were dominated by complaints of watery ears (95.3%), followed by hearing disturbances (53.5%).[12] Increased secretion may be caused by upper respiratory tract infection, focal infection or permanent tympanic membrane perforation

causing the middle ear to directly communicate with outer ear, making it easier for bacteria to enter the middle ear.

The majority of CSOM patients frequently ignore these complaints due to several factors starting from lack of knowledge until the low economic ability to seek medication and obtain complete health facilities, therefore prolonging the complaint of the patient.[14]

In this study, from the 40 respondents diagnosed with atticoantral CSOM accompanied by cholesteatoma, the most frequent complication encountered was retroauricular abscess (10 respondents) followed by retroauricular fistula in eight subjects, facial nerve paralysis in seven respondents, and labyrinthitis in five respondents. Although brain abscess was only present in one respondent, this finding shows the wide variety of complications that occur.

Complications of CSOM have different patterns. Viswanatha and Naseeruddin (2014) in India showed that the most frequent complication of CSOM is temporal lobe abscess, which was found in 24 patients (33.3%), whereas most least frequent complications were extradural and occipital lobe abscess.[5]

The variation in these complications may occur due to differed destruction to the bone structure surrounding the cholesteatoma, that includes the ossicle, otic capsule fascial nerve canal, tympanic and mastoid tegmen.[9]

In this study, the majority of respondents with overexpressed MMP-2 (36 samples/90%) with only four samples showing no overexpression (10%). Based on the degree of bone destruction, 19 sample were categorized into the moderate category and 18 sample into the severe category with only three samples in the mild category.

After analysis, MMP-2 expression was significantly associated with the degree of bone destruction with $p < 0.05$. Therefore, it could be concluded that MMP-2 expression was associated with bone destruction degree, the higher MMP-2 was expressed, the more severe bone destruction due to cholesteatoma would be.

Banerjee, James and Narula (1997) using the western blotting technique, revealed the presence of MMP-2 in cholesteatoma. Metalloproteinase 2 or Gelatinase A or 72 kDa collagenase is a protein induced by the cell nucleus and is associated with several characteristics such as tumor progression that include growth, invasion, metastasis, and angiogenesis (blood vessel growth). Zhu, Xie, and Wang (2001), in his study using immunohistochemical methods for MMP-2 and 9, with 36 cholesteatoma specimens, 10 outer ear skin fragments, and 16 middle ear cancer fragments, observed a direct association between cholesteatoma and MMP-2 and MMP-9, and concluded that a disturbance between metalloproteinases and their inhibitors is one reason for bone resorption in cases of middle ear cancer and cholesteatoma.[4]

In this study, a correlation test was also performed to assess the association between MMP-2 expression and the occurrence of complications. Results showed that MMP-2 expression was significantly associated with the occurrence of complications. The more intense MMP-2 was expressed, the more complications occurred. Morales et al. (2007) in his study using immunohistochemical methods showed high expression of MMP-2 in seven samples of cholesteatoma (87.5) from eight samples of invasive cholesteatoma and also high expressions of MMP-2 (27.3%) in cases of latent cholesteatoma. It was concluded that MMP-2 expression was more associated with cholesteatoma than latent cholesteatoma.[4]

CONCLUSION

This study found that increased MMP-2 expression was found in patients diagnosed with atticoantral CSOM and was associated with degrees of bone destruction.

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