Research

The relationship between IL-6 expression and ossicular destruction in CSOM with cholesteatoma

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ABSTRACT

Background: Chronic suppurative otitis media (CSOM) is a disease that affects the middle ear, leading to perforation of the tympanic membrane. This condition involves chronic inflammation in the middle ear and mastoid cavity. A CT scan often reveals destruction to the ossicles and cholesteatoma. **Purpose:** To determine the relationship between increased IL-6 expression and ossicular destruction in CSOM patients with cholesteatoma. **Method:** This observational analytic study with a cross-sectional comparative design was conducted at the Anatomy Pathology Laboratory of Prof. Ngoerah General Hospital, Denpasar. The study used cholesteatoma tissue paraffin blocks from patients with the dangerous type of CSOM who had undergone tympanomastoidectomy from January 2022 to December 2023. Sampling was carried out through consecutive sampling, which included all cholesteatoma tissue paraffin blocks from patients with the dangerous type of CSOM that met the inclusion criteria, amounting to 17 blocks. **Result:** This study found a p-value >0.05, indicating no association in the bivariate analysis between the percentage, intensity, and overall score of IL-6 pathology results and ossicular destruction due to cholesteatoma, in CSOM patients. **Conclusion:** This study concluded that there was no significant relationship between IL-6 expression and hearing bone destruction caused by cholesteatoma in CSOM patients.

Keywords: chronic suppurative otitis media, cholesteatoma, IL-6, ossicular destruction

ABSTRAK

Latar belakang: *Otitis media supuratif kronis (OMSK) merupakan suatu penyakit yang berlangsung pada telinga tengah, dan menyebabkan perforasi pada membran timpani. Kondisi ini merupakan proses radang yang terjadi pada telinga tengah dan rongga mastoid, yang berlangsung cukup lama. Pada pemeriksaan penunjang* CT scan *sering kali ditemukan adanya kerusakan tulang pendengaran, serta adanya kolesteatoma.* ***Tujuan:*** *Untuk mengetahui hubungan antara peningkatan ekspresi IL-6 dengan kerusakan tulang pendengaran, pada pasien OMSK dengan koleteatoma.* ***Metode:*** *Suatu penelitian observasional analitik dengan rancangan studi banding potong lintang, yang dilakukan di laboratorium Patologi Anatomi RSUP Prof Ngoerah, Denpasar, dengan menggunakan blok parafin jaringan kolesteatoma pasien OMSK tipe berbahaya, yang telah dilakukan tindakan timpanomastoidektomi pada Januari 2022-Desember 2023. Pengambilan sampel dilakukan dengan* consecutive sampling*, yaitu semua blok parafin jaringan kolesteatoma penderita OMSK tipe berbahaya yang memenuhi kriteria inklusi sejumlah 17 blok.* ***Hasil:*** *Pada penelitian ini ditemukan p>0,05, dimana secara uji bivariat tidak ditemukan adanya asosiasi antara persentase, intensitas dan skor keseluruhan hasil uji patologi interleukin 6 terhadap kerusakan tulang pendengaran akibat kolesteatoma pada pasien OMSK.* ***Kesimpulan:*** *Pada penelitian ini tidak didapati adanya hubungan yang signifikan antara IL-6 dengan kerusakan tulang pendengaran akibat kolesteatoma pada pasien OMSK.*

Kata kunci: *otitis media supuratif kronis, kolesteatoma, IL-6, kerusakan tulang pendengaran*

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INTRODUCTION

Chronic suppurative otitis media (CSOM) is a chronic infection that affects the middle ear, accompanied by perforation of the tympanic membrane. This infection impacts the middle ear and mastoid cavity, causing persistent discharge and hearing loss.[1,2] According to the Global Burden of Disease (GBD) 2021, the number of otitis media cases globally increased from 322.1 million in the year 1992 to 391.3 million in 2021. The age standardized incidence rate (ASIR) also rose from 5,345.09 to 5,529.1 per 100.000 population.[3] In Indonesia, there were 3% out of 19,375 cases of CSOM occurred. A retrospective study at the ENT outpatient clinic of Sanglah General Hospital Denpasar identified 98 cases of CSOM in 2020. Majority of patients were female (51%), with the most common age group being 21-30 years (24.5%).

The most frequent clinical symptom was otorrhea (95.9%) and the most commonly observed type of CSOM was the tubotumpanic type (73.5%).[4] Patient complaints typically include ear pain (otalgia), persistent or intermittent ear discharge (otorrhea) lasting over two months, and hearing loss. There may also be a history of ear discharge episodes, often associated with cold symptoms, sore throat, cough, or other upper respiratory infections, which increase the likelihood of CSOM. Physical examination commonly reveals tympanic membrane perforation.[1,2]

Cholesteatoma is an abnormal condition characterized by the growth of keratinized squamous epithelium in the middle ear, external auditory canal, mastoid, and petrosal bone. Cholesteatomas are generally classified into two types: congenital and acquired. A study conducted in Brazil found that 24.5% of CSOM patients developed cholesteatoma, with an average age of 34.49 years; the majority were female, and had lesions in the right ear. The process of bone destruction and ossicular invasion is a crucial factor associated with cholesteatoma formation. One of the inflammatory cells found in cholesteatoma is Interleukin-6 (IL-6). IL-6 is a well-known pro-inflammatory cytokine with multifaceted functions. It plays a significant role in host defense due to its extensive immunological and hematopoietic activities, and its strong ability to induce an acute-phase response. [1,2]

Cholesteatoma also requires confirmation through radiological examination. A computed tomography scan CT scan is used to confirm the presence of cholesteatoma lesions and assess temporal bone abnormalities. Additionally, a CT scan helps determine the location of the cholesteatoma, identify complications, and evaluate potential ossicular destruction. The imaging findings may show a well-defined soft tissue lesion with an expansile nature, tympanic membrane retraction, scutum blunting, and erosion of the tegmen tympani and ossicles. [1,2]

The ossicular destruction observed in patients with the dangerous type of CSOM with cholesteatoma is one of the motivations for this study. Numerous studies had previously been conducted to identify the inflammatory cells present in cholesteatoma, however, the result had generally been inconclusive. To date, there remains a limited number of investigations that specifically examine the correlation between IL-6 expression in cholesteatoma, assessed

through immunohistochemical methods and ossicular destruction. Therefore, this research aimed to investigate the relationship between IL-6 expression in cholesteatoma, using immunohistochemical methods of CSOM patients with ossicular destruction at the ENT Clinic of Prof. Ngoerah General Hospital, Denpasar.

METHOD

This was an observational analytic study with a cross-sectional comparative design, conducted at the Anatomical Pathology Laboratory of Prof. Ngoerah General Hospital, Denpasar. The research was performed on paraffin blocks of cholesteatoma tissue from patients with the dangerous type of chronic suppurative otitis media (CSOM) who had undergone tympanomastoidectomy from January 2022 to December 2023. Sample collection was taken through consecutive sampling, including all paraffin blocks of cholesteatoma tissue from dangerous-type CSOM patients who met the inclusion criteria, totaling 17 blocks. The study used Stored Basic Materials (BBT), specifically paraffin blocks of cholesteatoma tissue from dangerous-type CSOM patients stored in the Anatomy Pathology Department, and secondary data in the form of medical records accessed through SIMARS. Ethical clearance was submitted to the Research Ethics Committee of the Faculty of Medicine, Udayana University - Prof. Ngoerah General Hospital.

IL-6 levels were assessed using immunohistochemical staining with a rabbit anti-IL-6 monoclonal antibody. Analysis was conducted under a microscope at 100x and 200x magnifications. Cells with uniform red granules were considered positive cells. The percentage of positive cells was scored as follows:

- 0: Negative staining
- 1: Staining < 30%
- 2: Staining 30-60%
- 3: Staining > 60%

Staining intensity was scored as follows:

- 0: Negative staining
- 1: Light pink staining
- 2: Red staining
- 3: Dark red staining

The overall score for each specimen was calculated by multiplying the percentage score by the intensity score. The final result was recorded as follows:

- Negative: Overall score 0
- Weak positive (+): Overall score 1-2
- Moderate positive (++): Overall score 3-5
- Strong positive (+++): Overall score 6-9

The degree of hearing bone destruction was assessed using Saleh and Mill's criteria:

- Grade 0: Intact ossicular chain
- Grade 1: Eroded incus without ossicular chain discontinuity
- Grade 2: Eroded incus and stapes superstructure
- Grade 3: Absence of malleus head and incus
- Grade 4: Eroded stapes superstructure

Data distribution was assessed for normality using the Shapiro-Wilk test, as the sample size was fewer than 50. Analysis was conducted using SPSS version 27.

RESULT

This study was conducted using observational methods at Prof. Ngoerah General Hospital, Denpasar, utilizing medical record data and histochemical-stained paraffin blocks from patients with dangerous-type CSOM between January 2022 and December 2023. During this period, a total of 17 paraffin blocks were collected as research samples, with 7 blocks from male patients and 10 blocks from female patients.

The average age of the study subjects was 28.88±14.43 years, categorized as young adults. This population was considered ideal for assessing the effect of cholesteatoma in CSOM patients concerning IL-6 expression, as this age group generally possessed a robust immune system.

The paraffin blocks examined in the Anatomy Pathology section were evaluated for percentage and intensity using immunohistochemical staining. None of the samples showed negative staining results. Majority of samples (12 subjects, or 70.6%) displayed staining coverage greater than 60% in terms of percentage findings. In terms of intensity, most patients (8 patients, or 47.1%) had light pink staining intensity.

The percentage and intensity findings were multiplied to generate an overall score, which was then categorized. Most samples showed a strong positive result, with 8 patients (47.1%). However, weak positive and moderate positive results were also present, with 4 subjects (23.5%) and 5 subjects (29.4%), respectively.

Ossicular destruction was assessed on a scale from Grade 0 to Grade 4. In this study, no samples were found in Grade 1 or Grade 3. The majority of patients showed Grade 4, with 8 patients (47.1%).

Table 1. Demographic data of research subjects

Variable	N (%) or mean standard deviation	Normality test (Shapiro-Wilk)
Subjects		
Male	7 (41.2%)	
Female	10 (58.8%)	
Age	28.88±14.43	
Pathological anatomy findings (Percentage)		
Negative staining	0 (0%)	
< 30% positive cells	2 (11.8%)	
30-60% positive cells	3 (17.6%)	
>60% positive cells	12 (70.6%)	
Pathological anatomy findings (Intensity)		
Negative staining	0 (0%)	
Light red	8 (47.1%)	
Red	4 (23.5%)	0.034
Dark Red	5 (29.4%)	
Total Score		
1–2 Weak positive	4 (23.5%)	
3–5 Moderate positive	5 (29.4%)	
6–9 Strong positive	8 (47.1%)	
Bone Destruction		
Grade 0	4 (23.5%)	
Grade 1	0 (0%)	
Grade 2	5 (29.4%)	
Grade 3	0 (0%)	
Grade 4	8 (47.1%)	

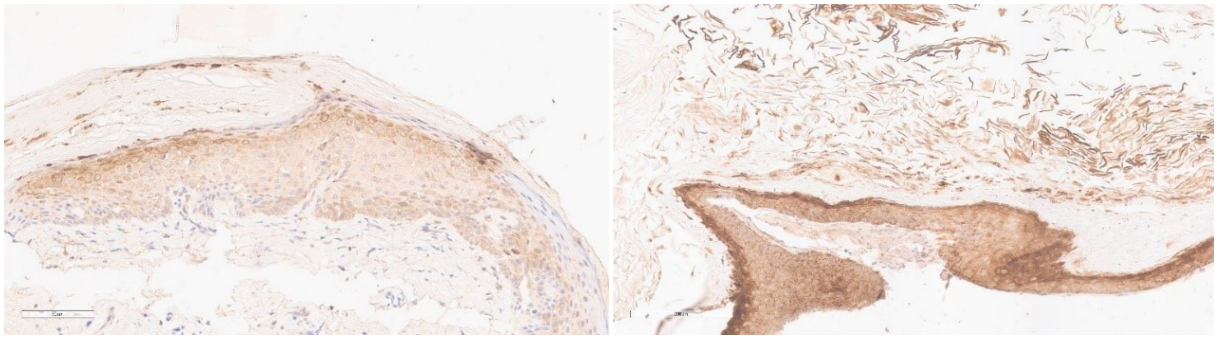

Figure 1. Anatomy pathology of cholesteatoma

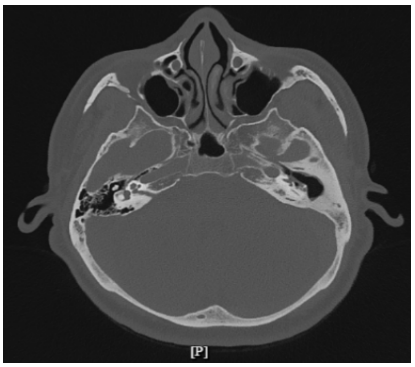

Figure 2. Temporal CT scan image showed the ossicular destruction at left ear

Based on the demographic data, bivariate analysis was performed on the study data, considering the limited sample size. Table 2 showed the results of this bivariate analysis, indicating no association between the percentage, intensity, or overall score of the IL-6 immunohistochemistry results and hearing bone destruction caused by cholesteatoma in CSOM patients.

Table 2. Bivariate test results for IL-6 and bone destruction

Variable	Degree of bone destruction			p-value
	Grade 0	Grade 2	Grade 4	
Percentage				
<30%	0 (0%)	0 (0%)	2 (100%)	0.321
30-60%	0 (0%)	3 (100%)	0 (0%)	
>60%	4 (33.3%)	2 (16.7%)	6 (50%)	
Intensity				
Light red	2 (25%)	3 (37.5%)	3 (37.5%)	0.357
Red	1 (25%)	2 (50%)	1 (25%)	
Dark red	1 (20%)	0 (0%)	4 (80%)	
Total Score				
Weak	0 (0%)	2 (50%)	2 (50%)	0.983
Moderate	2 (40%)	2 (40%)	1 (20%)	
Strong	2 (25%)	1 (12.5%)	5 (62.5%)	

This lack of association was evidenced by the p-values in all three tests, each showing no statistically significant results, with all yielding p>0.05. The IL-6 immunohistochemistry percentage test for hearing bone destruction had a p-value of 0.321. For example, in the group with <30% IL-6 staining, 2 patients (100%) still had Grade 4 hearing bone destruction, while in the >60% staining group, Grade 0 was still observed in 4 patients (33.3%).

A similar trend was observed in the staining intensity results. In the light pink staining group, many patients had Grade 2 and Grade 4, with 3 patients (37.5%) in each category. This test also produced a non-significant p-value of 0.357. The previous two variables were combined into an overall score, which similarly showed no association between the overall IL-6 immunohistochemistry score and hearing bone destruction, with a test result of p=0.983.

The result of this study found no significant relationship between IL-6 expression and hearing bone destruction, with a p-value of 0.983. However, the analysis was limited by the relatively small sample size.

DISCUSSION

Based on the analysis conducted, there was no significant relationship between IL-6 levels and the degree of ossicular destruction in patients with chronic suppurative otitis media (CSOM). The results of this study were consistent with Widyatama et al.[6] where majority of participants were female, and no relationship between IL-6 levels and the degree of ossicular destruction.

The process of resorption of the auditory ossicular involves several inflammatory factors such as interleukin-1 (IL-1), IL-6, IL-8, tumor necrosis factor-alpha (TNF-α), neurotransmitters, prostaglandins, interferon-α, parathyroid hormone-related protein, and receptor activator of nuclear factor-κB (RANKL). Various pro-inflammatory cytokines, such as IL-6, can lead to oxidative stress. Interleukin-6 is a cytokine involved in several processes, including inflammatory reactions, immune responses, and cell proliferation. This cytokine plays a significant role in the pathogenesis of cholesteatoma, such as hyperproliferation and destruction of the auditory ossicles, although its mechanisms in the pathogenesis of cholesteatoma remain unclear. Additionally, IL-6 is crucial in inflammation associated with otitis media. Embed et al. in their 2018 publication stated that IL-6 regulates the secretion of mucin from epithelial cells in the middle ear, which is part of the pathogenesis of serous and mucosal otitis media. The formation of osteoclasts is also triggered by IL-6. High concentrations of IL-6 are associated with the erosion of the ossicular and the presence of abundant granulation tissue found during surgery. [6,8]

Although no significant relationship was found, this study indicated that patients with severe ossicular destruction from CSOM tended to have higher IL-6 levels. The destruction of the ossicular is an important characteristic of cholesteatoma. Patients with CSOM tend to have higher levels of IL-1α, IL-6, and IL-8. A study by Liu et al.[10] stated that IL-6 was not related to the degree of ossicular destruction in CSOM. However, another study by Kuczkowski et al.[11] indicated that IL-6 had a strong positive relationship with the severity of ossicular destruction in CSOM. Interleukin-6 is suspected to be related to the pathology of ossicular destruction associated with cholesteatoma. Liu et al.[10] study showed that the expression of IL-6 and phosphorylated signal transducer and activator of transcription 3 (p-STAT3) was not related to the degree of ossicular destruction in CSOM, consistent with this study, which did not find a significant relationship between IL-6 and the severity

of ossicular destruction in CSOM. Inhibition of IL-6 can hinder osteoclastogenesis, facilitating chronic otitis. [6,8]

Liu et al.[10] stated that IL-6 levels and p-STAT3 expression were not associated with hearing bone destruction, possibly indicating that there were other signaling pathways apart from the IL-6/JAK/STAT3 cascade involved in the destruction of auditory ossicles in CSOM. Increased IL-6 is also associated with osteoclastogenic cytokines such as TNF-α and IL-1, which are produced by macrophages during inflammation. Both cytokines play a role in the pathogenesis of resorption of the auditory ossicles in cholesteatoma. Interleukin-1 stimulates fibroblasts and macrophages to produce collagen and prostaglandins (PGE2), leading to bone destruction. [5]

Interleukin-6 also activates cells on the bone surface, leading to an increase in RANKL expression and the release of Macrophage-Colony Stimulating Factor (M-CSF). RANKL binds to its receptors, while M-CSF causes the activation and differentiation of pro-osteoclasts into osteoclasts, destroying auditory ossicles. In addition to inflammation, the increase in IL-6 is also caused by the virulence factors of pathogens responsible for CSOM. The presence of IL-6 can accelerate the lysis of the auditory ossicles and enhance the destructive effects in the middle ear.[5,6]

Many studies had shown an increase in IL-6 levels in cholesteatoma, but not a few stated that the increase in IL-6 levels was not related to the degree of severity of hearing bone destruction in CSOM. Therefore, further studies with larger sample sizes and analyses of other confounding factors, such as antibiotic consumption, that could reduce pro-inflammatory cytokines like IL-6 due to the inhibition of macrophage induction by endotoxins, are needed.

In conclusion, this research found no significant relationship between IL-6 and hearing bone destruction, with a p-value of 0.983. This study was still limited by the small sample size. Statistical tests aimed at proving relationships require a large sample size with diverse subjects and additional variables. Further research is needed with a larger sample size, diverse subjects, and additional variables.

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