Research

Middle ear pepsin and IL-8 in active chronic suppurative otitis media with laryngopharyngeal reflux

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ABSTRACT

Background: Chronic suppurative otitis media (CSOM) is a chronic inflammatory condition of the middle ear, characterized by tympanic membrane perforation and persistent or recurrent ear discharge. The active phase of CSOM refers to a period of ongoing intense inflammation, marked by purulent exudate production, active infection, and heightened immune response. One of the contributing factors in CSOM pathogenesis is laryngopharyngeal reflux (LPR). Studies suggest that refluxed pepsin from LPR can reach the middle ear through the Eustachian tube, triggering local inflammation and exacerbating tissue damage during the active phase. Pepsin plays a role in upregulating pro-inflammatory cytokines, such as interleukin-8 (IL-8), thereby intensifying the inflammatory response and worsening CSOM symptoms in this stage. **Purpose:** To determine the correlation between pepsin and IL-8 levels in middle ear secretions of patients with active CSOM and LPR. **Method:** This cross-sectional study involved 32 patients with active CSOM and clinical signs of LPR. Middle ear secretions were analyzed for pepsin and IL-8 concentrations using enzyme-linked immunosorbent assay (ELISA). Pearson correlation was used to assess the relationship. **Result:** The study population was predominantly aged 36-45 years (28.12%) and female (59.4%). The mean pepsin concentration in middle ear secretions was 71.52±84.64 ng/mL, while the mean IL-8 level was 33.47±17.56 pg/mL. A strong and statistically significant positive correlation was observed between pepsin and IL-8 levels (r = 0.682; p = 0.001). **Conclusion:** Elevated pepsin levels due to LPR, strongly correlate with increased IL-8 expression, suggesting a reflux-mediated inflammatory mechanism in the pathogenesis of active CSOM.

Keywords: pepsin, interleukin-8, chronic suppurative otitis media, laryngopharyngeal reflux

ABSTRAK

Latar belakang: *Otitis media supuratif kronik (OMSK) merupakan kondisi inflamasi kronik pada telinga tengah, yang ditandai dengan perforasi membran timpani dan keluarnya cairan dari telinga secara terus-menerus atau berulang. Fase aktif OMSK mengacu pada periode inflamasi yang sedang berlangsung secara intens, yang ditandai oleh peningkatan produksi eksudat purulen, adanya infeksi aktif, dan respons imun yang lebih tinggi. Salah satu faktor yang berkontribusi dalam patogenesis OMSK adalah refluks laringofaring (LRF). Penelitian menunjukkan bahwa refluks pepsin yang berasal dari RLF dapat mencapai telinga tengah melalui tuba Eustachius, yang memicu inflamasi lokal dan memperburuk kerusakan jaringan pada fase aktif. Pepsin berperan dalam meningkatkan kadar sitokin pro-inflamasi interleukin-8 (IL-8) yang memperkuat respons inflamasi dan memperparah gejala OMSK pada fase ini.* ***Tujuan:*** *Mengetahui hubungan antara kadar pepsin dan kadar IL-8 di sekret telinga tengah pada pasien OMSK fase aktif dengan RLF.* ***Metode:*** *Penelitian cross-sectional ini melibatkan 32 pasien OMSK fase aktif disertai dengan gejala klinis RLF. Sampel sekret telinga tengah dianalisis untuk kadar pepsin dan IL-8 menggunakan metode ELISA. Uji korelasi* Pearson *digunakan untuk mengetahui hubungan antar-variabel.* ***Hasil:*** *Subjek penelitian didominasi kelompok usia 36-45 tahun (28,12%) dan perempuan (59,4%). Rata-rata kadar pepsin sekret telinga tengah adalah 71,52±84,64 ng/mL, sedangkan kadar IL-8 rata-rata sebesar 33,47±17,56 pg/mL. Ditemukan hubungan positif kuat dan signifikan antara kadar pepsin dan IL-8 (r = 0,682; p = 0,001).* ***Kesimpulan:*** *Peningkatan kadar pepsin akibat RLF berkorelasi kuat dengan peningkatan kadar IL-8, menunjukkan aktivasi jalur inflamasi yang dimediasi refluks pada patogenesis OMSK fase aktif.*

Kata kunci: *pepsin, interleukin-8, otitis media supuratif kronik, refluks laringofaring*

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INTRODUCTION

Chronic suppurative otitis media (CSOM) is a persistent inflammatory condition of the middle ear, typically characterized by tympanic membrane perforation and continuous or recurrent otorrhea.[1,2] It remains a major global health issue due to its potential to cause irreversible hearing loss, especially in developing countries.[3] In Indonesia, the prevalence of CSOM is estimated at 3.8%, with a higher burden among children and communities with poor access to health services.[4,5] The active phase of CSOM is marked by purulent discharge, persistent infection, and heightened mucosal immune response, making it an ideal period to evaluate the underlying pathophysiological mechanisms.[6,7]

CSOM has been linked to bacterial infections, particularly involving Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, and Klebsiella pneumoniae.[8] However, growing evidence suggests that non-infectious factors, such as laryngopharyngeal reflux (LPR), may contribute to the chronicity and severity of middle ear inflammation.[9] LPR allows retrograde movement of gastric contents into the upper aerodigestive tract and, potentially, through the Eustachian tube into the middle ear.[9]

Pepsin, a proteolytic enzyme derived from gastric refluxate, is considered a key mediator of extraesophageal reflux pathology.[10] Studies have identified pepsin in the middle ear effusions of patients with otitis media, including those with CSOM, indicating its ability to reach and persist in this region.[11,12] Pepsin may trigger local epithelial damage, activate immune cells, and modulate the expression of inflammatory cytokines, thereby worsening tissue injury and mucosal dysfunction.[13,14]

Among these cytokines, interleukin-8 (IL-8) plays a critical role. IL-8 is a chemokine involved in neutrophil chemotaxis, inflammatory amplification, and tissue remodeling.[15] Elevated IL-8 levels have been consistently detected in the middle ear fluid of patients with CSOM and are associated with disease severity and progression.[16] Mechanistically, pepsin may activate Protease-Activated Receptors (PARs), especially PAR-2, leading to NF-κB activation and IL-8 transcription in epithelial cells.[17]

Despite the increasing recognition of LPR and pepsin as contributors to upper airway inflammation, few studies have directly assessed the correlation between pepsin and IL-8 in CSOM, particularly during the active phase.[18] Existing research often addresses these markers in isolation, without integrating their interaction in the pathogenesis of middle ear diseases.[19]

This study aimed to fill that gap by investigating the relationship between pepsin concentration and IL-8 levels in middle ear secretions of patients with active-phase CSOM and clinical signs of LPR. Understanding this relationship could elucidate a reflux-mediated inflammatory pathway, supporting the development of novel diagnostic and therapeutic approaches for chronic middle ear conditions beyond bacterial etiology.

METHOD

This analytical observational study with a cross-sectional design, was conducted to evaluate the relationship between pepsin concentration and interleukin-8 (IL-8) levels in the middle ear secretions of patients with active-phase chronic suppurative otitis media (CSOM), who also exhibited clinical features of laryngopharyngeal reflux (LPR). The study aimed to clarify the role of reflux-mediated inflammatory mechanisms in the persistence and progression of CSOM.

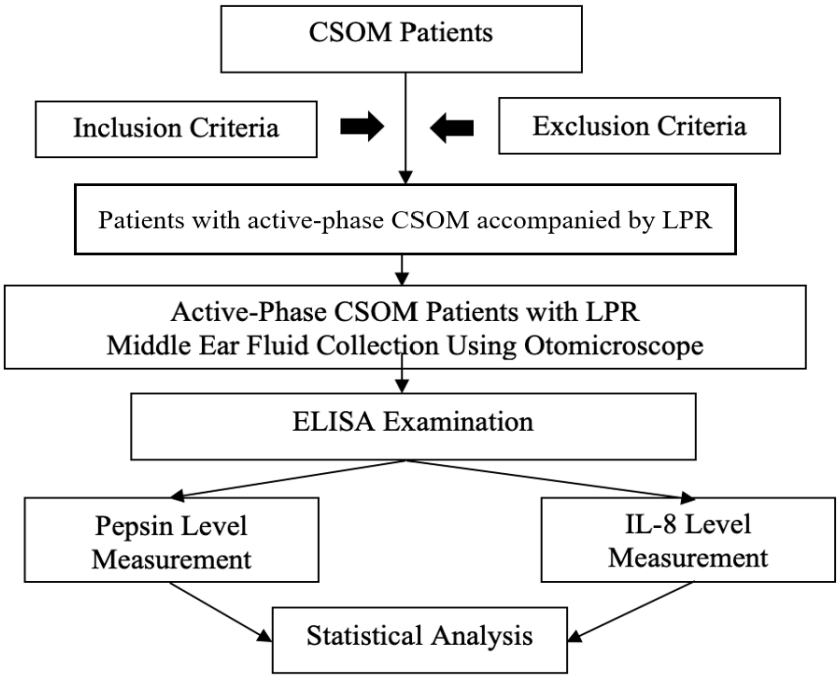

Figure 1. Study flow diagram

The study was conducted at the Department of Otorhinolaryngology – Head and Neck Surgery, Dr. Moewardi General Hospital, Surakarta, in collaboration with the Biomolecular Laboratory, Faculty of Medicine, Universitas Sebelas Maret, between January and December 2023. The overall workflow of this study, from subject selection through laboratory analysis and statistical testing, was illustrated in Figure 1.

A total of 32 adult patients diagnosed with active-phase CSOM were included through consecutive non-probability sampling. Diagnostic confirmation was based on otomicroscopic examination, which revealed persistent otorrhea, tympanic membrane perforation, and purulent discharge. The active phase was defined by the presence of mucosal inflammation and active infection.

The inclusion criteria for this study consisted of patients aged 18 years or older, who were diagnosed with active-phase Chronic Suppurative Otitis Media (CSOM).

Participants were required to have a Reflux Symptom Index (RSI) score of 13 or higher, indicating the presence of laryngopharyngeal reflux (LPR), and no history of antibiotic use within the preceding seven days.

The exclusion criteria included a history of ear surgery such as tympanoplasty or mastoidectomy, a diagnosis of cholesteatoma, or the presence of conditions that could affect immune function, including immunodeficiency, autoimmune diseases, or malignancies. Patients presenting with systemic infection or fever were also excluded to ensure the accuracy of local biomarker assessment in the middle ear.

Middle ear secretions were collected under direct otomicroscopic visualization using a sterile suction catheter connected to a microaspirator. The collected fluid was transferred into sterile microtubes and immediately stored at 4°C for transport, then kept at –20°C prior to analysis.

Quantitative analysis of pepsin and IL-8 levels was performed using Enzyme-Linked Immunosorbent Assay (ELISA) at the Biomolecular Laboratory of Universitas Sebelas Maret. The assays were conducted using validated commercial ELISA kits:

- Pepsin (Bioassay Technology Laboratory, Cat. No. E1581Hu), measured in ng/mL, and
- IL-8 (Bioassay Technology Laboratory, Cat. No. E0102Hu), measured in pg/mL.

All samples were analyzed in duplicate according to the manufacturer's instructions. Absorbance was read at 450 nm using a microplate reader. Quality control procedures were applied to ensure assay precision and accuracy.

Statistical analysis was performed using SPSS version 25.0. Descriptive statistics were used for demographic data and biomarker concentrations. The Shapiro-Wilk test assessed normality of data distribution. The correlation between pepsin and IL-8 levels was evaluated using the Pearson Correlation Test, with a significance level of $p < 0.05$.

This study was approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital (Reference No. 1261/IV/HREC/2023). All participants provided written informed consent. Anonymity and confidentiality of subject data were strictly maintained.

RESULT

Total 32 patients enrolled, 19 (59.4%) were female and 13 (40.6%) were male. Age distribution was skewed toward middle-aged adults: the largest proportion fell into the 36–45-year bracket (28.1%), followed by the >65-year group (18.8%). Most participants worked in the private sector (46.9%), or were housewives (34.4%), with the remainder in diverse occupations (students, traders, farmers, teachers). These data (Table 1) confirmed a broadly representative CSOM population, though a predominance of middle-aged women might reflect the increased health-seeking behavior in this subgroup.

Middle ear fluid analysis revealed a mean pepsin concentration of 71.52±84.64 ng/mL and a mean IL-8 concentration of 33.47±17.56 pg/mL. The large standard deviation for pepsin indicates marked interindividual variability, suggestive of heterogeneous reflux burden or differences in disease chronicity among subjects. Shapiro–Wilk testing showed both variables deviated significantly from normal distribution (IL-8: $W=0.555$, $p < 0.001$; Pepsin: $W=0.768$, $p < 0.001$).

Using Spearman's rank correlation, pepsin and IL8 levels demonstrated a moderate, statistically significant positive correlation ($r=0.42$, $p=0.016$). This finding underscoring a robust association between reflux-derived pepsin and local inflammatory activity.

Scatter plot showing individual patient values for IL8 (xaxis) and pepsin (yaxis) concentrations in middle ear fluid. While most values cluster at lower IL8 (<100 pg/mL), pepsin concentrations vary widely (Figure 2). The upward trend line highlighted that higher IL8 levels are generally accompanied by elevated pepsin, consistent with a refluxmediated inflammatory mechanism in CSOM.

Table 1. Participant characteristics

Characteristics	n (%)
Age (years)	
18 – 25	5 (15.62%)
26 – 35	4 (12.5%)
36 – 45	9 (28.12%)
46 – 55	4 (12.5%)
56 – 65	4 (12.5%)
>65	6 (18.75%)
Gender	
Male	13 (40.6%)
Female	19 (59.4%)
Occupation	
Private Sector	15 (46.9%)
Housewife	11 (34.4%)
College Student	3 (9.4%)
Trader	1 (3.1%)
Farmer	1 (3.1%)
Teacher	1 (3.1%)

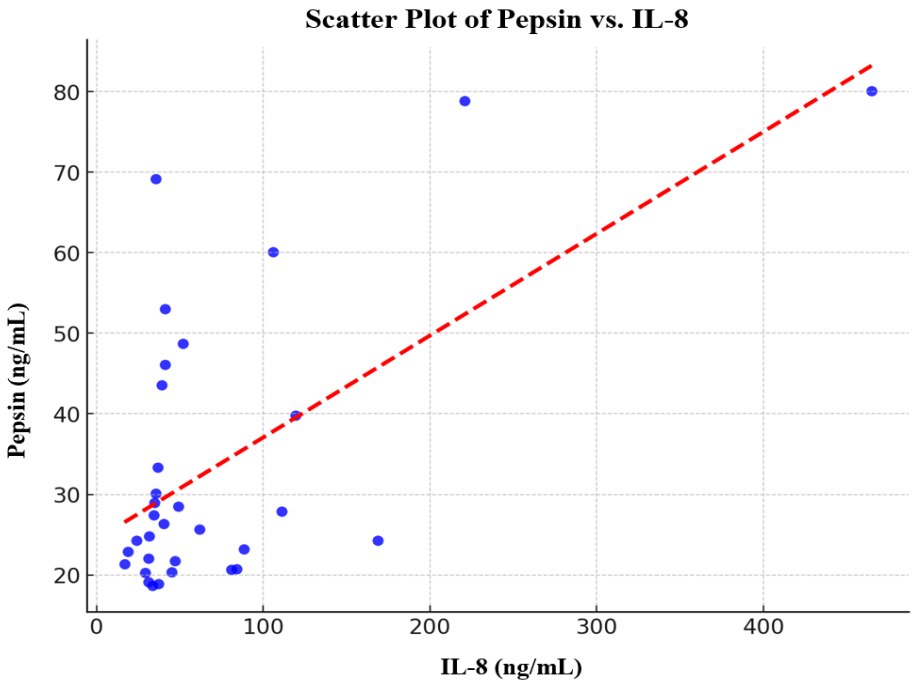

Figure 2. Study flow diagram

DISCUSSION

This study provided comprehensive evidence for a moderate, statistically significant positive correlation between pepsin and interleukin-8 (IL-8) levels in middle ear effusions of patients with active-phase chronic suppurative otitis media (CSOM) accompanied by laryngopharyngeal reflux (LPR) (r=0.42, p=0.016). By integrating demographic, socioeconomic, clinical, and molecular perspectives, our findings shed light on a reflux-mediated inflammatory axis contributing to CSOM pathogenesis. Furthermore, we would discuss these implications in depth.

Demographic and socioeconomic pattern

The predominance of middle-aged adults (36–45 years, 28.1%) and older adults (>65 years, 18.8%) parallels global epidemiological patterns indicating that CSOM often follows unresolved acute otitis media episodes and age-related declines in mucosal immunity.[20,21] In developing regions, where healthcare access may be limited, recurrent infections and delayed treatment accelerate progression to chronicity.[1] Furthermore, nearly half of our cohort worked in the private sector, and over one-third were housewives—a reflection of socioeconomic strata exposed to overcrowded housing, poor ventilation, and limited sanitary infrastructure.[22] Such environmental stressors predispose to repeated upper respiratory tract infections and impaired mucociliary clearance, compounding risk for persistent middle ear inflammation.

Although gender distribution was slightly skewed toward females (59.4%), neither pepsin nor IL-8 levels differed by sex, echoing prior studies that found no clinically significant gender bias in CSOM prevalence or inflammatory biomarker expression.[23] Occupation likewise did not influence biomarker levels, suggesting that individual exposome rather than job category per se, is central to LPR severity and subsequent pepsin deposition.[24]

Pepsin's mechanistic role in middle ear inflammation

Pepsin, an aspartic endopeptidase secreted as pepsinogen from gastric chief cells, retains activity when refluxed into the upper aerodigestive tract and middle ear.[24] The Eustachian tube, which normally equalizes middle ear pressure and drains secretions, can act as a conduit for refluxate when its function is compromised by inflammation or anatomical variants.[25] In the non-gastric milieu of the middle ear, pepsin retains enzymatic activity at pH values up to 6.5, promoting proteolysis of mucosal proteins and disruption of epithelial tight junctions.[26]

Beyond direct mucosal injury, pepsin exerts a pro-inflammatory signal through activation of Protease-Activated Receptor 2 (PAR-2) on epithelial cells.[27] PAR-2 engagement triggers intracellular cascades culminating in NF-κB nuclear translocation and transcription of multiple cytokines, notably IL-8.[28] Our observation that rising pepsin concentrations were mirrored by elevated IL-8 levels, provided in vivo corroboration of this pathway, highlighting pepsin as both a biomarker of reflux and a functional mediator of inflammation.

IL-8 as a central effector of neutrophil-mediated damage

IL-8, a CXC chemokine, orchestrates neutrophil chemotaxis, degranulation, and respiratory burst—key processes in acute inflammatory defense but deleterious when chronically activated.[29] In CSOM, persistent IL-8 elevation fosters a self-perpetuating cycle: neutrophils release proteases and reactive oxygen species that damage mucosal structures, which in turn sustain cytokine production.[18]

Our mean IL-8 level (33.47±17.56 pg/mL) aligned with pediatric and adult

CSOM cohorts, but the markedly higher standard deviation for pepsin underscored heterogeneity in reflux exposure.[30] Such variability may reflect differences in reflux frequency, volume, or concurrent factors such as Eustachian tube dysfunction and mucociliary impairment.[31] Further stratification by reflux severity would refine understanding of how pepsin load modulates IL-8 induction.

Clinical correlations and implications

Logistic and linear regression analyses from O'Reilly et al.[13] demonstrated that detectable pepsin conferred a four-fold increase in odds of elevated IL-8 (OR=3.96; 95% CI 1.3–12.0) and accounted for 25% of IL-8 variance (R^2=0.248; $p < 0.001$). Sriyana et al.[9] similarly reported that a positive Reflux Finding Score (RFS≥7) increased the risk of pepsin presence five-fold (95% CI 1.10–24.07; $p < 0.05$).[9] Lechien et al.[26] extended these insights, showing pepsin in 77% of middle ear effusions, with levels correlating to effusion viscosity and co-elevation of IL-6, neutrophil elastase, and mucin 5B.

Clinically, the detection of pepsin in ear fluid might serve as a screening tool to identify CSOM patients who could benefit from targeted anti-reflux therapy.[12] By attenuating pepsin exposure, such interventions may reduce IL-8–driven neutrophilic infiltration, diminished mucosal injury, and accelerated resolution of otorrhea. Moreover, anti-reflux measures could complement antibiotic regimens, potentially lowering the risk of antibiotic resistance by addressing a separate etiological factor.

Limitations and future directions

This study was not without limitations. Its cross-sectional design precluded causal interpretation between pepsin and IL-8 expression, and it did not include objective diagnostic tools such as 24-hour pH impedance monitoring, RFS, or RSI to quantify LPR severity. Confounding variables—including disease duration, prior use of anti-inflammatory or antibiotic medications, nutritional status, and environmental exposure—were not assessed and might have influenced the inflammatory markers measured. Furthermore, the absence of microbiological examination (e.g., bacterial culture or PCR) made it difficult to distinguish whether IL-8 elevation was driven solely by reflux-mediated mechanisms or also influenced by infection. These constraints limited the generalizability and precision of the conclusions, highlighting the need for more controlled study designs.

Future research should adopt a prospective, longitudinal approach, integrating reflux diagnostics, comprehensive biomarker panels, and microbiological profiling. Serial measurement of pepsin and cytokines over time, combined with intervention trials using anti-reflux therapies (e.g., PPIs, lifestyle modification), could help determine the true clinical impact of reflux in CSOM. In vitro and animal models should also be employed to dissect the underlying mechanisms by which pepsin induces IL-8 expression. Ultimately, defining pepsin threshold levels that trigger inflammation and linking them with clinical outcomes may facilitate development of diagnostic and therapeutic guidelines for reflux-associated CSOM.

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REFERENCE

1. World Health Organization. Chronic suppurative otitis media: burden of illness and management options [Internet]. Geneva: World Health Organization; 2004 [cited 2024 Jun 18]. Available from: http://www.who.int/pbd/publications/Chronicsuppurativeotitis_media.pdf
2. Mansour S, Al Shawabkeh MA, Nicolas K, Haidar H. Chronic suppurative otitis media (CSOM). In: Textbook of Clinical Otolaryngology. Cham: Springer International Publishing; 2021. p. 63-76. doi:10.1007/978-3-030-54088-3_5.
3. Bhutta MF, Leach AJ, Brennan-Jones CG. Chronic suppurative otitis media. Lancet. 2024;403(10441):2339-48. doi:10.1016/S0140-6736(24)00259-9.
4. Sari JTY, Edward Y, Rosalinda R. Otitis media supuratif kronis tipe kolesteatom dengan komplikasi meningitis dan paresis nervus fasialis perifer. J Kesehat Andalas. 2018;7(Suppl):88. doi:10.25077/jka.v7i0.931.
5. Ikhlas KA, Edward Y. Laporan kasus: penatalaksanaan otitis media supuratif kronis tipe kolesteatoma dengan komplikasi abses otak berulang. J Agromedicine Med Sci. 2022;8(1). doi:10.19184/ams.v8i1.2436.
6. Sudrajad H, Pratiwi D. The effect of zinc supplementation on non-cholesteatoma chronic suppurative otitis media. Jordan Med J. 2024;58(4).
7. Dewi MR, Pratiwi D, Kandhi PW. High TNF-α levels in active phase chronic suppurative otitis media caused by gram-positive bacteria. Mol Cell Biomed Sci. 2023;7(2):75. doi:10.21705/mcbs.v7i2.321.
8. Rizzo M, Varnier L, Pezzicoli G, Pirovano M, Cosmai L, Porta C. IL-8 and its role as a potential biomarker of resistance to anti-angiogenic agents and immune checkpoint inhibitors in metastatic renal cell carcinoma. Front Oncol. 2022;12:990568. doi:10.3389/fonc.2022.990568.
9. Sriyana AA, Tamin S, Hutahuruk SM, Bardosono S, Timan IS, Restuti RD. The relation of reflux finding score and reflux symptom index with middle ear pepsin level in chronic suppurative otitis media. J Int Dent Med Res. 2017;10:1048-51. Available from: http://www.ektodermaldisplazi.com/journal.htm
10. Li Y, Xu G, Zhou B, Tang Y, Liu X, Wu Y, et al. Effects of acids, pepsin, bile acids, and trypsin on laryngopharyngeal reflux diseases: physiopathology and therapeutic targets. Eur Arch Otorhinolaryngol. 2022;279(6):2743-52. doi:10.1007/s00405-021-07201-w.
11. Kowalik K, Krzeski A. The role of pepsin in laryngopharyngeal reflux. Otolaryngol Pol. 2017;71(6):7-13. doi:10.5604/01.3001.0010.7194.
12. Barona-Lleo L, Barona-De Guzman R, Krstulovic C. The diagnostic usefulness of the salivary pepsin test in symptomatic laryngopharyngeal reflux. J Voice. 2019;33(6):923-8. doi:10.1016/j.jvoice.2018.07.008.
13. O'Reilly RC, Soundar S, Tonb D, Bolling L, Yoo E, Nadal T, et al. The role of gastric pepsin in the inflammatory cascade of pediatric otitis media. JAMA Otolaryngol Head Neck Surg. 2015;141(4):350-7. doi:10.1001/jamaoto.2014.3581.
14. Wang J, Zhao Y, Ren J, Xu Y. Pepsin in saliva as a diagnostic biomarker in laryngopharyngeal reflux: a meta-analysis. Eur Arch Otorhinolaryngol. 2018;275(3):671-8. doi:10.1007/s00405-017-4845-8.
15. Tan JJ, Wang L, Mo TT, Wang J, Wang MG, Li XP. Pepsin promotes IL-8 signaling-induced epithelial-mesenchymal transition in laryngeal carcinoma. Cancer Cell Int. 2019;19:64. doi:10.1186/s12935-019-0772-7.
16. Elmorsy S, Shabana YK, Raouf AA, El Naggar ME, Bedir T, Taher S, et al. The role of IL-8 in different types of otitis media and bacteriological correlation. J Int Adv Otol. 2010;6(2).
17. Serban R, Filip C, Radulescu LM, Badescu MC, Badescu MM, Diaconescu BM, et al. IL-1α, IL-6 and IL-8 serum values in patients with chronic suppurative otitis media. Exp Ther Med. 2021;22(5):1226. doi:10.3892/etm.2021.10660.
18. Samuels TL, Khampang P, Espahbodi M, McCormick CA, Chun RH, McCormick ME, et al. Association of pepsin with inflammatory signaling and effusion viscosity

in pediatric otitis media. Laryngoscope. 2022;132(2):470-7. doi:10.1002/lary.29749.

19. Massa HM, Spann KM, Cripps AW. Innate immunity in the middle ear mucosa. Front Cell Infect Microbiol. 2021;11:764772. doi:10.3389/fcimb.2021.764772.

20. Schilder AGM, Chonmaitree T, Cripps AW, Rosenfeld RM, Casselbrant ML, Haggard MP, et al. Otitis media. Nat Rev Dis Primers. 2016;2:16063. doi:10.1038/nrdp.2016.63.

21. Hidayat R. Pathophysiological to clinical aspects of chronic suppurative otitis media (CSOM): narrative literature review. Arch Med Case Rep. 2022;3(2):246-55. doi:10.37275/amcr.v3i2.175.

22. Nath Prasad A, Shekhar R. Prevalence of chronic suppurative otitis media (CSOM) and associated hearing impairment among school age children in Muzaffarpur District, Bihar, India [Internet]. Int J Pharm Clin Res. 2024;16(1):1111-5. Available from: https://www.ijpcr.com

23. Abraham ZS, Ntunaguzi D, Kahinga AA, Mapondella KB, Massawe ER, Nkuwi EJ, et al. Prevalence and etiological agents for chronic suppurative otitis media in a tertiary hospital in Tanzania. BMC Res Notes. 2019;12(1):429. doi:10.1186/s13104-019-4483-x.

24. Suryani L, Widuri A. Chronic suppurative otitis media characteristic in secondary hospital in Yogyakarta. Open Access Maced J Med Sci. 2022;9(T5):152-6. doi:10.3889/oamjms.2021.7860.

25. Briddell JW, Levi JR, O'Reilly RC. Chronic otitis media. In: Infections of the Ears, Nose, Throat, and Sinuses. Cham: Springer International Publishing; 2018. p. 57-66. doi:10.1007/978-3-319-74835-1_5.

26. Lechien JR, Saussez S, Karkos PD. Laryngopharyngeal reflux disease: clinical presentation, diagnosis and therapeutic challenges in 2018. Curr Opin Otolaryngol Head Neck Surg. 2018;26(6):392-402. doi:10.1097/MOO.0000000000000486.

27. Juhn SK, Jung MK, Hoffman MD, Drew BR, Preciado DA, Sausen NJ, et al. The role of inflammatory mediators in the pathogenesis of otitis media and sequelae. Clin Exp Otorhinolaryngol. 2008;1(3):117-38. doi:10.3342/ceo.2008.1.3.117.