The Relation of Reflux Finding Score and Reflux Symptom Index with Middle Ear Pepsin Level in Chronic Suppurative Otitis Media

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Abstract

Laryngopharyngeal reflux is one of the factors that contribute to the development of chronic otitis media; however, its role in chronic suppurative otitis media (CSOM) remains unclear. Previous studies showed that refluxate could reach the middle ear, and reported the presence of pepsin, a marker of extraesophageal reflux, in the middle ear, where it may play inflammatory role. In CSOM patients with laryngopharyngeal reflux, refluxate may reach the middle ear and play a role in middle ear inflammation and infection. This study aimed to investigate the relation of reflux finding score (RFS) and reflux symptom index (RSI) with middle ear pepsin level as a predictor of reflux into the middle ear in CSOM patients. In this cross-sectional study, we analyzed RFS, RSI, and pepsin levels in the middle ear as markers of laryngopharyngeal reflux in CSOM patients. Forty-two subjects (21 male, 21 female) were enrolled via consecutive sampling. RFS was measured by two different blinded reviewers using recorded video. Middle ear pepsin levels were measured using a Human Pepsin ELISA Kit Cusabio (CSB-E08919h). **Results:** Pepsin was detected in the middle ear in 25 (59.5%) patients with CSOM. Mean middle ear pepsin levels were significantly higher in patients with positive RFS than in those with negative RFS ($p < 0.05$). Compared to patients with negative RFS, patients with positive RFS had a 5.13-fold higher risk of pepsin in the middle ear (CI 95%=1.095-24.073). RSI was not correlated with middle ear pepsin levels. Pepsin was detected in the middle ear in more than half the study participants, and RFS ≥ 7 was significantly correlated with increased risk of pepsin in the middle ear. These results suggest that the role of laryngopharyngeal reflux in CSOM warrants further investigation.

Clinical article (J Int Dent Med Res 2017; 10: (3), pp. 1048-1051)

Keywords: Reflux, Middle Ear. Pepsin Level, Chronic Suppurative Otitis Media.

Received date: 10 May 2017 **Accept date:** 13 July 2017

Introduction

Chronic suppurative otitis media (CSOM) is one of the major ear, nose, and throat problems in developing countries.[1] Many factors have been studied for their role in CSOM development. One such potential factor, laryngopharyngeal reflux,has been previously linked to otitis media;[2] however, very few studies have investigated its role in CSOM. Previous studies have reported that refluxate can reach the middle ear.[2-5] Pepsin, which is present in refluxate and is a reliable marker for the diagnosis of extraesophageal reflux disease, has been previously detected in the middle ear in patients with otitis media (or extraesophageal reflux disease).[6]

Previous studies have demonstrated the presence of pepsin in the trachea, lung, sinus, middle ear, combined sputum and saliva, and exhaled breath condensate.[6] Using a rabbit model, Basoglu *et al.* found that gastroesophageal reflux could induced middle ear inflammation andwas associated with increased expression of vascular endothelial growth factor, interleukin-1β, interleukin-17, and inducible nitric oxide synthase.[7] In CSOM patients with laryngopharyngeal reflux, refluxate may reach the middle ear and play a role in CSOM

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inflammation and infection. In this study, we aimed to investigate the relation of reflux finding score (RFS) and reflux symptom index (RSI), as predictors of reflux into the middle ear, with middle ear pepsin level in CSOM patients.

Materials and methods

Before initiating the study, we obtained written informed consent from the patients and ethical approval from the Ethics Research Committee Faculty of Medicine University of Indonesia. Inclusion criteria for the study were age over 18 years and a diagnosis of CSOM (without cholesteatoma). Exclusion criteria were ear tampon or ear drop use in the past 7days, proton pump inhibitor use in the past 4 days, H-2 blocker use in the past 12 hours, and antacid use in the past 6 hours. Consecutive sampling yielded 42 patients that matched the inclusion and exclusion criteria. This was a cross-sectional study to analyze laryngopharyngeal refluxin CSOM patients on the basis of RFS, RSI, and pepsin level in the middle ear. This study was conducted in the ENT Department of Cipto Mangunkusumo Hospital, Indonesia.

Patients were examined with otomicroscopy, and middle ear discharge was collected using a 3-ml syringe connected to a Spiggle and Theis microsuction tube. The middle ear was washed through the perforation with 1 ml of 0.9% NaCl by using the syringe connected to the microsuction tube, and the fluid was collected back into the syringe. The fluid was subsequently placed in a 2-ml Eppendorf tube. The sample was transported immediately to the lab and stored in a freezer at-80 °C. Middle ear pepsin levels were measured using a Human Pepsin ELISA Kit (CSB-E08919h; Cusabio Biotech, Wuhan, China) in the Pathology Clinic Laboratory of Cipto Mangunkusumo Hospital. Pepsin was analyzed quantitatively, and patients were then divided into a positive pepsin group (pepsin level > 108.1 pg/ml) and a negative pepsin group (pepsin level ≤ 108.1 pg/ml).[8]

A flexible endoscope Olympus 0tv-s7 and Olympus clv-s40 light source was used to examine the larynx. Laryngeal examinations were recorded on video and assessed by two blinded reviewers using the RFS developed by Belafsky.[9] RFS > 7 was considered as positive RFS. The reviewers were senior consultants in the Larynx, Pharynx, and Bronchoesophageal Division of the ENT Department at Cipto Mangunkusumo Hospital. The RSI questionnaire developed by Belafsky was used for the evaluation of reflux symptoms, and a score > 13 was categorized as positive RSI.[10] Data were collected and analyzed using SPSS *(Statistical Package for Social Science)* 20.0.

Results

Forty-two CSOM patients were enrolled in this study (21 male, 21 female; age range, 18–70 years; median, 36.5 years). Of the 42 participants, 39 (92.9%) experienced a >1-year history of CSOM. Disease recurrence occurred approximately 1–3 times a year in 64.3% of patients. Of the 42 CSOM patients, 30 experienced unilateral CSOM (71.4%) and 12 experienced bilateral CSOM (28.6%), with a total of 54 ears affected. Only 42 ears with discharge from 42 patients were analyzed for pepsin. Of these, 59.5% patients were positive for pepsin. Thirty-two (76.2%) patients had positive RFS but only five (11.9%) patients had positive RSI. Laryngopharyngeal reflux as determined by positive pepsin or positive RFS or both was detected in 35 (83.3%) of the 42 CSOM patients.

Characteristics	N	%
Sex		
Male	21	50
Female	21	50
Duration		
<1 Year	3	7.1
≥1 Year	39	92.9
Frequency		
1-3 times/year	27	64.3
≥4 times/year	15	35.7
CSOM unilateral/bilateral		
Unilateral	30	71.4
Bilateral	12	28.6
RSI		
Positive	5	11.9
Negative	37	88.1
RFS		
Positive	32	76.2
Negative	10	23.8
Pepsin		
Positive	25	59.5
Negative	17	40.5
Laryngopharyngeal reflux as determined by positive RFS and or pepsin	35	83.3
Positive		
Negative	7	16.6

Table 1. Patient clinicopathological characteristics

Middle ear pepsin level and RFS

The RFS from two blinded reviewers had statistically normal distribution, with the Spearman's correlation coefficient (R=0.814; p=0.001) revealing a high correlation. Inter observer agreement between the two reviewers was also analyzed for RFS (kappa agreement=1; p<0.001). Independent *t*-tests revealed that the mean middle ear pepsin level was higher in the RFS positive group (RFS >7) than in the RFS negative group (p<0.05; CI 95% = 22.1–190.4). Even though the Fisher exact test showed that RFS was not significantly correlated with middle ear pepsin level (p>0.05), the prevalence rate of patients positive for both RFS and middle ear pepsin was 5.13 (CI 95% 1.095-24.073). The positive predictive value of RFS for pepsin positivity in the middle ear was 68%. There was no significant difference in middle ear pepsin levels between patients with positive and negative RSI (p=0.722; CI 95%=-140-98.03; Table 4). No significant correlation was found between RSI and pepsin positivity/negativity in the middle ear (p=0.683; CI 95%=0.152–6.877; Table 5).

	N	Pepsin level (Mean± SD)	*P*	CI 95%
Positive RFS	32	185.715±127.2 pg/mL	0.015*	22.1–190.4
Negative RFS	10	79.46±54.4 pg/mL		

Table 2. Reflux finding score with middle ear pepsin level (n=42). *Independent sample *t*-test. CI, confidence interval; RFS, reflux finding score; SD, standard deviation

	Pepsin			*P*	PR	CI 95%
	Positive	Negative	Total			
RFS						
Positive	22	10	32	0.062*	5.133	1.095–24.073
Negative	3	7	10			
Total	25	17	42			

Table 3. Reflux finding score with positive/negative pepsin in the middle ear. *Fisher exact test. CI, confidence interval; PR, prevalence rate; RFS, reflux finding score

	N	Pepsin level (Mean± SD)	*P*	CI 95%
Positive RSI	5	141.8±90.2 pg/ml	0.722*	-140.2-98.03*
Negative RSI	37	162.9±126.9 pg/ml		

Table 4: Reflux symptom index and middle ear pepsin level (n=42). *Independent sample *t*-test.CI, confidence interval; RSI, reflux symptom index; SD, standard deviation

	Pepsin			*P*	PR	CI 95%
	Positive	Negative	Total			
RFS						
Positive	3	2	5	0.683*	1.023	0.152-6.877
Negative	22	15	37			
Total	25	17	42			

Table 5. Reflux symptom index and positive/negative pepsin. *Fisher'sexact test.CI, confidence interval; PR, prevalence rate, RSI, reflux symptom index

Discussion

Middle ear pepsin level was calculated using a pepsin standard curve at concentrations of 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, and 31.25 pg/ml. In this study, the proportion of patients positive for pepsin (59.5%) was higher than that of patients negative for pepsin, and higher than that reported previously. In a previous study in adult csom patients, Taliyah *et al.* detected pepsin in only two out of a total of five samples, with levels of 43 pg/ml and 335.7 pg/ml.[11] The discrepancy between our results and those of previous studies could be due to an exclusion criterion in our study. Compared to the exclusion criteria used in previous research, our exclusion criteria were more restrictive to avoid the possibility of undetected pepsin due to various factors such as the use of eardrops and the consumption of proton pump inhibitors, h-2 blockers, or antacids. positive RFS was found in 32 of 42 patients (76.2%) in the present study. using ph monitoring, Bercin *et al.* found that more than 40% of patients with chronic otitis media experienced laryngopharyngeal reflux[12] rfs has been investigated in patients with RLF and compared with ph monitoring results.[12] A total RFS>7 has been shown to be a significantly better RLF diagnostic tool than ph monitoring, with a 95% confidence level.[9]

Taliyah *et al.* found that seven of 20 CSOM patients in their study experienced reflux symptoms.[11] In the present study, positive RSI was found in only five of 42 CSOM patients (11.9%). Another study conducted by Habesoglu *et al.* revealed that scores on a questionnaire for symptoms of gastroesophageal reflux disease were higher in CSOM patients with failed

tympanic membrane closure than in patients with successful tympanic membrane closure.[13] In the present study, the mean middle ear pepsin level was higher in the positive RFS group than in the negative RFS group (table 2; $p < 0.05$). Pepsin, a marker of extra esophageal reflux, is not produced in the middle ear; therefore, its presence in the middle ear is a sign that the refluxate can reach the middle ear mucosa.[14] Positive RFS was correlated with the presence of pepsin in the middle ear; patients with positive RFS had a 5.13-fold higher risk of pepsin presence in the middle ear (95% CI = 1.095-24.073). These results indicate that laryngopharyngeal reflux characterized by positive RFS may increase the risk of inflammation in the middle ear and Eustachian tube mucosa. The positive predictive value of RFS for positive pepsin in the middle ear was 68%.

In addition to its role in infection, laryngopharyngeal reflux may play a role in chronic inflammation associated with CSOM. Reflux is known to directly damage the supra esophageal mucosal structure, including the Eustachian tube and middle ear mucosa. A previous study showed increased inflammation and cytokine expression in rabbit middle ear mucosa after exposure to refluxate.[8] In the present study, the mean middle ear pepsin levels were not significantly different between positive and negative RSI groups, and the RSI was not significantly correlated with middle ear pepsin levels (p=0.683, 95% CI=0.152-6.877). He *et al*. showed that the presence of pepsin in the middle ear is not always associated with the classic symptoms of RLF.[4] Similarly, a study conducted in 509 children by O'Reilly *et al.* revealed that pepsin in the middle ear is not associated with reported of symptoms of gastroesophageal reflux disease.[5]

Conclusions

Mean middle ear pepsin levels were significantly higher in the positive RFS group than in the negative RFS group ($p < 0.05$). More than half of the patients were positive for pepsin in the middle ear, and RFS > 7 resulted in a 5.13-fold increase in the risk of positive pepsin in the middle ear (CI 95%=1.095-24.073).

The RFS is crucial for the diagnosis of laryngopharyngeal reflux in CSOM patients. Reflux ate may reach the middle ear and play a role in the chronic inflammation associated with CSOM. A cohort study, based on the results of the present study, is warranted to learn more about the role of RLF as a risk factor for CSOM.

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