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Analysis of Pepsin Levels in Tonsil Swab Patients of Chronic Tonsillitis on Larynx Pharynx Reflux

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| : Chronic tonsillitis is a condition of tonsil inflammation. There is some evidence that | | | |
| the condition laryngeal pharyngeal reflux (LPR) causes chronic tonsillitis, through pepsin which can | | | |
| pper and lower throat secretions. | | | |
| o analyze the pepsin levels of tonsil swab from chronic tonsillitis patients on LPR | | | |
| s research used a cross-sectional design which was conducted at Wahidin Sudirohusodo | | | |
| ital in June 2023 until the sample was met. The research sample is chronic tonsillitis will be divided into two groups (chronic tonsillitis with LPR and without LPR). Both went pepsin examinations from tonsil swab. All data was tested using SPSS version 25. ubjects with chronic tonsillitis were included. The LPR group consisted of 11 subjects LPR group consisted of 40 subjects. Pepsin levels were found in all chronic tonsillitis sin levels were higher in the LPR group compared to without LPR group with median 335.16(23.46-399.21) vs 330.06(15.68-407.90), but statistically not significant .05) Pepsin levels were higher in the chronic tonsillitis group with LPR compared to without | | | |
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1. Introduction

Chronic tonsillitis is an inflammation of the palatine tonsils that lasts for a long time, caused by bacterial or viral infections and can occur at any age, but is more prevalent in children.¹ Tonsillitis also can caused by laryngopharyngeal reflux (LPR). A study in Makassar of LPR patients stated that 48 of 51 samples detected pepsin in saliva.²

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Pepsin is a diagnostic sign of LPR, and non-acid reflux can cause damage to the larynx. Normally, pepsin will stabilize at a pH of 6.8 because of the larynx's job of maintaining this condition. However, in subsequent episodes of reflux, or by hydrogen ions from the stomach or food, pepsin can be reactivated. Apart from that, pepsin can also cause intracellular damage, where the Golgi complex and lysosomes are found to have a low pH, namely 5.0 and 4.0. The presence of pepsin in tissues has been associated with depletion of E-cadherin, Sep-7 (stress epithelial protein), and carbonic anhydrase. According to a new study, pepsin raises levels of genetic markers linked to cancer.³

Reflux of gastric contents containing pepsin can cause various symptoms and cause inflammation, as well as worsen tonsil hypertrophy.⁴ The aim of this study was to analyze the pepsin levels of tonsil swab from patients with chronic tonsillitis regarding laryngopharyngeal reflux

2. Methods

Participants and Research Design

This research is an analytical study with a cross-sectional study design, which was conducted at Wahidin Sudirohusodo General Hospital from June 2023 until the sample size was met. The sample for this study was chronic tonsillitis patients who visited the ENT-HNS polyclinic at Wahidin Sudirohusodo Hospital. Patients aged 15–60 who were willing to take part in the research by providing written consent were included in this study. Patients who did not follow all study procedures and

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consumed proton pump inhibitors (PPI), antacids, and histamine-2 antagonists within 2 weeks before the tonsil swab examination were excluded.

Measurement Method

Reflux symptoms index (RSI) and reflux finding score (RFS) scores were assessed for all study samples and categorized into PLR and not PLR. A score of RSI > 13 and RSF > 7 is said to be PLR. Both groups had tonsil swab taken to check pepsin levels. Pepsin levels were determined by ELISA examination using the ELISA Kit Cat. No. MBS162469 method.

Statistic analysis

After all the data was obtained, data analysis was carried out using SPSS version 25. Subject characteristics in the form of age and gender were displayed in the form of frequencies and percentages. Pepsin levels in chronic tonsillitis patients were assessed as median, minimum, and maximum, and categorically as present or absent. Differences in tonsil swab pepsin levels in chronic tonsillitis patients based on the presence of PLR and without PLR were tested using Mann-Whitney U.

Ethical Clearance

Every action in this study was carried out only after information was provided and with parental consent. This research with human subjects was conducted in accordance with the ethical guidelines of each institution. In accordance with ethical guidelines, the Ethics Commission of the Research and Development Agency of Wahidin Sudirohusodo Hospital and the Hasanuddin University Faculty of Medicine support this research protocol.

3. Results

Fifty-one patients with a diagnosis of chronic tonsillitis met the inclusion and exclusion criteria, so they were included in this study.

Subject Characteristics

Of the 51 research subjects, 29 subjects(56.9%) were women. Based on the age range, the majority were in the 25-55 years with totaling 42 subjects (82.4%), and a small portion were in the 16-24 years, 6 subjects (11.8%), and in the >55 year age group, 3 subjects (5.9%). Based on PLR, the majority of subjects did not experience PLR, namely 40 subjects (78.4%). The results of the pepsin examination were obtained by as many as 51 subjects (100%) who detected pepsin in the research subjects. (Table 1).

Table 1. Subject Characteristics

| Characteristics | | n | % |
|------------------|-------------|----|------|
| Age | 16-24 years | 6 | 11.8 |
| | 25-55 years | 42 | 82.4 |
| | >55 years | 3 | 5.9 |
| Gender | Men | 22 | 43.1 |
| | Women | 29 | 56.9 |
| LPR Complaint | Yes | 11 | 21.6 |
| | No | 40 | 78.4 |
| Pepsin | Yes | 51 | 100 |
| | No | 0 | 0.0 |
| Total | | 51 | 100 |

Differences in Pepsin Levels in Tonsil Swab in Chronic Tonsillitis Patients Based on the Presence of LPR and Without LPR

Of the 51 research subjects, pepsin was found in all tonsil swab of chronic tonsillitis patients, where pepsin levels in tonsil swab of chronic tonsillitis patients ranged from 15.68 to 407.90 pg/ml with a median of 293.40 (table 2).

 Table 2. Pepsin Levels in Tonsil Swab of Chronic

 Tonsillitis Patients

| | Pepsin (pg/ml) | | | Min |
|---------------------------------|----------------|-----------------|--------|------------------|
| Variable | Detected | Not Detected | Median | Max |
| Chronic tonsilitis (n=51) | 51 | 0 | 293.40 | 15.68- 407.90 |
| Total | 100% | 0% | | |

The pepsin levels of tonsil swab from chronic tonsillitis patients with PLR complaints ranged from 23.46 to 399.21 pg/ml with a median of 335.16 pg/ml, while the pepsin levels from chronic tonsillitis patients who did not experience LPR ranged from 15.68 to 407.90 pg/ml with a median of 330.06 pg/ml (table 3).

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| Table 3. Differences in Pepsin Levels in the LPR and | ł |
|--|---|
| Withour LPR Groups | |

| LPR | N | modian | Min- | D voluo |
|------------------|----|--------|--------|---------|
| Complaint | IN | median | Max | P-value |
| Yes | 11 | 335.16 | 23.46- | 0 662* |
| | | | 399.21 | 0.003* |
| No | 40 | 330.06 | 15.68- | |
| | | | 407.90 | |
| *Monn Whitney II | | | | |

*Mann-Whitney U

The results of the Mann-Whitney U analysis showed that there was no significant difference in pepsin in tonsil swab, p = 0.663 (p > 0.05) between chronic tonsillitis patients with LPR and chronic tonsillitis patients without LPR, but based on the pepsin levels of chronic tonsillitis patients with LPR, it showed a value that was higher compared to those without LPR (median 335.16 vs. 294.97 pg/ml) but not statistically significant (table 3 and figure 1).



Figure 1. Box Plot of Pepsin Levels in Tonsil Swab in Chronic Tonsillitis Patients

4. Discussion

Ages 25–55 years were found to be more common in this study. In accordance with research by Silvoniemi et al. (2020), chronic tonsillitis was also found in patients over 30 years old. Overall, the age range of chronic tonsillitis patients can vary, but it generally affects those aged over 10 years.⁵ This study shows the presence of pepsin levels in all tonsil swab of chronic tonsillitis patients. Kim et al. (2018) found that in vitro, pepsin-positive cells were discovered around hypertrophic tonsil cells. These cells did not co-localize with CD20 or CD45 cells, but they did with CD68+ cells. This study demonstrates that pepsin from extraesophageal reflux may aggravate

tonsillar hypertrophy, and that the administration of pepstatin, a pepsin inhibitor, may be protective.⁴

Kim et al. (2016) found that pepsin was expressed at different levels in the tonsil tissue of each pediatric and adult patient. This suggests that pepsin may be involved in the development and progression of tonsillar hypertrophy. The presence of pepsin in the tonsils may be related to the presence of bacterial biofilm in chronic tonsil disease.⁶ Bakar MA et al. (2018) provides anatomical evidence of the presence of bacterial biofilm in chronic tonsil disease. These biofilms may explain the chronic and recurrent nature of some forms of tonsillitis, as the sessile bacteria in the biofilm are resistant to the body's defenses and antibiotics. Besides pepsin, other factors may contribute to the pathogenesis of chronic tonsillitis.7 Garca et al. (2014) found that increased adenosine deaminase (ADA) activity may be effective in the pathogenesis of chronic tonsillitis by damaging tissue structure and contributing to the generation of oxidative stress.⁸ Free radicals and scavenging enzymes are also involved in the pathophysiology of chronic tonsillitis.9

The results of the study showed that pepsin levels in tonsil swab from chronic tonsillitis patients with PLR were higher compared to chronic tonsillitis patients without PLR. However, this difference was not significant (p > 0.05). There is research evidence that shows differences in pepsin levels between individuals who have complaints related to PLR and those who do not have complaints. Wood et al. (2011) explained that pepsin, which is potentially found in the larynx, can cause damage during reflux episodes. Pepsin is actively carried into laryngeal epithelial cells and can cause intracellular damage even when only inactive pepsin is present. This shows that pepsin may play a role in the development of PLR symptoms.¹⁰ Li Y. (2022) supports this idea by stating that the laryngeal mucosa is more sensitive to acid and pepsin than the esophagogastric junction mucosa. This suggests that pepsin may have a greater impact on the larynx, potentially resulting in symptoms in individuals with PLR.¹¹

In the context of PLR, pepsin has been shown to cause inflammation regardless of reflux pH, even in the presence of acid suppression therapy. Therefore, measuring pepsin levels in the tonsillar swab of chronic tonsillitis patients with PLR may provide important insight into the role of pepsin in the pathogenesis of the condition.¹² Enzymatically active pepsin is required to

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deplete protective proteins in the laryngeal mucosa. Although the average pH of the laryngopharynx was 6.8, which is above the optimal pH for pepsin activity, the researchers suggested that pepsin was still stable and could potentially be reactivated after a decrease in pH caused by a subsequent acid reflux event. This suggests that pepsin may play a role in the pathogenesis of PLRrelated laryngeal disease.^{12,13} There are limited studies specifically investigating the correlation between chronic tonsillitis and PLR. However, there are several studies that provide insight into related factors. One study by Shen H et al. (2022) explored the role of gastric pylori pylori) Helicobacter (H. infection in laryngopharyngeal reflux disease.¹⁴ Gastric fluid contaminated with H. pylori can enter the pharynx through pathological reflux and colonize the tonsils, thereby potentially triggering pathological changes. This suggests that there may be a link between chronic tonsillitis and the presence of H. pylori in tonsillar tissue.15

Although there is evidence regarding differences in pepsin levels in tonsil swab in patients with chronic tonsillitis between patients with and without PLR, it has not been proven that the presence of PLR can contribute to the development or severity of chronic tonsillitis. Further research is needed to explore this relationship in more detail.

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