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Analysis of *Helicobacter pylori* in Saliva of Patients with Laryngopharyngeal Reflux and Non-Laryngopharyngeal Reflux

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Abstract

BACKGROUND: *Helicobacter pylori* is a Gram-negative bacteria known as the causative agent of chronic gastritis, peptic ulcer, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma. Several studies have correlated *H. pylori* in the pathogenesis of the upper airway diseases. *H. pylori* can be detected in saliva, oropharyngeal aphthae, nasal and sinus mucosa, secretions from the tympanic cavities, larynx, and pharyngeal lymphoid tissue. The diagnosis of LPR can be made simply by examining saliva.

AIM: The aim of the study is to analyze the presence of *H. pylori* in the saliva of LPR and non LPR patients.

METHODS: This study is an analytic and observational study with a case–control design. The research was conducted in the ENT-KL Department of Dr. M. Djamil Hospital, Padang, Indonesia. PCR examination was carried out at the Biomedical Laboratory of the Faculty of Medicine, Andalas University on saliva samples to detect the presence of *H. pylori*. The study was conducted on 22 LPR patients and 22 control subjects.

RESULTS: LPR patients are more common in women than men, which were 12 women and 10 men. The average age of LPR patients is 43.7 years. The presence of *H. pylori* in LPR patients was 86.4%, while in the non-LPR group was 50%. After being tested using the Chi-square test, a significant difference was found between the presence of *H. pylori* and the incidence of LPR ($p = 0.010$).

CONCLUSION: There was an increase in the frequency of the presence of *H. pylori* in patients with LPR compared to Non-LPR patients after statistical analysis.

Introduction

Laryngopharyngeal reflux (LPR) is the backflow of gastric and or duodenal fluid into the larynx, pharynx, trachea, and bronchus [1]. The fluid would make contacts with the upper airway and gastrointestinal (*Aerodigestive*) tract, causing symptoms such as hoarseness, cough, globus sensation, throat clearing, and postnasal drip [2]. The prevalence of LPR is difficult to determine due to the limited gold standard and the large variety of LPR symptoms. The exact prevalence of LPR is unknown, but it is estimated that 20–30% of patients with complaints in the larynx are LPR patients [3].

LPR is established based on medical history, clinical symptoms, laryngoscopic examination, and determining the presence of gastric backflow fluid into the laryngopharynx. The Reflux Symptom Index (RSI) questionnaire has been used to measure LPR symptoms severity and to observe the response toward treatments given to the patients, but it cannot distinguish LPR from any upper respiratory tract symptoms caused by other conditions. The Reflux Finding Score (RFS) indicates the severity of inflammation in laryngoscopic findings, but the findings may also occur in other types of chronic laryngeal irritation [4]. Ambulatory 24-h double-probe pHmetry examination is the gold standard to diagnose of LPR. However, the sensitivity of pH metry examination is only 50−80%. At present, a combination of 24-h double-probe pHmetry with multichannel intraluminal impedancemetry (MII) has been developed for the diagnosis of LPR. This combination can identify reflux in the form of liquid, gas, or both and can detect both acidic and non-acidic reflux [5].

Helicobacter pylori is a gram-negative bacteria known as the causative agent of chronic gastritis, peptic ulcer, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma. Despite of the fact that the stomach is the primary domain of this bacteria, several studies have shown the involvement of *H. pylori* in the pathogenesis of the upper airway diseases. Some studies have also shown that *H. pylori* can be detected in saliva, oropharyngeal aphthae, nasal and sinus mucosa, tympanic cavities secretes larynx, and pharyngeal lymphoid tissue [6], [7].

The oral cavity is believed to be the initial site of *H. pylori* infection. This organism can be detected in low amount in the oral cavity of healthy subjects without causing any symptoms [8]. The fecal-oral route is the main method of transmission of *H. pylori*. The oropharynx is a reservoir for *H. pylori* infection, as the genotypes of *H. pylori* isolated from saliva, stomach, and feces are similar [9]. Acidic fluid of the gastric contaminated with *H. pylori* enters the pharynx and oral cavity through reflux and infects the teeth, adenoid, tonsil tissue, and saliva. The infection can also spread from these locations to other sites of the upper airway and can trigger several pathological changes [7]. In Sayed *et al.* study, the frequency of *H. pylori* in the LPR group reached 86.4%. Detection of *H. pylori* in saliva by PCR gives results which vary between 0 and 80%. Detecting the presence of *H. pylori* in saliva can be used as one of the variables in diagnosing patients with LPR [10].

Methods

This study is an analytic and observational study with a case–control design. The study was conducted in the ORL-HNS Department of Dr. M. Djamil Hospital, Padang, West Sumatra, Indonesia.

The sample of the saliva was tested with PCR examination at the Biomedical Laboratory of the Faculty of Medicine, Andalas University to detect the presence of *H. pylori*. The population in this study were patients suspected with LPR who came to the Broncho-Exophagology and Laryngeal Pharynx Division in ORL-HNS Polyclinic of the Dr. M. Djamil Padang Hospital. The sample was divided into two groups, the LPR group and the control.

Based on the formula, a minimum sample size of n1 = n2 = 17 people was obtained, with the total sample size 34.

Subjects of this study were LPR patients with the symptoms of laryngopharyngeal reflux with RSI >13 and RFS >7. The inclusion criteria were patients with LPR who did not have any history of diseases; such as asthma, pulmonary tuberculosis, chronic obstructive pulmonary disease, laryngeal diseases, including polyps, nodules, vocal cord paralysis, and laryngeal carcinoma.

Informed consent was obtained from all subjects and the protocol of the study was approved by the Ethics Committee of the Faculty of Medicine, Andalas University with Number 315/UN.16.2/KEP-FK/2021. The sample size of this study is 44 participant, where 22 is LPR patient, and 22 is a healthy control called non-LPR patient.

Saliva samples (2−3 ml) were collected in a sterile container in the endoscopy procedure. DNA extraction was performed with MagNA pure compact Nucleic Acid Isolation Kit (Roche Diagnostics Nederland BV) following the manufacturer's instructions. Real-time PCR was performed with a Light Cycler (Roche, Mannheim, Germany).

The first set of primers, namely Primers Hp23S 1835F (5′-GGTCTCAGCAAAG AGTCCCT-3′) and Hp23S 2327R (5′-CCCACCAAGCATTGTCCT-3′) were used for the first PCR, and primers Hp23S 1942F AGGATGCGTCAGTCGCAAGAT and Hp23S 2308R CCTGCCAGTAC. The second primer set, namely primer Hp23S nF2, Forward sequence (5' to 3'): TGCTCGAAGGTTAAGAGGATG. The second primer, reverse Hp23S-nR2, (5' to 3'): was GCTAACAGAAACATCAAGGGTG, with an Amplicon Size of 359 bps.

Results

This study was conducted to analyze the presence of *H. pylori* by PCR examination in LPR and non-LPR groups. From patients who met the inclusion criteria, 44 people were obtained as research subjects, 22 people in the LPR group and 22 people in the non-LPR group. The study started from January 2021 to January 2022. The obtained data were statistically processed with the SPSS program using a computer and presented in text and tables.

Based on Table 1, females outnumbered males in both group; there were 12 females in the LPR group and 15 female patients in the non-LPR group. In the LPR group, the mean age is 43.7 years, the average RSI score is 18.54 and RFS score is 12. Meanwhile, in the non-LPR group, the average age is 24.6 years, the average RSI score is 1.45, and RFS score is 0.23.

Table 1: Characteristics of respondents based on gender, age, and basis of diagnosis

Characteristics	LPR	Non LPR
Gender		
Man	10	7
Woman	12	15
Age (Year)	43.7	24.6
Diagnosis		
RSI	19	1.45
RFS	10.2	0.23

LPR: Laryngopharyngeal reflux, RSI: Reflux symptom indeks, RFS: Reflux finding score.

Based on Table 2, *H. pylori* in saliva is higher in the LPR group compared to the non-LPR group. LPR group has the frequency of 86.4%. While in the non-LPR group only 50%.

Table 2: Comparison between the presence of *H. pylori* in LPR and non-LPR

Subject	*H. pylori*		p*
	Frequency	Percentage	
LPR	19/22	86.4%	0.010
Non LPR	11/22	50%	

LPR: Laryngopharyngeal reflux.

After Chi-square test, a significant difference was found between the presence of *H. pylori* and the incidence of LPR (p = 0.010).

The presence of *H. pylori* is 6x higher in risk to develop LPR compared to control, with an Odds Ratio of 6.33.

Discussion

In this study, it was found that LPR patients were more common in women than men, with 12 in women and 10 in men. From this data, the percentage ratio between women and men is 54%: 45%. This result is similar to a previous study by Junaid *et al*., which found that the incidence of LPR is higher in women, which is 56.9%. Junaid *et al.* concludes that gender differences are not significantly related to the development of LPR disease [11].

The mean age of LPR patients in this study was 43.7 years (range: 23−66 years). This number is higher than the research of Montasir *et al*. who found the average age of LPR patients was 41.8 ± 10.1 years. Another study conducted by Silva *et al*. found a higher average age in LPR patients, which was 47.2 years, from the age of 29 years to 73 years. All of these studies shown that the average age of LPR patients globally is above 40 years [12], [13].

The average RSI value was 19 with the highest value of 45 and the lowest value 14. Meanwhile, the average RFS value was 12 with the highest value of 18 and the lowest was 8. A similar study was conducted by Nunes *et al*., comparing RFS and RSI values in patients with RSF, with the average RSI value was 20.7 with the lowest value of 13 and the highest was 42 [14].

Assessment using RSI was conducted by the patients by questionnaire with nine symptoms generally complained by patients. RSI values above 13 and RSF values above 7 are categorized as positive. RFS and RSI values can be used to diagnose LPR and can be implemented in ENT clinics for subjective and objective assessment of LPR 11].

The previous literature suggested that LPR is associated with the presence of *H. pylori*. The presence of *H. pylori* infection affects the clinical symptoms of LPR. *H. pylori* is associated with various pathogenesis of the upper airway diseases. Several studies have reported that *H. pylori* can be found in saliva, oropharyngeal, nasal and sinus mucosa, tympanic cavity secretions, larynx, and lymphoid pharyngeal tissue with oropharynx as a reservoir for *H. pylori* infection [7].

In this study, the amount of *H. pylori* frequency in the LPR group was 86.4%, while in the non-LPR group was 50%. Chi-square test showed a significant difference of the presence of *H. pylori* and the incidence of LPR ($p = 0.010$). It showed that the presence of *H. pylori* gives a person a 6x higher risk of developing LPR compared to control group, with an Odds Ratio of 6.33.

H. pylori infection is caused by a Gram-negative, urease-producing, curved rod, or spiral-shaped bacteria. *H. pylori* grows in the microenvironment near by the gastric epithelial cells. *H. pylori* affects epithelial cell kinetics and is known to be an etiologic factor in the development of gastritis, peptic ulcer, MALT lymphoma, and gastric cancer [7], [10].

The virulence factors of *H. pylori* are the cytotoxin-associated gene A product (CagA), vacuolating cytotoxin (VacA), outer inflammatory proteins, and duodenal ulcer promoting, VacA and CagA have been investigated most thoroughly in an attempt to understand the pathogenicity of this bacteria. VacA is a toxin that disrupts cell polarity in the gastric mucosa, promotes epithelial cell apoptosis, and inhibits T cell proliferation. CagA is an immunodominant antigen that is translocated into gastric epithelial cells through the Cag-encoded secretion system. *H. pylori* strains that express CagA are associated with an increased risk of developing ulcers to gastric cancer [7].

The oral cavity is implicated as a potential *H. pylori* reservoir. This study showed that the oral cavity may act as the initial site of *H. pylori* infection. The organism can persist in low amount in the oral cavity of healthy subjects without causing any symptoms [8].

H. pylori can be found in non-LPR individuals. This is associated with the diversity of human salivary nitrite concentrations. The variation of nitrite concentration is affected by dietary intake from 0.05 to 1.0 mmol/L. *Nitric oxide* which has been recognized for its anti-bacterial function is a derivative of nitrate [15].

Conclusion

From the results, it was found that the higher presence of *H. pylori* in patients with LPR compared to non-LPR was statistically significant. Further research is needed to detect *H. pylori* with other tests, as well as its relationship with LPR.

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