



# Overview of *Helicobacter pylori* Detection Using Rapid Urease Test and Giemsa Modification Staining in Chronic Tonsillitis Patients

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## Abstract

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**BACKGROUND:** *Helicobacter pylori* (*H. pylori*) is a Gram-negative bacteria and has been known for its role in causing gastric infection aused diseases such as gastric ulcer. *H. pylori* also implied to play a role in chronic tonsillitis, but this theory remains controversial. Many researches have different and contradictory results due to difficulty to accurately detect *H. pylori* in tonsillar tissue. There is still no appropriate method that able to detect *H. pylori* in tonsil tissue.

**AIM:** The aim of the study was to detect *H. pylori* colonization in chronic tonsillitis and understand some of the methods of examination that can be done to detect *H. pylori* in tonsillar tissue.

**METHODS:** This study is a descriptive study conducted on 25 respondents. Each sample was taken from patients with chronic tonsillitis who underwent tonsillectomy. Then, the rapid urease test (RUT) and the Giemsa modification staining were carried out to determine the presence of *H. pylori*.

**RESULTS:** There were 19 people (76%) positive and 6 people (24%) negative for *H. pylori* using RUT. On examination with Giemsa modification staining obtained 19 people (76%) positive and 6 people (24%) negative for *H. pylori*.

**CONCLUSION:** *H. pylori* can be found in most of chronic tonsillitis. Combination RUT and Giemsa modification staining examination can be a good option in detecting *H. pylori* in chronic tonsillitis.

## Introduction

Warren and Marshall discovered *Helicobacter pylori* (*H. pylori*) in 1983 and reported it in 1984 and in 2005, and they were awarded the Noble Prize for this important discovery. *H. pylori* is prevalent throughout the world but is especially more endemic in developing countries [1]. *H. pylori* is a Gram-negative bacterium, which produces urease, in the form of a curved rod or spiral which is one of the bacterial infections that often occur in humans. This infection affects more than half of the world's population. It has been proven that *H. pylori* has a significant role in the pathogenesis of chronic gastritis, peptic ulcer, gastric cancer, and lymphoma in gastric lymphoid mucosal tissue [2], [3].

There are several opinions regarding the way of transmitting *H. pylori* to the upper airway. Gastrointestinal route is assumed to have an important role in the transmission of this bacterium, mainly through gastroesophageal reflux disease (GERD) or laryngopharyngeal reflux (LPR). *H. pylori* which contaminates gastric fluid enters the pharynx through pathological reflux which will then colonize on dental plaque, adenoid tissue, and tonsils. Infection can spread

from this location to other locations in the upper airway and can trigger pathological changes. The systemic immune response to the virulence of *H. pylori* from the gastric can also be a cause of upper airway disease, but the mechanism has yet to be ascertained [2], [4], [5].

Several studies have implicated that tonsils can be a potential colonization site for *H. pylori*. Ozgun *et al.* [6] reported that out of 100 patients who underwent tonsillectomy, 48 (48%) were *H. pylori* positive and 52 (52%) were negative. Lin *et al.* [7] reported that of 94 patients who were treated for tonsillitis, 33 (35%) were positive for *H. pylori* and 61 (65%) were negative. Wibawa *et al.* [8] reported that from 19 patients with chronic tonsillitis, 15.7% were positive for *H. pylori*. Therefore, more researchers are currently investigating the prevalence and role of *H. pylori* in the pathogenesis of chronic tonsillitis. Allegedly that the presence of *H. pylori* in the tonsillar tissue can activate the inflammatory process in the tonsils which can cause re-infection of the gastric mucosal and act as an etiopathogenetic factor in chronic tonsillitis [2], [9].

Various mechanisms are used to detect the presence of *H. pylori*. Rapid urease test (RUT), specimen culture, histology, and polymerase chain reaction (PCR) are the methods that currently used

and entirely accepted because the methods are quite reliable for *H. pylori* although there is currently still no gold standard for the *H. pylori* test in the oral cavity [9]. The discovery of *H. pylori* at oropharyngeal tissue even though there is no infection in the gastric mucosal confirm that *H. pylori* can colonize in the oropharyngeal tissue permanently and the discovery of this bacteria in the oropharynx is not always caused by contamination by gastric fluid. This theory is further proven by the findings of different strains of *H. pylori* between the gastric and oropharyngeal specimens of the same individual. Although tonsillar tissue can act as an extra gastric reservoir of *H. pylori*, several studies have shown results that refute the findings [7], [10], [11].

The impact of *H. pylori* infection in the pathogenesis of chronic tonsillitis remains controversial. Some researchers found that *H. pylori* had a role in tonsillar tissue infections, but other researchers did not find a link between *H. pylori* colonization with chronic tonsillitis and LPR [2], [12].

## Methods

This research is a descriptive study to find out the overview of *H. pylori* in chronic tonsillitis. The study was conducted from August to October 2018 at the Department of Otorhinolaryngology-Head and Neck Surgery (ORL-HNS), Dr. M. Djamil Hospital in Padang, West Sumatera, Indonesia. The samples obtained were examined at the Anatomic Pathology Department and Microbiology Laboratory, Faculty of Medicine, Andalas University, Padang. The population was patients with chronic tonsillitis who had been diagnosed based on history taking and physical examination at ORL-HNS outpatient clinic of Dr. M. Djamil Hospital, Padang. Samples were taken by consecutive sampling from all chronic tonsillitis patients who underwent a tonsillectomy at the Department of ORL-HNS and are willing to participate in research by filling out informed consent sheets. Specimens for RUT were taken from all patients with chronic tonsillitis before tonsillectomy procedure, conducted using the HelicotecUT® Plus RUT kit. Tissue was taken with sterile scissors at the tonsil's lower pole with the amount of 3–4 mm and dried with sterile gauze. Result of RUT determined by comparing the color shown by the patient's specimen with the color control specimen after 24 h, positive is pink-magenta and negative is yellow.

The research minimal sample size is determined by:

$$n = \frac{Z\alpha^2PQ}{d^2}$$

$$n = \frac{1.96^2 \times 0.35 \times 0.65}{0.2^2}$$

n = 22 samples

n = sample size

Z $\alpha$  = alpha standard deviation, degree of confidence

95% = 1.96

P = *H. pylori* proportion in tonsils = 35%

Q = 1-P = 1-0.35 = 0.65

d = precision 20% (0.2)

Specimen for Giemsa modification staining examination was the tonsillar tissue taken by tonsillectomy procedure. Specimens taken from tonsillar tissue were stored in 10% formalin solution and sent to the Anatomic Pathology Department. This specimen was labeled, then cut into 3 mm thickness. These pieces were placed in an automatic processor machine to be dried, cleaned, and given a layer of wax (processing) for 14 h. The tissue was then affixed to paraffin wax and then divided into paraffin blocks. Thin pieces, measuring 5 mm cut from paraffin wax, were placed on the slide. The finished slides were placed in xylene and irrigated using alcohol with smaller concentrations, which were 100%, 90%, and 70%. The slide was then rinsed with water, then given ethanol liquid, and then placed on a drying rack. This slide was then given a Giemsa 1% for 10 min. The slides were then rinsed with water, dried, cleaned, and placed back on a rack. Then, the slides were sent to the Microbiology Laboratory for microscopic examination.

The process of maintaining data quality was carried out by means of (1) history taking and physical examination including pharyngoscopy were carried out by researchers, (2) removal of tonsillar tissue was carried out in the operating room and then a RUT is conducted by the researcher. Giemsa modification staining examination was done in the Anatomic Pathology department, the specimens examined by the experts in the Microbiology Laboratory of the Faculty of Medicine, Andalas University, (3) data from each patient were recorded in the particular research form.

## Results

A total of 25 respondents were included in this study. RUT and Giemsa modification staining were done to all respondent's tonsils. Tonsils that had been carried out by tonsillectomy were taken to the Anatomic Pathology Department, Faculty of Medicine, Andalas University, to make microscopic slides and then examined by the experts in the Microbiology Laboratory of the Faculty of Medicine, Andalas University, Padang. In this study, women had a higher *H. pylori* frequency in chronic tonsillitis than men, which was 52%. Based on the age group (Table 1), we found that the age group

**Table 1: Characteristics of respondents by gender and age group**

Characteristics	Chronic tonsillitis	%	Confirmed <i>H. pylori</i> at tonsils	%
Gender, n (%)				
Male	9 (36)	36	6	24
Female	16 (64)	64	13	52
Age group (years)				
5-14	11	44	10	40
15-24	5	20	3	12
25-34	5	20	4	16
35-44	2	8	1	4
45-54	1	4	1	4
55-64	1	4	0	0

*H. pylori*: *Helicobacter pylori*.

with the highest frequency of suffering from chronic tonsillitis ranged from 5 to 14 years, with 11 patients (44%).

Repeated swallowing pain  $\geq 7\times$  per year was the most common symptom, complained by 12 patients (48%). Lumps sensation was found in all patients of chronic tonsillitis (Table 2). Twenty-four patients (96%) complained about bad odor breath (halitosis).

**Table 2: Distribution of clinical symptoms in chronic tonsillitis**

Clinical symptoms	Frequency (n = 25)	%
Repeated swallowing pain		
$\leq 3\times$ per year	10	40
4-6 $\times$ per year	3	12
$\geq 7\times$ per year	12	48
Lump sensation	25	100
Bad odor breath	24	96

In this study, the highest frequency of tonsillar enlargement was T3-T3, found in 13 patients (52%) (Table 3). The frequency of Reflux Symptom Index score that  $\geq 13$  was found in 5 patients (20%) (Table 4).

**Table 3: Distribution of tonsil enlargement measures in chronic tonsillitis**

Tonsils enlargement	Frequency (n = 25)	%
T2-T2	4	16
T2-T3	2	8
T3-T2	1	4
T3-T3	13	52
T3-T4	2	8
T4-T3	1	4
T4-T4	2	8

**Table 4: Distribution of the total frequency of RSI in chronic tonsillitis**

RSI	Frequency (n = 25)	%
<13	20	80
$\geq 13$	5	20

RSI: Reflux symptom index.

The frequency of *H. pylori* colonization location in the tonsillar tissue categorized as *deep* was found in 19 patients (100%) (Table 5). From the RUT result, there were 18 patients (72%) with *H. pylori* tested positive in tonsils of chronic tonsillitis (Table 6).

**Table 5: Distribution of *H. pylori* colonization location in tonsil tissues**

Location of colonization	Frequency (n = 19)	%
Deep	19	100
Floating	0	0

*H. pylori*: *Helicobacter pylori*.

**Table 6: Distribution of *H. pylori* in chronic tonsillitis with RUT**

RUT	Frequency (n = 25)	%
Positive	18	72
Negative	7	28

*H. pylori*: *Helicobacter pylori*, RUT: Rapid urease test.

From the Giemsa modification staining examination, there were 19 patients (76%) with *H. pylori* tested positive in tonsils with chronic tonsillitis (Table 7).

**Table 7: Distribution of *H. pylori* in chronic tonsillitis by Giemsa modification staining examination**

Giemsa modification staining	Frequency (n = 25)	%
Positive	19	76
Negative	6	24

*H. pylori*: *Helicobacter pylori*.

## Discussion

*H. pylori* has been shown to be a significant pathogenesis factor in gastric diseases such as chronic gastritis, gastric ulcer, duodenum ulcer, and gastric cancer. Although the gastric has been proven to be the main site of *H. pylori* colonization, recent studies indicate the role of *H. pylori* in various upper respiratory tract infections [2]. One of diagnostic method that is often used is the RUT. The RUT is easy to use and has been widely used as one of the most conventional methods for diagnosing *H. pylori* infection in clinical practice. The principle works of this test are based on the activity of *H. pylori*'s urease [13], [14]. From the RUT, it was found 18 patients (72%) with positive *H. pylori* and 7 patients (28%) had negative results.

Until now, existing studies have shown RUT on tonsillar tissue with varying results. The results obtained in this study are in accordance with other studies that show positive results on the RUT which is significant. Lin *et al.* studied 94 adult patients (44 patients in the tonsillitis group and 50 patients in the control group). *H. pylori* infection rates identified using the RUT were significantly higher in patients with chronic tonsillitis compared to the control group (48% vs. 24%) [7]. Other study examining 285 children using the RUT in which the *H. pylori* was identified in 39.6% of cases of chronic tonsillitis [15]. Unver *et al.* examined the colonization of *H. pylori* in adenotonsillectomy specimens using the RUT and found the presence of *H. pylori* at 57.89% [16].

Histological identification of *H. pylori* infection is currently used as a diagnostic method. Several staining methods have been used. Among them are Giemsa modification, Warthin-starry, Gimenez, Genta, and the immunohistochemical staining of *H. pylori* antibodies. Thus far, immunohistochemical staining has been agreed as a "gold standard" in the histological examination, because of its sensitive and specific nature [17]. In this study, the examination was done using Giemsa modification staining. The results obtained 76% were positive *H. pylori* in patients with chronic tonsillitis. This is convenient with the results of the Ozgun *et al.* report of 100 cases studied, where 48 cases (48%) showed a depiction of *H. pylori* from a tonsillar mucosal surface specimen by Giemsa examination [6].

The combination of two or three methods can confirm true positive results and then can be used as a “gold standard” in the methodology survey for the detection of *H. pylori* [18]. Therefore, this study was examined with two diagnostic methods, RUT and Giemsa modification staining of tonsillar specimens for detection of *H. pylori* in patients underwent tonsillectomy. In the examination using RUT and Giemsa modification staining, we obtained positive *H. pylori* results in 76% while 24% patients were negative.

There is a possibility of a small number of *H. pylori* in the tissue cannot produce enough urease to make color changes in the RUT. It was shown in the result of RUT in one specimen (female, 18 years) that was negative, but *H. pylori* was found by Giemsa modification staining. It takes a minimum of 105 *H. pylori* bacteria to make positive results [19]. Down to date, there has not been found a standard inspection method used in upper airway tissue. In addition, the results of a recent study report that in cases of tonsillitis, the prevalence of *H. pylori* in tonsillar tissue varies greatly, with ranges from 8.3% to 80% [20], [21], [22].

In a recent study that comparing the sensitivity and specificity of Urea Breath Test, culture, RUT, and histology in the diagnosis of *H. pylori* infection reported a small difference in specificity, but histological examination using Giemsa modification staining with a sensitivity of 98% was significantly more sensitive than other tests. Giemsa modification staining needs to be done aside from the RUT as a tool that allows definitive diagnosis and can be considered as a feasible method for diagnosing *H. pylori* infection [17]. As in this study, the results of both RUT and Giemsa modification staining showed the results of true positive and statistically significant in the detection of *H. pylori* in patients with chronic tonsillitis.

## Conclusion

*H. pylori* was found in the tonsil tissue of chronic tonsillitis by means of the RUT and Giemsa modification staining in more than half of the total specimens. Further research is needed in detecting *H. pylori* with other tests such as PCR and culture, as well as its relationship with LPR and GERD.

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