RESEARCH ON THE DETECTION OF *HELICOBACTER PYLORI* IN SALIVA OF GASTRITIS AND DUODENITIS PATIENTS BY REAL-TIME PCR TECHNIQUE

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ABSTRACT

Background: *Helicobacter pylori plays an important role in the etiology and pathogenesis of gastritis and duodenitis. There are two groups of test methods to detect Helicobacter pylori infection: invasive methods and non-invasive methods. Testing for Helicobacter pylori in saliva by real-time PCR technique belongs to group of non-invasive test methods.* ***Objectives:*** *To determine the detection rate of Helicobacter pylori in saliva by real-time PCR technique in gastritis and duodenitis patients and to investigate some factors related to Helicobacter pylori infection detected in saliva using real-time PCR technique in gastritis and duodenitis.* ***Materials and method:*** *A cross-sectional descriptive study on 91 patients after convenient sampling was preliminarily diagnosed with gastritis and duodenitis with indications for endoscopy and biopsy. Biopsy specimens were used for urease and histopathology tests. Saliva is taken directly from the mouth after spitting into a dedicated bottle and conducting a real-time PCR test. Gene amplification will be performed with a PYLORI-PCR mix containing primers and for the expected target product of 203 base pairs length: 5' - AGCGCTCTCACTTCCATAGGC - 3' and 5' -*

TCTTCGGTTAAAAAAGCGAT - 3'. Install the program "Protocol" for the Real-time PCR machine to operate with the following cycles: 1 cycle: 95^{0}C for 5 minutes; 40 cycles: 95^{0}C for 15 seconds, 55^{0}C for 1 minute and 72^{0}C for 20 seconds. ***Results:*** *The positive rate for Helicobacter pylori in saliva by real-time PCR technique, urease test and histopathology were 20.9%, 19.8% and 46.2% respectively. The detection rate of H. pylori by real-time PCR in saliva and the detection rate on urease test as well as histopathology had a statistically significant correlation. A few important factors related to Helicobacter pylori-positive patients in saliva as follows: personal history suffer from gastritis and duodenitis 40.7%, reflux esophagitis 67%, and congestive inflammation 75.8%.* ***Conclusions:*** *The rate of detecting Helicobacter pylori in saliva by real-time PCR technique in gastritis and duodenitis patients in Can Tho University of Medicine and Pharmacy Hospital is higher than urease test and lower than histopathology.*

Keywords*: Helicobacter pylori, gastritis, duodenitis, real-time PCR.*

I. INTRODUCTION

Helicobacter pylori infection is a common chronic gastrointestinal infection in the world, and different epidemiological studies have identified about 50% of the population infected with this bacterium [1], [2], [3]. *H. pylori* plays an important role in the etiology and pathogenesis of gastritis and duodenitis [4], [5], [6]. Accurate diagnosis of the cause of disease caused by *H. pylori* is currently mainly based on tests with two methods: invasive and non-invasive. Rapid urease test is often indicated because this test has the advantage of providing rapid results with a specificity of 95-100%; however, the disadvantage is that it has a false negative result when using proton pump inhibitors, antibiotics, anti-inflammatory and must be combined with invasive endoscopy. Other tests such as pathology, diagnostic serology, breath test, and fecal microbial antigen test are also applied in current medicine [2], [6], [7]. With the desire not to use invasive methods, PCR (polymerase chain reaction) technique has been applied to detect *H. pylori* in the saliva of patients with or without gastritis or duodenitis. This technique obtained very interesting results. On that basis, we conducted a study on the detection of *H. pylori* in saliva by real-time PCR technique on patients with gastritis or duodenitis aimed at:

1. To determine the detection rate of *Helicobacter pylori* in saliva by real-time PCR technique in patients with gastritis and duodenitis.

2. To study the relationship between the detection rate of *Helicobacter pylori* in saliva and influencing factors in patients with gastritis and duodenitis.

II. MATERIALS AND METHOD

2.1. Materials

Patients with clinical diagnosis of gastritis and duodenitis were assigned endoscopy and biopsy at the Center for Gastrointestinal Endoscopy of Can Tho University of Medicine and Pharmacy Hospital.

Selection criteria: Patients with clinical diagnosis of gastritis and duodenitis were assigned endoscopy and biopsy.

Exclusion criteria: Patients who had used antibiotics or *H. pylori* treatment regimens within 14 days prior to their visit were not included in the study; the patient was not able to answer the interview and was not eligible to perform endoscopy as indicated.

2.2. Methods

Study design: a cross-sectional study.

Sampling method: convenient sampling: select the patients meeting the selection criteria until the sample size. In fact, we collected 91 patients that were consistent with the sampling criteria from January 2021 to Februay 2022.

Study content

Characteristics of age, sex of study subjects, some related factors and clinical manifestations of patients with gastritis and duodenitis who were positive for *H. pylori* in saliva, positive rate for *H. pylori* in saliva, positive rate with urease test, positive rate for *H. pylori* by histopathology test. Patient information and some factors related to gastritis and duodenitis collected by interviewing questionnaire.

Collect patient saliva by instructing the patient to spit directly into the sample vial. Saliva samples were preserved at -20^{o}C, and DNA was extracted using the *H. pylori* ^{iVA}HP rPCR Kit of Viet A Technology Joint Stock Company. Gene amplification will be performed with a PYLORI-PCR mix containing primers and for the expected target product of 203 base pairs length: 5' - AGCGCTCTCACTTCCATAGGC - 3' and 5' - TCTTCGGTTAAAAAAGCGAT - 3'. Install the program "Protocol" for the Real-time PCR machine to operate with the following cycles: 1 cycle: 95^{o}C for 5 minutes; 40 cycles: 95^{o}C for 15 seconds, 55^{o}C for 1 minute and 72^{o}C for 20 seconds.

Two biopsies in the same position during endoscopy to check for urease and histopathology. The urease test results were read after 30 minutes. Biopsy specimens for histopathology were preserved in 10% formol solution, paraffin casting was performed, 3 - 5 µm lamella was thinly cut and Giemsa stained for *H. pylori*. Record the results of histopathological examination with or without *H. pylori* based on the improved Sedney system. The results were determined by Master Hoang Duc Trinh, Department of Pathology, Can Tho University of Medicine and Pharmacy.

Statistical analysis: Statistical analysis was performed using SPSS Statistics version 16.0.

III. RESULTS

3.1. Characteristics of the study subjects

Distribution of study subjects according to age and sex: Research results on 91 patients, in which men accounted for 42.9%, women accounted for 57.1%, average age was 40.24 ± 13.67 (minimum age was 15 years old, oldest age was 68 years old).

Factors affecting the detection rate of *H. pylori* saliva in patients with gastritis and duodenitis:

Table 1. Distribution of factors affecting *H. pylori* infection in saliva

Factors affecting	Frequency (n)	Rate (%)
Have a family history	30	33
Have a personal history	37	40.7
Has smoking	18	19.8
Has alcohol	20	22
Congestive inflammation	69	75.8
Slippery inflammation	19	20.9
Reflux esophagitis	61	67
Stomach-duodenal ulcer	4	4.4

The influencing factor accounting for the highest rate was congestive inflammation (75.8%), reflux esophagitis (67%), having a personal history of inflammation or ulcer of

stomach – duodenum (40.7%), having a family history of inflammation or ulcer of stomach – duodenum (33%), having alcohol (22%); having smoking was the lowest (19.8%).

Clinical characteristics:

Table 2. Distribution of clinical symptoms in study subjects

Factors affecting	Frequency (n)	Rate (%)
Epigastric pain	64	70.3
Belching and heartburn	47	51.6
Dyspepsia	35	38.5
Anorexia	14	15.4

The distribution of common clinical symptoms of study subjects such as epigastric pain, belching, heartburn, dyspepsia and anorexia were 70.3%, 51.6%, 38.5%, and 15.4%, respectively.

3.2. *Helicobacter pylori* detection rate

Table 3. The rate of *H. pylori* in saliva, urease test and histopathology

Test	Number (n = 91)	Rate (%)
Real-time PCR	19	20.9
Urease test	18	19.8
Histopathology	42	46.2

The rate of *H. pylori* detection by histopathology accounted for the highest rate 46.2%, followed by the rate of detection in saliva by Real-time PCR technique accounted for 20.9% higher than urease test (19.8%).

Table 4. Correlation between detection rate of H. pylori by real-time PCR in saliva and urease test

Histopathology	Real-time PCR		Total	p	Kappa
	Positive n (%)	Negative n (%)			
Positive	7 (38.9)	11 (61.1)	18 (100)	p = 0.036	Kappa = 0.22
Negative	12 (16.4)	61 (83.6)	73 (100)		

There was a correlation between the rate of H. pylori detection in saliva by real-time PCR and the rate of *H. pylori* in tissue samples by urease test and the consensus between these two methods was at the weak level.

Table 5. Correlation between detection rate of *H. pylori* by real-time PCR in saliva and histopathology

Histopathology	Real-time PCR		Total	p	Kappa
	Positive n (%)	Negative n (%)			
Positive	15 (35.7)	27 (64.3)	42 (100)	p = 0.001	Kappa = 0.29
Negative	4 (8.2)	45 (91.8)	49 (100)		

There was a correlation between the detection rate of *H. pylori* in saliva by real-time PCR technique and the rate of *H. pylori* in gastric tissue samples by histopathology with p = 0.001 and the consensus between these two methods was at the weak level.

IV. DISCUSSION

4.1. Clinical characteristics

The study recorded the rate of clinical manifestations of study subjects including: epigastric pain was 70.3%, belching and heartburn was 51.6%, and dyspepsia was 38.4%. This result in the study of Tran Thi Nhu Le (2019) were 72.3%, 59.6% and 40.4%, respectively [8]. Our research results are quite similar to Tran Thi Nhu Le.

4.2. *Helicobacter pylori* detection rate in saliva by real-time PCR technique

Our detection rate of *H. pylori* in saliva by real-time PCR technique recorded was 20.9%, this rate was lower than the study of Sekhar Goud et al. 2019 and Trieu Tien Sang et al. 2019 were 55% and 63%, respectively [9], [10]. These authors used PCR technique and read the results on electrophoresis, which was different from real-time PCR technique in our study. On the other hand, Trieu Tien Sang had not fully described the time of saliva sampling before or immediately after performing endoscopy. This difference may be due to the time of saliva sampling, taking a saliva sample immediately after endoscopy may increase the possibility of saliva containing gastric juice, leading to an increase in the positive rate. Thus, studies in the country and in the world had shown the same result that there was the existence of *H. pylori* in saliva.

4.3. The relationship between the *H. pylori* detection rates of the tests

The positive rate for *H. pylori* in saliva by real-time PCR technique, urease test and histopathology were 20.9%, 19.8% and 46.2% respectively. The positive rate for urease test in our study was 19.8%, lower than the study by Tran Thi Nhu Le et al. at Tien Giang Central General Hospital in 2020 is 24% [8]. For the results of histopathological examination by Giemsa staining technique to detect *H. pylori*, our study recorded a detection rate of 46.2%. Our results were quite similar to the results of the study by Sekhar Goud recorded on subjects with symptoms of inflammation or ulcer of stomach – duodenum was 50% [9]. However, our results were higher than those of Tran Minh Luan's research conducted at Can Tho University of Medicine and Pharmacy Hospital in 2018 [11]. With a sensitivity and specificity >95%, this method could be considered the gold standard in testing for the detection of *H. pylori* [12].

In our study, the detection rate of *H. pylori* by real-time PCR in saliva and the detection rate on urease test as well as histopathology had a statistically significant correlation, especially in histopathology test. Therefore, real-time PCR could be used to detect the infection of *H. pylori* in saliva.

4.4. The relationship with factors affecting the rate of *H. pylori* infection in saliva

The relationship with factors affecting the rate of *H. pylori* infection in saliva in our study in order from high to low was congestive inflammation (75.8%), reflux esophagitis (67%), having a personal history of inflammation or ulcer of stomach – duodenum (40.7%), having a family history of inflammation or ulcer of stomach – duodenum (33%), having alcohol (22%), having smoking was the lowest (19.8%). In it, we emphasized a lot on inflammation or ulcer of stomach – duodenum on endoscopic images and hypothesized that inflammation or ulcer of stomach – duodenum could increase *H. pylori* in the saliva was not. Our study had not found a statistically significant correlation between the above influencing factors and the rate of *H. pylori* detection in saliva.

V. CONCLUSION

A few important factors related to *H. pylori*-positive patients in saliva as follows: personal history suffers from gastritis and duodenitis 40.7%, reflux esophagitis 67%, and congestive inflammation 75.8%. The rate of detecting *H. pylori* in saliva by real-time PCR technique in gastritis and duodenitis patients in Can Tho University of Medicine and Pharmacy Hospital is higher than urease test and lower than histopathology.

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