

Original Research Article

Characteristics of basal gastric juice in *Helicobacter pylori*-associated gastritis before and after eradication therapy

Sam Phan Hai Nguyen¹, Tho Chau Minh Vinh Do², Tam Thai Thanh Tran¹, Tho Kieu Anh Pham¹, Van De Tran^{3*}, Kien Trung Nguyen¹

¹Department of Physiology, Can Tho University of Medicine and Pharmacy, 179 Nguyen Van Cu Street, Can Tho 900000, Vietnam, ²Department of Drug Quality Control-Analytical Chemistry-Toxicology, Can Tho University of Medicine and Pharmacy, 179 Nguyen Van Cu Street, Can Tho 900000, Vietnam., ³Department of Drug Administration, Can Tho University of Medicine and Pharmacy, 179 Nguyen Van Cu Street, Can Tho 900000, Vietnam

*For correspondence: **Email:** tvde@ctump.edu.vn **Tel:** +84943848691

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Abstract

Purpose: To evaluate the characteristics of basal gastric juice in *Helicobacter pylori*-positive patients before and after *Helicobacter pylori* eradication therapy.

Methods: This was a cross-sectional descriptive study on 150 gastritis patients admitted at the Hospital of Can Tho University of Medicine and Pharmacy. The patients were divided into 2 groups: study group (*Helicobacter pylori* gastritis patients) and control group (non-*Helicobacter pylori* gastritis patients). The pH, HCO₃⁻ concentration, and activities and concentrations of pepsin, lipase, and amylase were determined before and after treatment in study group.

Results: Patients with abnormal gastric juice comprised 76 % of the study population. Mean gastric potential of hydrogen (pH) was 2.31 (range: 1.64 - 7.68), while median concentration of HCO₃⁻ was 4.06 mmol/L (range: 0 - 73.04 mmol/L). The concentrations of pepsin, lipase, and amylase were 8.93, 0.93 and 1.38 ppm, respectively. Activities of pepsin, lipase, and amylase were 2.23, 0.28 and 0.04 U/mL, respectively. After the successful eradication of *Helicobacter pylori*, pH and HCO₃⁻ levels decreased, and there were significant differences in activities of pepsin and lipase before and after treatment ($p < 0.05$). Moreover, the levels of these parameters differed between patients in whom successful eradication was achieved and those in whom eradication failed ($p < 0.05$). The concentrations and activities of pepsin and lipase were statistically different between pre-treatment and post-treatment stages in both successful and failed *Helicobacter pylori* eradication categories ($p < 0.05$).

Conclusion: Basal gastric juice differs significantly between *Helicobacter pylori*-positive and *Helicobacter pylori*-negative patients. Intra-gastric ammonia produced by *H. pylori* may have a role in the increased pH of gastric juice.

Keywords: Gastric juice, *Helicobacter pylori*, Gastritis, Intra-gastric ammonia, Enzyme activities

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INTRODUCTION

Gastritis is part of the global problem of gastro-

duodenal diseases, and more than 50 % of people have been affected by it [1]. The disease is caused by multiple factors. *Helicobacter pylori*

(*H. pylori*) has been accepted as the pathogen responsible for some gastro-intestinal diseases such as gastritis, peptic ulcers and gastric cancer [2]. Indeed, many Vietnamese researchers have reported a relationship between *H. pylori* and some gastroduodenal diseases [3].

Helicobacter pylori was identified by Warren and Marshall in Perth, Australia in 1982 [5]. This spiral gram negative microaerophilic bacterium affects the human gastric mucosa and gastric juice [4]. Since its discovery, *H. pylori* has received a lot of research attention as the most prevalent bacterial pathogen of humans. It affects about half of the world's human population [6].

Basal gastric output (BAO) shows the output of a fasting and unstimulated stomach [7]. Gastric acid plays a major pathophysiological role in human gastric diseases and in the evaluation of the effects of some factors on these diseases [8]. Basal gastric output is a complex mixture of hydrochloric acid (HCl), pepsin, lipase, electrolytes, and other components [9]. *Helicobacter pylori* is one of the common causes of gastritis and an important biological factor in gastric secretion. However, the link between this organism and gastritis/gastric secretion is not well understood.

Several studies on gastric juice evaluation in people have been carried out. In 2010, it was reported that healthy subjects had lower gastric pH than those who had gastritis [9]. In 2018, a study on 46 subjects showed that there was an association between gastric juice, pH \geq 3 and chronic *H. pylori* infection [10]. In recent years, scientists have begun to pay attention, not only to concentration of metabolites, but also to enzyme activities [11].

Until now, the association between *Helicobacter pylori* and changes in gastric juice has been established. However, no studies have been conducted to investigate changes in gastric juice in *H. pylori*-infected patients. Thus, the present study was carried out to investigate the effect of *H. pylori* infection on gastric juice secretion in human subjects.

METHODS

The investigation was a cross-sectional study. Data were collected between April 2018 and March 2019 from 150 patients who were subjected to upper endoscopy for diagnosis of gastritis and *H. pylori* infection at Hospital of Can Tho University of Medicine and Pharmacy. The study was conducted on patients who had *H.*

pylori status, as confirmed using CLO test and histology. The patients agreed to participate in the study. The patients were divided into two groups: a study group comprising 50 patients with *H. pylori*-positive infection, and a control group consisting of 100 patients who were *H. pylori*-negative.

Inclusion criteria

Patients in the following categories were included in the study: Patients with a history of gastritis without peptic ulcer disease, gastrointestinal malignancy, or upper gastrointestinal surgery, and patients who did not receive gastric anti-secretory medication or take any antimicrobial medication e.g., bismuth, within the previous 2 weeks.

Standard eradication therapy was given to the study group of 50 patients with *H. pylori*-positive infection. The therapy consisted of amoxicillin (1000 mg), clarithromycin (500 mg), and omeprazole (60 mg) for 10 - 14 days. Then, in the subsequent 3-week period, omeprazole was excluded from the treatment regimen. Success in eradication was determined after 4 weeks of eradication therapy. Then, the patients were subjected to another upper endoscopy. Patients who complied with these protocols were the only subjects included in the analysis.

Endoscopic and clinicopathological examinations

An electronic panendoscopy instrument (Olympus, Japan) was used to perform gastrointestinal endoscopy on the esophagus, the entire stomach, and the bulbar portion of the duodenum. Each examination was conducted by experienced endoscopists without knowledge of the serological information of the study participants.

The gastric juice was taken and frozen at -20 °C. The gastric juice was characterized using the highly sensitive method of ultra-performance liquid chromatography-tandem mass spectrometry UPLC-MS/MS [7]. The protocol allows for accurate quantification of very low levels of pepsin, lipase, and amylase in BAO.

Mucosal biopsy samples obtained from the patients were fixed in 10 % formalin. The intensity of gastritis in each biopsy was scored at four levels. These were level 0 (no inflammation), level 1 (mild inflammation), level 2 (moderate inflammation), and level 3 (severe inflammation). The results also showed the presence or absence of stainable *H. pylori* [12,16].

The present research was approved by the Medical Ethics Council of Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam (approval no. 021/PCT-HĐĐĐ). The authors hereby declare that all the procedures and experiments used in this study conformed with the ethical standards stipulated in the Helsinki Declaration of 1975, as revised in 2008 [5,20], as well as national laws. Informed consent was obtained from all the patients included in the study.

Statistical analysis

Data were analyzed using SPSS 13.0 software. Count data are presented as numbers and percentages (n (%)), while measured data are expressed as median and range. Differences in mean pH, and in concentrations and activities of pepsin, gastrin and amylase, as well as gastric acid secretion parameters between the 2 groups, and also between pre-treatment and post-treatment values, were determined using paired *t*-tests. Values of *p* < 0.05 were taken as indicative of statistically significant differences.

RESULTS

The characteristics of basal gastric juice (BAO) in two groups are presented in Table 1. The median

value of pepsin concentration in patients with *H. pylori*-positive was 8.93 ppm, with a range of 0.7 and 111.13, which was significantly higher than that of *H. pylori*-negative patients (*p* < 0.05). The median activity of pepsin in *H. pylori*-positive group was 2.23 U/mL (range: 0.17 - 27.78), and it was significantly higher than the corresponding control group value (*p* < 0.05).

There were higher percentages of abnormal basal acid output in the study group (*H. pylori*-positive) than in the control group. The percentages of bicarbonate ion, as well as the percentage activities and concentrations of lipase, pepsin and amylase in the 2 groups are presented in Table 2.

Only 32 (64 %) out of the 50 *H. pylori*-positive patients completed the eradication therapy, and had the second endoscopy. There was 65.5 % success in eradication of *H. pylori*. The characteristics of basal acid outputs which were evaluated after *H. pylori* eradication treatment are shown in Table 3. After the successful therapy, the median value of pH and concentrations of HCO₃⁻ and pepsin were significantly decreased (*p* < 0.05). Moreover, the median values of lipase concentration, pepsin, and lipase activity were increased 2 folds, when compared with pre-treatment values (*p* < 0.05).

Table 1: Basal acid outputs in *H. pylori*-negative and *H. pylori*-positive patients

Parameter	<i>H. pylori</i> -positive (n=50) Median (min-max)	<i>H. pylori</i> -negative (n=100) Median (min-max)	P-value
pH	2.31	2.21	0.875
Bicarbonate ion	4.06	12.66	0.865
Enzyme concentration (ppm)			
Pepsin	8.93 (0.7-111.13)	6.28 (0-150.7)	0.026
Lipase	0.93 (0-572.57)	1.11 (0-275.47)	0.671
Amylase	1.38 (0-794.24)	2.45 (0-501.72)	0.118
Enzyme activity (U/mL)			
Pepsin	2.23 (0.17-27.78)	1.57 (0-37.68)	0.026
Lipase	0.28 (0-171.77)	0.33 (0-82.64)	0.671
Amylase	0.04 (0-23.83)	0.74 (0-45.05)	0.118

Table 2: Degrees of basal gastric juice disorder in the two groups

BAO	<i>H. pylori</i> -positive (n=50)		<i>H. pylori</i> -negative (n=100)		P-value
	Normal n (%)	Abnormal n (%)	Normal n (%)	Abnormal n (%)	
pH	38 (76)	12 (24)	66 (66)	34 (34)	0.446
Bicarbonate ion	24 (48)	26 (52)	54 (54)	46 (46)	0.063
Enzyme concentration					
Pepsin	19 (38)	31 (62)	24 (24)	76 (76)	0.356
Lipase	05 (10)	45(90)	11 (22)	89(89)	0.923
Amylase	49 (98)	01 (02)	87 (87)	13 (13)	0.720
Activity					
Pepsin	08 (16)	42 (84)	10 (10)	90 (90)	0.862
Lipase	02 (04)	48 (96)	05 (5)	95 (95)	0.998
Amylase	0 (0)	50(100)	01 (1)	99 (99)	0.999

Except for amylase concentration, there were significant differences in the levels of these parameters between successful treatment and failed treatment groups.

There were significant differences in disorder in BAO between the successful treatment group with *H. pylori* eradication, and the failed treatment group. These results are shown in Table 4.

DISCUSSION

The spiral-shaped *H. pylori* induces damage in gastric epithelial cells by directly disrupting gastric mucus which protects the epithelia [13]. This characteristic of *H. pylori* lies in its potent urease activity which enables it to generate ammonia (NH₃), thereby adversely affecting the secretion of digestive enzymes and acid [14]. In this study, a higher rate of abnormal basal acid output was found in the study group (76 %) than in the control group (83 %). This disparity could be explained by the presence of gastritis in both groups, which is expected to alter the characteristics of the basal acid output. In physiology and laboratory investigations, normal gastric pH has been shown to range from 0.5 to 3 [15]. In this study, the pH of the study group was 2.31, while the pH of the control group was 2.21. The present research results are similar to those obtained in a previous study in Korea, which reported a higher pH in *H. pylori* infection group (4.77) than in the non-infected group (3.49) [16]. Bicarbonate ions (HCO₃⁻) are important in the maintenance of gastric acid

homeostasis. It is one of the essential factors that protect the stomach lining, in addition to gastric mucus. The concentration of HCO₃⁻ in the study group was 4.06 mmol/L, while that of the control group was 12.66 mmol/L. These results were within the normal range of 6.0 to 88.1 mmol/L. However, the HCO₃⁻ concentration was lower in the group having *H. pylori* infection, which led to a decrease in gastric mucosa protection, and accentuation of the possibility of gastric mucosal damage. The excretion of HCO₃⁻ is dependent on the acidity of the gastric juice. The lower the pH is, the more the HCO₃⁻ secreted in order to buffer the gastric mucosa, and vice versa, implying that high pH inhibits HCO₃⁻ secretion [11]. If the groups are categorized on the basis of gastric pH, it would be seen that the higher the pH, the lower the HCO₃⁻ ions secreted. These results are similar to previous findings in a study at the University of Texas [11].

There are many digestive enzymes in the gastric juice, but pepsin is the most important one, as it is the main protein hydrolase in the stomach. The concentrations of pepsin in the study group and in the control group were 8.93 and 6.28 ppm, respectively. In a previous study, the pepsin concentration was 12 µg/mL, which is equivalent to 5.47 ppm [17]. Thus, the present study obtained a higher pepsin concentration. This is probably due to the fact that the previous study referred to was conducted on healthy people without gastro-duodenal disease, while the present study was performed on patients with gastritis.

Table 3: Characteristics of basal gastric juice in patients after *H. pylori* eradication treatment

Characteristic	Successful treatment (n=21)			Failed treatment (n=11)			P _{(2) vs (4)}
	Before (1) (Median)	After (2) (Median)	P _{(1) vs (2)}	Before (3) (median)	After (4) (median)	p _{(3) vs (4)}	
pH	3.22	2.11	0.002	3.72	3.72	1.000	0.006
HCO ₃ ⁻ (mmol/L)	19.42	5.34	0.001	36.14	31.88	0.285	0.000
Enzyme concentration (ppm)							
Pepsin	17.84	2.58	0.007	9.30	4.91	1.000	0.027
Lipase	32.19	58.54	0.001	2.18	2.78	0.005	0.001
Amylase	2.34	6.31	0.149	75.47	1.02	0.386	0.217
Enzyme activity (U/mL)							
Pepsin	4.46	8.21	0.007	4.46	1.23	1.000	0.027
Lipase	9.66	17.56	0.01	9.65	0.84	0.02	0.001
Amylase	0.07	0.19	0.112	0.07	0.03	0.245	0.217

Table 4: Percentages of abnormalities in basal acid output after *H. pylori* eradication treatment

Basal acid output	Successful treatment (n=21)			Failed treatment (n=11)		
	Before (1)	After (2)	p ₁₋₂	Before (3)	After (4)	p ₃₋₄
Abnormal	66.67%	38.1%	0.07	81.81%	63.63%	0.784
Normal	33.33%	61.9%	0.09	18.19%	36.37%	0.235

The activity of pepsin in this study was 2.23 U/mL (in the study group), while pepsin activity was 1.57 U/mL in the control group. In another study [11], the mean activity of pepsin in normal people was 26.7 U/mL. Thus, the activity of pepsin was relatively low in both groups with gastritis, indicating that the disease decreased pepsin activity, with negative consequences for digestion of food in the stomach. In this study, the concentrations of lipase in the study group and control group were 0.93 and 1.11 ppm, respectively. This is in agreement with the results of a previous study [15]. After treatment, *H. pylori* was successfully eradicated in 65.6 % of the patients. This result is also consistent with results obtained in another study which reported 84.9 % eradication of *H. pylori* [18]. It has been reported that *H. pylori* is able to survive at external pH values between 4 and 6 [19,21].

In this study, gastric pH of the successful *H. pylori* eradication group (2.11) was lower than the gastric pH of the failed treatment group (3.72). Moreover, gastric pH is a contributing factor to the success of the *Helicobacter pylori* eradication regimen. The concentration of HCO_3^- in the successful treatment group was significantly decreased from 19.42 to 5.34 mmol/L. In contrast, the failed treatment group recorded only a mild decrease in the concentration of HCO_3^- ions. After the *H. pylori* eradication regimen, the concentration of HCO_3^- ions in the successful group was significantly lower than that of the failed treatment one. The eradication therapy resulted in decreased pepsin concentration, but it led to significant increase in pepsin activity. This was due to the increase in pH which activated pepsinogen into pepsin, resulting in decrease in pepsin concentration and an increase in pepsin activity. For lipase, there were increases, both in concentration and activity of lipase after treatment. Moreover, the degree of return to the normal basal gastric acid secretion in the successful group was 28.57 %, which was 18.18 % higher than that of the failed group. The differences in concentrations and activities of pepsin and lipase before and after the treatment, were statistically significant ($p < 0.05$) between the successful and failed treatment groups.

CONCLUSION

The status of *H. pylori* infection is affected by pH, HCO_3^- and digestive enzymes in gastric juice. Gastritis patients with *H. pylori* infection has abnormal basal gastric acid levels. The results of this study suggest that intragastric ammonia produced by *H. pylori* may have a role in the increased pH of gastric juice.

DECLARATIONS

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Ethical approval

This study was approved by the Medical Ethics Council of Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam (approval no. 021/PCT-HĐĐĐ).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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