

Comparison of Immunohistochemistry and Conventional Stains for *Helicobacter Pylori* Detection in Gastric Biopsies of Patients Receiving Proton Pump Inhibitors

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Abstract:

Objective: To evaluate the diagnostic performance of different staining methods for *Helicobacter pylori* (*H. pylori*) in patients treated with proton pump inhibitors (PPI)

Material and Methods: This is a retrospective study involving 75 gastric biopsies from patients with and without PPI therapy between October 2018 and September 2019. Slides were stained with hematoxylin and eosin (H&E); Giemsa and immunohistochemical staining (IHC) was done using polyclonal anti-*H. pylori* antibodies. Statistical analysis was performed to determine any association between the results of different staining methods and PPI consumption. Sensitivity, specificity and positive-negative predictive values of H&E and Giemsa stains were calculated.

Results: Overall, *H. pylori* infection was detected in 33.3% of patients using IHC, 26.7% using H&E, and 28.0% via Giemsa staining. A strong correlation was found between PPI consumption and low *H. pylori* density detected via IHC (p -value=0.015) but not using H&E and Giemsa staining. The sensitivity of H&E and Giemsa was markedly reduced as a result of PPI consumption (57.1% to 18.2% vs. 85.7% to 27.3%, respectively).

Conclusion: Identification of *H. pylori* using IHC in patients treated with PPI was superior to H&E and Giemsa stains. Both H&E and Giemsa stains showed a marked decrease in sensitivity in patients receiving PPI. The results indicate that IHC should be performed in patients with PPI therapy instead of H&E or Giemsa stains, and PPI should be discontinued for at least 14 days before the performance of endoscopy.

Keywords: conventional stains, *H. pylori*, immunohistochemistry, proton pump inhibitors

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Introduction

Diseases related to *Helicobacter pylori* (*H. pylori*) affect more people globally than any other disorder; it is believed that over 50.0% of the world's population is infected to date.¹ Warren and Marshall take credit for the discovery of *H. pylori* in 1983 when they examined the gastric mucosal biopsy specimens obtained from patients suffering from peptic ulcers or chronic active gastritis.² *H. pylori* is a Gram negative spiral organism³ believed to cause peptic ulcers, chronic gastritis, and gastric cancer. Moreover, it could be responsible for non-Hodgkin B cell lymphoma, especially mucosa-associated lymphoid tissue lymphoma. Clinically, a number of different invasive techniques (requiring endoscopy and biopsy) or non-invasive techniques (including serology, respiratory urea breath test, or the detection of fecal antigen) can be performed in order to detect *H. pylori* infection.

In routine practice, hematoxylin and eosin (H&E), Giemsa, and immunohistochemistry staining methods are commonly used in the identification of *H. pylori* after an endoscopic procedure. The H&E stain can detect *H. pylori* under light microscopy; however, it becomes difficult to identify *H. pylori* when it is present in low density. Although the Giemsa stain is a more popular method than H&E in many laboratories, it could lead to false negative results when the organisms are few.

Furthermore, *H. pylori* bacteria could exist in two forms—rod and coccoid shapes. The rod-shaped bacteria often change to coccoid-shaped ones when undergoing proton pump inhibitors (PPI) therapy.^{4,5} It is difficult to determine the presence of coccoid-shaped bacteria when the Giemsa staining technique is applied because it can be very hard to distinguish the coccoid-shaped bacteria from other bacterial forms or other matter. Consequently, the best approach to use if *H. pylori* is to be detected is immunohistochemistry staining. Moreover, it is believed that bacterial transmission could be facilitated by the coccoid-

shaped *H. pylori*, and that these bacteria could play a key role when infection recurs following the completion of anti-microbial therapy as well. Moreover, PPI could also lead to false-negative results in other tests.⁶ Owing to their in vitro anti-*H. pylori* activity,⁷ PPI could reduce the bacterial load⁸⁻¹⁰ and suppress the urease activity of *H. pylori*.¹¹ Overall, the Immunohistochemical (IHC) stain has advantages over the other methods when the biopsied specimens have a few coccoid or intracellular forms of *H. pylori* present. Furthermore, it has a high specificity as it can differentiate between *H. pylori* and other mimicking organisms.¹²

Hence, in this study, we aimed to evaluate the diagnostic value of the immunohistochemical method using polyclonal anti-*H. pylori* antibodies as a gold standard test for the identification of these organisms in PPI-treated and PPI-untreated patients and compare its results with those of routine histochemical methods like H&E and Giemsa stains. The histopathological examination of gastric biopsies was reported in accordance with the "Updated Sydney System" classification of chronic gastritis.¹³

Material and Methods

In this retrospective 1 year study, the gastric biopsies taken from patients with a histopathological diagnosis of gastritis, who underwent an endoscopic examination between October 2018 and September 2019 at the Vajira Hospital, Faculty of Medicine, Navamindradhiraj University, were studied. Patients were divided into two groups according to PPI therapy; patients receiving PPI during the 14-day period prior to the procedure (called the PPI-treated group) and those receiving no PPI for at least 14 days before endoscopy (called the PPI-untreated group). At least four biopsy specimens were collected from both the antrum and corpus during each endoscopy. The clinical data were reviewed and recorded. The study protocol complied with the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee of

Navamindradhiraj University. Four-micron-thick sections were placed on slides for histochemistry. After that, the histological sections were dyed with H&E and Giemsa following our routine protocols. Immunohistochemistry was performed on the Leica Bondmax platform (Leica Micro-systems, Buffalo Grove, Illinois, the United States of America) on 4- μ m-thick consecutive sections in accordance with the manufacturer's instructions using rabbit polyclonal anti-*H. pylori* antibodies (1:150, Novocastra). In the present study, IHC was considered as the gold standard for *H. pylori* detection. All samples were examined and diagnosed by two experienced histopathologists unaware of the patient's previous diagnosis. Specimens showing a positive result for IHC were deemed to be *H. pylori* positive. Gastritis was classified in accordance with the updated Sydney system.

Bacterial density was quantified by counting *Helicobacter*-like organisms on the mucosal surface under a high-power field. The four grades used for bacterial density were: normal (no bacteria); mild (individual bacteria or small groups on less than a third of the mucosal surface); moderate (bacteria present in numbers greater than 'mild' but fewer than 'severe'), and severe (large groups of bacteria on more than two thirds of the mucosal surface). These classifications were derived from the visual analogue scale of the updated Sydney system.

For statistical purposes, all cases were grouped according to the presence or absence of (1) chronic gastritis, (2) inflammatory activity, and (3) *H. pylori* and/or chronic gastritis-related morphological alterations of the gastric mucosa. The considered morphological changes included intestinal metaplasia, atrophy, ulcer and foveolar hyperplasia. Briefly, the different subgroups were as follows: (1) chronic active gastritis with morphological change, (2) chronic active gastritis without morphological change, (3) chronic nonactive gastritis with morphological change, and (4) chronic nonactive gastritis without morphological change.

The chi-square or Fisher's exact tests were used to evaluate differences between categorical variables. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the tests were also calculated. The statistical analysis was carried out using the Stata software (version 13, StataCorp LP, College Station, TX).

In the present study, 75 gastric biopsies obtained from October 2018 to September 2019 and histopathologically diagnosed as chronic gastritis via the H&E stain were included for investigation. The demographic and histopathological characteristics of the study population are illustrated in Table 1. Of the 75 studied patients, 35 (46.7%) were male and 40 (53.3%) female, the mean age was 64.7 ± 3.0 years, and 33 (44.0%) underwent PPI therapy and 42 (56.0%) did not.

The mean age was 64.0 ± 4.5 years for the PPI-treated patients, and 65.2 ± 4.0 years for the PPI-untreated patients. No statistically significant correlation was observed between PPI-treated and untreated groups with regard to patient age, sex, and histopathological characteristics as shown in Table 1.

Comparison of the diagnostic performances of the different staining methods

Among the 75 patients studied, the IHC stain could identify bacilli in 25 (33.3%) biopsies, and the Giemsa and H&E stains, in 21 (28.0%) and 20 (26.7%) biopsies, respectively (Table 2). In 10 cases, *H. pylori* was suspected by the H&E stain, but the results were negative in the IHC stain. Overall, the results of the H&E and Giemsa stains were consistent with those of the IHC stain in 50 (66.7%) and 59 (78.7%) specimens, respectively. There were 6 cases that showed no bacilli in the IHC stain although they were suspected to be positive by the Giemsa stain. The overall sensitivity and specificity of the H&E stain were 40.0% and 80.0%, respectively. The

Table 1 Classification of 75 patients according to age, sex, histopathological findings, and the results of *Helicobacter pylori* diagnosis according to hematoxylin and eosin stain, Giemsa and immunohistochemical staining

Characteristics	PPI-treated (n=33)	PPI-untreated (n=42)	p-value
Mean age (years)	64.0±4.5	65.2±4.0	
Gender			
Male	14 (42.4)	21 (50.0)	0.514
Female	19 (57.6)	21 (50.0)	
Histology			
Chronic active gastritis with morphological change	2 (6.1)	5 (11.9)	0.385
Chronic active gastritis without morphological change	11 (33.3)	9 (21.4)	
Chronic nonactive gastritis with morphological change	2 (6.1)	2 (4.8)	
Chronic nonactive gastritis without morphological change	18 (54.6)	26 (61.9)	
<i>H. pylori</i> diagnosis			
H&E			
Positive	6 (18.2)	14 (33.3)	0.141
Negative	27 (81.8)	28 (66.7)	
Giemsa			
Positive	4 (12.1)	17 (40.5)	0.009*
Negative	29 (87.9)	25 (59.5)	
IHC			
Positive	11 (33.3)	14 (33.3)	1.000
Negative	22 (66.7)	28 (66.7)	

Values are presented as number (%) or mean±standard deviation.

H&E=hematoxylin and eosin stain, IHC=immunohistochemical stain, PPI=proton pump inhibitors, *H. pylori*=*Helicobacter pylori*

*p-value<0.050 (statistically significant)

Table 2 Overall detection rate of *Helicobacter pylori* with hematoxylin and eosin stain, Giemsa and immunohistochemical staining

Methods	Number of patients (%)
H&E	
Negative	55 (73.3)
Positive	20 (26.7)
Total	75 (100.0)
Giemsa	
Negative	54 (72.0)
Positive	21 (28.0)
Total	75 (100.0)
IHC	
Negative	50 (66.7)
Positive	25 (33.3)
Total	75 (100.0)

H&E=hematoxylin and eosin stain, IHC=immunohistochemical stain

sensitivity (60.0%) and specificity (88.0%) of the Giemsa stain were higher than those of the H&E stain. However, in the subgroup analysis, using immunohistochemistry as the gold standard, we found that a significantly higher percentage of patients with PPI use had a mild *H. pylori* density when compared to patients without PPI use (81.8% vs. 28.6%, respectively; p-value=0.015). In contrast, no significant difference in *H. pylori* density was observed between the H&E and Giemsa stains (p-value=1.000 vs. p-value=1.000, respectively) (Table 3).

Considering IHC stain as the gold standard for the identification of *H. pylori* in gastric mucosal biopsies in the PPI-untreated group, the sensitivity and specificity of the H&E stain were 57.1% and 78.6%, respectively. The sensitivity (85.7%) and specificity (82.1%) of the Giemsa

stain were higher than those of the H&E stain. Similarly, the positive and negative predictive values of the H&E stain were lower than those of Giemsa stain (57.1% vs. 70.6% and 78.6% vs. 92.0%, respectively). In contrast, the sensitivity of the H&E and Giemsa stains dramatically

dropped to 18.2% and 27.3%, respectively, in the PPI-treated group, whereas their specificity increased slightly to 81.8% and 95.5%, respectively. The comparative results of the H&E, Giemsa, and IHC stains are shown in Figures 1A–C and summarized in Tables 4 and 5).

Table 3 *Helicobacter pylori* density comparison between hematoxylin and eosin stain and Giemsa with immunohistochemical as the gold-standard test

Method/ <i>H. pylori</i> density	PPI-treated	PPI-untreated	p-value
H&E			
Mild	3 (50.0)	7 (50.0)	1.000
Moderate/marked	3 (50.0)	7 (50.0)	
Giemsa			
Mild	1 (25.0)	7 (41.2)	1.000
Moderate/marked	3 (75.0)	10 (58.8)	
IHC			
Mild	9 (81.8)	4 (28.6)	0.015*
Moderate/marked	2 (18.2)	10 (71.4)	

Values are presented as number (%) or mean±standard deviation.

H&E=hematoxylin and eosin stain, IHC=immunohistochemical stain, PPI=proton pump inhibitors, *H. pylori*=*Helicobacter pylori*

*p-value<0.050 (statistically significant)

Table 4 Comparison of hematoxylin and eosin stain, Giemsa and immunohistochemical staining methods

Methods and results	H&E		Giemsa	
	Positive	Negative	Positive	Negative
All patients				
IHC				
Positive	10	15	15	10
Negative	10	40	6	44
PPI-treated group				
IHC				
Positive	2	9	3	8
Negative	4	18	1	21
PPI-untreated group				
IHC				
Positive	8	6	12	2
Negative	6	22	5	23

H&E=hematoxylin and eosin stain, IHC=immunohistochemical stain, PPI=proton pump inhibitors

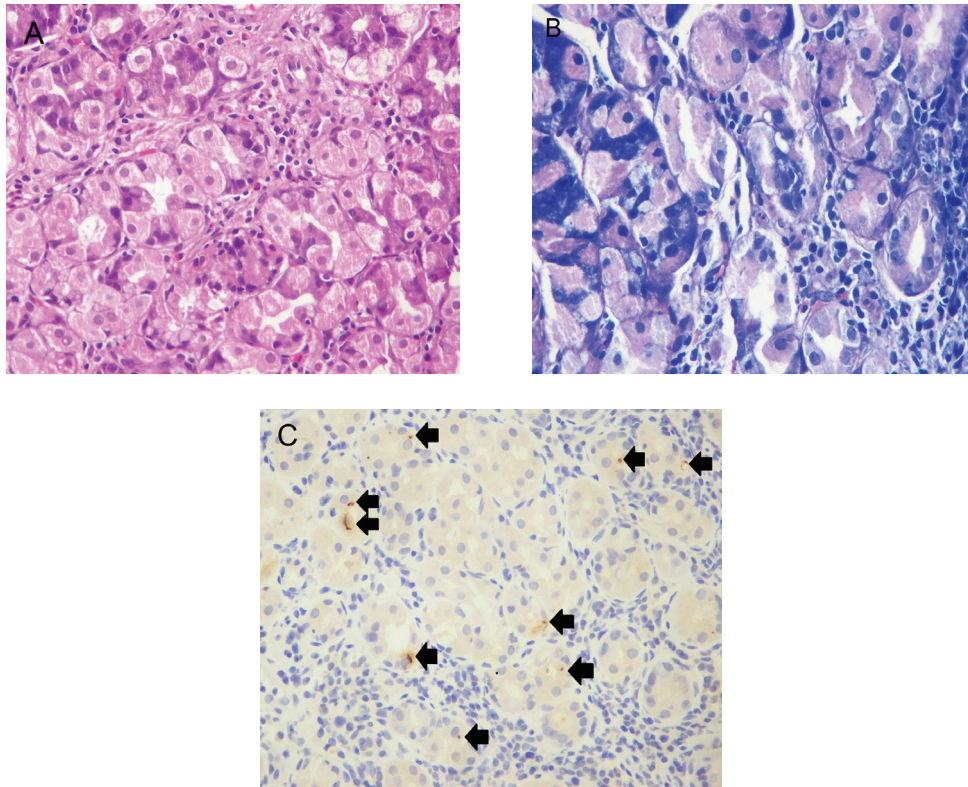


Figure 1 A case of PPI-treated patient showing no organisms on H&E (A) on Giemsa stain (B). IHC staining (C) showing focal positivity with few *H. pylori* (arrows) (original magnification × 40).

Table 5 Sensitivity, specificity, positive-negative predictive values of hematoxylin and eosin stain and Giemsa

All patients

Method	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
H&E	40.0	80.0	50.0	72.7
Giemsa	60.0	88.0	71.4	81.5

PPI-treated group

Method	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
H&E	18.2	81.8	33.3	66.7
Giemsa	27.3	95.5	75.0	72.4

Table 5 (continued)

PPI-untreated group

Method	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
H&E	57.1	78.6	57.1	78.6
Giemsa	85.7	82.1	70.6	92.0

H&E=hematoxylin and eosin stain, PPI=proton pump inhibitors

Discussion

In the present study, the *H. pylori* infection rate was 33.3% by immunohistochemistry, 28.0% by Giemsa, and 26.7% by H&E. Among the 75 patients, spiral bacterial cells were visualized in 20 (26.7%) cases using H&E, in 21 using Giemsa (28.0%), and in 25 (33.3%) using IHC. The *H. pylori* infection rate in the present study was relatively lower than those reported by previous studies. The study conducted by Pérez-Pérez et al.¹⁴ showed that the prevalence of *H. pylori* infection increased with age—from 17.5% among children aged 5 to 9 years old to 55.0% among those in their third decade of life; the infection peak was at 75.0% among those 30 to 49 years of age. Another study from Thailand conducted by Chinprasatsak et al.¹⁵ discovered a relatively high *H. pylori* infection rate, up to 73.8%, compared to our study. However, many recent studies have shown a decreasing trend for the *H. pylori* infection rate in some areas of the world. It has been hypothesized that this reduction might result from a better awareness of public health, improvements in personal hygiene, effective diagnostic tests as well as the eradication of *H. pylori*, which make it difficult to analyze the data between different populations.^{16,17}

Regarding *H. pylori* detection methods, the H&E, Giemsa and immunohistochemistry staining techniques have been commonly used for the identification of organisms in gastric mucosal biopsies. Though several methods for the identification of these bacilli have been investigated,

the identification of *H. pylori* by histological methods in gastric biopsies remains the most common and the most effective test.¹⁸ The H&E stain could detect *H. pylori* under high magnification, but it becomes difficult to identify *H. pylori* when it is present at a low density and when atrophic mucosal changes are present. The Giemsa stain is the preferred method over H&E in many laboratories; however, it could lead to false negative results when the organisms are few or in patients with a prior incomplete treatment for *H. pylori* gastritis. IHC stains enjoy some advantages when patients experience a partial treatment for *H. pylori* gastritis or either have few or coccoid forms of *H. pylori* present. This method has a high specificity because it can differentiate between other mimicking organisms and can, therefore, be considered the gold standard test.¹⁹ It is noteworthy that IHC has been found to show a good performance with a sensitivity of 89.6%, specificity of 100.0%, PPV of 100.0% and NPV of 84.1%.²⁰ Furthermore, the proportions of PPI patients with negative results via H&E and Giemsa stains (81.8% and 87.9%, respectively) were considerably higher than those of non-PPI patients (66.7% and 59.5%, respectively). Meanwhile, the IHC technique yielded the same proportions in both patients who received PPI (66.7%) and those who did not (66.7%). Considering *H. pylori* density, there was a discrepancy in *H. pylori* density when using IHC as the gold standard test. PPI-treated patients showed a higher prevalence of mild *H. pylori* density when compared to patients without

PPI treatment (81.8% vs. 28.6%; p -value <0.050). No such correlation was observed in the H&E or Giemsa staining methods.

In this study, on the basis of the results obtained from the H&E and Giemsa stains, the Giemsa staining had a higher sensitivity and specificity than H&E in patients who did not take PPI (sensitivity: 85.7% vs. 57.1%; specificity: 82.1% vs. 78.6%). However, we found that the sensitivity of the H&E and Giemsa stains dramatically dropped in patients with PPI use. The alterations in sensitivity were more prominent in both H&E and Giemsa (from 57.1% and 85.7% to 18.2% and 27.3%, respectively) due to PPI use. Our results indicated that in patients who did not receive PPI, Giemsa had the best performance of the two methods—sensitivity 85.7%, specificity 82.1%, PPV 70.6%, and NPV 92.0%.

In general, the Giemsa stain was superior to H&E in the detection of bacilli. The major disadvantage of this stain is that it produces little contrast between bacteria and the surrounding tissue. However, the presence of a few bacteria and the identification of coccoid forms of bacilli make identification difficult via both the routine and the histochemical methods. Nevertheless, these difficulties are not encountered when identifying bacilli using the IHC method. In the present study, among the stains utilized, the IHC stain identified bacilli in higher proportion of the PPI-positive biopsies. The IHC stain yielded a positive result in 33.3% of PPI-positive biopsies, whereas the H&E and Giemsa stains identified organisms in 18.2% and 12.1% of cases, respectively.

PPI are known to serve as bactericidal agents for *H. pylori*.²¹ The pH level in the stomach is increased by PPIs, whereupon the *H. pylori* urease produces ammonia, which is then accumulated and, thus, effectively curtailing the viability of the bacteria.²² As a consequence of exposure to PPI and antibiotics, the spiral forms of *H. pylori* will turn in a coccoid shape and exhibit a reduced urease

activity.²³ *In vitro* experiments have demonstrated that the inhibitory influence of PPI on *H. pylori* growth is dependent upon the concentrations involved,²¹ while the bacterium's virulence properties could have an effect as well.²⁴ At concentrations of ≤ 0.625 $\mu\text{g/ml}$, lansoprazole has been shown to exhibit bactericidal properties and be able to inhibit growth. Accordingly, it can be concluded that the effects of PPI on the *H. pylori* rapid urea test and culture are dependent upon the type of PPI and the dosage taken by patients.²⁵

In the present study, we found that the IHC staining method for *H. pylori* detection was significantly affected by PPI consumption. The IHC method revealed a low bacterial density in the majority of patients with PPI use. Consequently, *H. pylori* might not be detected by the H&E and Giemsa stains because of the rare bacteria. The IHC stain was considered the gold standard for the identification of bacilli in our study, while the H&E and Giemsa stains showed a sensitivity of 18.2% and 27.3% and a specificity of 81.8% and 95.5%, respectively.

In some countries, PPI are frequently prescribed, even in scenarios where it might not be necessary such as mild dyspepsia.²⁶ Thailand is one such country, with patients taking PPI for subclinical or mild gastritis. As a result, diagnostic testing (H&E, Giemsa stains) for *H. pylori* often generates false negative outcomes. It is, therefore, suggested that an endoscopy should be carried out no earlier than two weeks after the cessation of PPI consumption²⁷, and IHC should be performed in all cases instead of H&E or Giemsa in order to avoid false negative results, especially in patients with PPI use.

Conclusion

H&E and Giemsa stains showed a marked decrease in sensitivity to detect *H. pylori* in patients receiving PPI. The ability of IHC to identify *H. pylori* was superior to those of both H&E and Giemsa stains. Hence,

IHC should be preferred in patients treated with PPI instead of H&E and Giemsa stains.

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Conflict of interest

The authors declare that they have no conflict of interest.

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