

A comparative study between hematoxylin and eosin and immunohistochemistry in patients with chronic gastritis and gastric adenocarcinoma in Erbil City, Iraq

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ABSTRACT

Background and objective: *Helicobacter pylori* infection is the most common and treatable cause of chronic gastritis; it is strongly linked to peptic ulcer, low-grade gastric lymphoma, and adenocarcinoma. Infection with this microorganism affects more than half the world's population. The research aimed to compare sensitivity, specificity, and positive predictive value between two histopathological methods, hematoxylin and eosin (H&E) and immunohistochemistry (IHC), in patients with chronic gastritis and gastric cancer.

Methods: A total of 90 gastric biopsies were included in the study — 44 with chronic gastritis and 46 diagnosed with gastric adenocarcinoma — collected from the archive of the Histopathology Department at Erbil International Private Hospital between January 2024 and January 2025. Slides were stained with H&E, and IHC staining was performed using a polyclonal anti-*H. pylori* antibody. Sensitivity, specificity, and positive predictive value were calculated.

Results: More than two-thirds of cancer patients (67.4%) were aged ≥ 60 years, compared to 38.6% of the gastritis group ($p = 0.012$). More than half (52.2%) of cancer patients were male, compared with 43.2% of patients with gastritis. The two diagnostic techniques revealed a statistically significant difference ($p = 0.035$); the H&E stain showed 50% sensitivity, 46.9% specificity, and 47.7% overall agreement with IHC.

Conclusion: IHC staining is more accurate for determining *Helicobacter pylori* infection than the traditional H&E stain, especially in equivocal samples with a low bacterial load and inactive gastritis. IHC showed the highest sensitivity, specificity, and inter-observer agreement compared with H&E stain.

Keywords: Immunohistochemical test, Gastric biopsy, Histopathology, *Helicobacter pylori*, Gastric adenocarcinoma

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral-shaped, flagellated, Gram-negative bacterium. It is positive for catalase and oxidase and is a strong producer of urease. Patients with *H. pylori* have a 10-20% risk of developing gastric ulcer and gastric cancer (1). *H. pylori*-related gastritis is an important global health concern; more than fifty percent of the world's population carries *H. pylori*, often asymptomatically, with higher prevalence rates in developing than in developed countries (2, 3). *H. pylori* is known to occur in two forms, rod-shaped and coccoid-shaped, according to morphology. The rod-shaped form can convert to the coccoid form under several environmental conditions, such as alkaline pH, long-term treatment, oxygen exposure, inadequate proton pump inhibitor (PPI) or antibiotic therapy, and starvation (4). More people are now interested in accurately identifying *H. pylori* in gastric biopsy tissue samples, since various studies have shown the risk of *H. pylori* infection in causing gastric cancers and lymphomas (5). *H. pylori* has been classified as a type I carcinogen by the World Health Organization (WHO). It triggers inflammatory reactions in the gastric mucosa, causing multifocal gastritis, intestinal metaplasia, glandular atrophy, and adenocarcinoma in both the gastric corpus and antrum (6). Numerous methods are available for the diagnosis of *H. pylori*. They are classified into two groups: non-invasive methods, which include fecal antigen tests, serology, and urea breath tests; and invasive methods, which include gastric biopsy, rapid urease test, polymerase chain reaction, and tissue culture. Histopathological examination is considered the best way to identify *H. pylori* because it can detect the different harmful changes linked to this infection, such as inflammation, intestinal metaplasia, atrophy, and cancer (7).

Gastric cancer is the fifth most common malignancy and the third most common cause of cancer-related mortality in Iraq. Gastric adenocarcinoma accounts for 90% of all malignant neoplasms of the stomach. *H. pylori* gastritis has major complications, including gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma (8, 9). Several variables affect the diagnostic accuracy of *H. pylori* in gastric biopsy samples, including the type of diagnostic technique, low bacterial numbers, previous antibiotic use, the use of proton pump inhibitors, biopsy location, degree of infection, type of inflammatory changes in the tissue, the technique used to process the sample, and infection intensity (10). Therefore, this study focuses on two main points: using an immunohistochemical method to detect *H. pylori* in gastric biopsies, and comparing the results with the routine H&E stain.

METHODS

This is a retrospective study of 90 endoscopic biopsies with a low density of *H. pylori* organisms, retrieved from the histopathology lab; all cases were stained with the routine H&E method, followed by IHC staining to confirm the diagnosis. Endoscopic gastric biopsy reports with gastritis and gastric adenocarcinoma were collected from the archive of the Histopathology Department at Erbil International Private Hospital between January 2024 and January 2025. The medical record number, age, and gender of the patients were retrieved from the patients' record files. The histopathological examination of gastric biopsies was reported using the "Updated Sydney System" classification of chronic gastritis. All histological diagnoses were first made using the Updated Sydney System, and then a second qualified pathologist reviewed the results to confirm their accuracy and consistency.

Hematoxylin and Eosin Staining

Tissues were preserved in a 10% formaldehyde solution, embedded in paraffin using standard methods, cut into 2-3 µm slices for routine H&E staining, and then examined for *Helicobacter pylori* under a light microscope.

Immunohistochemical Staining

A standard three-step streptavidin-biotin complex staining procedure was used on charged slides after sectioning. The slides were deparaffinized in xylol for 3 to 10 minutes, then rehydrated in 100%, 96%, and 70% ethanol, and water. Endogenous peroxidase was blocked in a mixture of ethanol with 3% hydrogen peroxide for 10 minutes. After the slides were rinsed in water, antigen retrieval was performed by microwave heat treatment for 10 minutes in a citrate-buffered solution (10 mmol/L, pH 6.0), followed by rinsing in phosphate-buffered saline (PBS). Tissue sections were incubated for 1 hour at room temperature in 1.5% bovine serum

albumin (BSA) to block non-specific antigen sites. Sections were stained with a polyclonal *Helicobacter pylori* antibody (Vitro S.A. – Master Diagnóstica), an approach that provides high sensitivity and helps confirm *H. pylori* infection. The slides were then examined by the histopathologists.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 26, released 2019; IBM, New York, USA). The chi-square test of association was used to compare proportions. Fisher's exact test was used when the expected count in more than 20% of the table's cells was less than 5. The unpaired t-test was used to compare two means. The McNemar test was used to compare the H&E test results with the IHC test results of the same cases. The following table shows how the validity indicators of the H&E test were calculated:

Validity indicator formulas

	IHC Positive	IHC Negative	Total
H&E Positive	TP	FP	TP+FP
H&E Negative	FN	TN	FN+TN
Total	TP+FN	FP+TN	Grand total

TN = true negative; TP = true positive; FN = false negative; FP = false positive. Sensitivity = TP/(TP+FN)×100; Specificity = TN/(FP+TN)×100; PV+ = TP/(TP+FP)×100; PV- = TN/(FN+TN)×100; Total agreement = (TP+TN)/Grand total. A p-value ≤ 0.05 was considered statistically significant

RESULTS

Forty-four of the 90 patients had chronic gastritis, while 46 had gastric cancer. More than one-third (38.6%) of chronic gastritis patients were aged ≥ 60 years, compared with 67.4% of gastric cancer patients ($p = 0.012$). Around half (43.2%) of chronic gastritis patients were male, compared with 52.2%

of cancer patients, but the difference was not significant ($p = 0.393$), as presented in Table 1.

The prevalence of *H. pylori* (as detected by IHC) among patients with chronic gastritis was 27.3%, and among gastric cancer patients was 19.6%, but the difference was not significant between the groups ($p = 0.387$), as presented in Figure 1.

Table 1. Age and sex distribution

Variable	Chronic gastritis No. (%)	Gastric cancer No. (%)	Total No. (%)	p
<40y	10 (22.7)	2 (4.3)	12 (13.3)	
40-49y	11 (25.0)	6 (13.0)	17 (18.9)	
50-59y	6 (13.6)	7 (15.2)	13 (14.4)	
$\geq 60y$	17 (38.6)	31 (67.4)	48 (53.3)	0.012*
Mean (SD)	51.0 (17.1)	64.8 (16.1)		<0.001**
Male	19 (43.2)	24 (52.2)	43 (47.8)	
Female	25 (56.8)	22 (47.8)	47 (52.2)	0.393*
Total	44 (100.0)	46 (100.0)	90 (100.0)	

*Calculated by chi-square test. **Calculated by unpaired t-test.

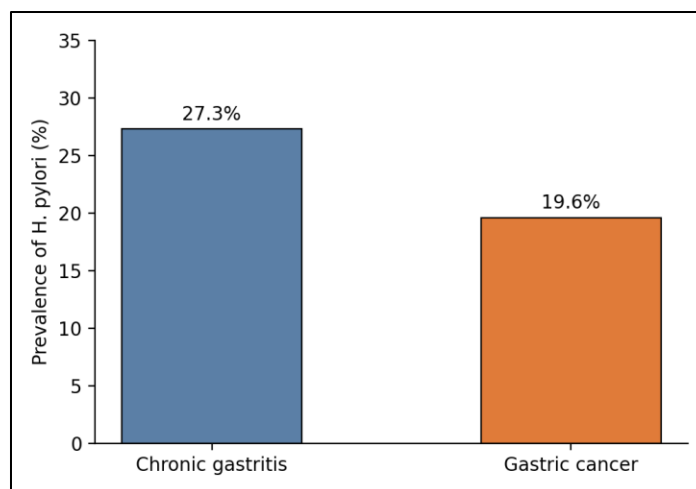


Figure 1. Prevalence of *Helicobacter pylori* in the study groups

No significant association was found between the prevalence of *H. pylori* and the following histological changes among patients with gastritis: chronic inflammation ($p = 1.000$), lymphoid aggregate ($p = 0.739$), activity ($p = 0.284$), glandular

atrophy ($p = 1.000$), and intestinal metaplasia ($p = 1.000$), as shown in Table 2.

Around two-thirds of gastric cancer patients were poorly differentiated, and the rest were moderately differentiated (Figure 2).

Table 2. Prevalence of *Helicobacter pylori* (by IHC) with histopathological changes in chronic gastritis

Histological change	Positive No. (%)	Negative No. (%)	Total No. (%)	p*
Mild chronic gastritis	3 (25.0)	9 (75.0)	12 (100.0)	1.000
Moderate chronic gastritis	9 (28.1)	23 (71.9)	32 (100.0)	
Lymphoid aggregate +	8 (29.6)	19 (70.4)	27 (100.0)	
Lymphoid aggregate –	4 (23.5)	13 (76.5)	17 (100.0)	0.739
Neutrophil absent	6 (20.7)	23 (79.3)	29 (100.0)	
Mild neutrophil activity	6 (40.0)	9 (60.0)	15 (100.0)	
Atrophy absent	7 (26.9)	19 (73.1)	26 (100.0)	1.000
Atrophy mild	5 (27.8)	13 (72.2)	18 (100.0)	
Metaplasia absent	11 (28.9)	27 (71.1)	38 (100.0)	
Metaplasia mild	1 (16.7)	5 (83.3)	6 (100.0)	1.000
Total	12 (27.3)	32 (72.7)	44 (100.0)	

*Calculated by Fisher's exact test.

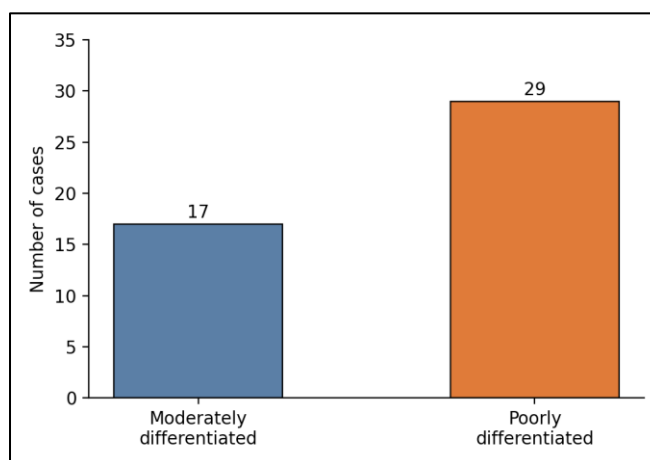


Figure 2. Grading of gastric adenocarcinoma cases

A significant ($p = 0.035$) difference was detected between the two tests for detecting *H. pylori*: the H&E test and the IHC test. The validity indicators of the H&E test were as follows: sensitivity 50%, specificity 46.9%, PV+ 26.1%, PV– 71.4%, and total agreement with IHC 47.7% (Table 3).

The prevalence of *H. pylori* among patients with poorly differentiated adenocarcinoma was 24.1%, compared with 11.8% among patients with moderately differentiated gastric cancer ($p = 0.450$) (Figure 3 & 4).

Table 3. Validity of H&E staining for *H. pylori* compared with IHC as a confirmatory test

H&E result	IHC Positive	IHC Negative	Total	p*
Positive	6	17	23	0.035
Negative	6	15	21	
Total	12	32	44	
Sensitivity	Specificity	PV+	PV–	Agreement
50.0%	46.9%	26.1%	71.4%	47.7%

*Calculated by McNemar test.

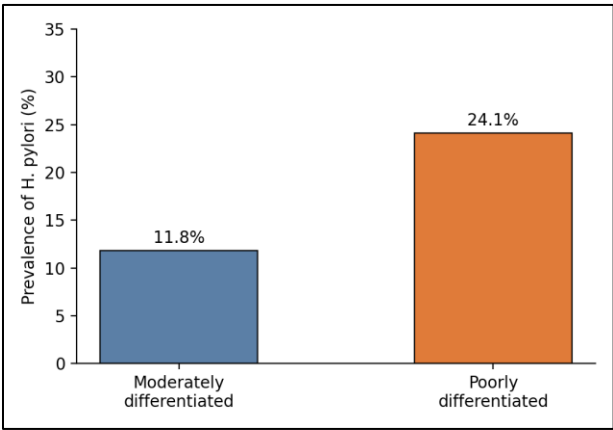


Figure 3. Prevalence of *H. pylori* by grade of gastric adenocarcinoma
*Calculated by Fisher's exact test.

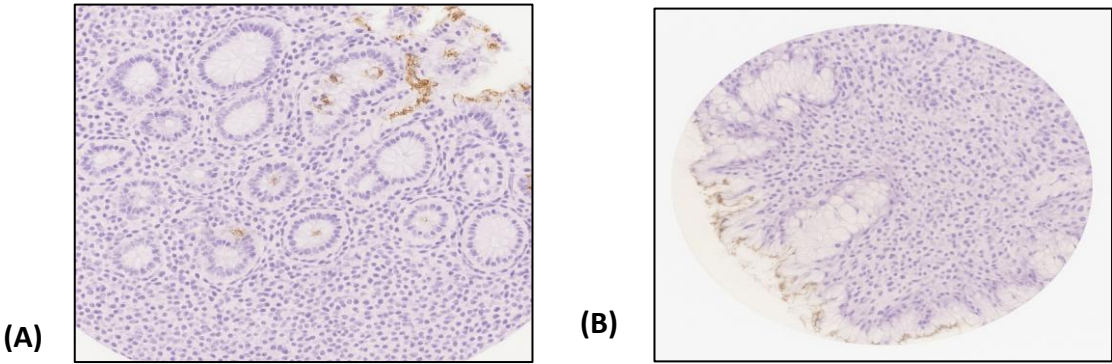


Figure 4. Immunohistochemical staining of *Helicobacter pylori* in (A) chronic gastritis and (B) gastric adenocarcinoma, poorly differentiated (signet ring morphology). Microscopy images, magnification 400x (*Helicobacter pylori* IHC).

DISCUSSION

This work aimed to assess the diagnostic accuracy for detecting *H. pylori* in patients with chronic gastritis and gastric cancer by comparing two staining methods, H&E and IHC. The study found notable variations in age distribution between the two groups: 44 of 90 cases were diagnosed with gastritis, and 38.6% of the gastritis patients were aged 60 or older, while more than two-thirds (67.4%) of the gastric cancer (CA) patients were in this age group ($p = 0.012$). More than half (52.2%) of CA patients were male, compared with 43.2% of patients with gastritis, but the difference was not significant ($p = 0.393$). The risk of gastric cancer increases with age; many studies have found that risk rises notably by age 60 (11), probably because of cumulative exposure to carcinogens, chronic inflammation, and age-related genomic instability. This highlights the importance of screening tests and early detection of gastric cancer in the elderly and in high-risk individuals. By contrast, the gender distribution between the two groups did not reach statistical significance, with 43.2% of gastritis patients and 52.2% of cancer patients being male ($p = 0.393$), despite some research suggesting a male predominance in stomach cancer incidence (12). This study found that not all patients with chronic gastritis showed significant histological alterations; hence, some people may remain asymptomatic or have only mild inflammation despite long-term infection (13, 14). Moreover, another study found that in some populations — especially East Asia — the presence of *H. pylori* was strongly linked to a greater prevalence of poorly differentiated stomach cancers, particularly those that had progressed to advanced stages (15).

Hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) were compared for their diagnostic performance in detecting *H. pylori*.

The two tests revealed a difference approaching statistical significance ($p = 0.035$), stressing their differing diagnostic accuracy. Alongside a modest total agreement (47.7%) with the IHC test, the validity indicators of the H&E staining test showed fairly poor sensitivity (50%) and specificity (46.9%). Immunohistochemistry is comparatively effective for differentiating *H. pylori* from other bacteria, especially at low bacterial counts or when coccoid forms are present. This step is especially important for patients taking proton pump inhibitors, since these can cause *H. pylori* to convert into coccoid forms and migrate deeper into the gastric foveolae (16). Unlike H&E staining, which is essentially a generic histological study and may not always reliably identify bacteria in every case, IHC can confirm infection that H&E might miss (17). In line with other investigations, we conclude that the capacity of H&E to identify *H. pylori* grew with the degree of inflammation; therefore, in cases of mild inflammation, IHC stains are necessary to detect cases of *H. pylori* infection undetected by H&E.

CONCLUSION

Immunohistochemical staining was better at accurately identifying *H. pylori* in gastric biopsies compared with H&E, and helped prevent missed diagnoses. This study strengthens the link between older age and gastric cancer, and shows that individuals with poorly differentiated gastric adenocarcinoma had a higher *H. pylori* burden. The rate of *H. pylori* infection increases with age and is linked to the development of chronic gastritis and gastric adenocarcinoma. In cases of mild inflammation, IHC stains identified instances that were undetected by H&E, consistent with several earlier investigations.

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COMPETING INTEREST

The authors declare that they have no competing interests.

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