# Efficacy of immunohistochemical staining in detecting *Helicobacter pylori* in Saudi patients with minimal and atypical infection

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**Research**

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## Abstract

# Background

Gastric *Helicobacter pylori* infection is diagnosed based on histopathological evaluation of gastric mucosal biopsies, urease test, urea breath test, *H. pylori* culturing, or direct detection using polymerase chain reaction (PCR). This study aimed to evaluate the efficacy of immunohistochemical (IHC) staining in detecting *H. pylori* in gastric biopsies from dyspeptic patients with minimal and/or atypical infection. Gastric biopsies from 50 patients with chronic gastritis were subjected to routine haematoxylin and eosin (H&E), modified Giemsa, and IHC staining. The results of staining were compared with those of quantitative real-time PCR (qRT-PCR).

# Results

The qRT-PCR analysis identified 32 (64%) *H. pylori*-positive cases, whereas IHC, H&E, and modified Giemsa staining identified 29 (58%), 27 (54%), and 21 (42%) positive cases. The false-positive rates of H&E and modified Giemsa staining were 16% and 14%, respectively. The sensitivity of IHC staining (87.50%) was higher than that of H&E (59.38%) and modified Giemsa (43.75%) staining. The specificity of H&E, modified Giemsa, and IHC staining was 55.56%, 61.11%, and 94.44%, respectively. IHC staining exhibited the highest diagnostic accuracy (90%), followed by H&E (58%) and modified Giemsa (50%) staining. Active gastritis, intestinal metaplasia, and lymphoid follicles were detected in 32 (64%), 4 (8%), and 22 (44%) cases, respectively, and all of these cases were *H. pylori* positive.

# Conclusions

*H. pylori* can be detected using routine H&E or modified Giemsa staining. However, the high sensitivity, specificity, and diagnostic accuracy of IHC staining minimise the false-positive/negative results and enable *H. pylori* detection in cases with minimal or atypical infection. Moreover, IHC can be an alternative diagnostic method to qRT-PCR for detection of *H. pylori* in such cases.

## Background

*Helicobacter pylori* is a gram-negative spiral-shaped flagellated bacterium that can be identified using routine haematoxylin and eosin (H&E) staining. *H. pylori* infects the gastric mucosal surface and it can be easily detected in the gastric pits of the mucosal surface [1]. The accurate diagnosis of *H. pylori* infection is important to devise specific antibiotic treatment and to treat subsequent complications, such as chronic gastritis, peptic ulcers, and gastric cancer. Various diagnostic techniques have been developed for *H. pylori* infection, including gastric biopsy-based invasive techniques, such as histological examination, bacterial culturing, rapid urease test, and polymerase chain reaction (PCR). The non-invasive diagnostic

techniques for *H. pylori* infection include serological detection of antibodies, urea breath test, and bacterial antigen detection in the stool [2].

The accuracy of *H. pylori* detection in the gastric biopsy specimens is dependent on several factors, including the degree of infection, previous administration of antibiotics that may clear the infection or decrease bacterial load in the test specimens, administration of proton pump inhibitors, type of diagnostic method, biopsy site, clinical sample processing method, and degree and type of tissue inflammatory changes [3–5]. *H. pylori* typically exhibits spiral morphology. However, *H. pylori* can exhibit atypical morphologies, such as coccoid forms under certain conditions, including exposure to antibiotics [6]. These atypical bacterial forms, which cannot be identified using routine staining methods, such as H&E and modified Giemsa staining, can be identified using immunohistochemical (IHC) staining as this method uses specific antibodies against *H. pylori* antigens [7, 8]. In H&E and modified Giemsa staining, structures resembling *H. pylori*, such as other bacteria or tissue debris may provide false-positive results. In contrast, IHC staining is associated with low false-positive results as this method does not analyse the bacterial morphology [8]. IHC staining is reported to be more sensitive than H&E, Giemsa, and Warthin-Starry silver staining to detect *H. pylori* in gastric biopsies [9–11]. The drawbacks of IHC staining include the need for specialised equipment and high analytical cost. However, other routine staining methods are associated with high false-positive results. Hence, IHC staining is a preferred diagnostic method for *H. pylori* infection owing to its high sensitivity, high specificity, and the ease of result interpretation.

This study, which is a part of our research project on *H. pylori* infection in Jazan, Saudi Arabia [12], aimed to evaluate the diagnostic efficacy of IHC staining in direct detection of *H. pylori* in gastric biopsy specimens obtained from Saudi patients with dyspepsia and minimal and/or atypical *H. pylori* infection. The diagnostic accuracy of IHC staining was compared with that of routine H&E staining, modified Giemsa staining, and quantitative real-time PCR (qRT-PCR), which was considered a diagnostic gold standard in this study. Additionally, the histopathological changes associated with *H. pylori*-positive biopsies were evaluated.

## Results

The gastric biopsy specimens obtained from 50 Saudi patients with dyspepsia using upper gastrointestinal endoscopy were subjected to H&E staining, modified Giemsa staining, IHC staining, and qRT-PCR analysis to detect *H. pylori*.

# qRT-PCR analysis

The qRT-PCR analysis detected 32 (64%) *H. pylori*-positive cases and 18 (36%) *H. pylori*-negative cases (Table 1).

# Histopathological microscopic examination

H&E staining revealed that all cases included in the study exhibited chronic gastritis (n = 50, 100%). Based on the Sydney classification and grading system of gastritis, 12 (24%), 24 (48%), and 14 (28%) cases were classified as mild chronic, moderate chronic, and severe chronic gastritis, respectively. Additionally, H&E staining detected 27 (54%) *H. pylori*-positive cases with varying degrees of colonisation in the gastric mucosa and 23 *H. pylori*-negative cases (46%). Among the *H. pylori*-positive cases, 35%, 60%, and 5% cases exhibited mild, moderate, and severe chronic gastritis, respectively. Furthermore, the active and inactive forms of gastritis were observed in 32 (64%) and 18 (36%) cases, respectively. Gastric ulcers were detected in 15 (30%) cases, which were all *H. pylori*-positive. Glandular atrophy and IM were detected in 2 (4%) and 4 (8%) cases, respectively. All 4 cases with IM were *H. pylori*-positive, while the 2 cases with glandular atrophy were *H. pylori*-negative. Lymphoid follicles (with or without germinal centres) were detected in 22 (44%) cases, which were all *H. pylori*-positive (Table 1, Table 2, Fig. 1).

Comparison with qRT-PCR results revealed that 8 (16%) and 13 (26%) H&E staining results were false-positive and false-negative, respectively. The sensitivity and specificity of H&E staining were 59.38 and 55.56%, respectively (Table 1).

Modified Giemsa staining detected 21 (42%) *H. pylori*-positive cases. Comparison with qRT-PCR results revealed that 7 (14%), 11 (22%), and 18 (36%) results were false-positive, true-negative, and false-negative, respectively. The sensitivity and specificity of modified Giemsa staining were 43.75% and 61.11%, respectively (Table 1, Fig. 2A, B&C).

IHC could detect 29 (58%) *H. pylori*-positive cases. In comparison with the results of qRT-PCR, 1 (2%) and 4 (8%) results of IHC staining were false-positive and false-negative, respectively. The sensitivity and specificity of IHC analysis were 87.5% and 94.44%, respectively (Table 1, Fig. 2D, E&F).

IHC staining exhibited the highest diagnostic accuracy (90%), followed by H&E (58%) and modified Giemsa (50%) staining (Table 1).

# Discussion

*H. pylori* infection is associated with several gastrointestinal complications, including mild chronic gastritis, gastric carcinoma, and gastric lymphoma. The accurate diagnosis of *H. pylori* infection is important to devise an effective treatment for these gastric complications. Among the several diagnostic methods developed for detecting *H. pylori*, direct detection of *H. pylori* in the gastric mucosa is the most accurate method.

This study aimed to evaluate the efficacy of IHC staining to directly detect *H. pylori* in the gastric biopsies with minimal and/or atypical infection obtained from 50 Saudi patients with chronic gastritis. The diagnostic accuracies of IHC staining were compared with those of routine H&E staining, modified Giemsa staining, and qRT-PCR, which was considered as the diagnostic gold standard in this study.

Previous studies have reported that qRT-PCR analysis is one of the most sensitive diagnostic tools for the detection of *H. pylori* [13–18]. The high specificity of qRT-PCR analysis is due to the use of specific primers and internal controls, which minimise false-positive results. Additionally, the ability of qRT-PCR to detect low counts of *H. pylori* minimises false-negative results. In this study, *H. pylori* detection was performed using primer-probe-based qRT-PCR targeting the RNA polymerase beta-subunit (*rpo*B) gene of *H. pylori* along with suitable internal controls that ensured high specificity and sensitivity.

The qRT-PCR analysis of 50 biopsy specimens revealed an *H. pylori* infection incidence rate of 64%, which was higher than that in other studies, which reported *H. pylori* infection prevalence of 48.7% and 57% [19, 20]. Previous studies in Saudi Arabia have reported that the *H. pylori* infection prevalence rates ranged from 28–70% with an average of 50% [21]. The variation in *H. pylori* prevalence rates among different studies can be attributed to differences in patient characteristics, administration of antibiotic therapy, sampling conditions, and qRT-PCR modality.

The false-positive and false-negative rates for routine H&E staining were 16 and 26%, respectively. The high false-positive rates associated with H&E staining can be attributed to the inability of this method to differentiate *H. pylori* from the surrounding gastric mucosa. Additionally, the inability of H&E staining to differentiate *H. pylori* from gastric secretions or eosinophilic debris may contribute to high false-positive rates. Of the 50 cases, modified Giemsa staining and H&E staining identified 21 (42%) and 27 (54%) *H. pylori*-positive cases, respectively. The specificity of modified Giemsa staining (61.11%) for *H. pylori* detection was higher than that of H&E staining (55.56%); this concurred with the results of Laine et al [22]. In addition to uniform staining, modified Giemsa staining enables the identification of distinctive morphologies of *H. pylori*. However, the sensitivity of modified Giemsa staining for the detection of *H. pylori* was lower than that of H&E staining. The degree of infection is reported to affect the sensitivity of modified Giemsa staining [10]. The low sensitivity of modified Giemsa staining can be attributed to the inability of this method to distinguish *H. pylori* adhering to the glandular epithelium, especially in cases with mild degree of colonisation. Both *H. pylori* and glandular epithelium appear blue upon Giemsa staining. *H. pylori* transforms its morphology from spiral form to atypical forms under harsh conditions, such as antibiotic treatment. These atypical forms of bacteria cannot be identified through modified Giemsa staining, which may contribute to low sensitivity of modified Giemsa staining.

The sensitivity (87.5%) and specificity (94.44%) of IHC staining were higher than those of H&E and modified Giemsa staining, and these values concurred with the results of previous studies [10, 23, 24]. In contrast to H&E staining, the histopathological changes cannot be evaluated through IHC staining. However, IHC staining is considered the most accurate direct histopathological staining method for the detection of *H. pylori*. The high sensitivity and specificity of IHC staining are due to the use of specific antibodies, ability to detect atypical bacterial forms, such as coccoid forms, and low false-positive rates. Moreover, IHC is recommended when other routine methods fail to detect *H. pylori* in cases of chronic gastritis caused due to minimal infection or atypical distribution of bacteria in affected tissue [25]. The limitation of IHC staining for routine diagnostic application is high cost. Hence, IHC staining is recommended for cases with minimal *H. pylori* infection [26].

In this study, H&E staining revealed that 12 (24%), 24 (48%), and 14 (28%) patients exhibited mild, moderate, and severe chronic gastritis, respectively. Of the 27 *H. pylori*-positive cases identified through H&E staining, 35%, 60%, and 5% cases exhibited mild, moderate, and severe infections, respectively. These results concurred with those of Siddiqui et al [27] who reported 45 (24.4%), 137 (74%), and 3 (1.6%) cases of mild, moderate, and severe chronic gastritis, respectively, among 185 chronic gastric cases, with *H. pylori*-positive rates of 17.5%, 80.7%, and 1.8%, respectively. In this study, *H. pylori* was majorly associated with moderate inflammation. The section must be carefully examined for the presence of *H. pylori* in cases of moderate inflammation associated with active chronic gastritis. Previous studies have reported the correlation between chronic active gastritis and presence of *H. pylori* infection [28–30].

In this study, gastric ulcer was detected in 30% of cases. *H. pylori* is reported to be the major risk factor for the development of gastric ulcers as more than two-thirds of patients with gastric ulcer test positive for *H. pylori* infection [31]. Additionally, *H. pylori* infection is an important risk factor for the development of gastric atrophy and IM [32–34]. This is consistent with the results of this study in which all four detected IM cases were *H. pylori*-positive. Previous studies have reported that the presence of lymphoid follicles in the gastric mucosa is a common response to *H. pylori* infection and that the lymphoid follicles are detected in the gastric mucosa in 27–100% of cases [29, 35]. In this study, 22 (44%) out of the 50 studied cases were associated with lymphoid follicles with or without germinal centres and all 22 cases were *H. pylori*-positive. The presence of lymphoid follicles in the gastric mucosa appears to be a strong predictor of *H. pylori* infection. These results were consistent with those of Afzal et al. [36] who examined 500 cases of chronic gastritis and reported that 95.8% of cases with follicular gastritis tested positive for *H. pylori*. Moreover, another study examining the histopathology of gastric biopsies reported that the presence of lymphoid follicles indicates high probability of *H. pylori* infection [37]. Under physiological conditions, the histological structure of stomach does not contain lymphoid follicles [38]. Hence, the presence of *H. pylori*-associated lymphoid tissue is considered as an important pathological feature in the stomach. The development of mucosa-associated lymphoid tissue (MALT) precedes the development of primary MALT lymphoma.

# Conclusion

In conclusion, H&E and modified Giemsa staining methods are cost-effective methods with comparable diagnostic accuracy. H&E staining has the added advantage of analysing the inflammatory changes in the tissues. *H. pylori* can be directly detected in gastric biopsies using routine H&E or modified Giemsa staining. However, IHC staining has high sensitivity and specificity, which minimise the false-positive and false-negative rates. Additionally, IHC staining enables accurate diagnosis in cases of minimal or atypical *H. pylori* infection, which cannot be detected using H&E or modified Giemsa staining. The high diagnostic accuracy of IHC enables its application as an alternative to qRT-PCR for direct detection of *H. pylori* in the gastric biopsies.

# Methods

# Clinical specimens

The presence of *H. pylori* was analysed in 50 gastric biopsies using qRT-PCR, H&E staining, modified Giemsa staining, and IHC staining. These 50 specimens were selected from 402 specimens obtained from 402 Saudi patients with dyspepsia at the general hospitals in Jazan, Saudi Arabia (study population of our research project on *H. pylori*). The biopsy specimens were collected using upper gastrointestinal endoscopy. First, all 402 gastric biopsies were examined microscopically using H&E staining, then, the specimens of the current study were selected to include cases that presented atypical and/or minimal infection, in addition to negative cases. The atypical infection cases represented coccoid or irregular bacterial forms, while the minimal infection cases had few scattered bacteria. The negative cases were included to test the ability of IHC staining to detect *H. pylori* in H&E staining false negative cases.

# qRT-PCR

The qRT-PCR analysis was considered as the gold standard diagnostic method in this study. The results of qRT-PCR analysis were compared with those of other diagnostic methods. DNA was extracted from the gastric biopsy specimens using the DNeasy blood & tissue kit (Qiagen), following the manufacturer's instructions.

All DNA samples extracted from the gastric biopsies were subjected to qRT-PCR analysis using the "genesig Quantification of *Helicobacter pylori*" kit (PrimerDesign Ltd. Southampton, United Kingdom), which is primer-probe-based kit and targets the RNA polymerase beta-subunit (*rpo*B) gene of *H. pylori*, according to manufacturer's instructions of RT-PCR detection and amplification protocols [39]. PCR was performed in a 20-μL reaction volume containing 10 μL of "oasig™ 2X qPCR Mastermix" (PrimerDesign Ltd.), 1 μL *H. pylori*-specific primer/probe mix, 1 μL internal control primer/probe mix, 2 μL of internal control DNA, 3 μL of sample DNA, and 3 μL RNase/DNase-free water. The PCR analysis was performed using the Smart Cycler (Cephied, Italy). Positive control (*H. pylori* DNA supplied with the kit) and negative control (contains RNase/DNase-free water) reactions were included in each PCR run. The PCR conditions were as follows: 50 cycles of denaturation at 95 °C for 10 s and 60 °C for 60 s. The fluorescence cycle threshold of each sample was determined.

# Histopathological microscopic examination

The specimens were fixed in 10% formalin overnight, processed, and embedded in paraffin wax. The specimens were then sectioned into 5-μm thick tissue sections, which were subjected to routine H&E staining for histopathological evaluation and *H. pylori* detection. Additionally, the sections were subjected to modified Giemsa (Sheedhan's modified method) and IHC staining to detect *H. pylori*. The H&E-stained and modified Giemsa-stained slides were classified and graded according to the Sydney classification and grading system [40]. Additionally, the following histopathological features were examined: a) *H. pylori*-associated gastritis, b) chronic gastritis, and c) chronic active gastritis. The sections were classified based on the following parameters: degree of inflammation (mild, moderate, and severe), presence or absence of *H. pylori*, presence or absence of active chronic gastritis, and presence of peptic ulcer,

lymphoid follicles, atrophy, and intestinal metaplasia (IM). Chronic active gastritis was confirmed based on the density of neutrophilic infiltration in the gastric mucosal crypts. IM was assessed based on the amount of glandular tissue replaced by the intestinal epithelium.

For IHC staining, the serial sections of the same blocks were stained using immunostaining with specific antibodies (Polyclonal Rabbit Anti-*Helicobacter pylori*, DAKO, CA, USA), following the manufacturer's instructions. Patients with positive results in IHC staining were considered to be *H. pylori*-positive. The results of IHC analysis were compared with those of routine H&E staining, modified Giemsa staining, and qRT-PCR.

# Statistical analysis

All statistical analyses were performed using the SPSS software version 20 and OpenEpi Version 3.03 software. Based on *H. pylori* detection, the sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated using the standard statistical formula. Each calculated indicator was described at 95% confidence interval.

# Declarations


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### Authors' contributions

MA, AE, AS, EE, HA and TA conceptualized and designed the research project, carried out the practical work, and wrote the manuscript. MSM performed the statistical analysis and finalized the manuscript. All authors provided significant input in the manuscript, read and approved the final version of it.


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### Availability of data and materials

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

### Ethics approval and consent to participate

Informed written consent was obtained from all enrolled patients, per the ethics guidelines in Saudi Arabia. Ethical approval for the current study was obtained from the Ethics Committee of the Faculty of Medicine, Jazan University.

### Consent for publication

Not applicable.

### Competing interests

The authors have no actual or potential competing financial or non-financial competing interests.

# References


1. Shimizu T, Akamatsu T, Ota H, Katsuyama T. Immunohistochemical detection of Helicobacter pylori in the surface mucous gel layer and its clinicopathological significance. Helicobacter. 1996;1(4):197–206.
2. Garza-Gonzalez E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol. 2014;20(6):1438–49.
3. Megraud F, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. Clin Microbiol Rev. 2007;20(2):280–322.
4. Saniee P, Shahreza S, Siavoshi F. Negative Effect of Proton-pump Inhibitors (PPIs) on Helicobacter pylori Growth, Morphology, and Urease Test and Recovery after PPI Removal–An In vitro Study. Helicobacter. 2016;21(2):143–52.
5. Graham DY, Miftahussurur M. Helicobacter pylori urease for diagnosis of Helicobacter pylori infection: A mini review. *J Adv Res.* 2018. 13: 51–57.
6. Kusters JG, Gerrits MM, Van Strijp JA, Vandenbroucke-Grauls CM. Coccoid forms of Helicobacter pylori are the morphologic manifestation of cell death. Infect Immun. 1997;65(9):3672–9.
7. Lee JY, Kim N. Diagnosis of Helicobacter pylori by invasive test: histology. Ann Transl Med. 2015;3(1):10.
8. Jonkers D, Stobberingh E, de Bruine A, Arends JW, Stockbrugger R. Evaluation of immunohistochemistry for the detection of Helicobacter pylori in gastric mucosal biopsies. J Infect. 1997;35(2):149–54.
9. Ashton-Key M, Diss TC, Isaacson PG. Detection of Helicobacter pylori in gastric biopsy and resection specimens. J Clin Pathol. 1996;49(2):107–11.
10. Kocsmar E, et al. Sensitivity of Helicobacter pylori detection by Giemsa staining is poor in comparison with immunohistochemistry and fluorescent in situ hybridization and strongly depends on inflammatory activity. *Helicobacter.* 2017; 22 (4).

11. Lash RH, Genta RM. Routine Anti-Helicobacter Immunohistochemical Staining is Significantly Superior to Reflex Staining Protocols for the Detection of Helicobacter in Gastric Biopsy Specimens. Helicobacter. 2016;21(6):581–5.
12. Akeel M, et al. Helicobacter pylori vacA, cagA and iceA genotypes in dyspeptic patients from southwestern region, Saudi Arabia: distribution and association with clinical outcomes and histopathological changes. BMC Gastroenterol. 2019;19():16.
13. Ramirez-Lazaro MJ, et al. Real-time PCR improves Helicobacter pylori detection in patients with peptic ulcer bleeding. PLoS One. 2011;6(5):e20009.
14. Saez J, et al. Real-time PCR for diagnosing Helicobacter pylori infection in patients with upper gastrointestinal bleeding: comparison with other classical diagnostic methods. J Clin Microbiol. 2012;50(10):3233–7.
15. Ierardi E, et al. Detection of Helicobacter pylori DNA sequences in gastric biopsy samples to refine the diagnosis and therapy. J Med Microbiol. 2015;64(7):788–9.
16. Redondo JJ, Keller PM, Zbinden R, Wagner K. A novel RT-PCR for the detection of Helicobacter pylori and identification of clarithromycin resistance mediated by mutations in the 23S rRNA gene. Diagn Microbiol Infect Dis. 2018;90(1):1–6.
17. He Q, Wang JP, Osato M, Lachman LB. Real-time quantitative PCR for detection of Helicobacter pylori. J Clin Microbiol. 2002;40(10):3720–8.
18. Bazin T, et al. Contribution of genetic amplification by PCR for the diagnosis of Helicobacter pylori infection in patients receiving proton pump inhibitors. United European Gastroenterol J. 2018;6(8):1267–73.
19. Gonzalez-Hormazabal P, et al. Prevalence of clarithromycin resistance in Helicobacter pylori in Santiago, Chile, estimated by real-time PCR directly from gastric mucosa. BMC Gastroenterol. 2018;18(1):91.
20. Raaf N, et al. Prevalence, antibiotic resistance, and MLST typing of Helicobacter pylori in Algiers. Algeria *Helicobacter*. 2017;22(6):e12446.
21. Akeel M, et al. Prevalence and factors associated with H. pylori infection in Saudi patients with dyspepsia. Electron Physician. 2018;10 :7279–86.
22. Laine L, Lewin DN, Naritoku W, Cohen H. Prospective comparison of H&E, Giemsa, and Genta stains for the diagnosis of Helicobacter pylori. Gastrointest Endosc. 1997;45(6):463–7.
23. Tajalli R, et al. The immunohistochemistry and toluidine blue roles for Helicobacter pylori detection in patients with gastritis. Iran Biomed J. 2013;17(1):36–41.
24. Hartman DJ, Owens SR. Are routine ancillary stains required to diagnose Helicobacter infection in gastric biopsy specimens? An institutional quality assurance review. Am J Clin Pathol. 2012;137(2):255–60.
25. Benoit A, Hoyeau N, Flejou JF. [Diagnosis of Helicobacter pylori infection on gastric biopsies: Standard stain, special stain or immunohistochemistry?]. Ann Pathol. 2018;38(6):363–9.

26. Wang XI, Zhang S, Abreo F, Thomas J. The role of routine immunohistochemistry for Helicobacter pylori in gastric biopsy. Ann Diagn Pathol. 2010;14(4):256–9.
27. Siddiqui ST, et al. Frequency of Helicobacter pylori in biopsy proven gastritis and its association with lymphoid follicle formation. J Pak Med Assoc. 2011;61(2):138–41.
28. Toulaymat M, Marconi S, Garb J, Otis C, Nash S. Endoscopic biopsy pathology of Helicobacter pylori gastritis. Comparison of bacterial detection by immunohistochemistry and Genta stain. Arch Pathol Lab Med. 1999;123(9):778–81.
29. Stolte M, Stadelmann O, Bethke B, Burkard G. Relationships between the degree of Helicobacter pylori colonisation and the degree and activity of gastritis, surface epithelial degeneration and mucus secretion. Z Gastroenterol. 1995;33(2):89–93.
30. Baako BN, Darko R. Incidence of Helicobacter pylori infection in Ghanaian patients with dyspeptic symptoms referred for upper gastrointestinal endoscopy. West Afr J Med. 1996;15(4):223–7.
31. Amieva MR, El-Omar EM. Host-bacterial interactions in Helicobacter pylori infection. Gastroenterology. 2008;134(1):306–23.
32. Cho SJ, et al. Randomised clinical trial: the effects of Helicobacter pylori eradication on glandular atrophy and intestinal metaplasia after subtotal gastrectomy for gastric cancer. Aliment Pharmacol Ther. 2013;38(5):477–89.
33. Matsuhisa T, Miki M, Yamada N, Sharma SK, Shrestha BM. Helicobacter pylori infection, glandular atrophy, intestinal metaplasia and topography of chronic active gastritis in the Nepalese and Japanese population: the age, gender and endoscopic diagnosis matched study. Kathmandu Univ Med J (KUMJ). 2007;5(3):295–301.
34. Park YH, Kim N. Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer. J Cancer Prev. 2015;20(1):25–40.
35. Genta RM, Hamner HW, Graham DY. Gastric lymphoid follicles in Helicobacter pylori infection: frequency, distribution, and response to triple therapy. Hum Pathol. 1993;24(6):577–83.
36. Afzal M, Baez-Giangreco A, Joarder MA, Laajam MA. Association of gastric lymphoid follicles and Helicobacter pylori infection. Saudi J Gastroenterol. 1999;5(1):15–7.
37. Genta RM, Hamner HW. The significance of lymphoid follicles in the interpretation of gastric biopsy specimens. Arch Pathol Lab Med. 1994;118(7):740–3.
38. Owen DA. Normal histology of the stomach. Am J Surg Pathol. 1986;10(1):48–61.
39. Quantification of Helicobacter pylori RNA polymerase beta-subunit (rpoB) gene. genesig Advanced kit handbook HB10.03.05. PrimerDesign$^{TM}$Ltd., UK.
40. Dixon MF, Genta RM, Yardley JH, Correa P Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol.* 1996; **20** (10): 1161–1181.

# Tables

le 1: Comparison of H&E, modified Giemsa and immunohistochemical staining in reference to RT-PCR for detection of *H. pylori*

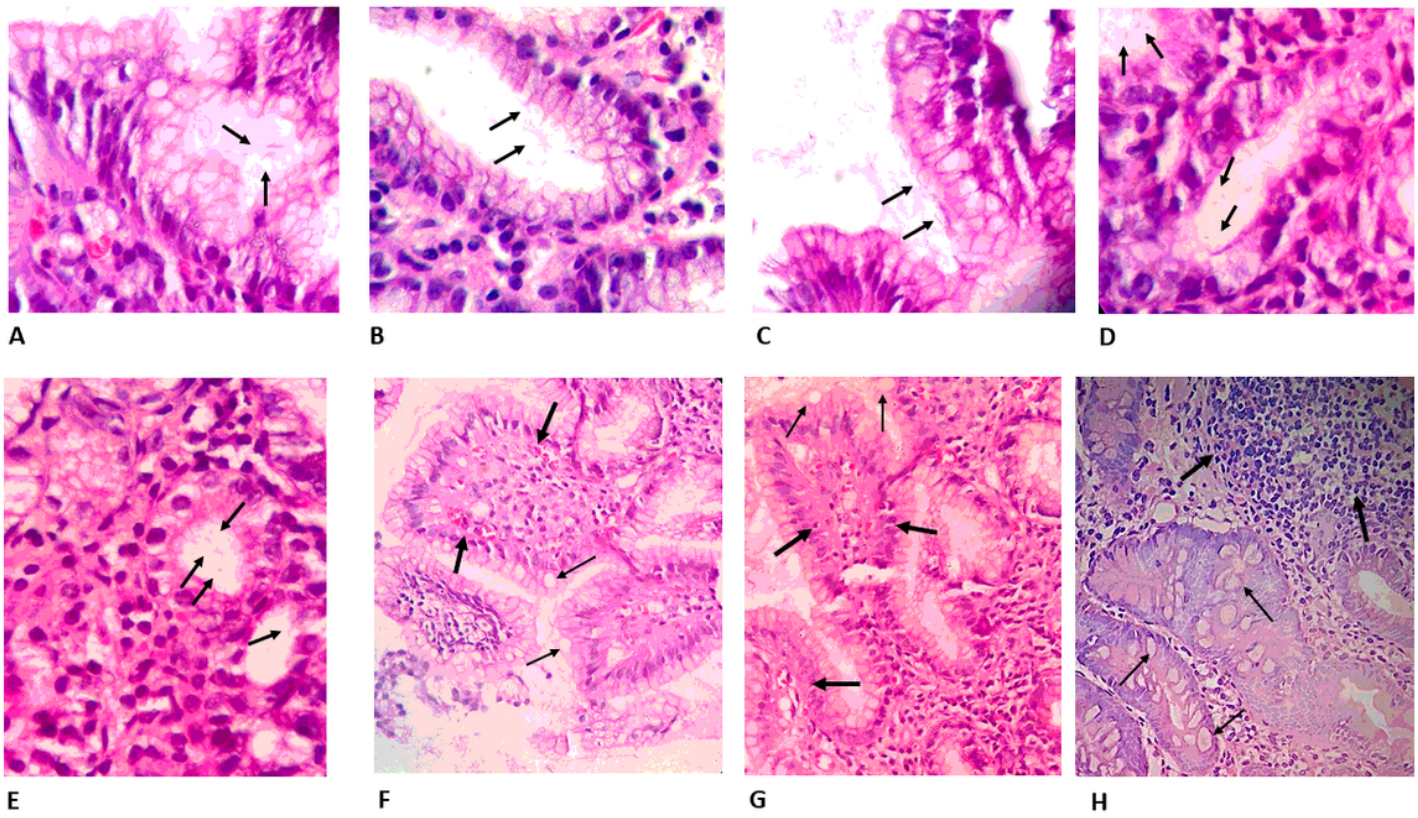
| | | RT-PCR | | | Sensitivity | Specificity | Positive Predictive Value | Negative Predictive Value | Diagnostic Accuracy |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive N | Negative N | Total N | | | | | |
| H&E | + ve | 19 | 8 | 27 | 59.38% | 55.56% | 70.37% | 43.48% | 58% |
| | -ve | 13 | 10 | 23 | | | | | |
| Modified Giemsa | + ve | 14 | 7 | 21 | 43.75% | 61.11% | 66.67% | 37.93% | 50% |
| | -ve | 18 | 11 | 29 | | | | | |
| Immunohistochemistry | + ve | 28 | 1 | 29 | 87.50% | 94.44% | 96.55% | 80.95% | 90% |
| | -ve | 4 | 17 | 21 | | | | | |
| Total | | 32 | 18 | 50 | | | | | |

N*: number

Table 2: Histopathological findings detected by H&E examination

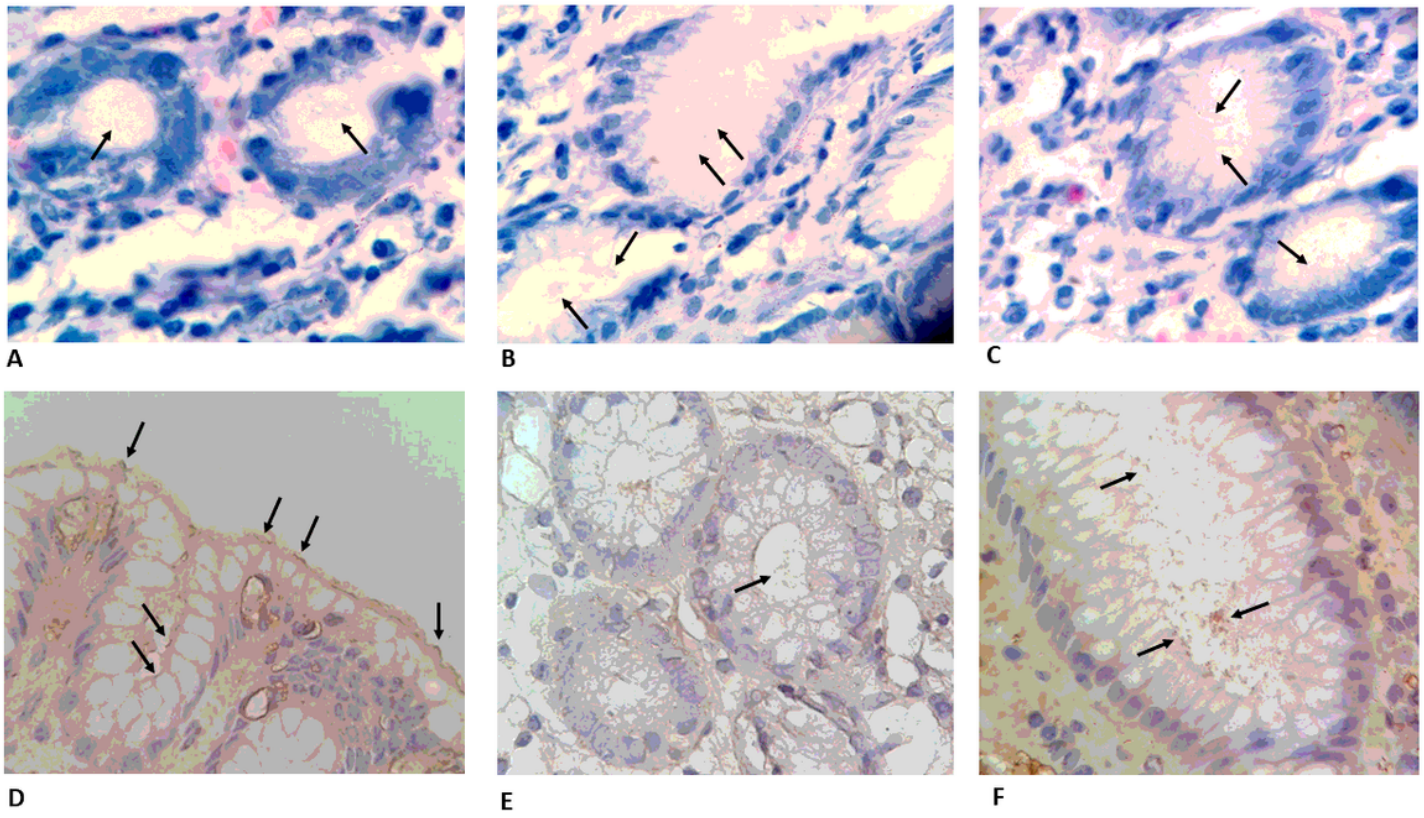
| Finding | | N(%) from total 50 cases |
|---|---|---|
| Chronic gastritis | | |
| | Mild | 12 (24%) |
| | Moderate | 24 (48%) |
| | Severe | 14 (28%) |
| Activity of gastritis | | |
| | Active | 32 (64%) |
| | Non-active | 18 (36%) |
| *H. pylori* | | |
| | Positive | 27 (54 %) |
| | Negative | 23 (46%) |
| Others | | |
| | Lymphoid follicles | 22 (44%) |
| | Glandular atrophy | 2 (4%) |
| | Intestinal metaplasia | 4 (8%) |
| Gastric ulcer | | 15 (30%) |

# Figures




## Figure 1

Gastric biopsies specimens showing: (A, B&C) superficial gastritis with minimal colonisation of gastric mucosal glands with few scattered Helicobacter pylori taking typical S-shaped bacilli (arrows). (D&E) superficial gastritis with minimal colonisation with H. pylori taking atypical coccoid and irregular shapes (arrows) (H&E; Magnification, 400X). (F&G) chronic gastritis with intestinal metaplasia and presence of papillary configuration (thick arrows) with mucous-secreting cells (thin arrows). (H) H. pylori associated follicular gastritis with lymphoid follicles (thick arrows) situated deeper in the gastric mucosa and associated with intestinal metaplasia (thin arrows) (H&E; Magnification 200X). H&E, haematoxylin and eosin.




## Figure 2

Gastric biopsies specimens showing: (A, B&C) gastric mucosal glands with minimal colonisation with Helicobacter pylori (arrows) (Modified Giemsa stain; Magnification 400X). (D, E&F): H. pylori observed using immunohistochemistry staining. (D) minimal colonisation with typical spiral S-shaped bacilli (arrows). (E) minimal colonisation with atypical coccoid forms. (F) enhanced colonisation with coccoid and irregular forms (IHC, Magnification, 400X).