(RESEARCH ARTICLE)

Detection of *Helicobacter pylori (H. pylori)* by histochemical stains in gastric biopsy comparing to immunohistochemistry

Mohammed Abdalla MA [1, *], Osman Amir [2], Elnazir Mohammed E [3]

[1] Department of Histopathology & Cytology, Faculty of Medical Laboratory Science, University of Medical Science and Technology, Khartoum, Sudan.
[2] Department of Hematology, Faculty of Medical Laboratory Sciences, Al-Neelain University, Khartoum, Sudan.
[3] Department of Histopathology & Cytology, Khartoum Oncology Center, Khartoum, Sudan.

Abstract

Aim: A reliable diagnosis of *Helicobacter pylori* (*H. pylori*) is important in clinical practice and research. The aim of this study was to compare the sensitivity and specificity of Giemsa stain with hematoxylin and eosin (H&E), Toluidine blue, Gimenez and Warthin-Starry stain in detection of *Helicobacter pylori* (*H. pylori*) in gastric biopsy and also with immunohistochemistry (IHC) stain in detection of *H. pylori* organism in gastric biopsy.

Method: A retrospective cross-sectional included 200 formalin-fixed paraffin-embedded (FFPE) gastric biopsies of age between 25 to 80 years in histopathology Laboratory at Alzaytouna Specialist Hospital, Khartoum, Sudan. The samples were sectioned and stained with H&E, Giemsa, Toluidine blue, Gimenez, Warthin-Starry silver, and IHC stains.

Result: Our study showed IHC yielded (40%) positive cases while (60%) were negative. all stains had 100% sensitivity in detection *H. pylori*. The specificities were 100% for toluidine blue and Gimenez stains, 92.3% for Warthin-Starry stain, 85.7% for Giemsa stain, and 82.8% for H&E stain.

Conclusion: Toluidine blue and Gimenez stain are more specific in detecting *H. pylori* organism than Giemsa, H&E and Warthin-Starry but are less sensitive than the later.

Keywords: *H. pylori*; Gimenez; Haematoxyline & Eosin; Immunohistochemistry; Toluidine blue; Warthin-Starry

1. Introduction

Helicobacter pylori (*H. pylori*) is spiral-shaped, gram-negative bacterium causing the onset of a number of gastric pathologies ranging from mild gastritis to gastric cancers [1]. *H. pylori* was first discovered and defined by Marshall et al in 1985 [2]. Then, many works have been conducted to find out this type of bacterium and to emphasis more about its significance in gastric pathology [3,4]. All at once, other investigators evaluated the most appropriate methods of detecting this infection in order to manage the patients and protect them from severe complications [3].

There are two methods used to identify *H. pylori* in routine clinical assessment: invasive and non-invasive techniques. In invasive methods, an upper endoscopic gastric biopsy is taken to be evaluated histologically and cultured, with distinctive stains, immune stains, molecular examinations and a rapid urase test (*Campylobacter*-like organism-CLO). The non-invasive means comprise several examinations like the urea breath test (UBT), serological techniques and

* Corresponding author: Mohammed Abdalla MA
Department of Histopathology & Cytology, Faculty of Medical Laboratory Science, University of Medical Science and Technology, Khartoum, Sudan

the *H. pylori* antigen in urine, blood and stool specimens. Each technique has its own benefits and weaknesses. Diagnosis of *H. pylori* in gastric biopsies is the gold standard technique of identifying *H. pylori* infection, but precisely what should be used as a panel of examinations in these biopsies remains debatable [4].

Various histopathological staining methods have been used for many years to detect *H. pylori* in stomach biopsies, including the combination of the hematoxylin and eosin (H&E) stain and other special stains, like the Giemsa, methylene blue and Warthin-Starry silver stains [3,5,6]. On other side, approaches such as immunohistochemistry (IHC) and fluorescent in-situ hybridisation-based staining techniques are accessible for *H. pylori* assessment [7-9]. Molecular biology methods, like polymerase chain reaction (PCR) tests, are also used to diagnose *H. pylori* [5,10].

The role of histological stains in the diagnosis of *H. pylori* still requires further studies, as the selected panel of staining for identifying *H. pylori* in gastric biopsies seems unclear. Therefore, this study is designed to investigate the sensitivity, specificity and applicability of Giemsa with H&E, Toluidine blue, Gimenez and Warthin-Sttary stains in detection of *H. pylori* in gastric biopsies comparing to IHC techniques

2. Material and methods

This is a retrospective cross-sectional study using archival histopathological material from paraffin-embedded gastric biopsies samples in histopathology Laboratory at Alzaytouna Specialist Hospital, Khartoum, Sudan. A total of 200 samples of formalin-fixed paraffin-embedded (FFPE) gastric biopsies previously sectioned were examined. The study included all gastric biopsies with chronic inflammation and/or activity and excluded other biopsies with no histological evidence of chronic inflammation.

The samples were sectioned and stained with H&E (REACTIFS RAL, FRANCE), Giemsa, Toluidine blue (RAL DIAGNOSTICS, FRANCE), Gimenez and Warthin-Starry silver stains (DAKO, USA), and then carefully examined microscopically to evaluate the features of chronic gastritis and presence of coma shaped rods in the lamina propria using Primo Star Zeiss microscope with x40 objective and x10 eye piece.

For IHC stain, procedure was carried out using polyclonal rabbit anti –*Helicobacter pylori* (DAKO, USA). Each slide was evaluated with investigator then the results were confirmed by consultant histopathologist.

2.1. Data analysis

Data was analyzed by using a computer program Statistical Package for Social Sciences (SPSS V. 21.0). Frequencies with proportions were reported for categorical variables and means with standard deviations (SDs) for continuous variables. The analyzed data presented in tables and figures designed by Microsoft Excel 2010.

Sensitivity and specificity were calculated based on the following formulae:

- Sensitivity= (True positive / (True positive + False negative)) * 100
- Specificity= (True negative / (True negative + False positive)) * 100

3. Results

Table 1 The sensitivity and specificity of H&E, Giemsa, Warthin-Starry, Toluidine blue and Gimenez staining methods for the detection of *H. pylori* in gastric biopsies as compared to the IHC staining (N= 200)

	Total +ve cases (n)	Sensitivity (%)	Specificity (%)
H&E	105	100	82.8
Giemsa	100	100	85.7
Warthin-Starry	90	100	92.3
Toluidine blue	80	100	100
Gimenez	80	100	100

As shown in table (1), all stains had 100% sensitivity in detection *H. pylori*. The specificities were 100% for toluidine blue and Gimenez stains, 92.3% for Warthin-Starry stain, 85.7% for Giemsa stain, and 82.8% for H&E stain.
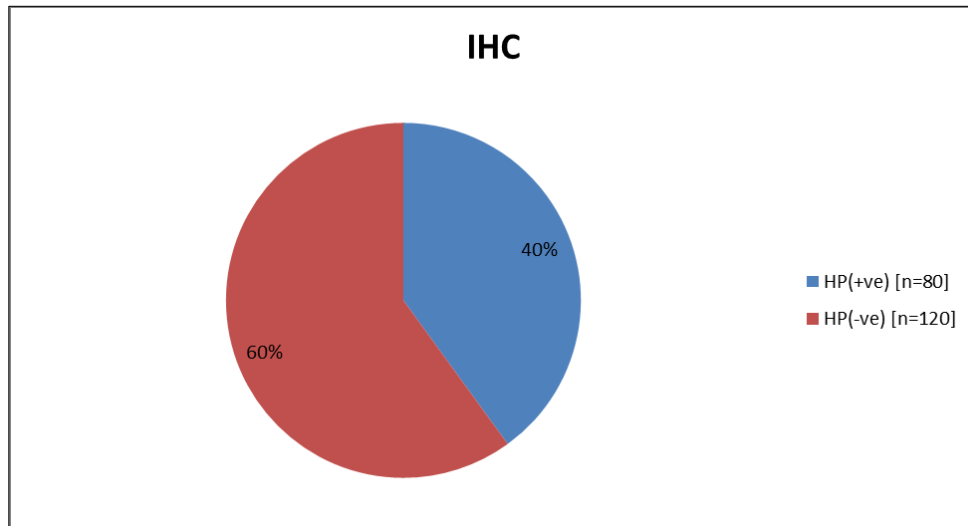

Figure 1 The distribution of positive and negative *H. pylori* results in gastric samples using immunohistochemistry (IHC) technique (N=200)

Among 200 patients, the ages of the patient were ranged from 25 to 85 years with mean 45.5± 14.8 years. Based on IHC technique, 80(40%) samples were positive and remaining 120(60%) were negative (figure 1)

4. Discussion

The detection of *H. pylori* infection through endoscopic features alone is not appropriate for a diagnosis. Therefore, these must be combined with the histopathological report [11, 12]. This is necessary because: i) the *H. pylori* microorganism cannot be seen endoscopically; ii) there are many types of mucosal alteration that can be associated with *H. pylori* infection (such as gastritis, mucosal atrophy, ulceration, erosion, polyps and neoplasms) [13]; and iii) there is no correlation between endoscopic findings and histopathological findings in patients with *H. pylori* infection [14]. However, gastric biopsies are the gold standard method to detect this infection; the question is which panel to use in these biopsies.

According to our research, the sensitivity and specificity of H&E in *H. pylori* detection were as high as 100% and 82.8%, respectively. Correspondingly, our findings were in the range reported by Laine Let al, Fallone C et al and Lee J et al those reported *H. pylori* can be seen in hematoxylin and eosin (H&E) staining with sensitivity and specificity of 90-100% and 69-93%, respectively [15-17]. Also, in the study of Carlo A et al, H&E stain showed sensitivity of 93% (87%–97%), and specificity of 87% (69%–96%) [18]. However, in the study of Abdullah S et al, the specificity of H&E in *H. pylori* detection is high (91.18%); however, its sensitivity is low (66.67%) [19].

Giemsa stain is a simple, rapid and affordable stain that has good sensitivity, specificity and consistency in the detection of *H. pylori* infection [5, 15, 20, 21]. Therefore, it is routinely used in some institutions. According to many scientific papers, Giemsa stain is superior to H&E in the detection of *H. pylori* [5, 15, 16, 20, 21]. Based on our findings, the sensitivity of Giemsa stain in the detection of *H. pylori* is 100% while its specificity is 85.7%, which makes it better than H&E stains. In the study of Kocsmár É et al, Giemsa revealed an overall sensitivity of 83.3% and a specificity of 98.8% [22].

Among the methods first used to demonstrate H pylori in tissue sections it should be mention the Warthin-Starry and. The first one was used in the early discovery of H pylori in gastric mucosa by Marshall and Warren. The Warthin-Starry method gradually was give H pylori significant contrast when lying free on the gastric mucus. However, the major disadvantage of this method is to hide the morphology of the gastric mucosa [23]. In our study, Warthin-Starry stain showed the overall sensitivity of 100% and its specificity is 92.3%. Although, in the study of Farouk WI et al, the sensitivity was only 50% while specificity was 92.4% [24]

Interestingly, this study demonstrated that, Toluidine blue and Gimenez stains showed overall sensitivity and specificity of 100%, for each. Consistently, Sakonlaya D et al reported that, the overall sensitivity and specificity of Toluidine blue for detection H pylori were 99% and 96%, respectively [25]. However, in the study of Arachchi PS et al, the sensitivity of Toluidine blue staining for diagnosis of *H. pylori* infection was 57.1% while the specificity was 97.9% [26]. Also, in the study of Raziye T et al, the sensitivity and specificity of toluidine blue were 76.74% and 100%, respectively [27]. In Gimenez stains, our results were sharple similar to the study of Kassa E et al who also found the overall sensitivity and specificity of Gimenez were 100% [28], while Kazim M et al reported the overall sensitivity of Gimenez stains was 75% [29].

5. Conclusion

This study concludes that, all stains had 100% sensitivity in detection *H. pylori*. But, the specificity was superior in Toluidine blue and Gimenez followed by Warthin-Starry, Giemsa and then H&E stains. Also, the data from this study suggest a recommendation to use H& and Giemsa as a routine panel of stains in all gastric biopsies with considerations of false positive results due to bacterial contaminants or debris.

Compliance with ethical standards

Acknowledgments

Disclosure of conflict of interest

There are no conflicts of interest.

Statement of informed consent

The authors certify that they have obtained all appropriate patient consent forms. Informed consent obtained for participation.

References

[1]   Crowe SE. *Helicobacter pylori* infection. N Engl J Med. 2019, 380(12):1158–1165.

[2]   Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfil Koch's postulates for pyloric Campylobacter. Med J Aust. 1985, 142(8):436–439.

[3]   Abadi ATB. Diagnosis of *Helicobacter pylori* using invasive and noninvasive approaches. J Pathog. 2018, 2018 doi: 10.1155/2018/9064952. 9064952.

[4]   Alkhamiss AS. Evaluation of Better Staining Method among Hematoxylin and Eosin, Giemsa and Periodic Acid Schiff-Alcian Blue for the Detection of *Helicobacter pylori* in Gastric Biopsies. Malays J Med Sci. 2020, 27(5):53-61.

[5]   Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G. Diagnosis of Helicobacter pylori: what should be the gold standard? World J Gastroenterol. 2014, 20(36):12847–12859.

[6]   Pajares-García JM. Diagnosis of Helicobacter pylori: invasive methods. Ital J Gastroenterol Hepatol. 1998, 30(Suppl 3):S320–S323.

[7]   Kocsmár É, Szirtes I, Kramer Z, Szijártó A, Bene L, Buzás GM, 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)

[8]   Miftahussurur M, Yamaoka Y. Appropriate first-line regimens to combat *Helicobacter pylori* antibiotic resistance: an Asian perspective. Molecules. 2015, 20(4):6068–6092.

[9]   Miftahussurur M, Shiota S, Suzuki R, Matsuda M, Uchida T, Kido Y, et al. Identification of *Helicobacter pylori* infection in symptomatic patients in Surabaya, Indonesia, using five diagnostic tests. Epidemiol Infect. 2015, 143(5):986–996.

[10] Rimbara E, Sasatsu M, Graham DY. PCR detection of *Helicobacter pylori* in clinical samples. Methods Mol Biol. 2013, 943:279–287.

[11] Khakoo SI, Lobo AJ, Shepherd NA, Wilkinson SP. Histological assessment of the Sydney classification of endoscopic gastritis. Gut. 1994, 35(9):1172–1175.

[12] Makristathis A, Hirschl AM, Mégraud F, Bessède E. Review: diagnosis of *Helicobacter pylori* infection. Helicobacter. 2019, 24(Suppl 1):e12641.

[13] Rudnicka K, Graczykowski M, Tenderenda M, Chmiela M. *Helicobacter pylori* morphological forms and their potential role in the transmission of infection. Postepy Hig Med Dosw. 2014, 68:219–229.

[14] Dominis M, Dzebro S, Gasparov S, Buljevac M, Colić-Cvrlje V, Banić M, et al. Morphology of gastritis and *Helicobacter pylori* infection. Lijec Vjesn. 2002, 124(Suppl 1):36–42.

[15] Laine L, Lewin DN, Naritoku W, et al. Prospective comparison of H&E, Giemsa, and Genta stains for the diagnosis of Helicobacter pylori. Gastrointest Endosc 1997, 45:463-7.

[16] Fallone CA, Loo VG, Lough J, et al. Hematoxylin and eosin staining of gastric tissue for the detection of Helicobacter pylori. Helicobacter 1997, 2:32-5.

[17] Lee JY, Kim N. Diagnosis of *Helicobacter pylori* by invasive test: histology. Ann Transl Med. 2015, 3(1):10. doi:10.3978/j.issn.2305-5839.2014.11.03

[18] Carlo A, Vivian G, John L, Alan N. Hematoxylin and Eosin Staining of Gastric Tissue for the Detection of Helicobacter pylori. Helicobacter. 1997, 2(1):32-35

[19] Abdullah S. Evaluation of Better Staining Method among Hematoxylin and Eosin, Giemsa and Periodic Acid Schiff-Alcian Blue for the Detection of *Helicobacter pylori* in Gastric Biopsies. Malays J Med Sci. 2020 Oct, 27(5): 53–61.

[20] Mawlood AH, Kawther RS, Balaky STJ. Evaluation of invasive and non-invasive methods for the diagnosis of *H. pylori* in dyspepsia patients. Diyala Journal of Medicine. 2019, 16(2):55–63.

[21] 34. Rotimi O, Cairns A, Gray S, Moayyedi P, Dixon MF. Histological identification of Helicobacter pylori: comparison of staining methods. J Clin Pathol. 2000, 53(10):756–759.

[22] Kocsmár É, Szirtes I, Kramer Z, Szijártó A 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 Aug, 22(4).

[23] Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984 Jun 16; 1(8390):1311-5.

[24] Farouk WI, Hassan NH, Ismail TR, Daud IS, Mohammed F. Warthin-Starry Staining for the Detection of *Helicobacter pylori* in Gastric Biopsies. Malays J Med Sci. 2018, 25(4):92-99.

[25] Sakonlaya D, Apisarnthanarak A, Yamada N, Tomtitchong P. Modified toluidine blue: an alternative stain for *Helicobacter pylori* detection in routine diagnostic use and post-eradication confirmation for gastric cancer prevention. Asian Pac J Cancer Prev. 2014, 15(16):6983-7.

[26] Arachchi PS, Weerasekera MM, Seneviratne B et al. Imprint cytology: a useful screening test for diagnosis of *Helicobacter pylori* in resource poor settings. BMC Res Notes. 2018, 11:481

[27] Raziye T, Maliheh N, Hajar M. The Immunohistochemistry and Toluidine Blue Roles for *Helicobacter pylori* Detection in Patients with Gastritis. Iran Biomed J. 2013 Jan, 17(1): 36–41.

[28] Kassa E, Tsega E, Gebre W. Comparison of diagnostic methods for detection of Helicobacter pylori. East Afr Med J. 1996 Apr, 73(4):239-41.

[29] Kazim M S, Alneil A. Reliability of histological staining methods in the detection of Helicobacter pylori: comparison of three staining methods. Int. J. of Adv. Res. 2015, 3 (Nov). 1163-1169.