

Diagnosis for H-Pylori by Various Staining Methods in Gastric Biopsy Specimens

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ABSTRACT

Objective: To run a comparison between specificity, sensitivity, PPV (positive predictive value) (PPV) & NPV (negative predictive value) of the three varied H. pylori detection methods used in the study.

Materials and Methods: It is a retrospective descriptive study that was carried out at Histopathology wing of Pathology Department, Sahiwal Medical College, Sahiwal. It comprised of entire cases of gastritis which were histopathologically proven by using Hematoxylin and Eosin, Giemsa and Immunohistochemical stains on biopsies taken by endoscopy and were sent from Gastroenterology Department Govt. Haji Abdul Qayyum Teaching Hospital, Sahiwal from January 2020 to the month of December 2020. SPSS version 20 was employed to evaluate all the received data.

Results: Amongst 95 samples, IHC (Immunohistochemistry) was ranked highest, displaying 100% specificity, 100% positive predictive value and 94.74% sensitivity. The H&E stain followed IHC, displaying 100% and 94.44% specificity and sensitivity respectively. The Giemsa stain was the runner up, displaying 95.65% specificity and 94.12% sensitivity.

Conclusions: Histologically, H. pylori infection was associated more with chronic active gastritis as compared with chronic gastritis. The three dissimilar staining methods achieved sensitivity, specificity, positive predictive value and negative predictive values equally well. IHC is a precise method for detection of H.Pylori in gastric biopsies with high sensitivity and specificity. It is suggested to use more than one staining method where H.pylori level is low, it will minimize false-negative rate.

Keywords: Helicobacter pylori, Diagnosis, Staining, IHC, H&E, Giemsa

INTRODUCTION

Gastric infection caused by the spiral, gram-negative H. pylori (*Helicobacter pylori*) is established to have a connection with a host of upper gastrointestinal diseases. In fact, H. pylori is held responsible for half of the infections caused in the world, being labelled by the WHO as a "definite biological carcinogen" in 1994¹. H. pylori has a definite role in about 80% of carcinomas of stomach as well as 92% of low-grade gastric MALT (mucosa-associated lymphoid tissue)². Presently, a combination of non-invasive and invasive diagnostic tools have been established to be able to better diagnose H. pylori³. An array of histochemical stains is employed to better recognize the samples generated from gastric biopsies and resections. They include altered Giemsa, Gimenez, cresyl violet, acridine orange, Ziehl-Nielsen (ZN stain), Genta, half Gram and H. pylori stain that is silver and modified Genta⁴. The use of different histochemical stains leads to enhanced visualization of the system in contrast to the hematoxylin and eosin (H&E) stain. Nonetheless, numerous studies have indicated that staining with immunohistochemistry (IHC) with precise Polyclonal H. pylori antibodies yields the sensitivity with highest specificity and improved variation of results between different observers in contrast to the use of different histochemical stains alone^{5,6,7}.

This study focused on carrying out a histological analysis of the gastric biopsies with the correlation to the evaluation of infection by H. pylori. We elaborate on the sensitivity, specificity, PPV, NPV of Giemsa, H & E as well as IHC in H. pylori positive infection.

MATERIAL AND METHODS

This was a retrospective descriptive investigation carried out at histopathology wing of Pathology Department, Sahiwal Medical College, Sahiwal. It comprised of entire cases of gastritis which were histopathologically proven by using Hematoxylin and eosin,

Giemsa and Immunohistochemical stains on biopsies taken by endoscopy sent from the Department of Gastroenterology, Govt. Haji Abdul Qayyum Teaching Hospital, Sahiwal from January 2020 to the month of December 2020. SPSS version 20 was used to analyze all the data received.

RESULTS

Total 95 gastritis cases were registered in this study and comparison was done using three distinct stains, H&E, Giemsa and IHC between H.Pylori positive & H.pylori negative endoscopic biopsies (Tables 1, 2 and 3). Sensitivity, specificity, PPV and NPV values were calculated. IHC (Immunohistochemistry) was ranked highest, displaying 100% specificity, 100% positive predictive value and 94.74% sensitivity. The H&E stain followed IHC, displaying 100% and 94.44% specificity and sensitivity respectively. The Giemsa stain was the runner up, displaying 95.65% specificity and 94.12% sensitivity (Table 4).

Table 01: H & E stain of H.Pylori on Endoscopic Biopsies

| Stain | H.pylori +ve | H.pylori -ve |
|-----------|--------------|--------------|
| H & E +ve | 68 | 0 |
| H & E -ve | 04 | 23 |

Table 02: Giemsa stain of H.Pylori on Endoscopic Biopsies

| Stain | H.pylori +ve | H.Pylori -ve |
|------------|--------------|--------------|
| Giemsa +ve | 66 | 01 |
| Giemsa -ve | 04 | 23 |

Table 03: Results of IHC stain of H.Pylori on Endoscopic Biopsies

| Stain | H.pylori +ve | H.pylori -ve |
|---------|--------------|--------------|
| IHC +ve | 48 | 0 |
| IHC -ve | 04 | 43 |

Table 04: Sensitivity, Specificity, PPV, and NPV of three Analytical Staining Methods for H. pylori Infection in 95 Patients

| Diagnostic Methods | Sensitivity in % | Specificity in % | Positive predictive value (PPV) | Negative predictive value (NPV) |
|--------------------|------------------|------------------|---------------------------------|---------------------------------|
| H & E stain | 94.44% | 100% | 100% | 85.19% |
| Giemsa stain | 94.12% | 95.65% | 98.46% | 84.62% |
| IHC | 94.74% | 100% | 100% | 94.12% |

DISCUSSION

Ever since this spiral germ was discovered in gastritis patients, pathologists of every era have poured in their heart and soul to come up with the best diagnostic techniques⁸. As a result, various invasive and non-invasive tools have been established to identify the *H. pylori* efficiently and have become a part of the routine practice⁹. Staining has been regarded as one of the most valuable diagnostic tools when it comes to *H. pylori* associated gastritis, and various types of stains are being used to complement such diagnosis¹. Our research is also aimed at further streamlining the diagnostic process by checking the performance of different stains in terms of specificity and sensitivity, with their positive & negative predictive values.

Our study has been featured with the specificity and sensitivity values of different stains. Most hospitals rely on the staining of gastric antral biopsies with H&E stain as it is the most reasonable and reliable analytical method for *H. pylori*. We recorded 100% specificity and 94.4% sensitivity for the H & E stain in our study, while this range falls between 87-90% and 69-93% respectively in various other studies. The use of high magnification H & E stain results in making the *H. pylori* directly identifiable to evaluate the degree of inflammation. However, seeing the organism becomes more of a challenge when there is a combination of low-density *H. pylori* and atrophic mucosa^{10,11}.

We recorded the specificity and sensitivity of Giemsa, another popular stain for histological examination of gastric biopsies, to be at 95.65% in our study. Many researchers prefer Giemsa staining because it offers ease of use, affordability and consistency in results¹².

IHC stain tops the ladder in this study with 100% specificity and 94.74% sensitivity, followed by the H & E and Giemsa stain. IHC has been considered as the most sensitive and reliable stain by many researchers, as also indicated by the results of our study¹³. IHC stain is considered especially advantageous in partially treated patients of *H. pylori* gastritis, a setting with the possibility of resulting in un-common (including coccoid) forms, capable of mimicking bacterial micro-organisms or cellular debris on H & E staining. Less screening time and high specificity features serve as the biggest benefits of IHC stain, helping it exclude other similar-shaped organisms^{14,15}.

Moreover, the interpretation of rare *H. pylori* in a smear is quite problematic, more so when the background is dirty¹⁶.

As demonstrated in one of the studied cases, *Helicobacter* is mostly hosted inside or under the surface mucus layer, an area susceptible to partial loss while processing the sample for histological examination. This probability further increases when there is a low bacterial count^{7,17}. These findings suggest that in case of low bacterial loads, using more than one analytical method is more feasible to suppress the false-negative rate.

CONCLUSION

Histologically, the appearance of *H. pylori* was more frequent in chronic active gastritis cases as compared to chronic gastritis cases. The three different staining methods resorted to in this study displayed equal efficiency in terms of specificity, sensitivity, PPV and NPV. With 100% PPV, IHC stain came up with the highest sensitivity amongst all stains. Similarly, the value of the H & E stain remained much closer to the IHC stain. Thus, in order to cut on the false-negative rate, more than one diagnostic method should be used for cases having low amounts of *H. pylori*.

Author's Contributions: RAL: designing the study and data analysis. QAT: Microscopic examination. SS: Manuscript drafting. SS: help in final draft and approval from all authors. SHS: provision of endoscopic biopsies. NN: manuscript revision and critical analysis

REFERENCES

- Lee JY, Kim N. Diagnosis of *Helicobacter pylori* by invasive test: histology. *Annals of translational medicine*. 2015 Jan; 3(1).
- Shukla S, Pujani M, Agarwal A, Pujani M, Rohtagi A. Correlation of serology with morphological changes in gastric biopsy in *Helicobacter pylori* infection and evaluation of immunohistochemistry for *H. pylori* identification. *Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association*. 2012 Nov; 18(6):369
- Best LM, Takwoingi Y, Siddique S, Selladurai A, Gandhi A, Low B, Yaghoobi M, Gurusamy KS. Non-invasive diagnostic tests for *Helicobacter pylori* infection. *Cochrane Database of Systematic Reviews*. 2018 (3).
- Garza-González E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ. A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication. *World journal of gastroenterology: WJG*. 2014 Feb 14; 20(6):1438.
- Miftahussurur M, Yamaoka Y. Diagnostic methods of *Helicobacter pylori* infection for epidemiological studies: critical importance of indirect test validation. *BioMed research international*. 2016 Jan 19; 2016.
- Aziz ZW, Saleem SH, Al-Nuaimy HA. *Helicobacter pylori* in gastric biopsy: a histochemical and immunohistochemical assessment. *Annals of the College of Medicine, Mosul*. 2020 Jan 29; 41(2):139-47.
- Trevisani L, Sartori S, Ruina M, Caselli M, Abbasciano V, Grandi E, Forini E. Touch Cytology (A Reliable and Cost-Effective Method for Diagnosis of *Helicobacter pylori* Infection). *Digestive diseases and sciences*. 1997 Nov; 42(11):2299-303.
- Smith SB, Snow AN, Perry RL, Qasem SA. *Helicobacter pylori*: to stain or not to stain? *American journal of clinical pathology*. 2012 May 1; 137(5):733-8.
- Wroblewski LE, Peek Jr RM, Wilson KT. *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. *Clinical microbiology reviews*. 2010 Oct; 23(4):713-39.
- Cha MS. Comparative analysis of histochemical stains about detection of *H. pylori* in gastric mucosa. *Korean Journal of Clinical Laboratory Science*. 2007; 39(3):223-30.
- El-Zimaity HM, Segura AM, Genta RM, Graham DY. Histologic assessment of *Helicobacter pylori* status after therapy: comparison of Giemsa, Diff-Quik, and Genta stains. *Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc*. 1998 Mar 1; 11(3):288-91.
- Wabinga HR. Comparison of immunohistochemical and modified Giemsa stains for demonstration of *Helicobacter pylori* infection in an African population. *African health sciences*. 2002; 2(2):52-5.
- Jonkerst D, Stobberingh E, De Bruine A, Arends JW, Stockbrügger R. Evaluation of immunohistochemistry for the detection of *Helicobacter pylori* in gastric mucosal biopsies. *Journal of Infection*. 1997 Sep 1; 35(2):149-54.
- Wang Xi, Zhang S, Abreo F, Thomas J. The role of routine immunohistochemistry for *Helicobacter pylori* in gastric biopsy. *Annals of diagnostic pathology*. 2010 Aug 1; 14(4):256-9.
- Loffeld RJ, Stobberingh E, Flendrig JA, Arends JW. *Helicobacter pylori* in gastric biopsy specimens. Comparison of culture, modified Giemsa stain, and immunohistochemistry. A retrospective study. *The Journal of pathology*. 1991 Sep; 165(1):69-73.
- Tzeng JE, Lin YL, Chung SM, Chu YT. Comparison of four diagnostic methods for *Helicobacter pylori*. *Tzu Chi Med J*. 2005 Jan 1; 17(5):339-43.
- Debongnie JC, Donnay M, Mairesse J. *Gastrospirillum hominis* (" *Helicobacter heilmanii*"): A Cause of Gastritis, Sometimes Transient, Better Diagnosed by Touch Cytology? *American Journal of Gastroenterology* (Springer Nature). 1995 Mar 1; 90(3).