Int.j.pathol.2021;19(1): 28-31

Original Article

Comparison of Different Staining Techniques for Identification of Helicobacter Pylori in Gastric Biopsies

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ABSTRACT

Objective: To compare the efficacy of different staining (H&E, modified Giemsa stain and Immunohistochemical stains) for detecting H pylori in gastric biopsies.

Methodology: This descriptive study was carried out at the Department of Pathology, Khyber Girls Medical College/ Hayatabad Medical Complex Peshawar. A non-probability convenience sampling technique was used to select study participants from December 2018 to July 2019. Total 104 gastric biopsies obtained through upper GI endoscopy were processed for identification of H pylori through various staining techniques. All the participants consented to participate in the study. The study was conducted after approval by institutional ethical committee.

Results: Among total 104 cases analyzed, 63 cases were found positive for h pylori infection using H and E stain. Based on Immunohistochemical analysis 101 turned out to be infected with H pylori. The third stain used for H pylori detection was Giemsa stain which revealed the highest percentage of H pylori positivity (100%) after IHC stain. All the 104 cases analyzed were found positive on Giemsa stain.

Conclusion: In daily practice modified Giemsa stain may be sufficient for diagnosis of H.pylori organism.

KEYWORDS: Giemsa Stain, Predictive values, Immunohistochemical stain, Helicobacter Pylori.

Introduction

Infection with *Helicobacter pylori* (*H.pylori*), a gram-negative bacterium is an important global health concern. More than fifty percent of the world's population carries *H.pylori*a symptomatically in their gastric mucosa with high prevalence rates in developing than in developed countries (1). Person to person transmission occurs commonly through oral to oral or fecal to oral route(2).Unless treated, individuals with long term colonization are at a risk of developing premalignant lesions including chronic atrophic gastritis, gastric metaplasia and gastric cancer(3).An estimated 80 % of gastric carcinomas and 92% of low-grade Gastric Mucosa Associated Lymphoid Tissue (MALT) lymphomas are H pylori associated.

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Due to vital role of H pylori in pathogenesis of gastric diseases management of chronically infected patients in terms of accurate diagnosis and effective eradications essential to prevent complications(4).

Since the discovery of H pylori in 1983, investigators have developed different methods for identification of H pylori in health care settings. Among the various diagnostic tests performed are both endoscopic and non-endoscopic techniques which have been categorized further as direct tests(histopathology, culture or bacterial antigen detection in the issue biopsy or stool) or indirect tests (urease breath test or an antibody response)(5). Active management of H. pylori associated gastro duodenal diseases depends on accurate diagnosis.(6). Identification of H pylori in clinical specimens using serological assays is indicative of exposure to bacteria but it fails to detect active infection. Selection of a suitable diagnostic test rests on various factors including availability, cost, any history of gastric carcinoma in the family, clinical situation and prior use of antimicrobials or proton pump inhibitors(7).

Although heavy bacterial load is readily apparent on routine Hematoxylin and Eosin (H&E) stained

specimens, detection of the presence of lower density of organisms requires special staining techniques. Although bacteriological confirmatory tests are ideal for H pylori diagnosis but are difficult to perform as they require specialized enrichment media, complicated incubation techniques and characterization of microbe is also a time-consuming process. Various special stains have been devised to detect *H. pylori* in histological sections. Modified Giemsa stain described by Grey et al has been forwarded by many researchers because of its convenience to perform also its availability in most diagnostic laboratories(8). However, it depends on the morphology of the bacterium for detection and it is possible to differentiate from H pylori. It is also known that H pylori bacterium may demonstrate variable morphology and therefore might not be a reliable marker for diagnosis(9). Immunohistochemical techniques have been developed in 1988 correlated well with the presence of bacteria. This study compares efficacy of three stains commonly used for H. pylori detection including H&E, Giemsa stain and IHC. A histological analysis of gastric biopsies was carried out simultaneously and correlated with H pylori infection.

Objective: To compare the efficacy of H&E, Modified Giemsa stain and Immunohistochemical stain for detecting H pylori in gastric biopsies.

Materials & Methods

This descriptive study was carried out at the Department of Pathology, Khyber Girls Medical College/ Hayatabad Medical Complex, Peshawar. A non-probability convenience sampling technique was used to select study participants from December 2018 to July 2019. Total 104 gastric biopsies obtained through upper GI endoscopy were processed for identification of H pylori through various staining techniques. All the participants consented to participate in the study. Ethical approval was obtained from institutional ethical committee.

Initially routine H and E stain was performed followed by modified Giemsa stain and IHC stain as described earlier. Gastric biopsies from patients suspected of having chronic gastritis were embedded in paraffin blocks. Three sections each having a thickness of about 3micrometers were stained and examined microscopically for H pylori, which appeared as light pinkish rods of variable sizes on the brushborder of mucosal cells in H and E stain. In Giemsa staining, H pylori appeared as dark blue rods against a bluish background, whereas in IHC bacteria appeared as light brown curved spiral rods by using anti H.pylori antibodies. Patients were labeled as having H Pylori infection if found positive on any of the three staining techniques. All the processing and staining was performed by same technician and slides were examined by same pathologist to avoid interoberver bias. Data was recorded on a predesigned proforma and results obtained in case of each staining technique were presented as percentages using SPSS version 21.

Results

Among 104 patients included in the present study 58 (60%) were female and 46 (40%) were male. In female patients the frequency of H pylori infection was high in patients having age more than 40 years (64%) as compared to patients < 40 years of age (36%). In male patients with age > 40years the proportion of H pylori infection was 54% as compared to 46% infection rate in patients with age < 40 years. H pylori detection using three stains yielded variable results. Among total 104 cases analyzed, 63(60.6%) cases were found positive for h pylori infection using H and E stain. Based on Immunohistochemical analysis 101(97%) turned out to be infected with H pylori. The third stain used for H pylori detection was Giemsa stain which revealed the highest percentage of H pylori positivity (100%) after IHC stain. All the 104 cases analyzed were found positive on Giemsa stain. Frequency of H pylori positivity using three stains has been shown in Table 1.

Table 1: Showing frequency of H pylori positivity using three staining techniques

Total No of Cases (n=104)	H and E Stain	Giemsa Stain	IHC Stain
60	+	+	+
40	_	+	+
3	+	+	_
1	_	+	+

Staining Technique	Staining Result No (%)	
	Positive	Negative
H&E Stain	63 (60.6%)	41(39.4%)
Giemsa Stain	104 (100%)	0 (0%)
IHC stain	101 (97%)	3(2.8%)

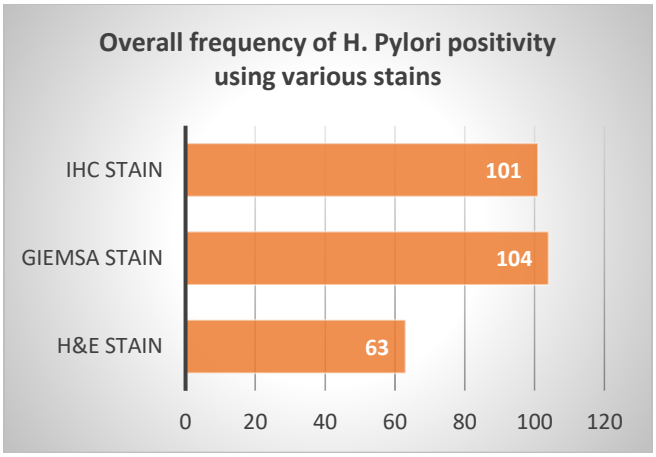

Figure: Demonstrate the overall rate of H.pylori detection using IHC: Immunohistochemical, Giemsa and H&E (Hematoxylin and Eosin Stain)

Discussion

Gastric ulceration is a frequent dilemma in Pakistan and in roughly all developing countries. (10) Bulk of these cases has a strong relationship with H.pylori infection. Helicobacter pylori infection is not only linked with high frequency of gastric ulceration but also has a positive association with gastric MALT lymphoma and some gastric carcinomas. (11) Therefore it is essential to recognize this infection at an early stage and to treat this state at an initial stage to avoid deadly complications.

Helicobacter pylori are a Gram –negative, curved organism which colonizes the gastric mucosa. H.pylori survives in the acidic environment of stomach by a number of mechanisms. It secretes the urease enzyme, which converts urea to ammonia. (12)

In our study out of 104 biopsies, 104cases were positive with Giemsa stain for H. pylori, while with IHC stains 101 showed H. pylori infection. Only 63 out of 104 cases were positive for h. pylori with H&E stain. These findings are comparable with other studies. A study done by Rotimi et al in 2000 showed that sensitivity of Giemsa stain is 98% and is much higher than H&E stain. (13) .Pandya et al in 2013 also found out sensitivity as high as 100% for Giemsa stain.(14)

In our study the use of Giemsa stain with significant sensitivity (100%) as compared to other methods is recommended due to its low cost and easy procedure. The use of Giemsa stain provided distinctive shape and uniform staining of the bacteria making their identification easy with high positivity rate. When H.pylori are present careful examination will almost always reveal them whatever stain is used however Giemsa stain is the method of choice because it is sensitive ,cost effective, easy to perform and reproduce.

Conclusion

In daily practice modified Giemsa stain may be sufficient for diagnosis of H.pyloriorganism in gastric biopsy. The efficacy of Giemsa stain (100%) is much better compared to other two stains. It is also cost effective, simple, easy and convenient to perform.

Declarations

Conflict of interest: None
Funding: None
Acknowledgments: None

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HISTORY	
Date received:	25-04-2021
Date sent for review:	21-05-2021
Date received reviewers comments:	24-05-2021
Date received revised manuscript:	30-06-2021
Date accepted:	30-06-2021

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C. Interpretation/ Analysis and Discussion