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ORIGINAL ARTICLE

Diagnostic Accuracy of Different Histological Stains for Helicobacter Pylori Detection, taking Immunohistochemistry as Gold Standard

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ABSTRACT

Aim: Helicobacter pylori infection has been ascertained to play pivotal role in the pathogenesis of chronic gastritis and gastric neoplasia.[1]The present study was performed to evaluate the diagnostic accuracy of H&E stain and Giemsa stain for the histological diagnosis of helicobacter pylori by taking immunohistochemical staining as a gold standard.
Methods: A total of 155 cases were included in our study. The received biopsies were fixed in 10% buffered formalin, grossed and stained with H&E and giemsa stain. A board of histopathologists analyzed the morphological details to ascertain the diagnosis. The biopsies were stained by using immunohistochemical techniques against H. pylori antigens, and the procedure was performed according to the guidelines provided by the manufacturer considering the appropriate positive and negative controls for staining. IHC staining was evaluated autonomously and recorded on the proforma as positive and negative cases.
Results: In our study, mean age was calculated as 38.4±11.57 years, 74(47.74%) were male and 81(52.26%) were females, frequency of H.Pylori on gold standard was recorded as 109(70.32%), the diagnostic accuracy of hematoxylin-eosin stain for helicobacter pylori detection by taking immunohistochemical staining as a gold standard measure was calculated as 63.30%, 65.22%, 81.18%, 42.86% and 63.87% for sensitivity, specificity, positive predictive value, negative predictive value and accuracy rate respectively, while these findings were recorded as 74.31%, 80.43%, 90%, 56.92% and 76.12% for Giemsa stain.
Conclusion: We concluded that the diagnostic accuracy of H&E and Giemsa stains for detection of HP is promising and cost-effective method in our population.
MeSH words: Helicobacter pylori, Immunohistochemistry, Hematoxylin, Pathology, Diagnosis

INTRODUCTION

Helicobacter pylori (HP) are curved, gram negative, basophilic, flagellated rods which harbor gastric mucosa[1]. HP comprises of huge variety of strains. HP was the very first bacterium observed to act as a cancer-causing agent. The contamination with HP brings about numerous upper gastrointestinal infections including HP related gastritis, gastric or duodenal ulcer, gastric adenocarcinoma and mucosa related lymphoid tissue (MALT) lymphoma[1,2]. On literature survey, it is inferred that almost half population of the world is contaminated with HP with the maximum burden of the infection in low income population[3,4].

The disease with HP is communicated through direct contact[5] and furthermore through contaminated food and water[6,7]. In Pakistan, its seroprevalence surpasses 58% of general population[4] and on histological examination; it is identified in 88.3% of biopsies of dyspeptic patients[6]. Treatment against HP is readily available so the accurate diagnosis and subsequent eradication therapy can diminish the possible progression of disease and neoplastic transformation in high risk groups[2, 7]. Therefore, a timely and reliable diagnosis is vital for patients with HP related diseases.[8]
Regardless of high frequency of HP related gastric issues, exceptionally restricted information is accessible from Pakistan on diagnostics of HP disease.

Diverse histologic stains are utilized in histopathology for diagnosing various diseases. Among them, immunohistochemistry has been demonstrated to be a dependable strategy for analysis of HP[9,10] and is viewed as a superior diagnostic tool with 100% sensitivity and 100% specificity[11]. Anyway immunohistochemistry is an expensive procedure and is likewise not accessible at most labs in Pakistan. Other histological stains, Hematoxylin and Eosin (H&E) and Giemsa are less expensive [2, 12] and are easily accessible in Pakistan.

There is significant variability seen in the sensitivity and specificity of Hematoxylin and Eosin stains and Giemsa stain for the recognition of HP on literature review[2,8,9]. The sensitivity and specificity of H&E vary from 41-92% and 89-100% according to different studies[2,8,9]. The sensitivity and specificity of Giemsa stain vary from 53.49-88% and 95-98% [2,8,9] and relies upon the density of bacilli infesting the gastric biopsies. The rationale of our study is that if diagnostic accuracy of H&E and Giemsa stains for HP is proved in comparison to immunohistochemistry then we'll be able to use these stains with confidence as a substitute to immunohistochemistry in our population for a practical, cheaper, readily accessible and effective mode of diagnosing the HP related gastric infections as accurate and timely diagnosis plays vital role in timely eradication therapy as well as it decreases the chances of neoplastic transformation.

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METHODS

This Descriptive, Cross Sectional Study was conducted in the Department of Pathology, Fatima Memorial Hospital, Lahore for a period of 6 months from 05-06-2016 to 05-12-2016 after approval from hospital ethical committee. One hundred and fifty five cases were taken as sample. Sample size is calculated with 95% confidence level, 9% margin of error for sensitivity of Giemsa stain i.e. 80.4%[1] and 14% margin of error for specificity of Giemsa stain i.e. 84.55%[3] in the detection of HP by taking immunohistochemistry as gold standard with an expected percentage of HP i.e. 88.3%[6]. Non-probability consecutive sampling technique was used.

Inclusion Criteria

1. Gastric biopsies referred with clinical suspicion of helicobacter pylori.
2. Patients of age range (20-60 yrs) from both genders.

Exclusion Criteria

1. Poorly preserved and poorly fixed specimen.
2. Specimen with scanty tissue.

Data collection procedure: In our study, a total of 155 cases were included which fulfilled our inclusion criteria. A case number and a medical record number were assigned to individual cases. Data including name, sex and age of each patient was gathered. The biopsy specimens received in histopathology lab were fixed in 10% buffered formalin. Processing and staining with H&E and Giemsa stain was done to record the histopathological details. A board of histopathologists analyzed the morphological details to ascertain the diagnosis. The biopsies were stained by using immunohistochemical techniques against H.pylori antigens, and the procedure was performed according to the guidelines provided by the manufacturer considering the appropriate positive and

negative controls for staining. IHC staining was evaluated autonomously and recorded on the proforma as positive and negative cases.

Data analysis: The collected information was entered into designed proforma and analyzed by using computer software SPSS version 18. The quantitative variables like age were presented in terms of mean standard deviation. The qualitative variables like gender were presented in terms of frequencies. The results of expression of IHC staining were compared for accuracy, sensitivity, specificity and predictive values with H&E staining and Giemsa staining. 2x2 tables were applied. Data was stratified for age and gender.

RESULTS

According to inclusion/exclusion criteria, total 155 selected cases were examined to conclude the diagnostic precision of hematoxylin-eosin stain and giemsa stain for the identification of helicobacter pylori in gastric biopsies by taking immunohistochemical staining as gold standard measure. Cases were distributed among groups according to their age, which reveals that 97(62.58%) cases were between 20-40 years of age while 58(37.42%) were between 41-60 years, mean+sd was determined as 38.4+11.57 years. Gender distribution of cases revealed that 74(47.74%) were male and 81(52.26%) were females among the patients. Frequency of H.Pylori infection on gold standard measures i.e. IHC was recorded as 109(70.32%) while 46(29.68%) had no findings of the disease (Table 1).

Table 1: Frequency of H.pylori on immunohistochemistry (Gold Standard) (n=155)

H.Pylori	n	%age
Yes	109	70.32
No	46	29.68
Total	155	100

Table 2: Diagnostic accuracy of hematoxylin-eosin stain for H. pyloridetection (n=155)

Hematoxylin -Eosin Stain	Immunohistochemical staining		Total
	H.Pylori (Positive)	H.Pylori (Negative)	
Positive	True positive(a) 69 (44.52%)	False positive (b) 16 (10.32%)	a + b 85(54.84%)
Negative	False negative(c) 40 (25.81%)	True negative (d) 30 (19.35%)	c + d 70 (45.16%)
Total	**a + c 109 (70.32%)**	**b + d 46 (29.68%)**	**155 (100%)**

Sensitivity = a / (a + c) x 100 =63.30%
Specificity = d / (d + b) x 100 = 65.22%
Positive predictive value = a / (a + b) x 100 =81.18%
Negative predictive value = d / (d + c) x 100 =42.86%
Accuracy rate = a + d / (a + d + b + c) x 100 = 63.87%

Table 3: Diagnostic accuracy of giemsa stain for H. pylori detection (n=155)

Giemsa Stain	Immunohistochemical staining		Total
	H.Pylori (Positive)	H.Pylori (Negative)	
Positive	True positive(a) 81 (52.26%)	False positive (b) 9 (5.81%)	a + b 90(58.06%)
Negative	False negative(c) 28 (18.06%)	True negative (d) 37 (23.87%)	c + d 65 (41.94%)
Total	a + c 109 (70.32%)	b + d 46 (29.68%)	155 (100%)

Sensitivity = a / (a + c) x 100 =74.31%
Specificity = d / (d + b) x 100 = 80.43%
Positive predictive value = a / (a + b) x 100 =90%
Negative predictive value = d / (d + c) x 100 =56.92%
Accuracy rate = a + d / (a + d + b + c) x 100 = 76.12%

Diagnostic accuracy of hematoxylin-eosin stain for helicobacter pylori detection by taking immunohistochemical staining as a gold standard measure was calculated as 63.30%, 65.22%, 81.18%, 42.86% and 63.87% for sensitivity, specificity, positive predictive value, negative predictive value and accuracy rate respectively (Table 2).

Diagnostic accuracy of Giemsa stain for helicobacter pylori detection by taking immunohistochemical staining as a gold standard measure was calculated as 74.31%, 80.43%, 90%, 56.92% and 76.12% for sensitivity, specificity, positive predictive value, negative predictive value and accuracy rate respectively (Table 3).

DISCUSSION

Helicobacter Pylori infection has been proved to be one of the most significant causes in the pathogenesis of chronic gastritis as well as other gastro duodenal illnesses like peptic ulceration, gastric lymphoma and gastric tumors[13,14]. Hence, the precise diagnosis of this bacillary infection plays a fundamental role in subsequent eradication therapy and prevention of progression of disease.[15]Different procedures are indicated for HP detection including, serology, culture, fast urease test, C-urea breath test and histology. The histological measures for the detection of HP are considered to be the reliable of all the above techniques and are commonly used[16,17].

The current study was planned with the view that if we are able to prove significant diagnostic accuracy of Hematoxylin- Eosin stains and Giemsa stains for HP, we can confidently use these stains as an alternative to immunohistochemistry in our population for a cost effective, easily available and reliable diagnosis of HP associated gastric diseases as timely diagnosis has a key role in timely management and progression to malignancy can be prevented.[18,19]

In our study, mean age was calculated as 38.4±11.57 years, 74(47.74%) were male and 81(52.26%) were females, frequency of H.Pylori on gold standard was recorded as 109(70.32%), the diagnostic accuracy of Giemsa stain for helicobacter pylori detection by taking immunohistochemical staining as a gold standard measure was calculated as 74.31%, 80.43%, 90%, 56.92% and 76.12% for sensitivity, specificity, positive predictive value, negative predictive value and accuracy rate respectively, while all of these parameters were recorded as 63.30%, 65.22%, 81.18%, 42.86% and 63.87% for Hematoxylin-eosin stain.

Several previous studies showing the sensitivity and specificity of H&E ranges from 41- 92% and 89- 100%.[2,8,9,20] The sensitivity and specificity of Giemsa stain ranges from 53.49- 88% and 95-98%[2,8,9] and depends on the density of organism in gastric biopsies, the findings of our study show similar results as concluded by the above studies.

A relatively new study[26] has compared the diagnostic accuracy and utility of two histological staining techniques regularly performed in labs for H. pylori detection and presumed that in reference to Modified Giemsa stain results, the sensitivity, and accuracy ratio and NPV of the Gimenez stain were (75%, 93.3and 91.7) as compared to (50 %, 86.6% and 84.6%) for H&E stain. It was concluded that Giemsa stain proved better in comparison to H&E stain in detection of HP in gastric biopsies. While there's no statistical disparity found but Giemsa stain has been proved to be a be favored stain over H&E, widely used to detect HP in gastric biopsies and this preference is given because of its better sensitivity, diagnostic accuracy and negative predictive value.

Though, in above mentioned study Gimenez stain was proved better as compared to H&E stain in order to dectect H.pylori in tissue biopsies, however both the stains share almost same range of sensitivity, specificity in agreement with the above magnitude.

Ju Yup Lee and others[8,27] presented their idea about importance of accurate as well as timely diagnosis of HP which is proved to play a significant role in multiple gastric diseases including gastric carcinoma and MALT lymphoma. Histopathological evaluation holds a significant role in diagnosing HP infested gastric among all the other diagnostic tools because it provides additional information about the area involved, the extent

of associated inflammation and erosions and also helps in diagnosing related conditions like atrophic gastritis (AG), intestinal metaplasia (IM), and gastric cancers or lymphomas. Routine staining by H&E stains can definitely helps in diagnosis of HP but use of other specific stains for example, modified Giemsa, Warthin-Starry silver, Genta, and immunohistochemical (IHC) stains can improve the quality of diagnosis due to increased specificity. Consequently, use of H&E stains is suggested for routine lab use while special stains like Giemsa appears to have edge over routinely used techniques due to its specificity for HP organisms as well as its simple lab techniques,

However, the findings recorded in our study are also encouraging and after validation with the help of some other multi-center trials, we can confidently use these stains as an alternative to immunohistochemistry in our population for a cost effective, easily available and reliable diagnosis of HP associated gastric diseases as timely diagnosis has a key role in timely management and progression to malignancy can be prevented.

CONCLUSION

We concluded that the diagnostic accuracy of H&E stain and Giemsa stain for detection of HP in gastric and intestinal biopsies by taking immunohistochemical staining as a gold standard measure is promising and cost effective method in our population and can be used confidently as an alternative to immunohistochemistry, however, other multi-center trials from other areas of country may validate our findings.

Conflict of interest: Nil

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