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EVALUATIONOF THE ROLE OF IMMUNOHISTOCHEMISTRY FOR IDENTIFICATION OF HELICOBACTER PYLORI ON UPPER GASTROINTESTINAL ENDOSCOPIC BIOPSIES AT A TERTIARY CARE CENTER

Histopathology

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ABSTRACT

H.pylori is known to cause peptic ulcers. Many histochemical stains are used for its detection in gastric biopsies and resections. H&E and Giemsa are routinely staining methods. Various arguments have been put for the use of IHC for detecting H. pylori. This study aims to evaluate the role of immunohistochemical marker for identifying H. pylori, and to compare with conventional histochemical staining techniques used for detecting H. pylori with IHC in biopsy specimens.38 formalin- fixed paraffin blocks of gastric biopsies were retrieved from archives for a period of one year. 5-µm histological sections were prepared and stained with H&E, Giemsa and IHC.Giemsa positive cases were 23.5% and negative cases were 76.5%.IHC was positive in 55.5% and negative in 44.5%.12 additional IHC positive cases were identified in comparison to Giemsa. Coccoid form of organisms was easily identified by IHC. Considering IHC to be gold standard for the identification of H. pylori; Giemsa has a sensitivity of 34% and specificity of 58%, positive predictive value of 0.82 and negative predictive value of 1.12. Upfront use of IHC may not be cost effective and hence can be indicated in the following settings- negative results with other histochemical stains, post-treatment when the bacilli decrease in number and when the bacilli appear in coccoid form.

KEYWORDS

Coccoid, Giemsa, Helicobacter pylori, H pylori, Immunohistochemistry

INTRODUCTION

Helicobacter pylori (H. pylori) is a Gram-negative bacteria which colonises the mucosal lining of the human digestive tract, especially the stomach and duodenum. [1] Infection by H. pylori infection involves 5% to 10% of the population in developed countries and 80% to 90% in developing countries, thus accounting as one of the most common infections.[2] Most of the cases are exposed to the infection in childhood which reaches to 80% of the general population in adulthood.[2] Various epidemiological surveys indicate a sero-prevalence of 20%-50% in children under the age of five, increasing to 80%-90% by the age of twenty, and remaining constant thereafter.[2-6] It is transmitted by feco-oral route. It is known to cause chronic active gastritis, peptic ulcer disease, atrophic gastritis, gastric carcinoma, MALToma.[6] A wide range of laboratory investigations are available for diagnosis of H. pylori. The tests can be divided into non-invasive tests and invasive tests. Non-invasive tests include urea breath test, serological immunoglobulin G and immunoglobulin M detection, saliva and urinary antibody test, and stool antigen test. The invasive tests are endoscopy-based tests, which include histopathological examination, rapid urease test and polymerase chain reaction.[7,8] Several histochemical stains used for histological detection of H. pylori in gastric biopsies and resections, including H&E, Modified Giemsa, Toluidine Blue, Warthin's Silver Stain and Immunohistochemical (IHC) staining. H&E and Giemsa are routinely staining methods, which are used in pathology laboratories for detection of H. pylori.[9] Various arguments have been put forth for the use of IHC for detecting H. pylori.

AIMS

1. To evaluate the role of immunohistochemical marker for identifying H. pylori
2. To compare with conventional histochemical staining techniques used for detecting H. pylori in biopsy specimens.

MATERIALS AND METHODS

1. Selection

This study was retrospective carried out on 38 endoscopically obtained gastric biopsies retrieved from archives of Department of Histopathology from June 2022 to June 2023. A tissue block of each case was chosen for H&E Giemsa and IHC. Relevant clinical information consisting of age, sex, clinical presentation was also retrieved from the archives.

Patients with chronic gastritis, chronic duodenitis, chronic gastric ulcer, normal gastric mucosa, squamous cell carcinoma, adenocarcinoma were also included.

2. Histochemical And IHC Staining

5-µm histological sections were prepared and stained with H&E, modified Giemsa and IHC. Modified Giemsa with 1:9 dilution was used. Immunohistochemistry was performed using PathnSitu H. pylori polyclonal antibody (PathnSitu Biotechnologies Pvt. Ltd.). The sections were stained according to the instructions by the manufacturer. Positive control was a Giemsa positive gastric biopsy and negative control samples were prepared by omitting the primary antibody.

3. Interpretation

The results were analysed by two Pathologists independently. Giemsa and IHC were categorised as either positive or negative. The presence of any stained organisms resembling H.pylori bacteria designated as positive. The lack of any H. pylori like microbe stain assigned as negative. H. pylori is typically a curved rod microbe that is observed on the lumen or epithelial surface of the gastric mucosa. The organisms are very rarely seen in within epithelial cells or gastric crypts. IHC results were considered as the gold standard in this study.

4. Statistical Analysis

SPSS v20 was used to analyse the data and to perform Pearson Chi-Square test for statistical significance namely Sensitivity, Specificity, Positive likelihood ratio and negative likelihood ratio.

RESULTS

The mean age of the study population was 49.47 ± 14.31 years, which included 28 (74%) males and 10 (26%) females.

The symptoms with which patients presented were as follows- 20 (52.63%) with abdominal pain, 8 (21.03%) gastritis, 6 (15.79%) dysphagia, 2 (5.26%) loss of weight, 2 (5.26%) with generalised weakness and labelled as anemia under evaluation.

The histopathological diagnosis of the upper GI endoscopies consisted of squamous cell carcinoma 8 (22%), chronic gastric ulcer 7 (19%), normal gastric mucosa 5 (14%), adenocarcinoma (14%), chronic duodenitis (14%), dysplasia (6%), chronic gastritis (11%).

Giemsa positive cases were 9 (23.5%) and Giemsa negative cases were 29 (76.5%). (Table 1)

IHC was positive in 21 (55.5%) and negative in 17 (44.5%). (Table 2) 12 additional IHC positive cases were identified in comparison to Giemsa which consisted 1 case of normal gastric mucosa, 1 of chronic gastritis, 3 of chronic gastric ulcer, 3 of chronic duodenitis, 2 of adenocarcinoma and 1 of squamous cell carcinoma. IHC could identify higher number of bacilli when compared to Giemsa in a few cases (Figure e,f). IHC additionally identified coccoid forms of organisms which were not identified by Giemsa (figure g).

Considering IHC to be gold standard for the identification of H. pylori in gastric mucosal biopsies; Giemsa has a sensitivity of 34% and specificity of 58%, positive predictive value of 0.82 and negative predictive value of 1.12 (*P 0.09*). We could not compare H&E with IHC as the true negative cannot be zero to calculate specificity and sensitivity.

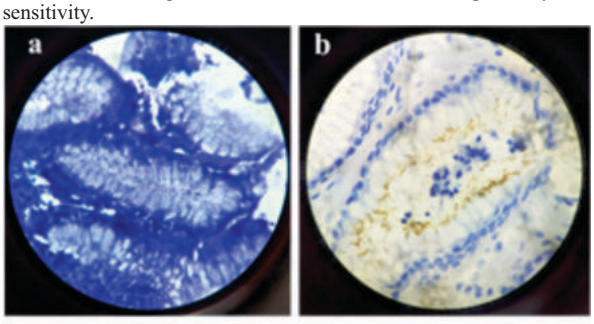

Figure (a,b) Bacilli stained by both Giemsa and Immunohistochemistry. (a) Giemsa 100x. (b) IHC 100x

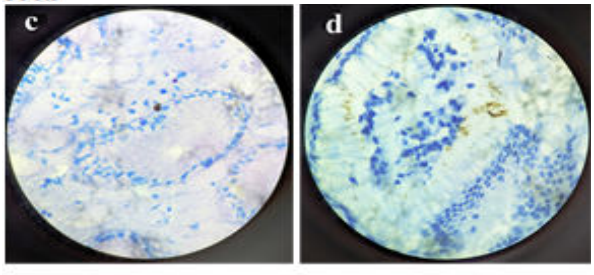

Figure (c,d) Bacilli not stained by Giemsa but stained by Immunohistochemistry. (c) Giemsa 100x. (d) IHC 100x

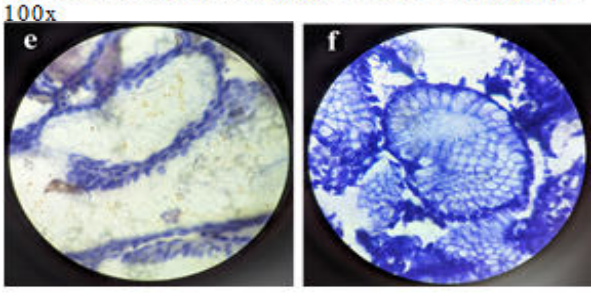

Figure (e,f) Scant number of bacilli identified by Giemsa but higher number identified by Immunohistochemistry. (f) Giemsa 100x. (e) IHC 100x

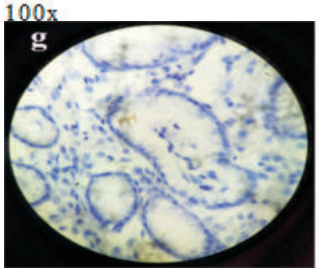

Figure g. Coccoid forms of bacilli. IHC 100x

DISCUSSION:

H. pylori gastritis is acquired in early childhood in most of the cases, but its severe symptomatic sequel of chronic gastritis appears in adults [10] because of many co-morbid conditions and lifestyle, [11] in addition its role as a Grade I carcinogen in the gastro-intestinal tract has been established.[12,13] Therefore, it is crucial to document the presence of H. pylori in a gastric mucosal biopsy for giving timely and appropriate patient care. Among all tests and methods of diagnosis, the most preferred one is the histological examination (antral biopsy).[14] Although it is slow, laborious, more expensive and associated with high observation variability [15], it gives useful information about the conditions associated with bacteria. Furthermore, histological examination of biopsies gives plausible information about the development of disease status, response to treatment, and provide a good opportunity for detecting post treatment form of bacteria (coccoid form). [16]

Table 1 Variables Of Giemsa

Giemsa	Number	Percentage
Negative	29	76.32
Positive	9	23.68

Table 2 Variables of H. Pylori Immunohistochemistry

IHC	Number	Percentage
Negative	17	44.74
Positive	21	55.26

The classic example of H. pylori gastritis consists of the following characteristic histological findings- a superficial band-like lymphoplasmacytic infiltrate that appears snug beneath the surface foveolar epithelium, acute inflammation, and prominent lymphoid aggregates. The bacilli appear as curved rods. Occasionally a lymphocytic pattern can be seen.

Important points of distinction from oral and gastrointestinal flora include the following: [17]H. pylori are closely situated near the surface epithelium, the clusters have same uniform morphology and the bacilli are always found against a background of active inflammation. Whereas normal gastrointestinal flora is found away from the surface epithelium, the morphology of the organisms in a clusters is not uniform and they are not strictly found along with inflammation.

In the present study, very few bacilli were identified on H&E sections. Hence, Giemsa stain was performed parallelly to identify the organisms. 29 cases (76.32%) were Giemsa positive, whereas 9 (23.68%) were Giemsa negative. Though bacilli can be identified on routine H&E sections, it leads to many false-positive and false-negative cases [18,19].

Immunohistochemistry was performed to compare the results with Giemsa. 17 (44.74%) of cases were negative on IHC, whereas 21 (55.26%) of cases turned out to be positive on IHC (Figure a,b,c,d). 12 additional IHC positive cases were identified in comparison to Giemsa. This is likely because there is very little contrast between the organism and the tissue on Giemsa. In the presence of scanty bacterial load, presence of coccoid forms (which are identified as small mucin globules or as extracellular mucin), prior incomplete treatment history it is difficult to identify the organisms on Giemsa.[13] Also, H. pylori antibodies cross-react with H. heilmannii, helping in their identification and differentiating them from H. pylori, as explained in the previous section. [17,20,21]

According to 2013 Rodger C. Haggitt Gastrointestinal Pathology Society Recommendations for the Appropriate Use of Special Stains for Helicobacter Detection,[18] assuming no Helicobacter organisms are seen on H&E, the indications for IHC are as follows-

1. Inflamed gastric biopsies, including cardiac mucosa and cases of reactive gastritis/gastropathy.
2. Lymphocytic gastritis pattern.
3. Chronic inactive gastritis with concomitant positive Helicobacter serologies, gastroduodenal ulcers, gastric MALToma or adenocarcinoma, duodenal lymphocytosis, prior Helicobacter infection.
4. Inflamed or ulcerated duodenal biopsies with gastric foveolar metaplasia.

In the present study, IHC was considered as the gold standard. Giemsa showed sensitivity of 34% and specificity of 58%, positive predictive

value of 0.82 and negative predictive value of 1.12, which was in concordance with studies by Dogar T et.al;[12] Bamanikar S. et.al;[13]Patnayak R et.al;[22] Kacar F. et.al;[23] Aziz et.al.[24]We could not compare H&E with IHC as the true negative cannot be zero to calculate specificity and sensitivity.

IHC was positive in 21 (55.5%) cases, which was in concordance with studies byDogar T et.al - 32.85%;[12] S. Bamanika et.al;- 52.69 %;[13] Patnayak R et.al- 62%;[22]Kacar F at.al - 85.71%;[23]Aziz et.al 75%.[24]

CONCLUSION

In routine practice, two kinds of stains, H&E and Giemsa are enough for the diagnosis because of the simplicity and consistency. Even though IHC has shown higher identification rates and more reliability, its use in routine practice may not be practical. However, IHC can be used in certain cases such as-

1. Negative results with other histochemical stains but a strong suspicion remains.
2. Post-treatment when the bacilli decrease in number.
3. Bacilli appear in coccoid forms.

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