Original Research Article

Detection of Helicobacter Pylori in Gastric Biopsies of Patients with Chronic Gastritis: Histopathological and Immunohistochemical Study

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ABSTRACT

Introduction: *H. pylori* infection is a major cause of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma and gastric B-cell lymphoma (MALT lymphoma). Infection with this organism affects more than half the world's population. Although, several special stains have been used to detect *H. pylori* in histological sections; however, their sensitivity and specificity vary greatly.

Aims and Objectives: This study was conducted to compare traditional staining methods with immunohistochemical method in diagnosing *H. pylori* in chronic gastritis.

Materials and Methods: This is a retrospective and prospective study, includes 985 gastric biopsies (471 retrospective and 514 prospective) with histopathological diagnosis of chronic gastritis. Slides were stained with Hematoxylin and eosin (H&E), modified Giemsa and immunohistochemical staining (IHC) was done using polyclonal antibody marker for *H. Pylori*. Sensitivity, specificity and positive predictive value of the H&E, Giemsa stains was calculated.

Results: Most of the biopsies were obtained from gastric antrum (895) by endoscopic method, followed by gastric body. Recurrent abdominal pain (92.79%) was the most frequently encountered clinical presentation. *H. pylori* was identified in 40.3% biopsies stained by H&E, followed by 49.2% of biopsies stained with modified Giemsa stain, the frequency of detection was greater with IHC stained sections 52.7%.

Conclusion: Although H & E and modified Giemsa stains, are standard stains for detection of *H. pylori*; the reliability and yield is better using IHC stains; especially when present even in coccoid forms or in small numbers.

Key Words: H. Pylori, Immunohistochemistry, Special stains, chronic gastritis.

INTRODUCTION

H. pylori-related diseases are the most prevalent in the world. *H. pylori* infect more than half the population in the world. [1] The bacilli were discovered in 1983 by Warren and Marshall from gastric mucosal biopsy specimens of patients with chronic active gastritis and peptic ulcer disease. [2] *H. Pylori* is a Gram negative, spiral organism, which colonizes the gastric mucosa. [3]

Now the organism is accepted as the causative agent of gastritis, peptic ulcer disease, gastric adenocarcinoma and gastric B-cell lymphoma (MALT lymphoma). *H. pylori* infection may also play a role in some cases of non-ulcer dyspepsia. [3] In developing countries, the infection rates are much higher and infection rate increases as the age advances.

Hence documenting the presence of *H. pylori* in gastric mucosal biopsies is essential for appropriate patient care. With

advances in endoscopic technique, biopsy can be easily obtained from the site intended with precision by using flexible fiber optic endoscope. H&E, Giemsa and Immunohistochemistry staining technique has helped in identification of *Helicobacter pylori*. H&E stain can directly identify *H. pylori* in a high magnification, but it becomes difficult to identify *H. pylori* when it is present in low density and when atrophic mucosal changes are present. Giemsa stain is the preferred method over H&E in many laboratories, but it gives false negative results when the organisms are few or in patients with prior incomplete treatment. IHC stains have advantages when patients are partially treated for *H. pylori* gastritis or have few or atypical forms (coccoid) of *H. pylori* present. It has high specificity as it can exclude other similar shaped organisms. [4]

Therefore, in this study we propose to use immunohistochemical method using polyclonal anti *H. pylori* antibody for identification of these organisms in gastric biopsies and compare the results with routine histochemical methods like H&E stain and Giemsa stain. The histopathological examination of gastric biopsies is reported using the "Updated Sydney System" classification of chronic gastritis. [5]

MATERIALS AND METHODS

This is a retrospective and prospective study, carried out from May 2012 to September 2017 at the Department of Pathology at Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune. The study was approved by the Institutional Ethics committee. All the gastric biopsies received from clinical department within this period with histopathological diagnosis of chronic gastritis were included in the study. Auto lysed specimens and sections not having enough material were excluded.

The data for retrospective study was retrieved from histopathology reports, slides and paraffin blocks from the department of

Pathology. For prospective study endoscopic gastric biopsies received for histopathological examination were included. First biopsies were fixed in 10% formalin overnight and were processed for approximately 12 hours in tissue processor to obtain paraffin blocks. The paraffin blocks were sectioned at 3-micron thickness and stained with H&E, modified Giemsa and IHC stains using Biogenex Anti-Helicobacter Pylori polyclonal marker for *H Pylori*. The sections were carefully examined for the presence of *H.Pylori* and were reported using the "Updated Sydney System" classification of chronic gastritis. [5] The results of H&E and Giemsa stain were compared with IHC stain findings in the identification of *H. Pylori*. Histopathological and immunohisto-chemical results were correlated with clinical parameters. Sensitivity, specificity and positive predictive value of the H&E, Giemsa and IHC stains was calculated. Statistical analysis was carried out using the SPSS 20 and Open Epi (version 3) software.

OBSERVATION AND RESULTS

In present study, total 985 gastric biopsies, histopathologically diagnosed as chronic gastritis on H&E stain were included. H. pylori infection in the age group 31- 40 years was 47.6% and showed male predominance (58%). Most of the biopsies were obtained from gastric antrum (90.8%) by endoscopic method, followed by gastric body. Recurrent abdominal pain (92.8%) was the most common clinical complain followed by dyspepsia (7.2%). Chronic gastritis was classified as per "Updated Sydney System of Classification" 2002. [5] (Table 1)

Table 1: Classification of chronic gastritis evaluated in our study (n = 985) according to Updated Sydney System of Classification

Type of gastritis	No. of cases	% of cases
Mild chronic gastritis	520	52.8%
Moderate chronic gastritis	395	40.10%
Severe chronic gastritis	70	7.10%
active gastritis	467	47.41%
Atrophy	328	33.29%
Metaplasia	126	12.79%
H. Pylori positive cases	519	52.69%

Out of 985 biopsies with chronic gastritis, 520 (52.8%) biopsies were reported as mild chronic gastritis. Moderate and severe chronic inflammatory infiltrate was observed in 395 (40.10%) and 70 (7.10%) biopsies respectively. Acute inflammatory infiltrate was encountered in 467 (47.41%) biopsies, whereas atrophic and intestinal metaplastic changes were seen in 328 (33.29%) and 126 (12.79%) biopsies respectively. Presence of *H. Pylori* was observed in 519 (52.69%) biopsies by IHC stain.

As per "Updated Sydney System" of chronic gastritis; active gastritis, atrophic and intestinal metaplastic changes were further subdivided as per degree of severity of these changes and *H. Pylori* positive biopsies were classified according to degree of bacillary colonisation. (Table 2)

Table2: Subdivision of gastric biopsies according to degree of Active Inflammation, Atrophic, Intestinal Metaplastic changes and Bacillary Colonisation (n = 985)

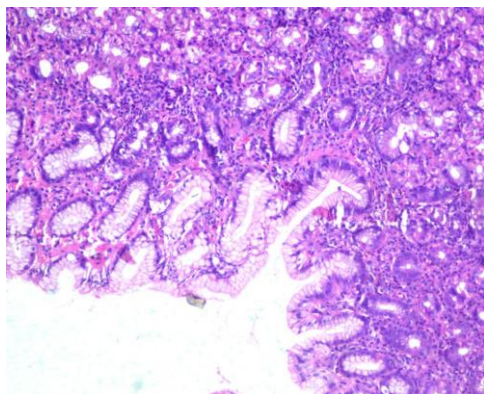|	Active inflammation	Atrophic changes	Intestinal Metaplasia	Bacillary colonisation
Nil (0)	518 (52.59%)	657 (66.70%)	859 (87.21%)	466 (47.30%)
Mild (1)	88 (8.93%)	167 (16.95%)	79 (8.02%)	127 (12.89%)
Moderate (2)	127 (12.90%)	106 (10.77%)	42 (4.26%)	187 (19.0%)
Severe (3)	252 (25.58%)	55 (5.58%)	05 (0.51%)	205 (20.81%)

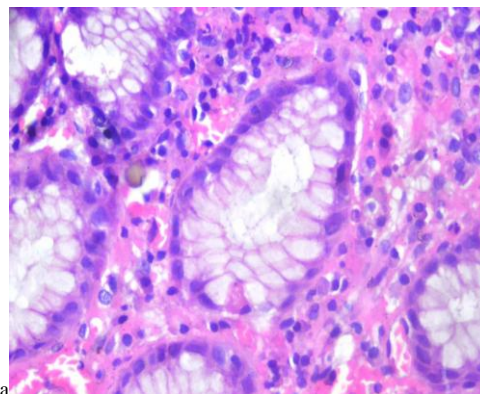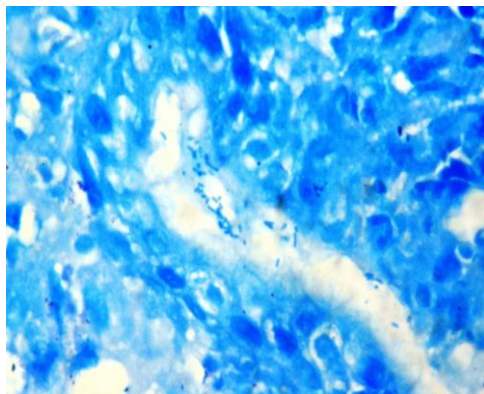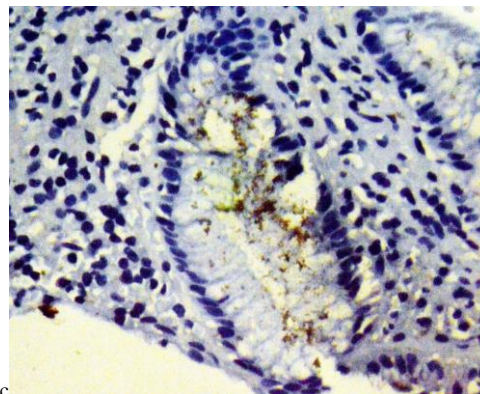**Fig.1a: Antral biopsy of a patient with chronic active gastritis - showing diffuse chronic active inflammation in the lamina propria (H&E, x100), Fig.1b: Presence of few coccoid forms of *H.pylori* in the antral glandular lumina (H&E, x400), Fig.1c: Coccoid forms of the organism in the glandular lumina, better visualised than in H &E (Modified Giemsa, x400), Fig.1d: Strong immunopositivity with numerous coccoid forms of H.pylori in the lumen of the gastric glands (IHC stain, x400)**

IHC stain could identify bacilli in 519 (52.69%) biopsies followed by Giemsa and H&E stain, in 484 (49.14%) and 397 (40.30%) biopsies respectively (Fig.1, 2). In 35 cases *H. Pylori* was suspected by H&E stain but were clearly negative by IHC stain. There were 6 cases which showed no bacilli by IHC stain, although they were suspected positive by Giemsa stain. (Figure3)
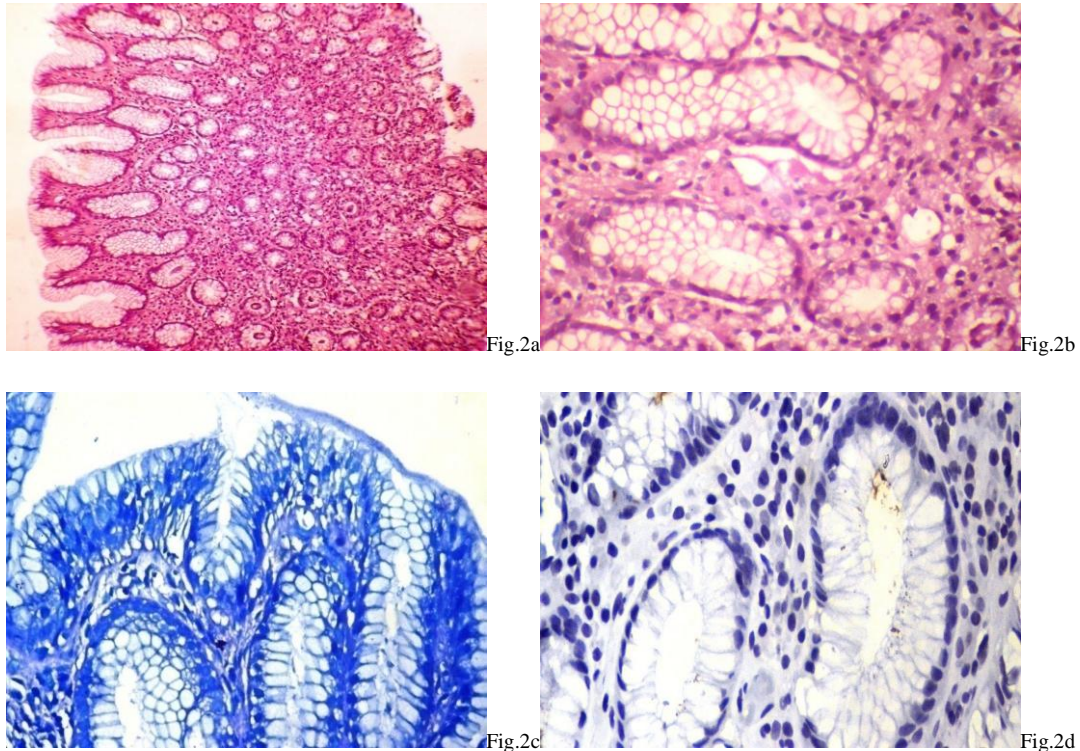
Fig 2a, b: A case of chronic active gastritis which showing no organisms on H&E (x100, x400), Fig 2c: nor on Giemsa stain (x400). However Fig 2d: in view of the marked active gastritis, IHC staining was done which revealed focal positivity with few H.pylori (x400)
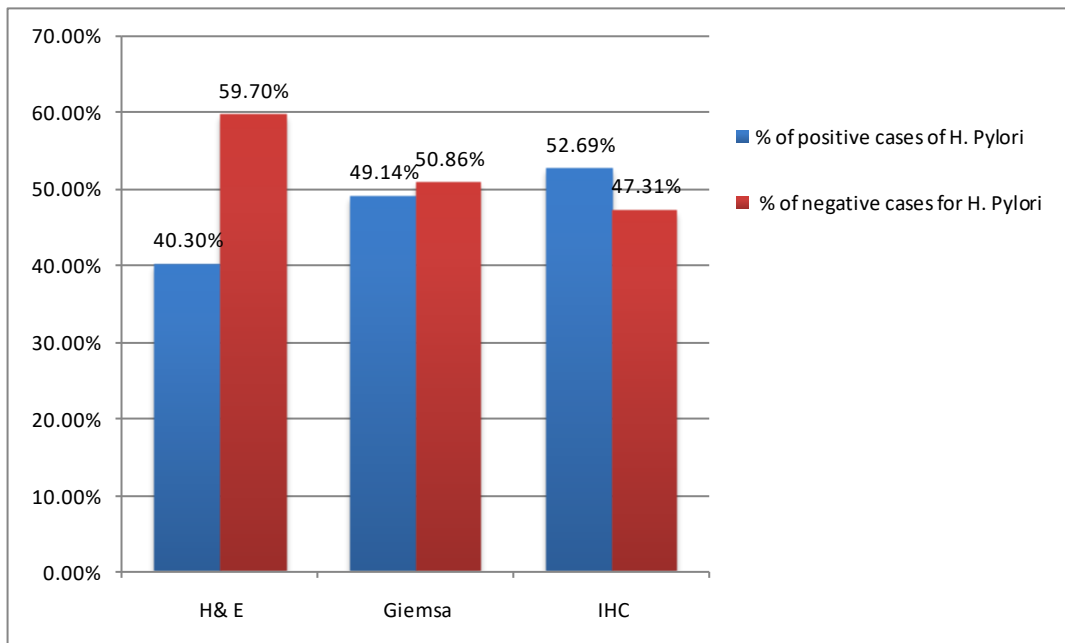

Figure 3: *H. Pylori* positive cases in gastric mucosal biopsies by various stains

In mild chronic gastritis, Giemsa and IHC stains identified organism in equal number of biopsies (20.57%), followed by H&E stain (10.76%). Whereas in moderate and severe chronic gastritis IHC stain demonstrated bacilli in maximum number of biopsies 87.34% and 95.71% respectively. Giemsa stain reported positivity (92.85%) almost equivalent to IHC stain in severe chronic gastritis, but more than those identified by (77.14%) H&E stain (Figure 4).
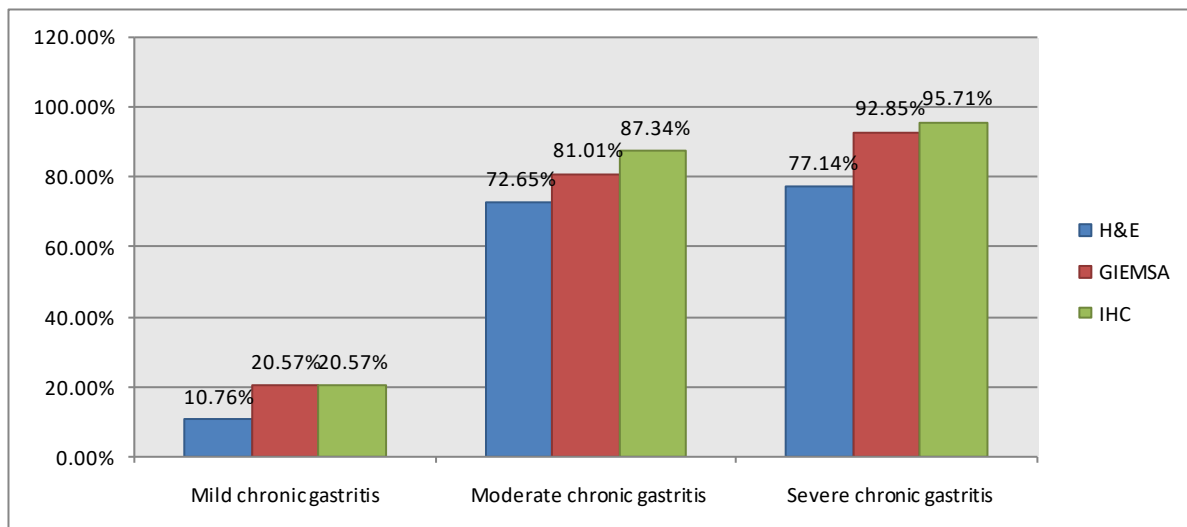
Figure 4: Frequency of *H. Pylori* positive cases detected using various stains- subdivided according to degree of chronic gastritis

In gastric biopsies associated with changes of active gastritis, atrophic and intestinal metaplastic changes, IHC stain reported highest positivity for identification of bacilli (61%, 67.98% and 73.01% respectively) followed by Giemsa and H&E stain. Bacilli were observed in almost equal number of biopsies of chronic active gastritis stained by Giemsa (54%) and H&E (52%) stain. However, in biopsies with atrophic and intestinal metaplastic changes, Giemsa stain reported higher positivity than H&E stain. (Table 3)

Considering IHC stain as gold standard for identification of *H. Pylori* in gastric mucosal biopsies, sensitivity and specificity of H&E stain was 69.74% and 92.48% respectively. Sensitivity of Giemsa stain (92.10%) was higher than H&E stain but specificity (88.40%) was found to be lower. Positive and Negative predictive values of H&E stain were higher than Giemsa stain. For H&E and Giemsa stains positive predictive values were 91.18% and 73.29% respectively and negative predictive values were 98.76% and 82.63% respectively. (Table 4)

Table 3: Frequency of *H. Pylori* associated changes identified by various stains

H.Pylori associated changes	H&E (397)	Giemsa (484)	IHC (519)
Active Gastritis (467)	242 (52%)	252 (54%)	284 (61%)
Atrophic gastritis (328)	141 (42.98%)	187 (57.01%)	223 (67.98%)
Intestinal metaplasia (126)	61 (48.41%)	79 (62.69%)	92 (73.01%)

Table 4: Sensitivity, Specificity, Positive predictive value and Negative value of H&E and Giemsa stains

Stain	Sensitivity	Specificity	Positive predictive value	Negative predictive value
H & E	69.74%	92.48%	91.18%	98.76%
Giemsa	92.10%	88.84%	73.29%	82.63%

The Cohens kappa coefficient (κ) for H&E stain was 0.61 (P = <0.001) and for Giemsa stain was 0.8116 (P = <0.001). Both stains were showing agreement with IHC stain but Giemsa stain imparting higher agreement.

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 [1] because of many co- morbid conditions and lifestyle, [6] in addition its role as a Grade I carcinogen in the gastro-intestinal tract has been established. [7] Therefore, it is crucial to document the presence of *H. pylori* in a gastric mucosal biopsy for giving timely and appropriate patient care.

H&E, Giemsa and Immunohisto-chemistry staining technique has helped in identification of organism in gastric

mucosal biopsies.

Though several methods have been described for identification of these bacilli (invasive and non-invasive methods), histological detection of *H. pylori* in gastric mucosal biopsies remains the most common and the most sensitive test. [8]

Present study is a five years retrospective and prospective study, which includes 985 chronic gastritis cases. Most common age of occurrence was 31-40 years (47.6%). In a study done by Garg B et al [3] and Dogar T et al [7] majority of patients were adults like in our study. Male patients were 58% in present study with M: F ratio was 1.4:1; similar observation was reported by Dandin AS et al, [9] Abu-Ahmad NM et al, [10] where percentage of male cases was 75%, 62% respectively. Several studies have suggested that oestrogen protects women from this kind of inflammation. Women with delayed menopause and increased fertility have a lower risk of gastric infections and cancer. [11]

In our study, recurrent abdominal pain (92.80%) was the most common clinical complain, followed by dyspepsia (7.20%). Similar observations were reported by a study, conducted on 7941 Shanghai population cases by Zhang C et al. [12] In our study, 895 biopsies (90.86%) were obtained endoscopically from gastric antrum and 90 (9.13%) from body; similar ratio was noted in a study by Toulaymat M et al. [13] Antral biopsies are adequate for *H pylori* detection because bacillary colonization is markedly more severe in the antrum than in the gastric body. [9]

According to "Updated Sydney System" of classification, [7] chronic gastritis was classified into mild, moderate and severe according to degree of chronic inflammatory infiltrate. In our study; mild, moderate and severe chronic gastritis was observed in 52.80%, 40.10% and 7.10% of biopsies respectively. Results of our study are in concordance with Wabinga HR [14] study, where 41.66%, 39.58% and 14.5% cases were reported as mild, moderate and severe chronic gastritis respectively.

In present study chronic active gastritis was encountered in 47.41% of biopsies, similar observations were reported by Toulaymat M et al [13] study (48.5% cases); whereas in Wang XI et al [15] study, it was 30.35% comparatively lower than present study.

Atrophic and intestinal metaplastic changes were reported in 33.29% and 12.79% of cases respectively in our study, whereas in Garg B et al [3] study, atrophic and metaplastic changes were reported in 12.33% and 7% of cases respectively. In another study, done by Zhang C et al [12] metaplastic changes was seen in 37% of cases, comparable with our study; on other hand atrophic changes was reported in 36.8% of cases which is higher than present study.

In the present study, IHC stain resulted in diagnosis of *H. Pylori* in 52.69% of biopsies, a finding that is almost similar to Key MA et al [16] study (58.0%), but less than that reported in Kacar F et al [17] study with 85.71% H.Pylori positive cases. This variation could be because of different sample size, variability in staining pattern and standards followed by various manufacturing kits.

In present study, H&E stain identified bacilli in 40.30% cases, whereas Giemsa stain reported higher positivity in 484 biopsies (49.14%), and maximum positive cases were reported by Immunohistochemical Stain (52.69%). Comparable results were reported in various other studies by Patnayak R et al, [8] Kacar F et al [17] and Dogar T et al. [8] (Table 5)

Giemsa stain was more reliable than H&E in detection of bacilli. The major disadvantage of this stain being, there is little contrast between organisms and tissue. However, the presence of scanty bacteria and identification of coccoid forms of bacilli was difficult to recognize by both routine and special histochemical methods. These difficulties are not encountered when identifying bacilli using IHC method.

Table 5: Comparison of various stains for *H. Pylori* positivity with other studies

Study	H&E	Giemsa	IHC
Patnayak R et al [16]	26 (32.9%)	26 (32.9%)	49 (62%)
Kacar F et al [15]	58 (82.85%)	58 (82.85%)	60 (85.71%)
Dogar T et al [14]	19 (27.2%)	-	23 (32.85%)
Present Study	397 (40.30%)	484 (49.14%)	519 (52.69%)

In present study, IHC stain identified bacilli in maximum number of biopsies irrespective of bacillary colonisation; similar findings were seen in Pity IS et al [18] study.

In our study, various stains could discern bacilli in highest number, when severe chronic gastritis was present and IHC stain has reported maximum positivity among various stains. In moderate chronic gastritis IHC stain reported bacilli in 87.34% of biopsies, comparable with 72.6% biopsies, reported in a study by Pity IS et al. [18]

In present study, IHC stain reported bacilli in 61% of active gastritis biopsies. In a study done by Pity IS et al, [18] 67.1% biopsies were positive with active inflammatory infiltrate, which is in accordance to our study. In our study, H&E stain identified bacilli in 52% of chronic active gastritis, which is lower than IHC stain. In a study done by Chitkara Y, [19] H&E stain (58.39%) reported higher positivity than IHC stain (45.98%) for identification of *H. Pylori* in chronic active gastritis. The possibility behind these results could be the presence of other organism like H. Heilimanii, H. Bizzozeroni, Pseudomonas fluorescence which has similar morphology and could be difficult to differentiate from *H. Pylori* on H&E and other routine histochemical stains.

In present study, among various stains, IHC stain identified bacilli in higher number of biopsies, with atrophic changes. Both routine and immunohistochemical stains have reported highest positivity for bacilli in moderate atrophic gastritis. [17] In present study, IHC stain has reported positivity for organism in 67.98% of biopsies with atrophic changes and almost

comparable results were observed in studies reported by Pity IS et al [18] and Zhang C et al. [12]

In our study, IHC stain revealed 73.01% positivity for identification of bacilli in biopsies showing features of intestinal metaplasia. In various other studies this percentage is lower for IHC stain; these findings could be because of large sample size in our study. In Pity IS et al, [18] Zhang C et al [12] and in Toulaymat M et al [13] studies, IHC stain was positive in 57.9%, 34.1% and 14.0% of biopsies respectively which were showing intestinal metaplastic changes.

In present study IHC stains is considered as gold standard for identification of bacilli, while H&E and Giemsa stains showed 69.74% and 92.10% sensitivity and 92.48% and 88.84% specificity respectively. For Giemsa stain Cohens kappa coefficient (κ) was 0.8116 (P = <0.001) which is higher than H&E stain 0.61 (P = <0.001), hence Giemsa stain is imparting strong agreement with IHC stain for recognition of bacteria. In a study done by Wabinga HR et al [14] κ for Giemsa stain is 0.69 which is in concordance with our results.

Routine and special stains are sufficient to identify organism when bacillary density is high with typical spiral morphology. However, these stains fail to demonstrate bacilli, when organisms are sparse, presence of coccoid forms or when patient has taken prior incomplete treatment. In such a scenario immuno-histochemistry using polyclonal antibody for *H. pylori* is superior to H&E as well as Giemsa stains for identification of these bacteria. [14]

CONCLUSION

IHC identification of *H. pylori* was more reliable than Giemsa stain for detecting *H. pylori*. Moreover, biopsies with non specific chronic active gastritis with absence of *H. pylori* on routine stains should have IHC done to detect possible scanty or coccoid forms of organisms. However, upfront use of IHC may not be cost effective

in a resource restricted setting, hence Albeit being considered as a gold standard in the detection of H. pylori, its use in second line in case of ambiguous H&E-Giemsa result is more appropriate.

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