



## Diagnostic Utility Of Immunohistochemistry In Comparison With Histochemical Stains In The Identification Of Helicobacter Pylori In Upper Gastro-Endoscopic Biopsies

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### Abstract

#### Background:

Helicobacter pylori infection is believed to be the most common infection of the stomach followed by that of the duodenum, once acquired, is believed to persist throughout life, unless treated. Infection with H. pylori is a leading cause of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and gastric B-cell lymphoma (MALT lymphoma). Although several special stains have been employed to identify H. pylori in histological sections, their specificity and sensitivity range widely.

#### Methods

This study was conducted on 56 upper gastro-endoscopic biopsies obtained from patients with chronic gastritis in a time period of 18 months from May 2021 to September 2022. Slides were stained with H&E stain, modified giemsa stain, half gram stain and Immunohistochemistry stain. Histopathological evaluation was done, and sensitivity and specificity of each stain was compared.

#### Results

Sensitivity and specificity of IHC was more as compared to other routine stains in histopathology. Helicobacter pylori colonization was increased when the gastritis was associated with neutrophilic infiltration, chronic inflammation, intestinal metaplasia and atrophic changes.

#### Conclusion

Immunohistochemistry was found to be the gold standard test in identification of Helicobacter pylori in gastritis patients. Histo-morphological details of the gastric biopsies were better visualized with Hematoxylin and Eosin stain. Modified Giemsa was observed to be the best alternative for IHC stain among routine stains when taking time and expense into consideration. Helicobacter pylori positivity was increased with active gastritis, atrophic gastritis, chronic inflammation and cases with intestinal metaplasia.

**Keywords:** H. pylori, Immunohistochemistry, H&E stain, Giemsa, Gastritis

### Introduction

Helicobacter pylori (H. pylori) is a comma-shaped gram-negative bacteria that affects up to 50 percent of the world's population, with a higher incidence in developing countries. It is the most significant contributing factor to peptic ulcer disease, gastric carcinoma, gastric lymphoma, and chronic or

atrophic gastritis. Hence it becomes to diagnose Helicobacter pylori organism at an early stage of infestation. [1] In routine clinical practice, combining the findings of two or more tests may be a sensible tactic to get the most trustworthy outcome to detect the organism. We have a spectrum of both invasive

for eg. Histological examinations, cultures and rapid urease tests and non-invasive diagnostic tests like serology, urine, urine and blood examination to aid the identification of *Helicobacter pylori*. In histological examination *Helicobacter pylori* has been identified using H&E, Giemsa, and Immunohistochemistry staining techniques. H&E stain can directly detect *H. pylori* at high magnification, but it becomes difficult to recognise *H. pylori* at low density and with atrophic mucosal changes. When patients have few or unusual forms of *H. pylori* (cocci) or are partially treated for *H. pylori* gastritis, IHC stains might be advantageous. It has a high specificity since it can eliminate other species with similar shapes. [2] In our study we have attempted to compare the sensitivity and specificity of routine stains like H&E, modified giemsa and half gram in comparison with immunohistochemistry and correlate the degree of colonization of the organism in different pattern of gastritis based on the Sydney system of grading.

### Materials And Methods

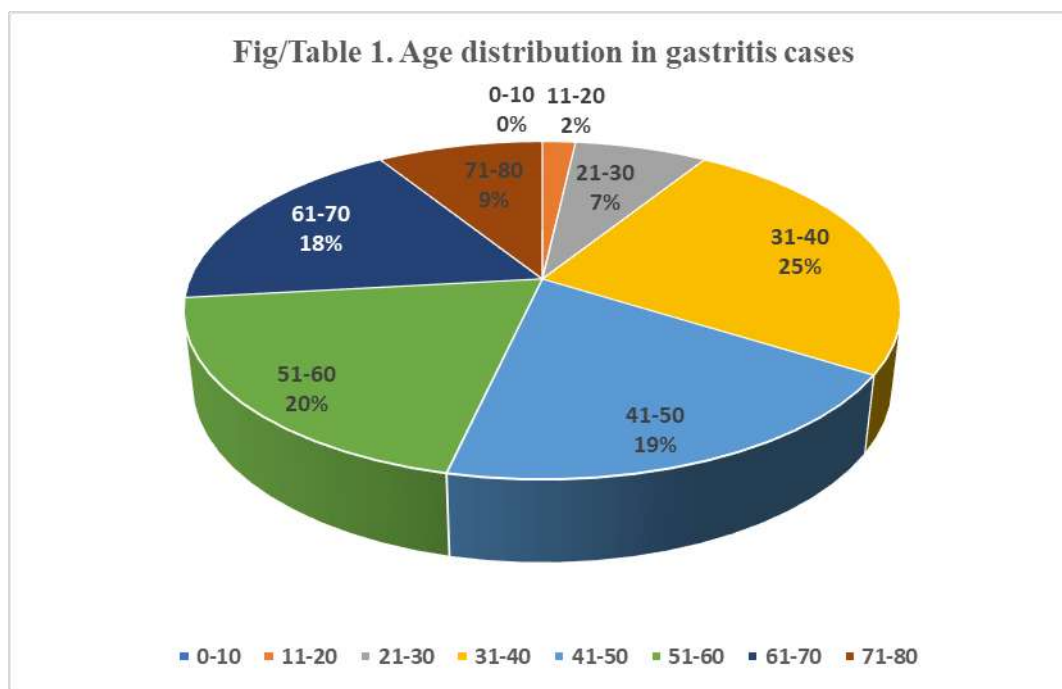
This study was carried out in the Departments of Pathology, BGS Global Institute of Medical Sciences, Bengaluru over a period of 18 months from March 2021 to September 2022 on 56 patients with symptoms of gastritis like epigastric pain, epigastric burning, postprandial fullness and early satiety. Endoscopic biopsies from antrum, pylorus, and body

regions of the stomach were collected and sent for histopathological assessment in a 10% buffered formalin filled sterile container to the histopathology lab. These were processed in an automated tissue processor. Dehydration, clearing and embedding was done in an automated tissue processor from Leica (TP 1020). Further on, embedding was done with paraffin wax and 4–5 micron thin sections were cut from the block using microtome (Leica).

These sections were stained with H&E, modified giemsa, half gram and IHC stain using Biogenex Anti-*Helicobacter Pylori* polyclonal marker. The sections were thoroughly examined for the presence of *H. Pylori* and reported using the "Updated Sydney System" categorization of chronic gastritis. H&E and Giemsa stain results were compared to IHC stain findings in the identification of *H. Pylori*. Clinical factors were associated with histopathological and immunohistochemical data. The H&E, Giemsa, and IHC stains' sensitivity, specificity, and positive predictive value were determined. The data was analysed using statistical software – Medcalc and SPSS.

### Results

Out of 56 patients, Gastritis was found to be more common after the age of 30 years, of which 25% were found in the age group of 31-40. [Graph 1] 59% of the patients in our study were male and 41% were female.



Out of 56 cases stained with H & E, a total of 44 cases showed positive results and 12 showed negative results. Out of the 44 cases that were positive, 20 were females and 24 were males. The positive and negative predictive values were 100% and 41.67% respectively with 87.50% accuracy.

Out of 56 cases stained with IHC, a total of 51 cases showed positive results and 5 showed negative results. Out of the 51 cases that were positive, 23 were females and 28 were males.

Out of 56 cases stained with modified giemsa, a total of 48 cases showed positive results and 8 showed negative results. Out of the 48 cases that were positive, 22 were females and 26 were males. The positive and negative predictive values were 100% and 62.50% respectively with 94.64% accuracy.

Out of 56 cases stained with Half gram, a total of 41 cases showed positive results and 15 showed negative results. Out of the 41 cases that were positive, 18 were females and 23 were males. The positive and negative predictive values were 100% and 33.33% respectively with 82.14% accuracy.

On comparing IHC stain with routine staining methods like H&E, modified giemsa and half gram stains it was observed that the IHC stain was more sensitive in detection of *H. pylori* than all the three stains combined. Although, out of 56 cases the routine stains were able to detect 48 positive cases as opposed to 51 positive cases by IHC stain. [Fig/Table 2 & 3]

**Fig/Table 2: Male and female distribution of positive & negative cases of *Helicobacter pylori* in H&E, Giemsa, Half gram and IHC**

Method	H and E stain		Giemsa		Half gram		IHC	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
<b>Male</b>	24	8	26	6	23	9	28	4
<b>Female</b>	20	4	22	2	18	6	23	1
<b>Total</b>	44	12	48	8	41	15	51	5

**Fig/Table 3. Sensitivity, specificity, positive predictive value and negative predictive value for Helicobacter pylori in H&E, modified giemsa and half gram stains**

Statistics	H&E	Modified giemsa stain	Half gram stain
Sensitivity	86.27%	94.12%	80.39%
Specificity	100%	100%	100%
Positive predictive value(*)	100%	100%	100%
Negative predictive value(*)	41.67%	62.50%	33.33%

Of the 56 cases studied, H&E stained sections revealed mild colonization in 9 (16%), moderate colonization in 44 (79%) and marked colonization in 3 (5%).

**Fig/Table 4: Pattern of gastritis of the studied cases**

Lesion	Number (%)
Inflammation	
Mild	9(16%)
Moderate	44(79%)
Marked	3(5%)
Activity	29(52%)
Chronic inflammation	15(27%)
Intestinal Metaplasia	9(16%)

When grouping the positive cases of helicobacter pylori according to the Sydney grading for gastritis, we observe that out of the biopsies associated with active gastritis, 52% of cases showed positive results for h. pylori. In 15 biopsies associated with atrophy, 13 showed positive results for h. pylori.

**Fig/Table 5: Colonization of H. pylori by parameters of gastritis according to the Sydney Grading System**

Pattern of gastritis	H pylori	
	Positive	Negative
Activity (29)	26	3
Metaplasia (9)	7	2
Atrophy (15)	13	2

**Fig/Table 6. Comparison of the age group affected by gastritis in different studies**

Study	Age group
Piotrowicz et al.	46-50 years (77%)
Anjana M. L. and Kavitha Yevoor	41-50 years (32.72%)
Present study	31-40 years (25%)

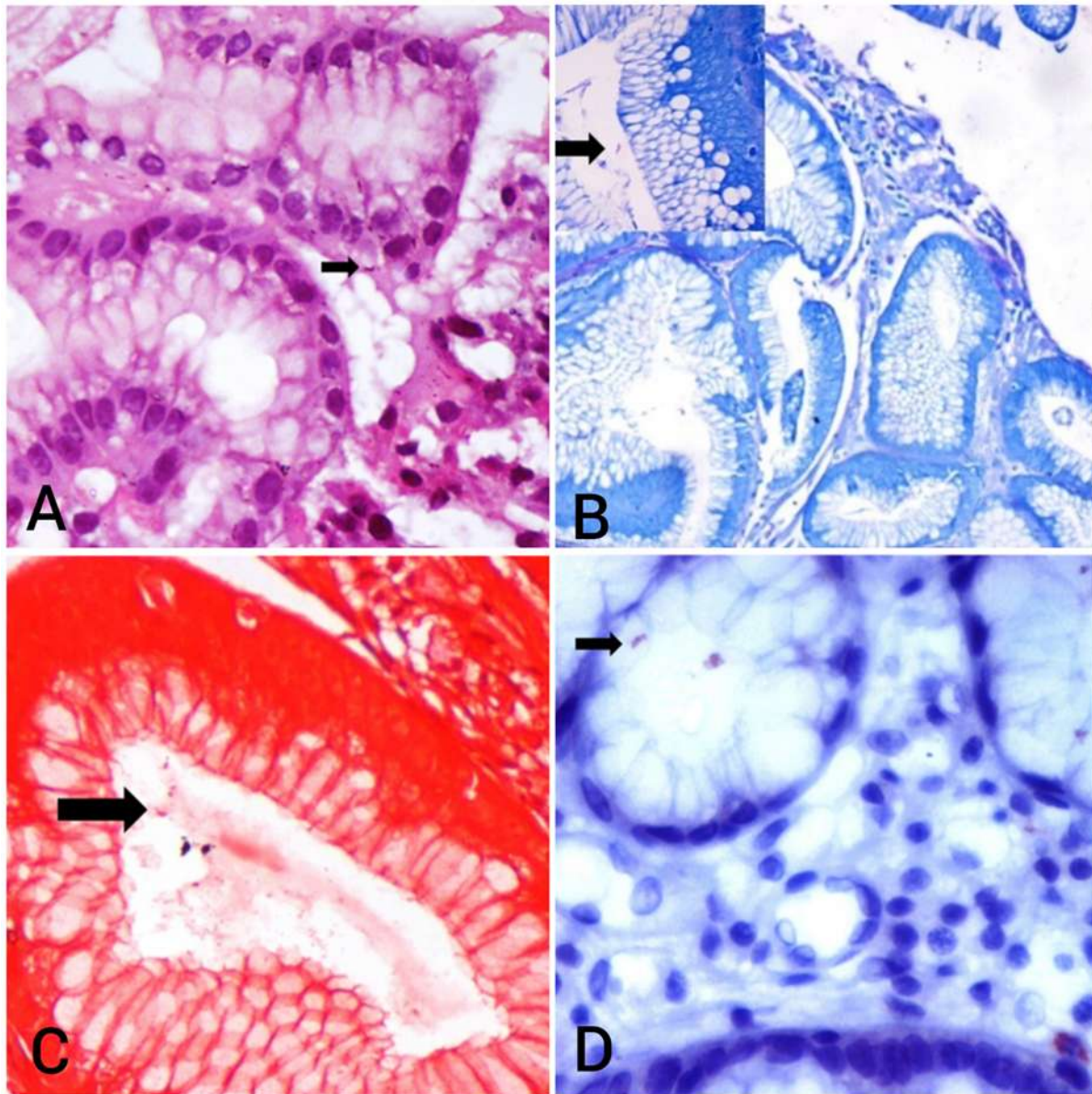
**Fig/Table 7. Comparison of Male and female distribution of H. pylori detection by different stains**

Method		H&E		Modified Giemsa		Half gram		IHC	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Narain A et al	Male	30	12	32	10			33	10
	Female	8	10	8	10			9	10
Present study	Male	24	8	26	6	23	9	28	4
	Female	20	4	22	2	18	6	23	1

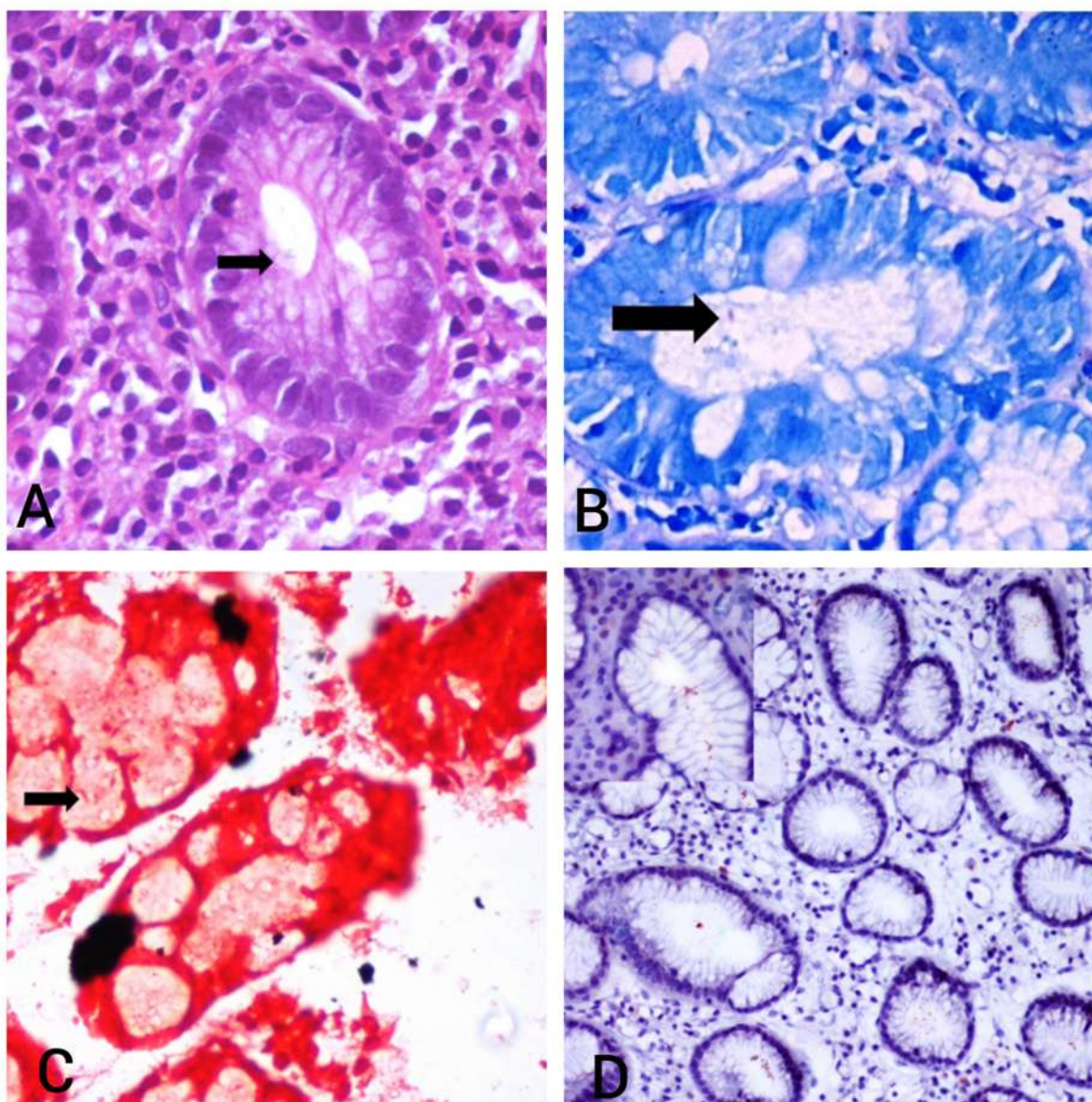
**Fig/Table 10. Comparison of gastritis classified according to Updated Sydney Scoring**

Lesion	Bamanikar S et al	Pity IS et al	Present study
Inflammation			
Mild	520 (52.8%)	35(33.3%)	9(16%)
Moderate	395 (40.1%)	51 (48.6%)	44(79%)
Marked	467 (47%)	19 (18.1%)	3(5%)
Activity	518 (52.59%)	76 (72.4%)	29(52%)
Chronic inflammation	-		15(27%)
Intestinal Metaplasia	126 (13%)	19 (18.1%)	9(16%)

Figure/Table 8 A. H&E stain. H pylori in pink. B. Modified giemsa stain. H pylori in purple. 100X. C. Half gram stain. H pylori in red. 100X. D. IHC. H pylori in brown. 100X.



**Figure/Table 9** A. H&E stain. H pylori in pink. B. Modified giemsa stain. H pylori in purple. 100X. C. Half gram stain. H pylori in red. 100X. D. IHC. H pylori in brown. 100X.



**Fig/Table 11. Comparison of various stains for H. Pylori positivity**

Study	IHC	H&E	Giemsa	Half Gram
Patnayak R et al	49 (62%)	26 (32.9%)	26 (32.9%)	-
Kacar F et al	60 (85.71%)	58 (82.85%)	58 (82.85%)	-
Dogar T et al	23 (32.85%)	19 (27.2%)	-	-
Present study	51 (91%)	44 (78.5%)	48 (85.7%)	41 (73%)

**Discussion**

H. pylori gastritis is most commonly acquired in childhood, but its severe symptomatic sequel of

chronic gastritis appears in adults due to many co-morbid conditions and lifestyle, and its role as a Grade I carcinogen in the gastro-intestinal tract has been established. [3]

As a result, documenting the presence of *H. pylori* in a Gastric mucosal biopsy is critical for providing early and appropriate patient management.

The use of H&E, Giemsa, and Immunohistochemistry staining techniques in stomach mucosal biopsies has aided in the identification of organisms.

Though numerous techniques for identifying these bacilli (invasive and non-invasive approaches) have been discovered, histological detection of *H. pylori* in gastric mucosal biopsies remains the most common and sensitive test. [4]

A study conducted by Piotrowicz *et al.* in a sample size of 230, most cases of gastritis was observed in the age group of 46-50 years (77%) followed by 31-45 years (55%). [5]

A total of 62 gastric biopsy samples of dyspepsia were included in the study conducted by Anjana M. L. and Kavitha Yevoor, out of which majority of cases were seen in the 41-50 years age group (32.72%) followed by 51-60 years age group (21.8%). [6]

In our study of 56 cases, the maximum number of gastritis patients were seen in the age group of 31-40 years (25%).

In a study by Narain A *et al*, the results were as follows:

H&E stain – Out of 60 positive cases, 38 were reported positive by H&E stain out of which 30 were male and 8 were female.

Modified Giemsa stain- Out of 60 positive cases, 40 were reported positive by Giemsa stain out of which 32 were male and 8 were female.

IHC stain - Out of 60 positive cases, 42 were reported positive by IHC stain out of which 33 of the cases were males and 9 of them were females. [7]

In our present study, 28 males were positive with IHC stain in comparison with 26, 24 and 23 of Giemsa, H&E and Half gram stains respectively. 23 females were positive with IHC stain in comparison with 22, 20 and 18 of Giemsa, H&E and Half gram stains respectively.

Because there is little difference between the bacteria and the surrounding tissue, the H&E stain has a poor sensitivity. Owing to its nonspecific staining of

bacteria other than *Helicobacter pylori* in the stomach, H&E has a low specificity. Giemsa is an inexpensive and rapid stain that takes about a total of 15 minutes to complete. The findings are reliable. The values for specificity and sensitivity are adequate. The Giemsa technique's lack of contrast is a drawback, yet careful inspection enables accurate "identifying" of the species. Immunohistochemistry for *Helicobacter pylori* is a costly and time-consuming operation, taking anything from one hour to twenty-four hours to complete. Regardless, IHC is the gold standard since it has a good sensitivity and specificity for the detection of *Helicobacter pylori*. [7]

Figure/Table 8 shows a biopsy from gastric antrum showing *helicobacter pylori* in the gastric mucosa in curvilinear form on Haematoxylin and eosin stain on 100X magnification. Similarly, it was seen in modified giemsa stain, half gram stain and Immunohistochemistry [Fig 2-4]

Figure/Table 9 shows a biopsy from gastric body showing *helicobacter pylori* in the gastric mucosa in rod form on Haematoxylin and eosin stain on 100X magnification. Similarly, it was seen in modified giemsa stain, half gram stain and Immunohistochemistry [Fig 6-8]

In a study by Bamanikar S *et al*, out of 985 samples with chronic gastritis, 520 (52.8%) were classified as having mild chronic gastritis. In 395 (40.10%) and 70 (7.10%) samples, moderate and severe chronic inflammatory infiltrates were found, respectively. Atrophic and intestinal metaplastic alterations were found in 328 (33.29%) and 126 (12.79%) samples, respectively, while acute inflammatory infiltrate was discovered in 467 (47.41%) tissues. [8]

According to a study by Pity IS *et al.* in which 105 cases of chronic gastritis patients were examined, inflammation was mild in 35 (33.3%), moderate in 51 (48.6%), and severe in 19 (18.1%) cases. 72.4% (n=76) of these were active forms. Intestinal metaplasia was detected in 19 (18.1%) patients. [9]

In our study out of 56 cases of gastritis patients, inflammation was mild in 9 (16%), moderate in 44 (79%), and marked in 3 (5%) cases. 52% of these were active forms. Intestinal metaplasia was detected in 9 (16%) patients.



It was observed that moderate cases of gastritis are more common as compared to mild and marked. Gastritis with marked inflammation is relatively rare. However, active forms of gastritis seem to constitute the majority in the pattern of gastritis. Intestinal metaplasia is rare when comparing gastritis patients in different studies.

The IHC stain used in the current study led to the diagnosis of *H. Pylori* in 91% of biopsies, a finding that is more than the studies published by Patnayak R *et al* (62%), Kacar F *et al* (85.71% *H. Pylori* positive patients) and Dogar T *et al* (32.85%). [10-12]

In the present study, H&E stain detected bacteria in 78.5% of instances, Giemsa stain detected more bacteria in 56 biopsies (85.7%), and immunohistochemical stain detected the most bacteria (91%).

Similar findings were observed by Patnayak R *et al*, Kacar F *et al* and Dogar T *et al* in several additional research.

It was observed that identification of *H. pylori* was more accurate with modified giemsa stain as compared to H&E stain. But the main drawback of this stain is the lack of contrast between tissue and organisms.

However, there is difficulty in detecting minimal bacteria present and the coccoid types of bacilli with both routine and specialized histochemical techniques. The IHC approach is not hampered by these issues while detecting bacilli and therefore it can identify maximum number of gastritis cases with *H. pylori* infection.

## Conclusion

IHC should be performed on biopsies with non-specific chronic active gastritis in the absence of *H. pylori* on standard stains to look for potential scanty or coccoid forms of organisms. Albeit being regarded as a gold standard in the resource-constrained scenario, upfront adoption of IHC may not be cost-effective. Use of IHC stain as a second line in the case of an equivocal H&E-Giemsa result is more suitable.

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