

# Histomorphological Interpretation of Gastric Endoscopic Mucosal Biopsies in Dyspeptic Patients with Special Reference to Immunohistochemical Demonstration of Helicobacter Pylori

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**Abstract:** ***Background & Objectives:** The aim of this study was to evaluate the role of endoscopic biopsies for detection of Helicobacter Pylori to see the frequency of this infection and histological changes that are strongly associated with it, in our setup. Fifty three cases of endoscopic biopsies were included in the study. These patients had a clinical history of dyspeptic symptoms, especially those with chronic gastritis. **Results:** Of the total 53 cases studied there was a clear male preponderance with M: F ratio of 2:1 with a mean age of 46.5 years. Of the 53 cases of studied most of them had chronic H.pyloric gastritis [n=20 (44.4%)] followed by chronic gastritis with non specific features [n=18]. 25 of the 53 cases showed positivity for H pylori which was confirmed with IHC of which 20 (32.7%) were chronic H.pyloric gastritis. Carcinoma was seen in 10 cases with diffuse type of adenocarcinoma in 7(70.0%) 2 (20.0%) cases of intestinal adenocarcinoma and 1 (10.0%) case of mucinous carcinoma of which 4 cases showed carcinoma positive for H.pylori. The modified Giemsa is the cheapest and easiest to perform technically but antibodies directed against specific antigens in IHC proved to be more specific in identifying H.Pylori than other staining techniques. **Interpretation and conclusion:** In conclusion Gastric biopsy brings important practical supplement to endoscopic examination in revealing the presence of H. pylori and also some serious pre-malignant dysplasia or malignant gastric lesions. Our study also highlighted the association of Helicobacter Pylori in patients with functional dyspepsia and proving Immunohistochemistry being gold standard in picking up Helicobacter Pylori with Geimsa being practically applicable in Indian set up keeping the cost factor in mind.*

**Keywords:** Dyspepsia, Endoscopy, Helicobacter Pylori, Geimsa, IHC

## 1. Introduction

*Dyspepsia* (dys + Greek peptin to digest) is defined as upper abdominal discomfort, often chronic or persistent, colloquially referred to as indigestion.

Endoscopy is the most appropriate investigation to detect pathological lesions in the alimentary tract as well as the presence Helicobacter pylori to ensure that the patient receives the most appropriate treatment.

The discovery of Helicobacter pylori (H.pylori) and the acceptance of its role in gastric pathophysiology represent a fundamental change in our understanding of gastroduodenal disease. H.pylori infection of the gastric mucosa can be found in majority of population and is associated with a range of pathologies, including chronic gastritis, peptic ulcer disease, atrophic gastritis, gastric MALT lymphoma and gastric adenocarcinoma. Infection with Helicobacter pylori is carcinogenic to humans (Group 1 carcinogen). The subsequent discovery of the pivotal role of Helicobacter pylori in a wide range of conditions has revolutionized our understanding of gastroduodenal diseases. Improvements in diagnostic and therapeutic options, combined with the gradual acceptance of the aetiological role of an infective agent in peptic disease, have led to a remarkable change in the management of gastroduodenal conditions in the past decade.

Another important cause for peptic ulcer disease can be attributed to NSAID induced mucosal damage.

In view of this pathogenetic importance, accurate diagnosis of infection is essential to institute eradication treatment in appropriate cases. Various techniques are used for this purpose, including serology, culture, rapid urease test, 13C-urea breath test, and histology. With advances in endoscopic techniques, biopsy can be easily obtained from the site intended with precision. Biopsies are obtained using flexible fiberoptic endoscopes provide with either an optical image or an electronic video image, which has aided in better diagnosis. The histological identification of H.pylori infection is now a widely used means of diagnosis. To achieve this, several staining methods are in use.

These include modified Giemsa, Warthin-Starry, Genta and immunohistochemical H.pylori antibody stains. Immunohistochemistry is the agreed “gold standard” for histology, being a highly sensitive and specific staining method. However, the modified Giemsa stain is the method of choice because it is sensitive, cheap, easy to perform, and reproducible.

The Sydney System for the classification of gastritis emphasized the importance of combining topographical, morphological and etiological information into a schema that would help to generate reproducible and clinically useful diagnoses. The Sydney system and its modifications

are the most widely used method for the standardized reporting of gastric biopsies.

The infection can be eradicated successfully with several regimens in which different drugs are combined. Eradication of *H. pylori* resolves gastritis, prevents recurrence of peptic ulcer disease.

In view of the above this study had been taken to find out various histopathological gastric mucosal lesions in patients with dyspepsia and compare the efficacy of H&E, Giemsa and immunohistochemical staining techniques in the detection of *H. pylori*.

### 1.1 Aims and Objectives

- 1) To study the morphological lesions associated with dyspepsia.
- 2) To study the association of *Helicobacter Pylori* in all biopsies using Hematoxyline and Eosin, Giemsa and Immunohistochemistry staining techniques and their efficacy.
- 3) To study the most common gastric pathological lesion associated with *Helicobacter Pylori*.
- 4) To provide vital data for subsequent treatment regimen.

## 2. Material and Methods

**Source of Data:** Study was done in Pathology department of Rajendra Institute of Medical Sciences, Ranchi, Jharkhand in collaboration with Department of Surgery, RIMS, Ranchi, Jharkhand.

**Collection of Data:** Biopsies were fixed in 10% formalin and histopathological slides were prepared for staining with Hematoxyline and Eosin, Giemsa and Immunohistochemical methods using paraffin blocks.

**Inclusion Criteria:** The study included all biopsies which were done for various chronic upper abdominal symptoms abdominal pain, dyspepsia, heartburn, nausea, vomiting and also for associated systemic manifestations like anorexia, weight loss.

**Exclusion Criteria:** Acute symptoms, Patients on *H. pylori* eradication therapy, autolysed specimens

**Study Sample Design:** Prospective study

**Method:** Grading of the variable were done in reference to Sydney classification as given by Aydin O et al. The data was analyzed using SPSS version 20. Microsoft word and Excel have been used to generate graphs, tables.

#### Number of Cases:

A total of 53 patients with symptoms of dyspepsia are selected for the study.

#### Staining Technique:

H&E stained sections of all the cases were reviewed. All endoscopic gastric mucosal biopsies taken from different sites were brought in 10% buffered formalin and were oriented with submucosa embedded downwards. After

overnight fixation in formalin, dehydration done with graded alcohol, clearing in chloroform followed by paraffin embedding and section cutting in rotary microtome. Sections of 3µm thicknesses will be made & stained with H&E, Giemsa and immunohistochemical stains. Sections for IHC were specially taken on poly-L-lysine(PLL) coated slides. Morphological changes in all cases were studied with special reference to atrophy, dysplasia, metaplasia, neutrophilic infiltration, lymphoid follicle formation, plasma cell infiltration & malignant changes along with identification of *H. pylori*.

#### Hematoxylin and Eosin: Procedure

- 1) Dewax sections, hydrate through graded alcohol to water
  - 2) Remove fixation pigments
  - 3) Stain in alum hematoxylin for 5 minutes
  - 4) Wash in running tap water until sections blue for 5 minutes or less
  - 5) Differentiate in 1% acid alcohol for 5-10 seconds
  - 6) Wash well in tap water until sections are again blue
  - 7) Blue by dipping in an alkaline solution (ammonia water) followed by a 5 min tap water wash
  - 8) Counter stain in 1% eosin Y for 30 seconds to 1 minute
  - 9) Dehydrate, clear and mount in DPX
- Result: Nucleus- Blue, Cytoplasm and connective tissue- Shades of pink

#### Giemsa Stain: Procedure

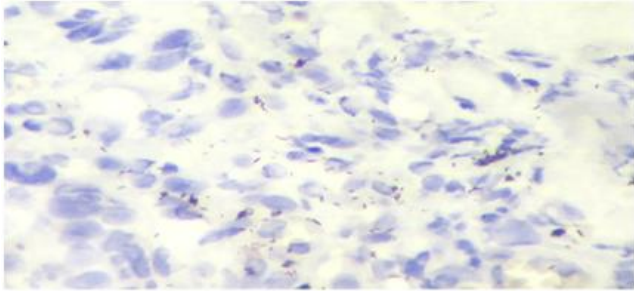
- 1) Bring section down to water
- 2) Giemsa stain -5min
- 3) Blot
- 4) Quick dehydration in alcohol
- 5) Clear in xylene
- 6) Mount in DPX

Results: *H. pylori*-Dark Blue, Background -Pink to pale blue

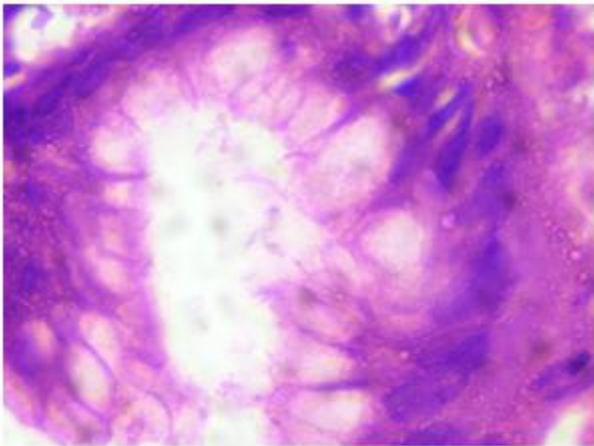
#### Immunohistochemistry

- 1) Formalin fixed paraffin embedded sections are taken
- 2) Incubate for few hours
- 3) Sections are deparaffinised 2 changes of xylene and rehydrated with 2 changes of alcohol
- 4) Primary blocking is done using hydrogen peroxide
- 5) Heat Antigen retrieval by using decloaking chamber
- 6) Secondary blocking done by bovine serum albumin
- 7) Incubate with mouse monoclonal primary antibody (specific for *H. pylori*) for 30 minutes followed by MACH2 secondary antibody
- 8) 3, 3-diaminobenzidine as chromogen for 5 minutes and Hematoxyline as counter stain
- 9) Counter stain with hematoxyline
- 10) Dehydrate in alcohol, clear in xylene and mount it

Results: Small brown curved shaped bacterium seen clearly using 100x oil objective under microscope.



Photomicrograph of IHC Positive H. Pylori in Adenocarcinoma (40X)



Photomicrograph of H. pylori positive in H&E with few small curved organisms (100X)

### 3. Results

A study of 53 cases of endoscopic biopsies was carried out in Pathology department of RIMS in collaboration with Department of Surgery, RIMS, Ranchi, Jharkhand. Biopsies were fixed in 10% formalin and histopathological slides were prepared for staining with Hematoxyline and Eosin, Geimsa and Immunohistochemical methods using paraffin blocks.

Analysis of the clinical and histopathological findings:

- 1) The present study included 53 gastric biopsy specimens
- 2) The mean age of presentation was 46.5 years and peak incidence was seen in 51-60 years.
- 3) The present study shows dyspepsia in 83%.
- 4) Most frequent site sampled for biopsy was from gastric antrum constituting 46 cases (86.8%).
- 5) Of the 53 cases of studied most of them had chronic H. pyloric gastritis [n=20 (44.4%)] followed by chronic gastritis with non specific features [n=18(38.6%)].
- 6) 25 of the 53 cases showed positivity for H. pylori which was confirmed with IHC of which 20 (32.7%) were chronic H. pyloric gastritis. 4 cases which showed carcinoma also were positive for H. pylori.
- 7) Carcinoma was seen in 10 cases with the most common being Diffuse type of adenocarcinoma with signet ring features 7(70.0%). 2 (20.0%) cases of Intestinal type of adenocarcinoma and 1 (10.0%) case of mucinous carcinoma.
- 8) Of the total 25 cases positive for H. pylori in IHC only 7 cases showed positivity in Hematoxyline and eosin. As compared to Giemsa which showed positivity in 23 cases of which 3 cases were negative in IHC.

- 9) Spiral type of distribution pattern was the most common type seen in 11 cases with small curved bacilli seen.

### 4. Conclusion

Study of gastric mucosal biopsies is useful in distinguishing non neoplastic, benign and malignant lesions and thereby aid in further treatment protocol.

With the diagnosis of various lesions like chronic non specific gastritis, H. pyloric gastritis, adenocarcinoma and other infectious causes like cytomegalovirus gastritis, it has become a requisite to undergo endoscopic evaluation of stomach with biopsy taken if indicated. This supplemented with special stains helps in prompt identification of the pathogen Helicobacter Pylori thereby alleviate the symptoms and prevents its sequelae.

Therefore this study highlights the importance of endoscopic biopsies, identification of the bacilli, various gastric lesions and gives a good comparison between different staining techniques with IHC being gold standard but Geimsa being relatively cheap and less time consuming than IHC in our set up.

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