



## A COMPARATIVE STUDY OF HEMATOXYLIN & EOSIN, SPECIAL STAINS AND IMMUNOHISTOCHEMISTRY FOR DETECTION OF HELICOBACTER PYLORI IN CASES OF GASTRITIS

**Dr. Umasankari. L. Ramasamy**

Assistant Professor, Department Of Pathology, Madurai Medical College.

**Dr. Manjula Rajendran\***

Assistant Professor, Department Of Pathology, Madurai Medical College.  
\*Corresponding Author

### ABSTRACT

**Background:** The role of Helicobacter Pylori in the pathogenesis of Gastritis-Peptic ulcer syndrome and its association with the development of upper gastrointestinal tract malignancy warrant efficient method for the identification of the bacteria in biopsy specimens. Four methods - Hematoxylin and Eosin, Giemsa, Toluidine Blue and Immunohistochemistry were compared to detect the presence of Helicobacter pylori. The prevalence of H.Pylori in gastric biopsies was also evaluated.

**Aim Of The Study:** The aim is to evaluate the prevalence of H.Pylori in cases of gastritis and compare the efficacy of Hematoxylin and Eosin, Giemsa, Toluidine blue and Immunohistochemistry for the detection of H.pylori in cases of gastritis.

**Materials And Methods:** This Retrospective study was done in the Department of Pathology. A total of 40 cases of gastritis were randomly selected for this study and all the four methods were applied.

**Results:** Helicobacter pylori infection showed an overall prevalence rate of about 75%. When compared with Immunohistochemistry, Sensitivity and specificity of Hematoxylin and Eosin was 66.67% and 100% respectively. Giemsa showed sensitivity of about 86.67% and specificity of about 100%. Toluidine blue showed sensitivity of about 80% and specificity of about 100%.

**Conclusion:** Hence in the present study Giemsa was more reliable and cost effective stain when compared with Hematoxylin & Eosin, Toluidine blue and immunohistochemistry. However, Immunohistochemistry carries the highest level of sensitivity in the detection of Helicobacter Pylori especially when the density of organism is low and in clinically suspected cases of Helicobacter Pylori with negative Giemsa staining

**KEYWORDS :** Helicobacter pylori, Hematoxylin & Eosin, Giemsa, Toluidine blue, Immunohistochemistry.

### INTRODUCTION:

Helicobacter pylori plays an important role in the causation of numerous benign, premalignant and malignant lesions of Gastrointestinal tract which include peptic ulcer, gastritis, intestinal metaplasia, gastric adenocarcinoma and Mucosa – associated lymphoid tissue lymphoma. H.pylori affects more than 50% of individuals in developed countries and 90% in developing countries. Numerous methods are available for the diagnosis of H.pylori. They are classified into 2 groups. Non invasive methods are urea breath test, serology and faecal antigen test. Invasive methods – include rapid urease test, Polymerase Chain Reaction, histopathological examination and culture. Histopathological examination remains the gold standard for the identification of H.pylori because it is possible to identify various pathogenic changes associated with this infection such as inflammation, intestinal metaplasia, atrophy and malignancy. In 1994 (WHO) World Health Organisation and the International Agency for Research on Cancer (IARC) classified H.pylori infection as a group I carcinogen in humans. The updated Sydney system graded gastritis based on several parameters which includes inflammation, activity, intestinal metaplasia, atrophy and density of H.pylori.

Several histochemical stains are available for detecting the presence of H.pylori in gastric biopsies and resection specimens which include Hematoxylin and Eosin (H&E), Toluidine blue, Modified Giemsa, Alcian yellow – toluidine blue, Warthin-starry, Modified Genta and Immunohistochemistry staining. H&E and Giemsa are more commonly used. Several studies have been conducted about the need for use of special stains and immunohistochemistry in H.pylori detection.

### AIM OF THE STUDY:

The aim of the present study is to evaluate the prevalence of H.Pylori in cases of gastritis and compare the efficacy of Hematoxylin and Eosin, Giemsa, Toluidine blue and

Immunohistochemistry for the detection of H.pylori in cases of gastritis.

### Materials And Methods:

This study is a retrospective study carried out in the Department of Pathology. A total of 150 cases of gastritis were received during the period of one year and out of this, 40 cases of gastritis were randomly selected for this study. Endoscopic biopsies and resected specimens of gastric lesions were included for this study. Relevant clinical details (age, sex) and investigations were collected from the medical records of Pathology. Corresponding histopathological slides prepared from formalin fixed paraffin embedded tissue of both endoscopic biopsies and resected specimens of gastric lesions were subjected to Hematoxylin & Eosin staining and studied. Sections from gastritis cases had been categorized using Sydney grading system and the results were tabulated. Special stains (Giemsa, Toluidine blue) and Immuno his to chemical study using Helicobacter pylori polyclonal antibody were done in 40 gastritis cases.

The distribution of infection with Helicobacter pylori among different races, age groups, different population among various countries and level of socio economic status and to detect the presence of Helicobacter pylori using Hematoxylin and Eosin, Giemsa, Toluidine Blue and Immuno his to chemistry and their difference in staining pattern of H.Pylori according to the grading features of gastritis.

### Definition Of Study Variable:

#### Sydney Grading System Of Gastritis

In the updated Sydney system Several histopathological variables were graded as mild, moderate and severe

- Graded variables include
- Neutrophilic infiltration
  - Mononuclear infiltration
  - Helicobacter pylori density
  - Atrophy
  - Intestinal metaplasia and dysplasia

For accurate detection of H.pylori ,biopsy from multiple sites including two corpus and two antral specimens recommended.

**GRADED VARIABLES :**

**A) H. Pylori Density :**

The variations in the density of H.pylori may have epidemiological importance and have an association with many diseases.

**B) Polymorphonuclear Neutrophil Activity :**

It measures the rate of acute inflammation. Tissue damage is usually caused by proteases and reactive oxygen species which are derived from neutrophils which are seen with in the epithelium, in lamina propria and also in the foveolar lumen forming "pit abscesses".

**C) Chronic Inflammation :**

Scattered chronic inflammatory cells are normally seen in the gastric mucosa. In H.pylori infection there is increase in the density of lymphocytes, monocytes, eosinophils, plasma cells and mast cells.

**D) Glandular Atrophy** which results from severe inflammation or follow ulceration or erosion of the mucosa and always look for evidence of intestinal metaplasia which replaces the antral epithelium and carries a higher level of risk for the development of gastric cancer .

**E) Intestinal Metaplasia** most common feature in all forms of chronic gastritis. Intestinal metaplasia is classified into 3 types based on its glycoprotein content and morphology by using mucin histochemistry .

**NON-GRADED VARIABLES:**

Surface epithelial damage, erosion and mucin depletion, Lymphoid follicles, Foveolar hyperplasia, Pseudopyloric metaplasia ,Pancreatic (acinar) metaplasia and Endocrine cell hyperplasia are the features seen in gastritis.

**Table-1: Sydney Grading System Of Gastritis**

Features	Grade		
Chronic Inflammation	Mild	Moderate	Severe
Atrophy	Mild	Moderate	Severe
Activity	< 1/3 of Pits mild	1/3 to 2/3 moderate	> 2/3 Severe
Intestinal Metaplasia	Mild	Moderate	Severe
H.Pylori colonisation	< 1/3 of surface mild	1/3 to 2/3 moderate	> 2/3 Severe

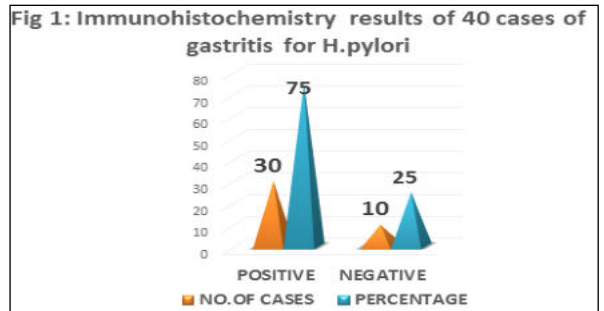
**OBSERVATION AND RESULTS:**

Out of 40 patients of gastritis , highest incidence (57.5%) was seen in the age group of 41-60 years (23 out of 40) in both gender which is a significant factor and shows a male predominance in this study (25 out of 40 about 62.5%). (13)

**Table - 2: Sydney Scoring In 40 Cases Of Gastritis Presented With Symptoms**

Sydney Score	Acti vity	Chronic Inflammation	Intestinal Metaplasia	Atro phy	Helicobacter Pylori
1	17	18	3	12	10
2	1	17	0	0	7
3	0	5	0	0	3

In this study of 40 cases of gastritis , maximum number of cases were seen in the Sydney score of 1 in all graded variables and the positivity of Helicobacter Pylori on H&E stain. Out of 40 cases of gastritis, positivity for H.Pylori was seen (50%) in H&E stain, (65%) in Giemsa stain, (60%) in Toluidine blue stain and (75%) in IHC which show maximum positivity .



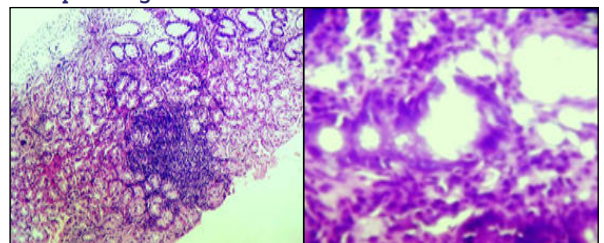
Out of 40 cases of gastritis studied for Helicobacter pylori with immunohistochemistry 30 cases showed positivity.

**Table -3: Grading Of H. Pylori Infection In Gastric Biopsies Using Sydney Scoring System In Various Staining Methods Like H&E, Giemsa, Toluidine Blue And IHC**

Staining Method	Total No. Of Cases	H.pyl ori Positive	Grade 1	Grade 2	Grade 3	H.pylori Negative
IHC	40	30(75%)	19(47.5%)	8(20%)	3(7.5%)	10(25%)
GIEMSA	40	26(65%)	15(37.5%)	8(20%)	3(7.5%)	14(35%)
TOLUIDINE BLUE	40	24(60%)	13(32.5%)	8(20%)	3(7.5%)	16(40%)
H&E	40	20(50%)	10(25%)	7(17.5%)	3(7.5%)	20(50%)

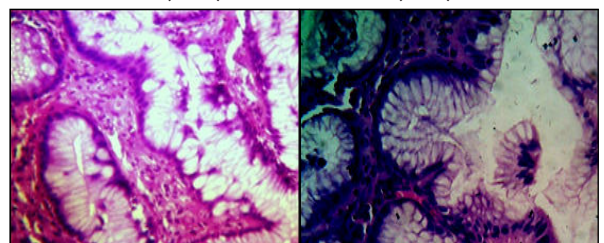
From this table it was shown that detection of Helicobacter pylori using H&E, Giemsa, Toluidine blue and IHC was identical in cases of grade 2 and grade 3 colonisation of H.pylori. Differences in staining pattern were observed in grade 1 when there is low colonisation of H.pylori. Out of 40 cases , IHC showed positivity for Helicobacter pylori in 30 cases,4 cases which were negative by Giemsa stain was found to be positive in IHC. Both Giemsa and IHC showed negativity in 10 cases. Out of 40 cases, IHC showed positivity for Helicobacter pylori in 30 cases,6 cases which were negative by Toluidine blue stain was found to be positive in IHC. Both Toluidine blue and IHC showed negativity in 10 cases. So overall sensitivity of IHC is more than 80% and specificity of 100%.

**Histopathological Examination Pictures:**



**Fig.2 :** Lamina Propria With Lymphoid Follicle Formation- H&E (100x)

**Fig.3 :** Presence Of Intraepithelial Neutrophils H&e (400x)



**Fig.4:** Intestinal Metaplasia In Antral mucosa- H&E (400x)

**Fig.5:** Helicobacter Pylori colonisation-H&E (400x)

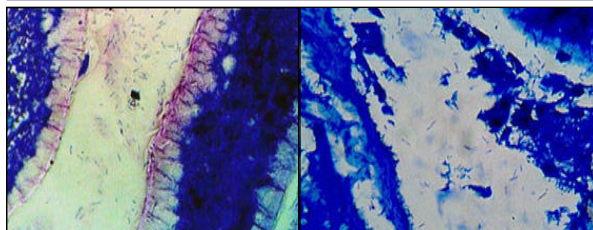


Fig.6 : Helicobacter Pylori Grade 3 Colonisation- Giemsa (400x)

Fig.7: Helicobacter Pylori Grade 2 Colonisation- toluidine Blue (400x)

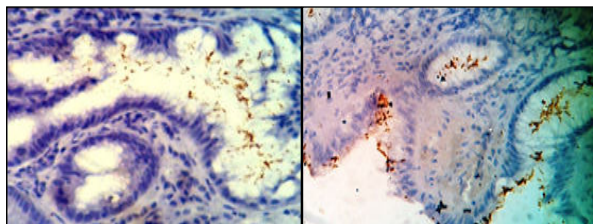


Fig.8: Helicobacter Pylori grade 2 colonisation- IHC (100x)

Fig.9: Helicobacter Pylori grade 3 colonisation- IHC (100x)

#### DISCUSSION:

Helicobacter pylori infection has an important role in the etiology of several diseases of the gastrointestinal tract which include chronic active gastritis, peptic ulcer, Gastric adenocarcinoma and Mucosa - associated lymphoid tissue lymphoma. It affects more than 50% of the world wide human population. In this study to detect the presence of Helicobacter pylori Giemsa, Toluidine blue and IHC were done for 40 cases of gastritis .

In the present study of 40 cases of gastritis ,30 cases were positive for Helicobacter pylori with a overall prevalence rate of 75% .this is comparable with other studies.

Out of 40 patients of gastritis , highest incidence (57.5%) was seen in the age group of 41-60 years (23 out of 40) which is also reported in other studies,in both gender which is a significant factor and shows a male predominance in this study (25 out of 40 about 62.5%).

The distribution of infection with Helicobacter pylori varies widely among different races, age groups, different population among various countries and level of socio economic status.

Due to lack of contrast between the surrounding tissue and the bacteria the H&E stain carries low sensitivity. The specificity is also low due to non specific staining of other bacteria which is seen in the stomach.Modified Giemsa is a simple, rapid procedure at low cost. It provides reliable results with acceptable levels of sensitivity and specificity.IHC carries high level of sensitivity and specificity for detection of Helicobacter pylori but it is a time consuming technique and also expensive.

In 40 cases of gastritis H&E showed 66.67% sensitivity and specificity of about 100%,Giemsa showed 86.67 % sensitivity and specificity of about 100%. Toluidine blue showed 80% sensitivity and specificity of about 100% which is comparable with the study by shukla et al for H.Pylori detection among 102 patients H&E showed sensitivity of about 72.5% and specificity of about 100%.Giemsa showed sensitivity of about 80.4% and specificity of about 100%. Another study among 111 patients for H.Pylori by J.E Tzeng et al the sensitivity of H&E was 98.5% and specificity was 100%. Giemsa showed sensitivity of about 98.5% and specificity of about 97.8%.Raziye Tajalli et al in their study among 54 patients for H.Pylori detection found that the sensitivity of H&E was 41.86% and specificity was 100%.

Giemsa showed sensitivity of about 53.49% and specificity of about 95.24%. Toluidine Blue showed sensitivity of about 76.74% and specificity of about 100%.

To summarise ,40 cases of gastritis were selected randomly for this study to detect the presence of Helicobacter pylori using Hematoxylin and Eosin, Giemsa, Toluidine Blue and Immunohistochemistry.

- In this study the most common age group infected with Helicobacter pylori was seen between 41-60 years in gastritis
- In the present study of 40 cases of gastritis, 30 cases were positive for Helicobacter pylori with a prevalence rate of about 75%
- Males showed a maximum percentage of positivity in this study
- From this study it was shown that detection of Helicobacter pylori using Hematoxylin & Eosin ,Giemsa, Toluidine blue and Immunohistochemistry were identical in cases of grade 2 and grade 3 colonisation of H.pylori. Differences in staining pattern were observed in grade 1 when there is low colonisation of H.pylori , where maximum number of cases were detected by immunohistochemistry.
- Hematoxylin and Eosin showed sensitivity of about 66.67% and specificity of about 100% .
- Giemsa showed sensitivity of about 86.67% and specificity of about 100%.
- Toluidine blue showed sensitivity of about 80% and specificity of about 100%.

#### CONCLUSION:

In histopathological sections the identification of Helicobacter pylori can be done by various staining methods among which Toluidine blue and Giemsa methods are inexpensive and reliable. The major disadvantage is little contrast between the tissues and the bacteria. Immunohistochemistry is the most sensitive technique but it is expensive and time consuming. It is not economical to use in all gastric specimens except certain specific situations like Low density or coccoid forms of organisms and inactive gastritis where the other stains carry low rate of detection for Helicobacter pylori. The cost, reliability and applicability of Giemsa and Toluidine Blue make them as suitable stains for identification of Helicobacter pylori in gastric biopsies. In this study Giemsa stain carries higher level of sensitivity over toluidine blue ,H&E and less time consuming procedure when compared with IHC. Hence in the present study Giemsa was more reliable and cost effective stain when compared with Hematoxylin & Eosin, Toluidine blue and immuno his to chemistry. However, Immunohistochemistry carries the highest level of sensitivity in the detection of Helicobacter Pylori especially when the density of organism is low and in clinically suspected cases of Helicobacter Pylori with negative Giemsa staining.

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