Original Research Paper

Volume - 12 | Issue - 05 | May - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pathology

COMPARING THE EFFICACY OF HEMATOXYLIN & EOSIN, GIEMSA , TOLUIDINE BLUE AND IMMUNOHISTOCHEMISTRY FOR DETECTION OF HELICOBACTER PYLORI IN CASES OF GASTRIC ADENOCARCINOMA

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ABSTRACT

BACKGROUND: 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. In histopathological sections Helicobacter pylori can be identified by various staining methods like Hematoxylin and Eosin (H&E), Toluidine blue, Modified Giemsa, Alcianyellow –Toluidine blue, Warthin-starry, Modified Genta and Immunohistochemistry.

AIM OF THE STUDY: The aim of the present study is to compare the efficacy of Hematoxylin and Eosin, Giemsa , Toluidine blue and Immunohistochemistry for the detection of H.pylori in cases of gastric adenocarcinoma.

MATERIALS AND METHODS: A total of 30 cases of gastric adenocarcinoma were randomly selected for this Retrospective study conducted in Department of Pathology and all the four stains were applied and Immunohistochemical study using Helicobacter pylori polyclonal antibody were also done in all cases.

RESULTS: Helicobacter pylori infection showed an overall prevalence rate of about 56.6%. When compared with Immunohistochemistry, Sensitivity and specificity of Hematoxylin and Eosin was 41.18% and 100% respectively. Giemsa showed sensitivity of about 88.23% and specificity of about 100%. Toluidine blue showed sensitivity of about 76.47% and specificity of about 100%.

CONCLUSION: Hence in the present study, Immunohistochemistry carries the highest level of sensitivity in the detection of Helicobacter Pylori. 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 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[1] .It plays main etiological role in the development of gastric adenocarcinoma .Gastric cancer was the second most common cancer in the world. 60% of cases were usually seen in developing countries [2,3]. Several studies emphazise that there is increased risk in patients who had anti-H.pylori antibodies in stored serum samples 10 or more years before the diagnosis of cancer [4,5]. H.pylori causes several phenotypic changes which leads to the development of adenocarcinoma.

These changes includes mucosal atrophy , intestinal metaplasia and Dysplasia. H-pylori also produces vac A which is a vacuolating cytotoxin which plays a role in gastric carcinogenesis and also causes epithelial cell damage. Inoculation of cag and vac A positive strain in Mongolian gerbils [6] produces intestinal metaplasia and gastric carcinoma which confirms the role of H.pylori in gastric carcinogenesis. In histopathological sections Helicobacter pylori can be identified by various staining methods like Hematoxylin and Eosin (H&E), Toluidine blue, Modified Giemsa, Alcianyellow – Toluidine blue, Warthin-starry, Modified Genta and Immunohistochemistry.

AIM OF THE STUDY:

The aim of the present study is to compare the efficacy of Hematoxylin and Eosin, Giemsa , Toluidine blue and Immunohistochemistry for the detection of H.pylori in cases of gastric adenocarcinoma.

MATERIALS AND METHODS:

This study is a retrospective study carried out in the Department of Pathology. A total of 90 cases of gastric adenocarcinoma were received during the period of one year and out of this, 30 cases of gastric adenocarcinoma were randomly selected 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. Cases of Gastric adenocarcinoma were graded into Well, Moderate and Poorly differentiated. Special stains (Giemsa, Toluidine blue) and Immunohistochemical study using Helicobacter pylori polyclonal antibody were done in 30 cases of gastric adenocarcinoma.

DEFINITION OF STUDY VARIABLE:

GRADING OF GASTRIC ADENOCARCINOMA:[3]

a. **Well differentiated:** It is composed of well formed glands which have the appearance of metaplastic intestinal epithelium.
b. **Moderately differentiated:** It forms the intermediate category between well and poorly differentiated forms.
c. **Poorly differentiated:** It is composed of tumor cells that are scattered singly or seen in small groups with mucin secretions. Glandular structures are rarely seen.

Well and moderately differentiated tumors are considered as low grade and poorly differentiated tumors as high grade.

ADENOCARCINOMA WITH H.PYLORI IN DIFFERENT STAINS

Fig 1: Adenocarcinoma Stomach

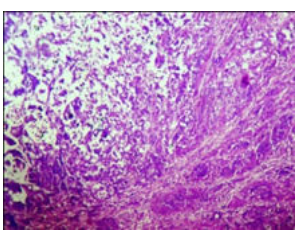

Fig 2: Infiltrating Adenocarcinoma Stomach - Moderately Differentiated- H&e (100x)

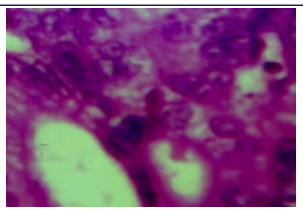

Fig 3: H.pylori Colonisation In Gastric Adenocarcinoma H&e (400x)

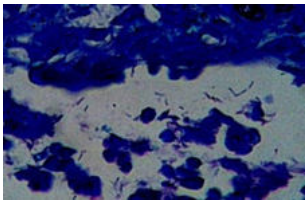

Fig 4: H.pylori Colonisation In Gastric Adenocarcinoma- Giemsa (400x)

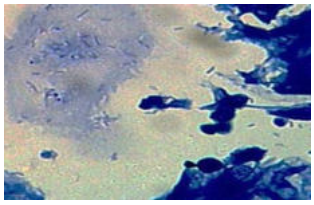

Fig 5: H.pylori Colonisation In Gastric Adenocarcinoma Toluidine Blue (400x)

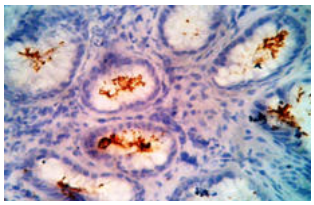

Fig 6: H.pylori Colonisation- Ihc (100x)

OBSERVATION AND RESULTS:

Table 1: Positivity Percentage Of H.pylori Among Various Stains

GENDER	STAINING METHODS							
	H & E		GIEMSA		TOLUDINE BLUE		IHC	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
MALE (23)	5	18	13	10	11	12	15	8
FEMALE (7)	2	5	2	5	2	5	2	5
TOTAL (30)	7 **(23%)**	23	15 **(50%)**	15	13 **(43%)**	17	17**(56.6%)**	13

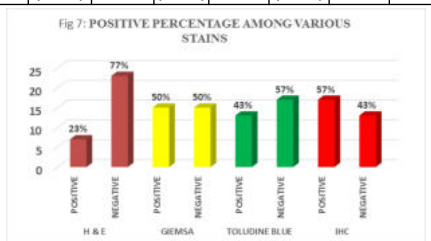

Fig 8: Grading Of Adenocarcinoma Among 30 Cases

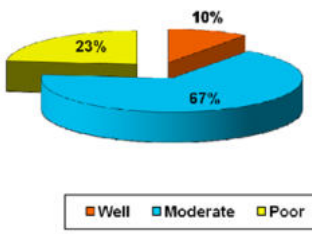

Table 2: Immunohistochamistry Results Showing The Prevalence Of H.pylori In Gastric Adenocarcinoma In Different Grades

GRADE	Total No. of Cases	H.Pylori Positive	H.Pylori Negative
Well Differentiated	3	2(66.6%)	1(33.4%)
Moderately Differentiated	20	11(55%)	9(45%)
Poorly Differentiated	7	4(57%)	3(43%)

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.

Out of 30 patients with gastric adenocarcinoma highest incidence was seen in the age group of 41-60 years (14 out of 30 about 46.7%) and positivity for H.Pylori was seen in 7 out of 30 (23%) in H&E stain,15 out of 30 (50%) in Giemsa stain 13 out of 30 (43%) in Toluidine blue stain,17 out of 30 cases (56.6%) in IHC. High prevalence of H.Pylori (56.6%) were found among adenocarcinoma in our IHC testing , the prevalence is the same as in a study conducted by Adisa et al [7] which showed positivity of 345 out of 603 cases and the prevalence rate (57.2%). In a study by Dr,VishwaPriya.M. Godkhindi et al [8] out of 110 cases Helicobacter pylori was positive in 69 cases with an overall prevalence rate of 62.72%. Javed et al [9] in his study found that the prevalence of Helicobacter pylori infection was maximum in the age group of 30 -50 years.In this present study the most common age group affected with Helicobacter pylori was seen between 41-60 years .In a study conducted by Dr.Vishwapriya. M. Godkhindi et al [8] among 14 cases of Adenocarcinoma (7.14%) 1 showed positivity for Helicobacter pylori. In the study by WYatt, JI Semin Diagn pathol [10] Helicobacter pylori positivity was seen in 50% of cases of Adenocarcinoma.In the present study among 30 cases of gastric Adenocarcinoma 17 cases (56.6%) showed Helicobacter pylori positivity.

Helicobacter pylori has an important role in the pathogenesis of upper gastrointestinal tract malignancy. So, early detection and eradication of this organism is essential for the prevention of gastric cancer. 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 30 cases of gastric adenocarcinoma comparing to Immunohistochemistry detection of H.Pylori, the H&E showed sensitivity of about 41.18% and specificity of about 100%.Giemsa showed sensitivity of about 88.23% and specificity of about 100%. Toluidine blue showed sensitivity of about 76.47 % and specificity of about 100% and these results are same as in the study by Raziye Tajalli et al[11] which showed among 54 patients for H.Pylori detection 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%. In a study by shukla et al[12] for H.Pylori detection among 102 patients H&E showed sensitivity of about 72.5% and specificity of about100%.Giemsa showed sensitivity of about 80.4% and specificity of about 100%.

CONCLUSION:

In histopathological sections Helicobacter pylori can be identified by various staining methods like Hematoxylin and Eosin (H&E), Toluidine blue, Modified Giemsa, Alcianyellow – Toluidine blue, Warthin-starry, Modified Genta and Immunohistochemistry. 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 Immunohistochemistry in all gastric specimens. It is used in certain specific situations like ; Low density of organisms,to identify coccoid

forms 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 and H&E Giemsa stain is also a 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 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.

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