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A Study On Different Histopathlogical Stains Used In Identification Of Helicobacter Pylori Infection In Association With Gastro-Duodenal Lesions

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Abstract

Introduction

Gastro duodenal disorders are a frequent cause of clinical disease, with inflammatory and neoplastic lesions being particularly common. Endoscopy helps to detect pathological lesions in the alimentary tract and to obtain mucosal biopsies for histopathological examination and presence of Helicobacter pylori to ensure that the most appropriate treatment is given to the patient. The main aim of the present study is to find the best special stain in the detection of H.pylori in biopsy specimens.

Objectives:

To evaluate the use of various histopathological stains in the detection of Helicobacter pylori in biopsy specimens also to study the association between Helicobacter pylori and the various gastroduodenal lesions.

Methodology

The present study is a prospective study which was conducted on the histopathologic and endoscopic assessment of 220 gastro duodenal endoscopic biopsies at the Department of Pathology of Tertiary care teaching hospital

Results

Out of 220 gastro duodenal biopsies, 204(92.73%) were from stomach and 16(7.27%) from duodenum. Age distribution ranged from 16-85 years, commonest age group was 41-50 years. 127(57.72%) were males and 93(42.27%) were females. In our present study, demonstration of H.pylori was possible by all the three staining methods, but were particularly appreciable in Warthin starry silver stain in 126 cases (57.27%) followed by Giemsa stain in 120 cases (54.54%). The overall H. pylori positivity was 57.27%.

Conclusion

In this study chronic active gastritis was highly associated with H.pylori. Warthin starry silver stain shows high H.pylori positivity followed by giemsa stain.

Keywords: Endoscopy, Gastro duodenal, Histopathology, H.Pylori

Introduction

Gastro duodenal diseases are the most common diseases in adult population world wide.1The stomach and the duodenal disorders are the frequent causes of clinical diseases with inflammatory and neoplastic lesions.

The most appropriate investigation is the endoscopy, which helps to detect pathological lesions in the alimentary tract and the presence of Helicobacter pylori to ensure that the proper treatment is given to the patient.

H.pylori infection of the gastric mucosa can be associated with a wide range of pathologies, which includes peptic ulcer disease, chronic gastritis, atrophic gastritis, gastric MALT lymphoma and gastric adenocarcinoma. In view of its pathogenetic importance, it is essential for the accurate diagnosis of H.pylori to institute eradication treatment in appropriate cases. Nowadays, the widely used means of diagnosis is the histological identification of H.pylori infection. To achieve this, several staining methods such as the modified Giemsa, Warthin-Starry, Genta and immunohistochemical H.pylori antibody stains, are in use. However, the modified Giemsa stain is the most preferred method, since because it is cheaper, easy to perform, sensitive and reproducible.1 In existence of the above conditions, this study has been taken to find out various gastroduodenal mucosal lesions and their association with H.pylori infection and to compare the efficacy of H&E, Giemsa and Warthin-Starry staining techniques in the detection of H.pylori. Hence objectives of this study are to evaluate the use of various histopathological stains in the detection of Helicobacter pylori in biopsy specimens also to study the association between Helicobacter pylori and the various gastroduodenal lesions.

Materials & Methods

The present study is a prospective study which was conducted on the histopathologic and endoscopic assessment of 220 gastro duodenal endoscopic biopsies at the Department of Pathology of Tertiary care teaching hospital after obtaining the approval of Institutional Ethics Committee. Patients presenting with dyspeptic symptoms in outpatient department of Gastroenterology were examined by upper GI endoscopy. A detailed clinical history was taken and endoscopic biopsies were obtained from the gastric and duodenal lesions if present after getting an informed written consent. All the clinical and endoscopic findings of these cases along with diagnosis were noted down.

Critically ill patients whom not fit for endoscopy and biopsy,pregnant women., Children less than 12 years and Patients diagnosed and treated for Helicobacter pylori infections for past 6 months were excluded from the study

Patients were advised not to take food overnight till the procedure is over. The endoscopic procedure and its risks were explained to the patients and a written consent was obtained from the patients prior to endoscopy. The endoscopic findings were noted down and small bits of tissue from the representative areas were obtained, using a punch biopsy forceps. Tissue bits were preserved in 10% formalin for histopathological examination.

The data was analyzed using SPSS (Version 21.0). Chi square test was used to test association between two categorical variables. Microsoft word and Excel have been used to generate charts and tables.

RESULTS

In our study 220 biopsy cases were reported out of which gastric biopsies were the highest 204 cases (92.73%) and rest were duodenal biopsies 16 cases (7.27%). Among the total 220 cases of gastro duodenal biopsies, only 93(42.27%) were females and the rest 127 (57.72%) were males. Gastric biopsies were most common in males 119 cases (58.33%) than females 85 cases (41.66%). In case of duodenal biopsies, there was equal distribution in both males 8 cases (50.00%) and females 8 cases (50.00%).

Gastro-duodenal biopsies were most commonly performed in 4th decade. Gender wise, in males (33 cases), it is higher in 3rd decade of age and in females (31cases) it's again higher in 4th decade of age. In males, Gastric lesions were observed more in 3rd decade of age. In case of females, it is observed high in 4th decade of age.

Of the 220 cases reported, Chronic superficial gastritis 80 cases (39.22%) was observed to be more followed by chronic active gastritis 74 cases (36.27%). And the least one was dysplasia 1 case (0.49%). Gastric Carcinoma was observed in 8 cases (3.92%). Chronic superficial gastritis and chronic

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active gastritis in histopathology were the most common lesions. Inflammation was the most common endoscopic feature followed by ulcer and nodule.

Of the total 120 cases which are positive for H.pylori in Giemsa stain, only 32 cases showed positivity in H & E stain. The H & E stain showed 100% specificity but very low sensitivity (26.67%) in comparison with Giemsa stain. All 32 cases which showed H.pylori positivity in H&E was confirmed after Giemsa staining which was positive in that particular patient, thereby giving 100% specificity.

Of the total 126 cases which are positive for H.pylori in WSS stain, 120 cases showed positivity in Giemsa stain. 6 cases not detected in Giemsa stain were positive in WSS stain. The WSS stain showed 94% specificity with high sensitivity (100%) in comparison with Giemsa stain. This indicates that WSS stain is highly sensitive in identifying H.pylori than Giemsa stain.

Table No 1 : Evaluation Of All 3 Staining Techniques

Stains	Positive	Negative
H& E	32 (14.55%)	188 (85.45%)
GIEMSA	120 (54.55%)	100 (45.45%)
WSS	126 (57.27%)	94 (42.73%)

Amongst all the 3 staining techniques, WSS stain (57.27%) was more sensitive in identifying H.pylori followed by Giemsa stain (54.55%).

Antrum with 171cases (83.82%) was found to be the most common site for gastric lesions followed by body 26 cases (12.74%). H. Pylori were more commonly found in antrum 109 cases (86.50%) followed by body 15 cases (11.90%). H. pylori positivity was found to be more common in males than females. H.pylori positivity was found to attain peak at 3rd and 4th decade of age and started declining after 5th decade of age.

Table No 11:Gastro Duodenal Lesion Associated With H.Pylori

Histopathological diagnosis	H.pylori		Total	*p* value
	Positive	Negative		
Chronic superficial gastritis	48	32	80	0.536
Chronic active gastritis	63	11	74	0.000
CSG with IM	4	8	12	0.085
CAcG with IM	7	2	9	0.204
Congestive gastropathy	0	2	2	0.100
Erosive gastritis	3	13	16	0.001
Hyperplastic polyp	1	1	2	0.835
Dysplasia	0	1	1	0.246
Carcinoma	0	8	8	0.001
Duodenitis	0	12	12	0.000
Duodenal ulcer	0	1	1	0.246
Duodenal adenoma	0	2	2	0.100

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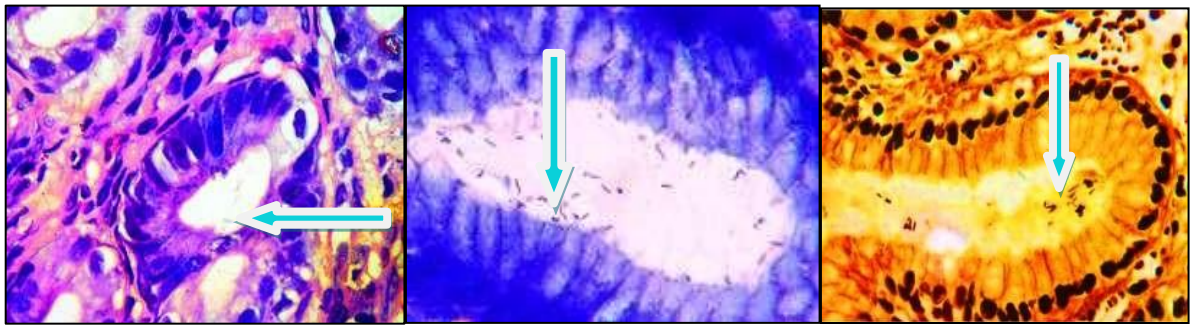
NSP	0	1	1	0.246
Total	126	94	220	

Of 220 cases studied, 63 cases were associated with chronic active gastritis with H pylori positivity with significant p value (p= 0.000). In 3 cases, there was a positive association (p=0.001) between H.pylori and Erosive gastritis. There was no association of H.pylori in all the 8 cases of Gastric carcinoma and 16 cases of duodenal lesions,

H.Pylori Positivity

Figure 1: H & E, GIEMSA, WARTHIN STARRY SILVER STAIN



Discussion

Endoscopic screening may detect gastro duodenal lesions at an early stage especially atrophy, intestinal metaplasia and dysplasia so as to prevent progress of these lesions to invasive cancer.[2] The upper GI endoscopic biopsy not only permits exact diagnosis of gastro duodenal lesions but also provides an opportunity to see H. pylori status and plan for specific medical or surgical therapy.

This study was conducted for period of two years in the department of pathology in a tertiary care teaching hospital. There were 220 gastro duodenal biopsies, of which 204 cases (92.70%) were gastric biopsies and the remaining 16 cases (7.27%) were duodenal biopsies. This study is comparable to the studies done by Al-Ezzy et al[3] and Gulia SP et al[4] which showed the most common site for endoscopic biopsies was stomach followed by duodenum.

In our present study, 59 cases (26.81%) were in the age group of 41-50 followed by 52 cases (23.63%) in the age group of 31-40.This study was comparable with studies by Sharma P et al[5] and Godkhindi VM et al[6] in which there was maximum distribution of gastro duodenal lesions in the age group of 31-40. This shows that gastro duodenal lesions are more common in 3rd decade and 4th decade of age. In our study, the youngest patient was 16 years old and the oldest patient was 85 years old. The age related difference could be due to variation in the risk factor among the different age groups.

The present study showed that among 220 cases of gastro duodenal biopsies studied, 127 (57.72%) were males and 93(42.27%) were females. There was more of male preponderance with M: F ratio of 1.36: 1 which was similar with the study done by Sharma P et al[5] and Godkhindi VM et al[6], which shows similar male preponderance. This reflects the fact that males are exposed to more risk factors than females.

In our present study, chronic superficial gastritis 175 cases (79.54%) was observed to be more followed by erosive gastritis 16 cases (7.27%), chronic duodenitis 12 cases (5.45%) and gastric carcinoma 8 cases (3.63%).This study is comparable to the study conducted by Godkhindi VM et al[86], in which chronic superficial gastritis (35.81%) was observed to be high followed by duodenal ulcer (20.90%), gastric ulcer and carcinoma (12.72%).

The similar trend was observed in the study done by Sharma P et al[5] where, Chronic superficial gastritis was the most common lesion (89%) followed by duodenitis (16%), duodenal ulcer (5%), gastric carcinoma (5%) and gastric ulcer (4%) .

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In our present study, majority of the samples with 2 to 3 biopsies were obtained from the antrum with 63.74% showing positivity for H.pylori which was similar to the findings of the study done by Khanna AK et al[7], Misra V et al [8] and Turkay et al[9].

Mishra J et al[99] also found H.pylori infection has a frequent association with chronic duodenal ulcer cases, with gastric antrum being the most common site for its colonization. Besides antrum (84%), H.pylori also affects fundus (41%) and body of the stomach, though in lesser number of cases.

In our present study, the most common histopathological diagnosis was chronic duodenitis which had endoscopic findings of ulcer (1 case), nodule (3 cases) and polypoidal growth (1 case). Duodenal adenoma was the second common histopathological diagnosis whose endoscopic finding was inflammation (2 cases). The *p* value for chronic duodenitis with endoscopy finding of inflammation was 0.318, therefore the association was found to be insignificant. This study is comparable to the study done by Lewis S et al which concluded that endoscopic appearance of duodenal mucosa is unreliable in determining the presence of histological inflammation[10.]

In our present study, out of 74 cases of chronic active gastritis 63cases(85.14%) showed H.pylori positivity. The H.pylori was significantly associated with chronic active gastritis (p=0.000) followed by erosive gastritis (p=0.001) in our present study. *H pylori* with a significantly higher frequency in those with chronic active gastritis compared to those with normal histology, showed a strong association with chronic active gastritis, which is suggestive of its causative role in chronic gastritis and gastritis activity.

This study was comparable to the study conducted by Godkhindi VM et al[6] and Sharma P et al[5] in which chronic superficial gastritis was positively associated with H.pylori(Table no-20). In our present study all the 8 cases of Gastric carcinoma and 16 cases of duodenal lesions, there was no association of H.pylori.

Our present study showed overall H.pylori positivity of 57.27% in 220 gastro duodenal cases. All our cases were confirmed by special stains – Giemsa and Warthin starry silver stain for H.pylori positivity. Our study correlated with similar other studies. Our present study was comparable to the study done by Yakoob Javed et al[11] which showed 57% overall H.pylori positivity.

Jhala et al[12] found 68.6% prevalence of H.pylori in patients which was statistically highly significant. Some researchers[6] have reported higher incidence of H. pylori in various gastroduodenal lesions than in the present study. The reason could be that the patients who were H. pylori negative in our study area had ingested acid suppressant drug and/or antibiotics which are known to suppress the organism although chronic inflammatory cells are slow to disappear after eradication of H. pylori and may take a year or more to fall to normal levels.

In our present study, demonstration of H.pylori was possible by all the three staining methods, but were particularly appreciable in Warthin starry silver stain in 126 cases (57.27%) followed by Giemsa stain in 120 cases (54.54%). This study is comparable to the study done by Ashton- Key M et al[13] demonstrated H.pylori positivity high in WSS stain followed by Giemsa stain (Table no-22).

In our present study, WSS stain showed 94% specificity with high sensitivity of 100% (Table no-9) similar to the study done by Cutler A F et al [14] which showed that warthin-Starry staining of antral biopsy specimens had the greatest combined sensitivity (93.1%) and specificity (99%) to establish a patient's *H. pylori* status.

In our present study, H.pylori positivity in giemsa stain was almost equal to H.pylori positivity in WSS stain. The study done by Lee JY et al[15] stated that in routine practice, at least two kinds of stain methods are recommended for detection of H.pylori positivity. H&E staining is usually adequate to detect H.pylori positivity in gastroduodenal biopsy specimens but it has low sensitivity, probably due to lack of contrast between the bacteria and the surrounding tissues. The Giemsa stain showed high sensitivity and specificity in detecting H.pylori positivity and it seems to have advantage over other stains because it is easy to use, inexpensive, and provides consistent results .The WSS stain also showed high sensitivity and specificity in detecting H.pylori positivity but it was expensive, technically demanding and frequently not reproducible particularly in our setup.

Conclusion

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Endoscopy with histopathological evaluation not only provides input to treating clinician regarding the existing pathology, it also helps in further management of such cases. The present study therefore highlights various gastroduodenal lesions and their association with H.pylori , giving a good comparision between different stains for the demonstration of H.pylori. The WSS stain showed high sensitivity and specificity in detecting H.pylori positivity but it was expensive, technically demanding and frequently not reproducible .Giemsa stain being relatively cheap and less time consuming than WSS stain showed consistent results particularly in our set up.

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