Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

STUDY TO EVALUATE THE USEFULNESS OF GIEMSA STAIN IN ADDITION TO HEMATOXYLIN AND EOSIN FOR HISTOPATHOLOGICAL IDENTIFICATION OF H. PYLORI

Dimple Elizabeth Thomas[1], Vineetha KV[2], Sruti Joy[3], Bithun Balan Kunnummal[4]

[1]Assistant Professor, Department of Pathology, Sree Uthradam Thirunal Academy of medical Sciences, India.
[2]Assistant Professor, Department of Pathology, Kannur Medical College, India.
[3]Assistant Professor, Department of Pathology Sree Uthradam Thirunal Academy of medical Sciences, India.
[4]Associate Professor, Department of Pathology, Kannur Medical College, India.

Received Date: 18/09/2024 Accepted: 19/10/2024 Published: 30/11/2024

Corresponding Author: Dr Bithun Balan Kunnummal, Associate Professor, Department of Pathology, Kannur Medical College, India.
Email: bithunbalan@gmail.com

ABSTRACT

Background: Histological identification of H pylori infection done by modified Giemsa, Warthin-Starry and immunohistochemical H pylori antibody stains. Present study was aimed to evaluate the usefulness of Giemsa stain in addition to Hematoxylin and eosin for histopathological identification of H. Pylori. **Material and Methods:** Present study was Hospital based Cross-sectional study, conducted in patients undergoing upper gastrointestinal endoscopy undergoing biopsies. Three biopsies were taken from stomach under endoscopic guidance and fixed in 10% formalin and subjected for tissue processing. **Results:** Out of 100 cases, 58 were males and 42 were females with male to female ratio of 1.4 :1 and majority of lesions were in males. The age ranged from 13 to 85 years with mean age of 53.6 years. Highest incidence of cases was seen in 5th, 6th and seventh decade. There were 78 cases of chronic gastritis, 3 adenocarcinoma, 3 cases of dysplasia, 1 case of eosinophilic gastritis, 5 gastric polyps and 10 cases with normal histology. Sensitivity of H&E stain was only 78 % and specificity was 88% when compared with Giemsa stain. H. Pylori was positive in 74 out of 100gastric biopsies. H. pylori was positive in 85.9% cases of chronic gastritis and its association with H. pylori was found to be statistically significant. It was seen in 60% of hyperplastic polyp and 66.7% of adenocarcinoma and 66.67% of dysplasia. H. pylori was completely absent in Fundic polyps , adenocarcinoma diffuse type and eosinophilic gastritis. **Conclusion:** Organisms are better appreciated using Giemsa stain, even when the density of organisms are low and should be used for the demonstration of H. Pylori as it is sensitive, cheap and reproducible.

Keywords: H. Pylori. Giemsa stain, endoscopy, gastritis, gastric carcinoma

INTRODUCTION

Barry Marshall and Robin Warren successfully isolated and cultured a spiral bacterial species known as Helicobacter pylori, from the human stomach.[1] Major diseases associated with H .Pylori include duodenal and gastric ulcers, chronic gastritis, atrophic gastritis, iron deficiency, MALT-type lymphomas of the stomach and gastric adenocarcinomas. H. Pylori is considered as class I carcinogen by World Health Organization.[2]

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

Helicobacter pylori persistently colonize the gastric mucosa of humans, infecting approximately 50% of the population worldwide. The prevalence of H. pylori is decreasing in most parts of the developed world due to the improving sanitation, reduced transmission and the frequent usage of antibiotics in childhood.[3,4]

Nearly all peptic ulcers are caused either by Helicobacter pylori infection or by use of non-steroidal anti-inflammatory drugs (NSAIDs), which include aspirin. NSAID ulcers are caused due to the suppression of gastro-protective cyclo- oxygenase 1 (COX-1).[4] The histological identification of H pylori infection is used for diagnosis. These include modified Giemsa, Warthin-Starry and immunohistochemical H pylori antibody stains. Immunohistochemistry is the agreed as gold standard for histology, being a highly sensitive and specific staining method.[5] Present study was aimed to evaluate the usefulness of Giemsa stain in addition to Hematoxylin and eosin for histopathological identification of H. Pylori.

MATERIAL AND METHODS

Present study was Hospital based Cross-sectional study, conducted in department of Pathology, Kannur Medical College, Anjarakandy, Kannur, Kerala, India. Study duration was of One and half year from 2018 to September 2019. Study was approved by institutional ethical committee.

Inclusion criteria

All patients undergoing upper gastrointestinal endoscopy at Kannur medical college

Exclusion criteria

- All patients undergoing duodenal biopsies.
- All patients undergoing esophageal biopsies.

Three biopsies were taken from stomach under endoscopic guidance and visualisation and tested for Rapid urease test using a commercially available test kit and result of the test was noted in the requisition form. Other biopsies were taken and fixed in 10% formalin and subjected for tissue processing. The processed tissue was embedded in paraffin wax and sections were cut serially at a thickness of 4-5 microns. Multiple sections were taken from each case.

The sections were stained with Haematoxylin and Eosin, Giemsa stain and observed under microscope. The various histopathological changes in the gastric mucosa were noted. The haematoxylin and eosin stained sections were examined for histomorphological parameters in the Sydney system associated with Helicobacter pylori infection namely activity, chronic inflammatory infiltrate, lymphoid aggregates, atrophy, intestinal metaplasia and graded according to visual analogue scale. All the sections were looked for the presence of H. pylori using the special stain Giemsa and correlated with the results of Rapid urease test. Statistical analysis was done using descriptive statistics.

Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables were tested using chi-square test or Fisher exact test as applicable. P value less than 0.5 was considered as statistically significant.

RESULTS

Out of 100 cases studied, 58 were males and 42 were females with male to female ratio of 1.4:1. Age ranged from minimum of 13 to maximum of 85 years with mean age of 53.6 years. Maximum number of cases were seen in the sixth decade with 22 cases.

Table 1: Age and Gender distribution of all cases.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

AGE GROUP	MALES	FEMALES	TOTAL
11-20	2	0	2
21-30	3	4	7
31-40	9	5	14
41-50	10	7	17
51-60	13	9	22
61-70	11	10	21
71-80	9	6	15
81-90	1	1	2
Total	58	42	100

Of the 100 cases studied, maximum number of cases were of chronic gastritis which was 78, followed by 5 gastric polyps out of which 3 were fundic polyps and 2 were hyperplastic polyps. There were 3 cases of carcinoma out of which 2 were intestinal type and 1 was diffuse type. There were 3 cases of dysplasia. 10 cases had no significant pathology. Maximum number of chronic gastritis cases were seen in the sixth and seventh decades.

Maximum number of cases were of chronic gastritis seen in the sixth, fifth and fourth decades with gradual decrease in the number of cases towards younger and older age groups. Majority of the gastric lesions were seen in males when compared to females. Chronic gastritis was the common lesion. Out of 78 cases of chronic gastritis, 46 were in males and 32 were in females. There was one case of eosinophilic gastritis which was seen in male. There were 5 cases of polyp in which 3 were in females and 2 were in males. Out of the 3 cases of dysplasia 2 were in females and one in male. Out of 3 cases of adenocarcinoma, 2 were diagnosed in females and one in male.

Table 2: Age distribution of all gastric lesions

Age Group	Normal	Chronic Gastritis	Eosinophilic Gastritis	Gastric Polyp	Dysplasia	Adeno Carcinoma	Total
11-20	0	2	0	0	0	0	2
21-30	1	6	0	0	0	0	7
31-40	1	10	0	2	1	0	14
41-50	1	14	1	1	0	0	17
51-60	4	16	0	1	0	1	22
61-70	1	17	0	0	1	2	21
71-80	2	12	0	1	0	0	15
81-90	0	1	0	0	1	0	2
Total	10	78	1	5	3	3	100

Out of 100 cases 81 cases were positive for Rapid Urease test. Out of 81 positive cases 47 were males and 34 were females

Table 3: Rapid Urease test

Rapid Urease test	MALES	FEMALES	TOTAL
Positive	47	34	81
Negative	11	8	19

Out of the 10 normal cases 9 were negative for RUT and 1 was false positive for RUT. 73 cases of chronic gastritis were positive for RUT but only 67 cases showed the presence of H. Pylori using Giemsa stain which were true positives and 5cases were negative. Eosinophilic gastritis was negative for RUT. All the fundic polyps were negative for RUT and 2

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

hyperplastic polyps were positive for RUT. Out of 3 cases of dysplasia 2 were positive for RUT. Both intestinal and diffuse variants of adenocarcinoma were tested positive for RUT but only one case of intestinal carcinoma showed the presence of H. pylori using Giemsa stain. Out of the 81 cases tested positive for RUT, 73 cases showed presence of H.pylori on histopathological study using special stain Giemsa (true positive). 8 cases which were RUT positive were negative for the presence of Pylori on histopathological examination (false positives).

Sensitivity of rapid urease test was 98.6%. Sensitivity is high hence rapid urease test can be considered as a good screening test for detection of H. pylori.

Specificity is only 69.2 %; hence Rapid Urease Test is not a good confirmatory test for detection of H. pylori.

Positive predictive value was 90.1%, this implies that when the patient is RUT positive there are 90.1% chances that the patient is suffering from H. Pylori. Negative predictive value was 95%, this implies that in a patient who is tested negative for RUT there is only 5% chance that he may be positive for H. Pylori.

Table 4: Sensitivity and specificity of Rapid Urease Test

RUT	Affected	Not affected
POSITIVE	73 (a)	8 (b)
NEGATIVE	1 (c)	18 (d)

a-true positives, b-false positives, c- false negatives, d-true negatives.

Out of 100 cases74 cases showed the presence of H. pylori using Giemsa stain and only 62 cases showed the presence of H. pylori using Hematoxylin and Eosin stain. The sensitivity and specificity of hematoxylin and eosin stain in detecting the presence of H. pylori was calculated by taking Giemsa stain as the specific test for detecting the presence of H. pylori

The sensitivity (78 %) and specificity (88 %) of Hematoxylin and Eosin in detecting H. Pylori is low. It is not useful in detecting H. Pylori especially in low density ,but when H. Pylori are present in high density, careful examination will almost always reveal them. Giemsa is the stain of choice in the detection of H. pylori because it is sensitive, cheap, easy to perform, and reproducible.

Table 5: Sensitivity and Specificity of Hematoxylin and Eosin

Hematoxylin & Eosin	GIEMSA	
	PRESENT	ABSENT
PRESENT	59	3
ABSENT	15	23

Out of 78 cases of chronic gastritis 67 i.e.; 85.9% cases were positive for H. pylori.

Chi square tests were done to find the association of various gastric lesions with H. pylori. It was found that chronic gastritis was associated with H. pylori with p value<.00001. Other lesions of the stomach did not show significant association with H. pylori. Eosinophilic gastritis was negative for H. pylori and 60% of polyps and 66.7% cases of dysplasia and adenocarcinoma were positive for H. pylori.

Out of 74 positive cases of H. pylori maximum number of cases were seen in the 6th decade. Prevalence of H. Pylori was seen to be increasing from 3rd to sixth decades after which there was a fall in the prevalence of H. pylori. Out of 100 cases 46 males and 28 females were found to have infection with H. pylori on histopathological examination.

Table 6: Helicobacter. Pylori positivity in various gastric lesions

Gastric lesions	Total	H.pylori	Percenta	Chi

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

		Positive cases	ge positivity	square P value
Chronic gastritis	78	67	85.90%	P<.00001
Eosinophilic gastritis	1	0	0	.02673
Polyps	5	3	60%	0.5362
Dysplasia	3	2	66.70%	0. 0864
Adenocarcinoma	3	2	66.70%	0. 0864

Out of 78 cases of chronic gastritis 73cases showed mild, 3 cases showed moderate and 2 cases showed severe/marked presence of lymphocytes. 24 cases showed activity with neutrophilic infiltration in the lining epithelium or in the lamina propria. Atrophy with loss of mucosal gland was seen in 2 cases of chronic gastritis and intestinal metaplasia was seen in 3 of cases and H. pylori were present in 70 cases of chronic gastritis.

Table 7: Grading of morphological variables in chronic gastritis by Updated Sydney system

Variables	Grading of morphological variables			
	Mild	Moderate	Severe/Marked	Total
Chronic inflammation	73	3	2	100%
Activity	20	2	2	30.7%
Atrophy	2	-	-	2.5%
Intestinal metaplasia	3	-	-	3.8%
H. pylori	52	16	2	89.7%

A Chi-square test of independence was performed to examine the relation between H. Pylori and other morphological variables of the Sydney system. Chronicity and activity was found to be significantly associated with the presence of H.Pylori.

Intestinal metaplasia and atrophy did not show association with the presence of H. Pylori. Highest degree of association of H. pylori was found with activity with 91.3% cases showing H. pylori positivity. Cases with chronic inflammation showed 86% positivity for H. pylori. 50% of cases with atrophy and 33.3% cases with intestinal metaplasia were positive for H. pylori.

Table 8: Association of H. Pylori with other morphological variables of Sydney system.

Mucosal changes	Number of cases	H. pylori positive cases	Percentage positivity	P value
Chronicity	86	74	86%	P<.00001
Activity	23	21	91.3%	p<.031075
Intestinal metaplasia	3	1	33.3%	P<.103004
Atrophy	2	1	50%	P<.4344223

DISCUSSION

Helicobacter pylori is present in approximately 50% of the human population worldwide and infection levels reach over 70% in developing countries. To understand how H. pylori can produce such diverse effects in the human host, several studies have focused on understanding the local and systemic effects triggered by this bacterium.[6]

In a study conducted by Aggarwal *et al.*,[7] the age range was 15–86 with mean age of 50.3 years (108). In a study conducted by S. Adlekha *et al.*,[8] most of the gastric lesions were seen in 3rd to 6th decade which is comparable with the present study where maximum number

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

of cases were seen in the 3rd to 6th decade with gradual decrease in cases after 6th decade. In the present study out of 100 cases studied 58 were males and 42 were females with male to female ratio of 1.4:1. Maximum cases were in the sixth decade with 22 cases. These findings were comparable with the studies conducted by S.Adlekha *et al.*,[7] and Aggarwal *et al.*,[8].

In the study done by S.Adlekha *et al.*,[8] out of 530 gastric biopsies 61.9% were in males and 38.1% in females. In the study conducted by Aggarwal *et al.*,[7] 63% were males and 37% were females. In the present study also, maximum number was in males 58% compared to females 42%, which is in agreement with these studies.

In a study by S.Adlekha *et al.*,[8] 10.5% (56/530) patients were found to have reactive gastritis and 62% had chronic gastritis. Normal gastric mucosa was evident in 8.4% (45/530) cases. 3.9% patients had dysplasia/cancer. In study conducted by Poonam Sharma *et al.*,[9] chronic gastritis was the most common lesion (89%) followed by duodenitis (16%), duodenal ulcer (5%), gastric carcinoma (5%), gastric ulcer (4%) and MALToma (1%), 5% cases had normal histology. In the present study also, majority were suffering from chronic gastritis with 78 % cases. 10 % cases were normal and there were 5 % polyps and 3 % dysplasia and 3 % adenocarcinoma.

S.Adlekha *et al.*,[8] study showed 89.4% for H. pylori, while in a study conducted by Poonam Sharma *et al.*,[9] H. pylori were seen in 47% patients. The present study showed H. pylori positivity in 74% of cases.

In a study conducted by Poonam Sharma *et al.*,[9] 50.56% cases with chronic gastritis were positive for H. pylori. 40% cases of gastric carcinoma of intestinal type were positive for H. pylori. The single case of diffuse type of gastric carcinoma was negative for H. pylori similar to the scenario in the present study. In a study conducted by S.Adlekha *et al.*,[8] H. pylori positivity was seen in 65.9% (292/443) patients with diagnosis of gastritis and there 90.5% (19/21) cases of dysplasia and carcinoma positive for H. pylori.

In a study done by Athavale *et al.*,[10] in 100 patients 83% were tested positive which is close to value in the present study. S. Adlekha *et al.*,[8] conducted a similar study in which only 57.7% were tested positive by RUT.

In a study conducted by Athavale *et al.*,[10] to find out the sensitivity and specificity of Rapid urease test it was found that the test had 96.38% sensitivity and only 74.47% specificity. The positive and negative predictive values of the test were 95.24% and 81.25% respectively while in our study it was 91% and 95% respectively. The findings were similar to the values in the present study

In a study conducted by Sonam Pruthi *et al.*,[11] the result of rapid urease test was available for 19 cases, and was positive in 8 cases. Among the positive urease test cases H. pylori was seen in 5 (62.5%) cases. However, in urease test negative 11 cases, H. pylori was present in 3 (27%) cases. Sensitivity and specificity of rapid urease test was 62.5% and 37.5%. The positive and negative predictive value of the test was 62.5% and 72.7%, respectively. The low sensitivity and specificity in this study is attributable to the smaller number of cases in which data of rapid urease test result was available.

Laine *et al.*,[12] compared three stains H&E, Giemsa and Genta and found the sensitivities were comparable at both low H. pylori density (H&E, 70%; Giemsa, 64%; Genta, 66%), and at high *H. pylori* density (H&E, 98%; Giemsa, 96%; Genta, 97%). Specificity was excellent (98-100%) for the Genta and Giemsa stains at both low and high H. pylori density and the H&E stain at a high density; however, specificity decreased (90%) in the H&E stain in low-density H. pylori.

In a study conducted by Sushna Maharjan *et al.*,[13] in 110 patients 59 cases had H. pylori colonization. Mononuclear cell infiltrate was detected in all the cases with H. pylori (53.6%) in 59 cases similar to the present study. Neutrophilic activity was observed in 37 cases out of

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

which twenty four (40.7%) had H. Pylori. Intestinal metaplasia and atrophy were observed in 19% and 5.5% of cases respectively.

In a study conducted by Sonam Pruthi *et al.*,[12] in 100 patients. H. pylori was detected in 34.2% of cases with mild neutrophilic activity, and 85.7% of cases with moderate neutrophilic activity. All six cases having marked neutrophilic activity showed H. pylori positivity. In 37.5% cases with mild, 33.3% of moderate and 80.6% of marked mononuclear cell or lymphocytic infiltrate, H. pylori was detected. The mononuclear cell infiltration was associated with plasma cells in all the cases. Helicobacter pylori was seen only in 32.2%cases with mild intestinal metaplasia which was similar to the present study. Eight cases showed gastric atrophy and H. pylori were demonstrable in 5 cases (62.5%).

In the present study H. pylori was seen in 82.7% cases with mild mononuclear cell infiltrates and in all cases (100%) with moderate and severe inflammation. H. pylori was seen in 77.27 % cases with mild neutrophilic infiltrate and in all cases with moderate and severe activity. Intestinal metaplasia showed the lowest association with H. pylori in the comparative studies which was similar to the present study.

Helicobacter Pylori infection is found to be associated with antral gastritis that can progress to intestinal metaplasia, dysplasia and carcinoma. Detection and eradication of Helicobacter Pylori in the early stages of the disease is an important part of gastroenterology. Effective regimes are available which will eliminate the organism in most of the patients. Rapid Urease test can be considered as a good screening test for detection of H. Pylori but histopathological identification of organisms using special stains is the confirmatory test. Clinicians cannot solely rely on Rapid urease test.

CONCLUSION

Hematoxylin and Eosin stained sections are good for the study of histomorphological features but is not recommended for the detection of H. Pylori. Sensitivity of H&E stain was only 78%and specificity was 88% when compared with Giemsa stain. Organisms are better appreciated using Giemsa stain, even when the density of organisms are low and should be used for the demonstration of H. Pylori as it is sensitive, cheap and reproducible. Endoscopy should be accompanied by biopsy for early detection of H.Pylori and assesment of other histomorphological features like intestinal metaplasia and atrophic gastritis which are the forerunners of gastric carcinoma.

Conflict of Interest: None to declare
Source of funding: Nil

REFERENCES

1. Backert S, Neddermann M, Maubach G, Naumann M. Pathogenesis of Helicobacter pylori infection. Helicobacter. 2016;21(3):19–25.
2. Batts KP, Ketover S, Kakar S, Krasinskas AM, Mitchell KA, Wilcox R, *et al.* Appropriate Use of Special Stains for Identifying Helicobacter pylori. Am J Surg Pathol. 2013;37(11):e12–22.
3. Calvet X, Sánchez- Delgado J, Montserrat A, Lario S, Ramírez- Lázaro MJ, Quesada M, *et al.* Accuracy of Diagnostic Tests for Helicobacter pylori: A Reappraisal . Clin Infect Dis. 2009;
4. Bang CS, Baik GH. Peptic ulcer. In: Helicobacter pylori. 2016.
5. Rotimi O, Cairns A, Gray S, Moayyedi P, Dixon MF. Histological identification of Helicobacter pylori: Comparison of staining methods. J Clin Pathol. 2000;53(10):756–9.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

6. Damjanov ivan james linder. Helicobacter pylori in human health and disease : Mechanisms for local gastric and systemic effects. Vol. 24. 2018. 3071–3089 p.
7. Agarwal PK, Badkur M, Agarwal R, Patel S. Prevalence of Helicobacter pylori infection in upper gastrointestinal tract disorders (dyspepsia) patients visiting outpatient department of a hospital of North India. J Fam Med Prim Care. 2018;7:577–80.
8. S.Adlekha, T Chadha, P Krishnan and BS. Prevalence of Helicobacter PyloriInfection Among Patients Undergoing Upper Gastrointestinal Endoscopy in a Medical College Hospital in Kerala, India. Ann Med Heal Sci Res. 2013;3(4):559–63.
9. Sharma P, Kaul KK, Mahajan M, Gupta P. Histopathological Spectrum of various gastroduodenal lesions in North India and prevalence of Helicobacter pylori infection in these lesions : a prospective study. 2015;3(5):1236–41.
10. Athavale VS, Singh V, Khandalkar SN, Nirhale DS, Lad A, Shetty I. Comparative study of rapid urease test and histopathological examination for detection of H . pylori infection. 2017;4(12):4071–5.
11. Pruthi S, Nirupama M, Chakraborti S. Evaluation of gastric biopsies in chronic gastritis : Grading of inflammation by Visual Analogue Scale. 2014;7(4).
12. Laine L, LewinDN NW *et al.* Prospective comparison of H&E, Giemsa, and Genta stains for the diagnosis of Helicobacter pylori. Gastrointest Endosc 1997;45:463-7. 1997;45:463–7.
13. Maharjan S, Ranabhat S, Tiwari M, Bhandari A, Osti BP. Helicobacter Pylori Associated Chronic Gastritis and Application of Visual Helicobacter Pylori Associated Chronic Gastritis and Application of Visual Analogue Scale for the Grading of the Histological Parameters in Nepal. 2017;(May):1–8.