www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i8.83

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Role of Special Stain Giemsa in Demonstration of Helicobacter Pylori in Gastric Biopsies

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ABSTRACT

Introduction: *Helicobacter pylori is a gram negative, curved bacillus, found in the gastric mucosa which was recognized as a Class I carcinogen. Majority of gastric adenocarcinomas and most gastric MALT lymphomas are related to chronic infection with this organism. Various invasive and noninvasive methods are available for the identification of H.pylori. The gold standard for the diagnosis remains to be histopathology and culture. But culture is a laborious and time consuming method. Histopathological identification of H.pylori can be improved with the use of special stains like Giemsa. The aim of this study was to evaluate the efficacy of the special stain (Giemsa) in the identification of H.pylori compared to the routine H &E stain (Hematoxylin and Eosin).*

Materials and Methods: *Gastric biopsies taken from dyspeptic patients were evaluated with routine H&E and Giemsa stains. The presence of H.pylori was analysed in both the groups.*

Results: *Out of 150 cases studied Giemsa stain revealed H.pylori in 58.67% of cases whereas routine H & E stain identified only 44.67% of cases. This was found to be statistically significant.*

Discussion and Conclusion: *Special stain like Giemsa should definitely be used as an adjunct to standard H &E stain for the identification of H.pylori in gastric biopsies. The depiction of H.pylori in gastric biopsies allows for the specific treatment by which the eradication of the organism can be done, thereby decreasing the incidence of gastric carcinoma and MALT Lymphoma.*

Keywords: *Giemsa, Gastritis, Hematoxylin and Eosin stain.*

INTRODUCTION

Helicobacter pylori is a Gram negative, microaerophilic, spiral shaped bacterium, measuring 0.5-1.0µm×2.5-5.0µm in size. It is motile, has 4-6 sheathed flagella, attached at one pole. The organismcan live in the acidic environment of the stomach by its special structure and virulence factors like vacuolatingcytotoxin (VacA), which causes cytoplasmic vacuolization in gastric epithelial cells and cytotoxin associated antigen (CagA). It has the ability to produce various enzymes like

hydrogenase, urease, oxidase and catalase, which also contributes to its existence in gastric mucosa. This organism was first discovered by Dr. Barry Marshall and Robin Warren in the gastric mucosa of patients with gastritis and ulcers in 1982.[1]In 1875 itself, German scientists found spiral-shaped bacteria in the lining of the human stomach, but they were unable to culture them. It was called Campylobacter pyloridis initially, then renamed C.pylori. Later in 1989, following the genetic studies it was named Helicobacter pylori. The prevalence of H. pylori in the India is as high as 80percent.[2]

The common diseases caused by H.pylori are chronic active gastritis and gastric and duodenal peptic ulcers, In a subset of patients, active gastritis can evolve in to chronic gastritis ,atrophic gastritis, intestinal metaplasia and precancerous states like dysplasia and finally to carcinoma over the course of many years. In India the most common manifestation of H. pylori infection is peptic ulcer disease, especially duodenal ulcer disease.[3]H. pylori was considered as a Class I (Definite)carcinogen by International Agency for Research on Cancer (IARC), a subordinate organization of WHO in 1994, based on epidemic-ological data .70% of gastric adenocarcinomas and most gastric MALT lymphomas are related to chronic infection with this organism.

Various methods are available for the identification of H.pylori in gastric biopsies. This includes non-invasive and invasive techniques. Noninvasive methods include urea breath test(UBT), Stool antigen test and serology(Anti-H. pylori IgG antibody). Invasive techniques include Rapid Urease Test, histopathology, culture and Polymerase Chain Reaction (PCR).Many studies were done around the world comparing the different methods with varying outcome.Among these, the gold standard for the diagnosis remains to be culture and histology. But culture is a laborious and time consuming method. Histology is highly sensitive and specific especially in combination with special stains. When compared to other methods, histology has the added advantage of directly visualizing the bacteria and surrounding mucosal changes can be found out ranging from inflammation to malignancy. Almost 100% diagnostic accuracy is reported when histology along with Immunohistochemistry is done, but the test is costly.

In a developing country like India where health fund allocation is minimal, a cost effective, yet highly sensitive and specific test need to be identified. Of all the special stains described in histology, Giemsa is simple, cheaper and give consistently good results for H. pylori.[4]This study was undertaken to compare the efficacy of the special stain Giemsa with the routine H&E staining for better identification of H.pylori.

MATERIALS & METHODS

150 consecutive biopsies from patients presented with dyspeptic symptoms like postprandial fullness, early satiation, and epigastric pain or burning were evaluated. Endoscopy was done and biopsies taken from antrum and or body were included in the study. Clinical details & other relevant information were obtained from the hospital records. Patients with gastrointestinal malignancy and gastrectomy specimens were excluded from the study. All specimens were Formalin fixed & Paraffin embedded. Minimum 2 sections were taken from each biopsy specimen and were stained with H&E and Giemsa. For preparing stock solution of Giemsa,4 gram of stain powder was dissolved in 250 ml glycerol at 60°C with regular shaking. 250 ml of methanol was added, shaken the mixture and allowed to stand for 7 days. Working Giemsa stain was prepared by adding 4ml of Giemsa stock solution to 96 ml of Acetate buffered distilled water,(pH 6.8). All cases were evaluated microscopically under oil immersion objective(1000 X)for the presence of H.pylori. Organisms showing typical morphology were selected. Doubtful isolated forms were excluded. The problematic cases were reevaluated by a second pathologist. All the data obtained were entered in a master sheet in Microsoft Excel. The results were compared and

p value less than 0 .05 was considered significant. Sensitivity, Specificity, Positive and Negative Predictive Values were calculated.

RESULTS

Total of 150 cases were evaluated. Age of the patients ranged from 7 to 82 years. Majority of the H pylori positive cases were in the age group of 51-60 years (20.45%).Table 1

Males were more affected than females. Male to female ratio was 3:2.Table 2

In H& E stained sections, curvilinear, eosinophilic organism was seen in the mucous layer of gastric lining epithelium. Figure 3.In Giemsa stained sections, the organism had blue colour in a pale blue back ground. Figure 4.

Out of 150 cases, H&E staining detected 67 cases (44.67%) of H.pylori. In 83 sections stained with H&E the organism was not identified. With Giemsa stain, 88 cases (58.67%) showed positivity for H.pylori while 62 cases were negative. In 1 case (0.67%), H.pylori was identified in H&E stain,but not visible with Giemsastain. The proportion of cases showing positivity with Giemsa stain was found to be significant, pvalue being 0.015. using N-1 Chi-squared test. Table 5

The sensitivity, specificity, positive predictive value and negative predictive value of H&E stain in comparison with Giemsa stain were also calculated. Sensitivity was found to be 75.86%, Specificity was 98.41%,Positive Predictive Value was 98.51% and Negative Predictive Value was 74.70%. Table 6

Table 1- Age distribution of patients showing H. pylori positivity

Age group	Total number of biopsies	Number of cases showing positivity for H .pylori
0-10	3	1
11-20	12	8
21-30	21	15
31-40	27	17
41-50	24	16
51-60	32	18
61-70	18	8
71-80	12	5
81-90	1	0

Table 2-Sex distribution of patients showing H. pylori positivity

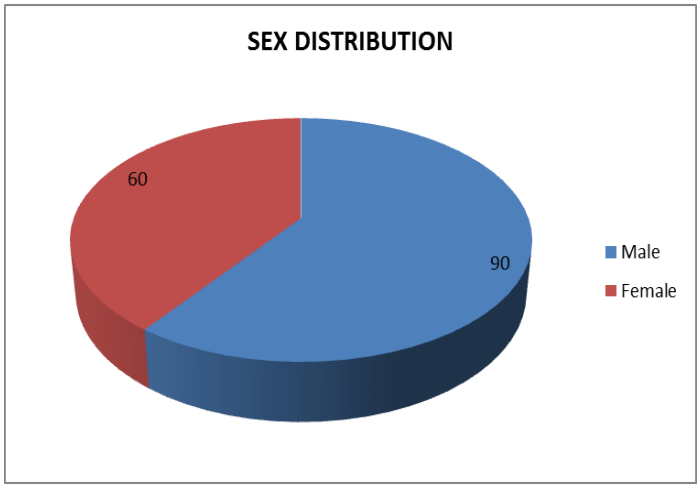

Figure 3-H. pylori : H& E stain

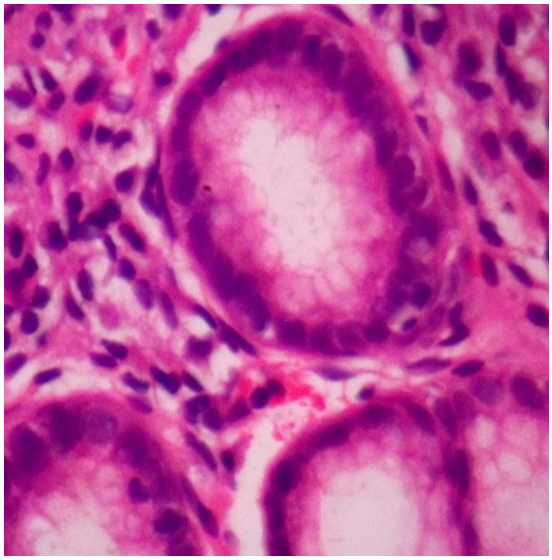

Figure 4-H.Pylori:Giemsa stain

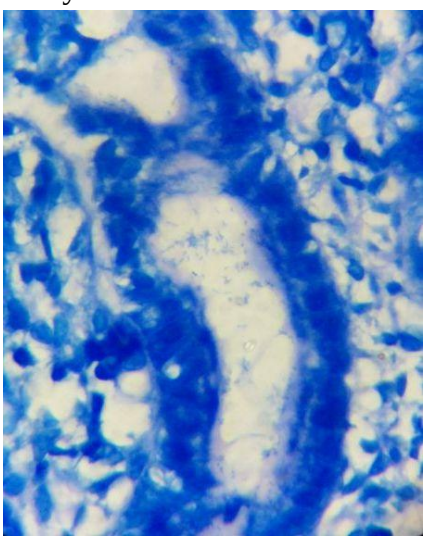

Table 5- Number of cases showing positivity with H & E stain and Giemsa stain

Identification Of H.pylori	H&E stain		Giemsa stain	
Positive	67	44.67%	88	58.67%
Negative	83	55.33%	62	41.33%
Total	150		150	

Table 6-Sensitivity, Specificity ,PPV and NPV of H &E stain

Sensitivity	75.86%,
Specificity	98.41%,
PPV	98.51%
NPV	74.70%.

DISCUSSION

The availability of an array of diagnostic methods for the identification of H.pylori denotes the incompetency of any one test to pinpoint the diagnosis. Non-invasive diagnostic tests included urea breath test, stool antigen test and serology. Invasive tests include endoscopy, histology, rapid urease test, culture, and molecular methods.

Non Invasive diagnostic tests

Urea breath test (UBT) is the most popular, accurate and reproducible noninvasive test useful for the diagnosis of H. pylori infection. The ingested 13C- or 14C-labeled urea is hydrolyzed to CO2 in stomach by the urease activity of H. pylori, then this labeled CO2 exhaled is measured. It has 95% sensitivity and specificity.

Stool Antigen Test(SAT) –It has got good sensitivity (95%) and specificity(98%).The test is based on enzyme immunoassay (EIA) orimmune chromatography assay (ICA) to detect H. pylori antigens in stool samples.No significant reduction in the sensitivity after treatment with Proton Pump Inhibitors.[5]

Serological tests based on the detection of anti-H.pyloriIg G antibody are available for H. pylori detection. Enzyme inked Immunosobent Assay is the most common and accurate technique. It is also used in screening for epidemiological studies. Ulcer bleeding, gastric atrophy or the intake of PPI or antibiotics will not affect the test result. The problem with serological test is that it do not differentiate between active infection and past exposure to H. pylori because even after successful eradication antibody levels remain in the blood for longer time. Sensitivity and specificity varies depending on the type of serological test performed.

Invasive diagnostic tests

RUT (Rapid Urease Test) is highly specific (95-100%),economical, simple and rapid method .More than 10,000 bacteria are required for a positive diagnosis. Drug intake can result in false-negative results. Other urease producing bacteria in the stomach causes false positive.[6]

Culture is the highly specific method for detection of H. pylori infection, but the sensitivity varies widely depending on the quality and transport of specimens, exposure to aerobic environment ,technical errors etc.[7] It is also used in population with high antibacterial resistance.

PCR is now used for the diagnosis of H. pylorinot only from gastric biopsy specimens, also from saliva, gastric juice, stool etc. It has higher sensitivity (95%)and specificity compared to other conventional tests. Advantages of PCR include, positive result even if fewer bacteria are present in the sample, faster results and no need for special processing or transportation. Test material for RT-PCR can be taken from tissue in paraffin blocks. Also, PCR detects specific mutations and virulence factors, such as CagA and VacA. This helps to understand the variation in clinical presentation with different strains of H. pylori. Many studies demonstrated that the presence of virulence factors, such as CagA and VacA gene, are associated with severe inflammation of the gastric mucosa and higher prevalence of peptic ulcer disease and gastric cancer. PCR also detect H. pylori in environmental samples for epidemiological studies. But some studies showed that blind rely on PCR data alone for treatment decisions on H. pylori is not advisable. Antigenic cross-reactivity can give rise to false positivity.8

Histology is considered to be the gold standard for direct demonstration of H. pylori infection and is also the first method used for the detection of H. pylori. But, the accurate diagnosis is influenced by

various factors like site, size and number of biopsies, staining methods, intake of proton pump inhibitor (PPI) and antibiotics and expertise of the pathologist. The updated Sydney system recommend to take five biopsy specimens from different sites. If a single biopsy is taken, the gastric body greater curvature is the preferred site.

Several stains were described for the better identification of the organism other than the routine H&E stain including Warthin- starry stain used originally by Marshall and Warren, Genta, silver stain, toluidine blue, acridine orange, McMullen, Dieterle and immunohistochemical stains. Warthin-Starry stain is expensive and the results not always reliable. Genta stain is a combination of silver, H&E, and Alcian blue stains which identifies the inflammatory cells and H. pylori. But it is time-consuming, complex and expensive method.[9]

The significance of using ancillary techniques is more important nowadays due to the widespread use of proton pump inhibitors, which can cause reduction in the number and change in the morphology of H pylori,the organism colonizing proximal stomach, that too in deeper layers. Ancillary techniques especially Immunohisto-chemistry showed near 100% sensitivity and specificity in many studies. But cost effectiveness of it is still under debate, especially in developing countries. So it's use is usually limited to those biopsy specimens which show moderate and severe chronic gastritis, but no H. pylori identified in H&E and special staining.

In this study, 44.67% of gastric biopsies revealed the presence of H pylori by H&E stain, while Giemsa stain showed 58.67% positivity. This result is comparable to findings in other studies which showed positivity of 54.5% by the routine H&E stain and 68.7% by Giemsa stain.[10]

When comparing the proportion of cases showing the organism by the two methods, p value was found to be significant (p value= 0.015). According to many studies, Giemsa stain is the method of choice when comparing different special stains because it is sensitive, cheap, easy to perform, and reproducible.[11] The prevalence of H pylori in gastric biopsy is 58.7 % similar to other studies in South India, showing 59.4 % prevalence.[12]

According to this study, the sensitivity and specificity of H&E stain were 75.86%, and 98.41%, respectively. This is in concordance with similar studies showing sensitivity of 70% - 98 % and specificity of 90- 98% with H&E stain .However in cases showing low density of organisms, the specificity of H&E was low compared to Giemsa.[13]Hartman and Owens review showed 83.3% sensitivity of H&E-stained sections.[11]Compared to that review, the sensitivity of H&E in this study was less, may be because organisms with typical morphology only were included in this study. Regarding limitation of this study, even though histopathology shows high diagnostic sensitivity and specificity for H pylori detection, various factors like biopsy sites and numbers, staining methods and intake of drugs like PPI can influence the results. Turnaround time for histological diagnosis was another drawback compared to noninvasive methods. Also IHC could have been done to increase the diagnostic accuracy.

CONCLUSION

Although several noninvasive and invasive methods are available for the detection of H pylori, histopathological evaluation of endoscopic biopsy still plays a prominent role in the diagnostic scenario. According to this study, the diagnostic yield of histology is improved with the help of special stain Giemsa which is cost effective, simple and give consistent results. It is a good practice to do Giemsa stain along with H&E stain for H pylori demonstration in routine gastric biopsies.

Source of support(grants)-None

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