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RESEARCH ARTICLE

HISTOPATHOLOGICAL GRADING AND COMPARISON OF STAINING METHODS FOR H PYLORI DETECTION

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ABSTRACT

Introduction: Helicobacter pylori (H pylori) is the most common bacterial infection in human. In our study we are studying histopathological changes in gastric mucosa in patient with H. pylori infection. Comparison of staining methods in terms of their cost, time required, reproducibility of techniques and ability to demonstrate H. pylori in Paraffine sections were also done.

Materials and methods: Prospective analysis of 100 patients presented with acid peptic disorder to our gastroenterology outpatient department for a period of 1 ½ year was carried out . Biopsies taken and were immediately subjected to Rapid urease test in endoscopy room. Thereafter the biopsy processed, standard routine histological section were cut at five micron thickness with the help of fully automated microtome. Sections were stained with routine H & E and special stains like Modified Giemsa, Gimenez, Warthin Starry stain re evaluated for the presence of H. pylori bacterial infection in the antral biopsies.

Observation and results: The maximum H. pylori positivity by histopathology was found in age group 21-40 years (60.2%) and followed by 41-60 years (58.2%) and then up to 20 year (57.1%). The grades of chronic inflammation and activity (infiltration by neutrophils) were higher with higher grades of H. pylori infection. Intestinal metaplasia, atrophy and lymphoid follicles aggregates were found mostly with grade I and grade II. Out of total 100 cases maximum number of H. pylori positive cases were detected by Warthin starry stain 55/100(55%) followed by Gimenez 53/100 (53%). On Giemsa staining 52/100 cases were detected. H and E could detect only 50 cases out of 100. Least number of cases were detected by H and E stain.

Conclusion: histopathology is the investigation of choice in detection of H pylori. It can detect carcinoma, lymphoma in early stages. Geimnez stain most useful as it is economical and quick, Modified Giemsa is also good but it requires careful search as good contrast is not provided. Warthin starry stain is time consuming, expensive, least reproducible so difficult to use.

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INTRODUCTION

Helicobacter pylori (H. pylori) is the most common bacterial infection in human. The isolation of Helicobacter pylori from clinical specimen by Marshall and Warren launched revolutions in gastroenterology and microbiology. H pylori is gram negative, highly motile, multi flagellated spiral organism. H pylori infection is the major cause of chronic gastritis and is important in pathogenesis of duodenal and gastric ulcers, distal gastic adenocarcinom. Carriage of H. pylori also increases the risk of developing primary non-Hodgkins lymphoma of stomach (MALTOMAS) by 6 folds. Accurate diagnosis of H. pylori infection is required to institute eradication treatment in appropriate cases.

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The investigations done to demonstrate H. pylori infection are, serology, culture, rapid urease test, C-urea breath test, PCR, and histology. Histological stains included are modified Giemsa, Warthin starry, Gimenez, Genta and Immunohistochemical H. pylori antibody stain. Advantages of histopathology include it documents H. pylori infection, degree of inflammation, presence of atrophy, intestinal metaplasia, activity and diagnosis of carcinoma and lymphoma in early stages.

In our study we are studying histopathological changes in gastric mucosa in patient with H. pylori infection. Comparison of staining methods in terms of their cost, time required, reproducibility of techniques and ability to demonstrate H. pylori in Paraffine sections were also done.

MATERIALS AND METHODS

Prospective analysis of 100 patients presented with acid peptic disorder to our gastroenterology outpatient department for a period of 1 ½ year was carried out and were evaluated for the presence of H. pylori bacterial infection in the antral biopsies . Various parameters like name, age, sex, detailed clinical history were recorded and endoscopic examination of stomach of all patients were performed. Two to three antral biopsies were obtained. Biopsies were immediately subjected to Rapid urease test in endoscopy room. Thereafter the biopsy was fixed in formalin and processed and embedded in paraffin wax . Standard routine histological section were cut at fine micron thickness with the help of fully automated microtome. Sections were stained with routine H&E and special stains like Modified Giemsa, Gimenez, Warthin Starry stain Patients with history suggestive of dyspepsia i.e. abdominal pain ,abdominal bloating and postprandial fullness etc of 4 weeks duration are included in this study.

Observation and results

Table 1. Age wise distribution of cases

Age group (years)	No. of cases	H. pylori positive on histology				H. pylori negative on Histology		
		Male	Female	Total	% +ve	Male	Female	Total
Up to 20	7	4	0	4	57.1	2	1	3
21-40	40	16	9	25	60.2%	10	5	15
41-60	35	15	5	20	58.1%	10	5	15
61 and above	18	4	2	6	33.3	10	2	12
Total	100	39	16	55	55.0	32	13	45

Table 2. Sex wise distribution of h. pylori positive cases

Sex	Total	H. Pylori positive on histology	H. Pylori negative on histology
Male	71	39(55%)	32(45.5%)
Female	29	16(53.9%)	13(51.9%)

Table 3. Correlation of H. pylori positivity by rapid urease test with histology

Rut	Total	H .pylori positive on histology		H .Pylori negative on histology	
No of RUT positive cases	42	38	90.4%	4	9.5%
No of RUT negative cases	58	12	26.3%	46	79.3%
Total	100	50		50	

Table 4. Relationship of H. pylori density to various histologic parameters

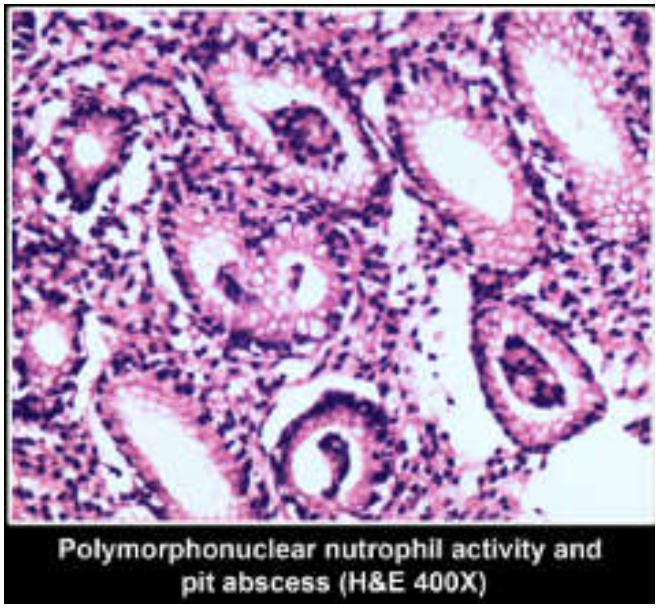
H. pylori density	Chronic inflammatory activity (Mean)	Activity (Mean)	Atrophy (Mean)	Intestinal Metaplasia (Mean)	Lymphoid Aggregates And Follicles (Mean)
Grade 0	3.1	1.2	0.5	0.6	1.9
Grade I	2.7	2.1	1.2	1.2	2.0
Grade II	2.3	1.9	1.2	1.1	2.0
Grade III	3.3	3.5	0.8	0.8	1.8
Grade IV	3.1	2.3	0.6	0.3	1.6
Grade V	3.3	2.3	0.6	0	1.7

100 patients with history of dyspepsia, who attended the Gastroenterology OPD were evaluated. The observations made during the study are as follows:- The most common age group in present study was 21-40 years (40 out of 100). The maximum H. pylori positivity by histopathology was found in age group 21-40 years (60.2%) and followed by 41-60 years (58.2%) and then up to 20 year (57.1%). The overall positive cases were 55 out of 100 .i.e 55% of the studied cases had H pylori gastritis. Out of the total 100 cases, 71cases were males and 29 were females. Thus M:F ratio was 2.4:1. Out of 71 males 39 (55%) were positive for H. pylori and 32% (45.5%) were negative for H pylori and out of 29 females, 16 (53.9%) showed H. pylori positive infection 13(51.9%) were negative for the same. RUT test was performed in all 100 cases, out of which 42 cases were positive and 58 cases were negative. 38/42 cases showed H. pylori on histology.

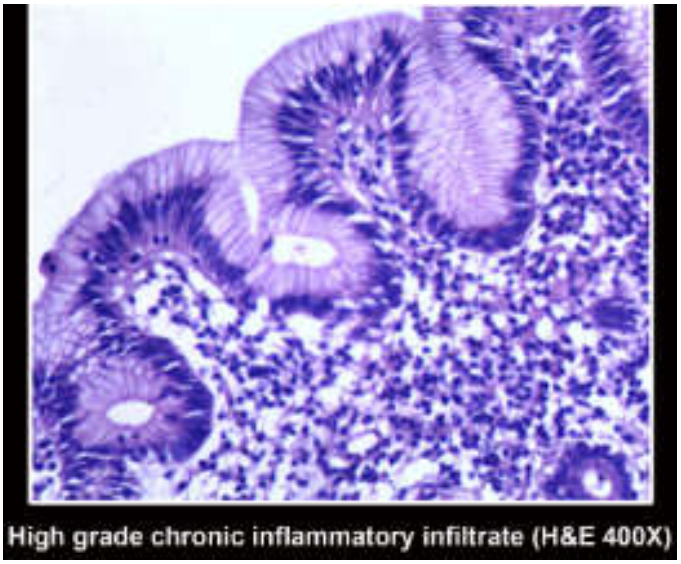
Out of 42 positive cases, 4 (9.5) RUT positive cases did not show any H. pylori on histopathology (False positive).Out of 58 RUT negative cases,12 cases (26.3%) were positive on histology (false negative).The sensitivity and specificity of RUT was calculated to be 76% and 92% respectively.

This table shows the relationship of H. pylori density to the histologic severity of gastritis. The grades of chronic inflammation and activity (infiltration by neutrophils) were higher with higher grades of H. pylori infection. Intestinal metaplasia, atrophy and lymphoid follicles aggregates were found mostly with grade I and grade II.

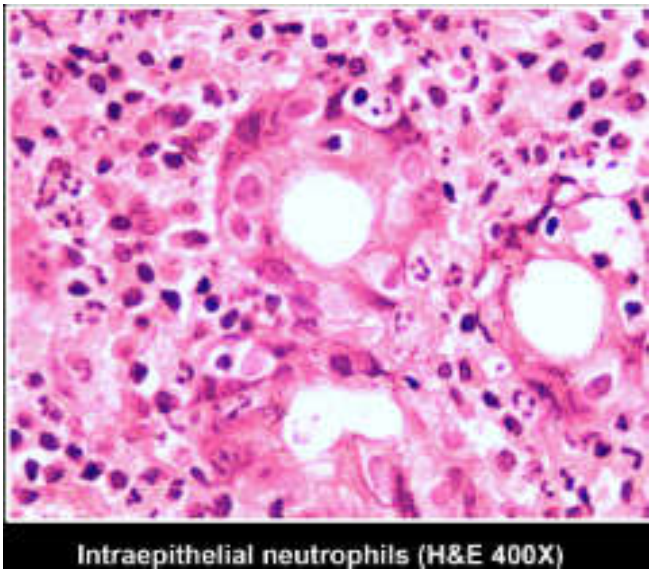
- The number of H pylori cases undetected by H and E staining were 5 i.e.10%. On Giemsa 6.7% and Gimenez 3.6% cases were undetected. None of the cases were undetected by Warthin starry stain.
- Grade 5 and 6 are more accurately detected by Warthin starry stain followed by Gimenez compared to H and E staining.
- Biopsies with low H. pylori densities were better demonstrated by Gimenez and Warthin starry stain than on H and E.



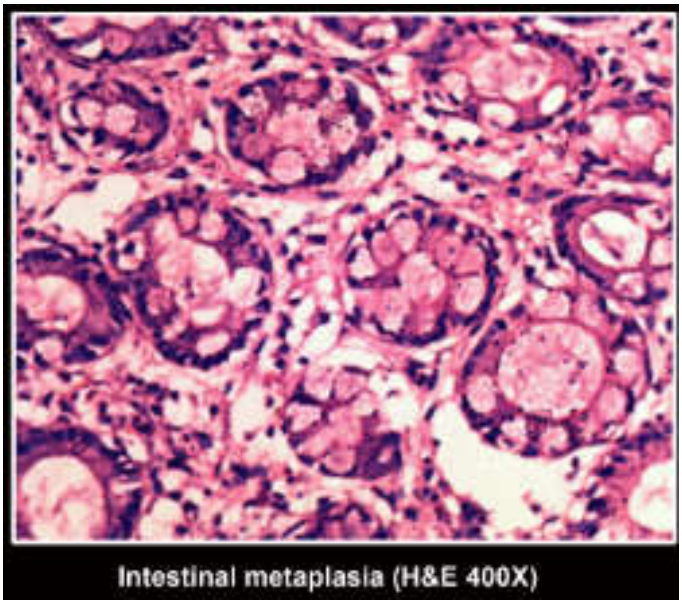
Polymorphonuclear nutrophil activity and pit abscess (H&E 400X)



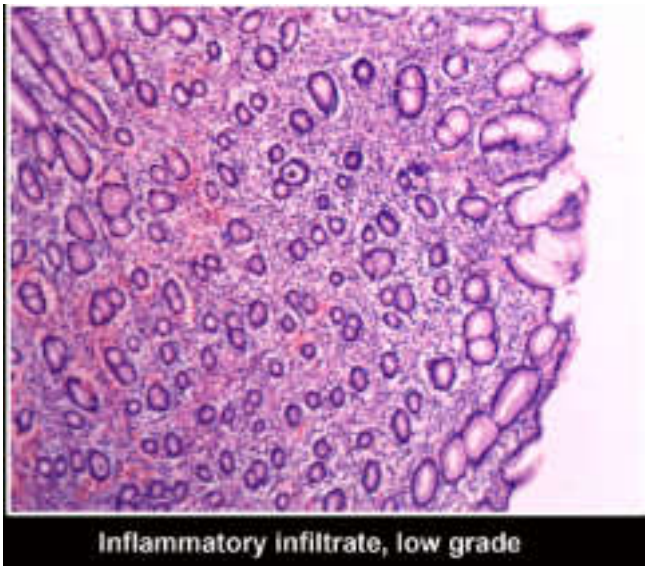
High grade chronic inflammatory infiltrate (H&E 400X)



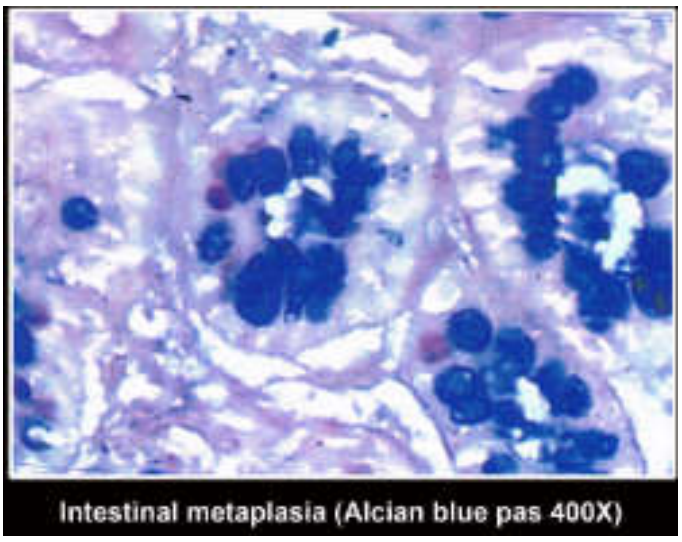
Intraepithelial neutrophils (H&E 400X)



Intestinal metaplasia (H&E 400X)



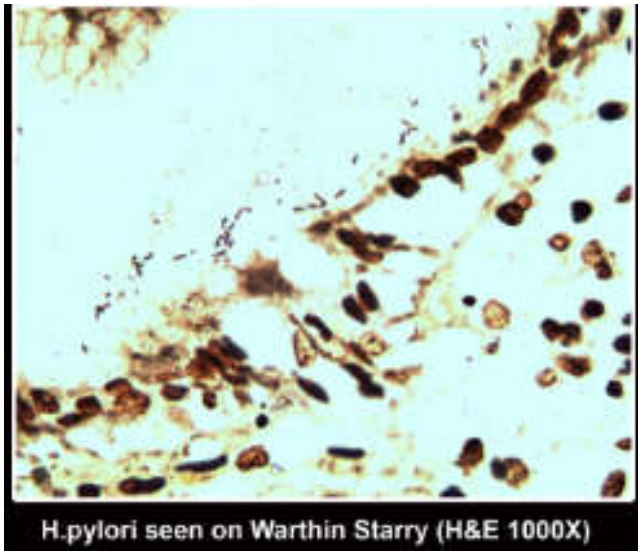
Inflammatory infiltrate, low grade



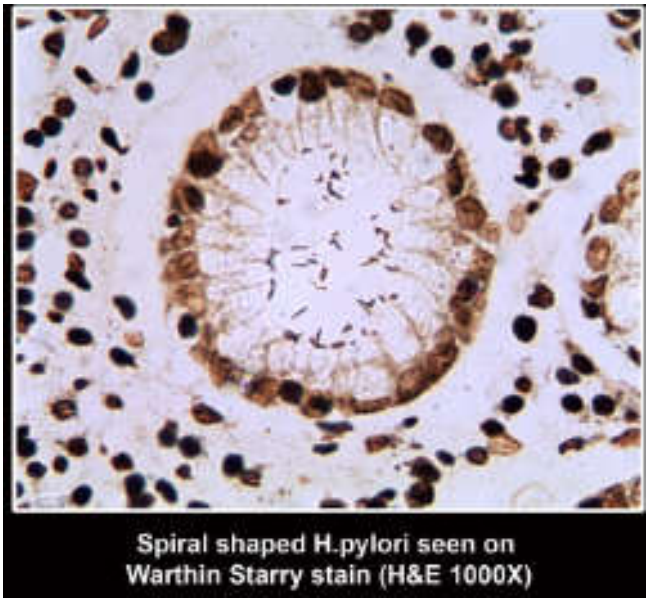
Intestinal metaplasia (Alcian blue pas 400X)

Out of total 100 cases maximum number of H. pylori positive cases were detected by Warthin starry stain 55/100(55%) followed by Gimenez 54/100 (53%).

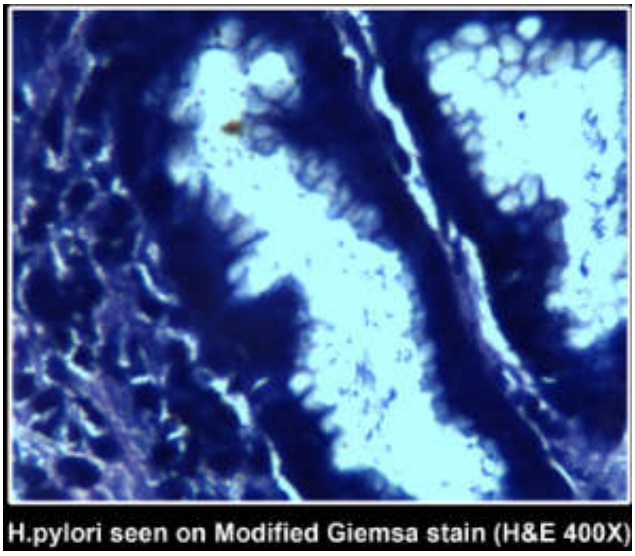
On Giemsa staining 53/100 cases were positive. H and E could detect only 50 cases out of 100. Least number of cases were detected by H and E stain.



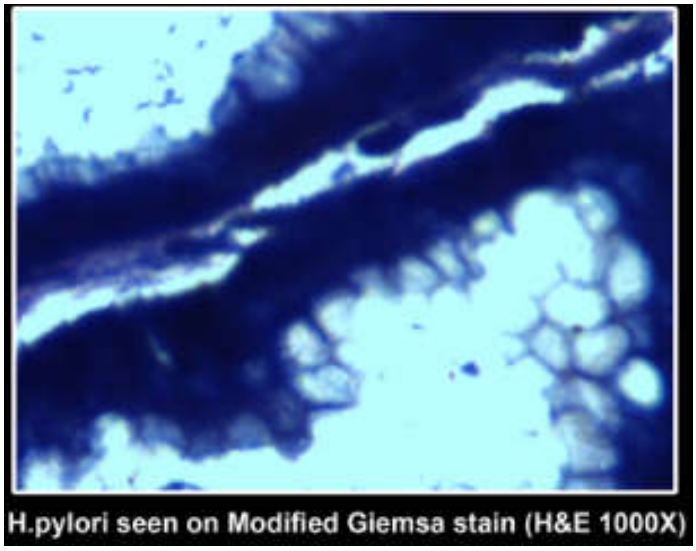
H.pylori seen on Warthin Starry (H&E 1000X)



Spiral shaped H.pylori seen on Warthin Starry stain (H&E 1000X)



H.pylori seen on Modified Giemsa stain (H&E 400X)



H.pylori seen on Modified Giemsa stain (H&E 1000X)

DISCUSSION

Astonishing success, though accidentally by Warren and Marshall in culturing H. pylori bacilli, opened new window of thinking of H. pylori as the important cause of gastritis. More than 50 % population is infected all over the world, more commonly victims belonging to underdeveloped nations. It is now crystal clear that this bacillus is the culprit of diverse gastric diseases like chronic atrophic gastritis and fore bringer of a gastric cancer, intestinal metaplasia, peptic ulcer disease, extranodal marginal zone B cell lymphoma and gastric adenocarcinoma.

Bhatia *et al.,* 1995 in their study on indian subjects found the peak prevalence of H. pylori infection in the age group of 20-40 years. In present study also peak incidence was found in age group of 21-40. Gill, *et al.* (1993) studied 340 Indian patients of dyspepsia in Mumbai and found that maximum prevalence of H. pylori infection was in 2nd decade (Gill *et al*., 1994), as was found in our study. In India exposure to H. pylori occurs early in life and is wide spread, about 83% of the population is exposed to H. pylori during 1st two decades of life (as evaluated by serological methods) The predominance of H. pylori infection in younger age group in our study might be due to the fact that the patients belonged to low socio-economic status with poor hygienic conditions in whom H. pylori infection is acquired at an early age as compared to those in the developed countries. Gill *et al.*

In their study also reported that a significant fall in the prevalence of IgA H pylori antibody from 100% in the 5th decade to 76% by the 7th decade (Ang *et al*., 2005). In the present study we found lower H. pylori positivity with increase in age beyond 6th decade. This may be the result of spontaneous clearance of H. pylori in a proportion of individuals perhaps due to local immunological response. In our study out of 100 cases, 71 were males and 29 were females, giving a M: F ratio of 2.4:1. Out of 71 males 39 (55%) were positive and 32 (45.5%) were negative. Out of 29 females 16 (53.1%) were positive, and 13 (51.9%) were negative. Gender difference found in the study is not stastically significant and bears no relation to positivity rate according to Gill HH *et al* and Graham Dx *et al*. (Ang *et al*., 2005; Jaber *et al*., 2008)

Table 5. Comparison of various staining methods with grades of h. pylori density

Histological grading parameters	Staining methods							
	H&E positive N = 50		Gimesa N =52number		Gimenez positive N =53		Warthins starry N =55	
Grade	No	%	No	%	No	%	No	%
Grade 1	26	52%	18	34.6	17	32.7	12	21.9
Grade 2	9	18%	16	30	14	26	8	14.5
Grade 3	4	8%	7	13.3	7	13.2	10	19
Grade 4	8	16%	8	15.3	10	18.9	13	23.5
Grade 5	3	6%	4	7.6	6	11.5	10	19
Grade 6	0	0%	1	1.9	1	1.8	2	3.6
No of H.Pylori not detected	5	10%	3	6.7%	2	3.6	0	0

Table 6. Statistical analysis of stains performed

Staining methods	No of cases examined	No of sample with H.pylori present	Percentage of H.pylori positivity on biopsy
H&E	100	50	50%
Gimesa	100	52	52%
Gimenez	100	53	53%
Warthin starry	100	55	55%

In our study, the most common clinical presentation in all patients with dyspepsia was pain in abdomen (55/100), followed by waterbrash and nausea and vomiting .We found the most common symptom in H. pylori infected patients was loss of appetite (62.5%) followed by pain in abdomen (54.5%). The other symptoms in decreasing order of frequency were nausea and vomiting (50%) water brash (25%) followed by belching (33.3%) which were also found in H. pylori negative cases. Anderson *et al* (1998) in their detailed analysis also showed no difference in the variety of symptoms between H. pylori infected and uninfected individuals (Anderson *et al*., 1988). At present, rapid urease testing of gastric biopsy specimens is considered the initial test of choice for the diagnosis of H. pylori at endoscopy. In our study, RUT was done on all 100 cases, and the sensitivity and specificity calculated was 76% and 92% respectively. The result were read at 1 min, 5 min, 30 min, 1 hour, 3 hours and 24 hours.

Various studies have described the role of RUT in the diagnosis of H. pylori with sensitivity and specificity of more than 90%. Laine *et al*. found 24 hr sensitivity of 75% with single biopsy (Laine *et al.,* 1996). Our sensitivity of 76% is comparable to Laine *et al*, as we also had also processed a single biopsy for RUT. Specificity of 92% is closest to that reported by Laine *et al*. (98%). Various reports confirm that histopathological examination of gastric mucosal biopsy specimens is generally considered to be the gold standard for the diagnosis of H. pylori infection. (Laine *et al*., 1997) On analysis of biopsies with reference to histologic grading of chronic inflammation , it was found that higher grades of chronic inflammation was seen in H. pylori positive cases than H. pylori negative cases. Out of 55 cases of H. pylori positive gastritis chronic inflammation was seen in 46 cases i.e.83.6% cases.

However 60% of H. pylori negative cases also revealed chronic inflammation of (27/45) amounting to 60%. Alan *et al*, (1995) in their study also found that chronic antral inflammation was the most frequently detected test parameters recorded in 205 of the 268 patients. (Alan *et al*., 1995) as found in our study. The number of H. pylori positive cases showing activity on histology was 22/55 i.e. 75%. Study conducted by Alhomsi *et al*. (Alhomsi and adeyemi, 1996) reported active inflammation in 74.5% as seen in our study but India and study conducted by Patkar *et al*. (Patkar *et al*., 2006) reported lower neutrophilic activity (61%). Atrophy was seen in 22 out of 55 (i.e. 40.2%) cases of H. pylori positive gastritis and 15 out 45 (i.e. 30.5%) in H. pylori negative gastritis. According to Oksanen *et al*. (2000), Kuipers *et al*. (1997), atrophic antral gastritis was significantly more often seen in H. pylori positive cases than in H. pylori negative cases as seen in our study. Intestinal metaplasia was found in (21 out of 55) cases of H. pylori gastritis.

In our study, atrophy and intestinal metaplasia showed similar results of positivity viz 40.2% for atrophy and 34.2% for intestinal metaplasia in H. pylori positive gastritis, and 30% and 31.1% respectively in H. pylori negative gastritis. These both parameters were found in low grade gastritis and were seen decreasing frequency with increase in the grade of gastritis. According to Genta, when metaplastic epithelium replaces the specialized epithelium of mucous glands in the antrum or the oxyntic glands in the corpus ,there is an actual loss of functional glandular tissue and therefore true atrophy. (Louw *et al*., 1993) In such instances, atrophy and intestinal metaplasia coincide, this must have happened in our cases. Superficial epithelial damage was seen in both types of gastritis i.e. H. pylori positive gastritis and H. pylori negative gastritis, but it was slightly more in H pylori negative cases (82.2%) as compared to H. pylori positive cases.(72.7%).

In our study we carried out various stains and compared them. Various stains compared for detection of H. pylori are H and E, Modified Giemsa, Gimenez and Warthin starry. H and E stain is routinely carried out stain in all histopathological laboratories and we found 50 out 100 cases were positive for H. pylori. 53 /100 cases were positive by Giemsa stain. 54/100 by Gimenez and 55/100 by warthin starry stain. Thus special stain like Modified Giemsa, Gimenez, Warthin starry were more sensitive in detecting H. pylori as compared to simple H and E. Low density H. pylori were not detected by H and E. The bacteria may be masked in H and E stained sections by inspissated mucous or being positioned flat ,closely opposed to the epithelial surface. O Rotimi *et al*. in there study ,also demonstrated that sensitivity of H. pylori is low on H and E if

density of H pylori is low. (Dunn *et al*., 1997) Laine *et al* (1997) (Midolo P. Marshall, 2000) and conduced similar study and found similar result. For low density, H. pylori sensitivity was only 70% and for high density H. pylori it was only 98%. But advantage of H and E stain is that it is cheap, easy to perform, eminently reproducible and available in every histological laboratory. When density of H pylori is high, it is readily detected by H and E. We found Modified Giemsa as a very suitable stain compared to other stains. The modified Giemsa stain is very straight forward, inexpensive, and takes about fifteen to twenty minutes to perform, and rarely requires repeat stains (none were required in our study). This method is easily reproducible. O Rotimi *et al*. (Rotimi *et al*., 2000) in their study also found similar results as in our study.

Number of organisms detected in our study by Giemsa (54/100) were more compared to H and E (50/100). Kolts *et al* also found better sensitivity of Giemsa (96%) compared to H and E (84%) (Kolts *et al*., 1993). The lack of contrast is a disadvantage of the Giemsa technique, but a careful observer should not have problems in identifying the organisms. When H. pylori are present, a careful examination will almost always reveal them, whichever of these stains is used. However, the modified Giemsa stain is the method of choice because it is sensitive, cheap, easy to perform, reproducible (Dunn *et al*., 1997) and the hands-on time required.

Conclusion

Histopathology is the investigation of choice in case of Helicobacter pylori infection as it not only detects H pylori but also provides grading of H pylori infection in terms of degree of inflammation, presence of atrophy, intestinal metaplasia, and activity. It can also detect carcinoma, lymphoma in early stages.

Special stains are required for detection of low density H pylori. Amongest this stain we found geimnez stain most useful as it is economical and quick, Modified Giemsa is also good but it requires careful search as good contrast is not provided. Warthin starry stain is time consuming, expensive ,least reproducible so difficult to use.

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