Comparative study of Hematoxylin & Eosin, special stains and Immunohistochemistry for detection of Helicobacter Pylori in both neoplastic and non neoplastic gastric lesions.

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ABSTRACT

Introduction:

Helicobacter pylori plays an important role in the causation of numerous benign, premalignant and malignant lesions of Gastrointestinal Tract.

Material and methods:

The present prospective study was carried out in the Department of Pathology, Government Medical College and Rajindra Hospital, Patiala during the duration of October 2019 to November 2021. 100 antral gastric biopsy were studied. The results of H&E, modified Giemsa (MG) and toluidine blue (TB) stain were compared to those of Immunohistochemistry which was considered as the gold standard method.

Results :

The results of the staining methods (H&E, MG, TB) in reference to that of IHC were considered false negative for any of the three tested staining methods (H&E, MG, TB), when the method

gave negative result while the gold standard (IHC) gave positive result, on other hand, the false positive results were considered when the staining method gave positive result while the gold standard (IHC) gave negative result. Sensitivity of Modified Giemsa (73.81%) was found out to be better than Toluidine blue (71.43%) and H & E (57.14%). p value was <0.001 for each stain and was found to be statistically significant.

Conclusion :

IHC is the gold standard and sensitive technique but its availability in all the health care center is not feasible so in all suspected cases of H. pylori infection Modified Giemsa should be preferred for the early detection and management of H. pylori infection.

Keywords : H. pylori, immunohistochemical, sensitivity

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]

H. pylori is a Gram-negative bacteria which has a spiral shape and affects more than 50 % and 90 % of individuals in developed countries and in developing countries respectively.[2] As eradication of this organism has become part of clinical practice, much research has been done in assessing the sensitivity and reliability of the available diagnostic method for the detection of this organism.[3]

Numerous methods are available for the diagnosis of H. pylori like non invasive methods – including urea breath test, serology and fecal antigen test and invasive methods – including rapid urease test, Polymerase Chain Reaction, histopathological examination (HPE) and culture. HPE remains the gold standard for the identification of H. pylori because it is possible to identify various pathogenic changes associated with this infection such as inflammation, intestinal metaplasia, atrophy and malignancy.[1,4]

H Pylori exhibit atypical morphologies, such as coccoid forms under certain conditions, including exposure to antibiotics. These atypical forms, which cannot be identified using routine staining methods, such as Hematoxylin & Eosin (H & E), Modified Giemsa (MG) and Toluidine Blue (TB), can be identified using Immunohistochemical (IHC) staining as this method uses specific antibodies against H. Pylori antigens. In H & E, Modified Giemsa (MG) and Toluidine Blue (TB), structures resembling H Pylori, such as other bacteria or tissue debris may provide false positive results. In contrast, IHC staining is associated with low false positive results as this method does not analyze the bacterial morphology. Therefore, IHC staining is considered to be more sensitive than other staining method to detect H. Pylori.[5]

The present study aimed to compare the diagnostic efficacy of IHC staining with that of routine Hematoxylin & Eosin (H & E), Modified Giemsa (MG) and Toluidine Blue

(TB). Additionally, the histopathological changes associated with H. Pylori positive biopsies were evaluated using Sydney system of scoring gastritis.

Material and methods:

Source of data : The present prospective study was carried out in the Department of Pathology, Government Medical College and Rajindra Hospital, Patiala with the help of Medicine department during the duration of October 2019 to November 2021. A total of 110 antral gastric biopsies were received using endoscope and out of those 100 biopsy with adequate data were randomly selected.

Inclusion criteria : All Endoscopic biopsies of gastric lesions.

Exclusion criteria : Patients who are uncooperative, less than 18 years and who are mentally unstable.

Method of data collection : Out of 110 cases, 100 cases with adequate data were randomly selected. 2 antral gastric biopsy were received for each case. Corresponding histopathological slides prepared from formalin fixed paraffin embedded tissue (4 micron thick) of endoscopic biopsies of gastric lesions subjected to H &E staining and studied. Total number of sections examined from each gastric biopsy were 12 (3 for each stain). Sections of gastric biopsy had been categorized using Sydney grading system based on activity, chronic inflammation, intestinal metaplasia, atrophy, H Pylori colonisation and the results were tabulated. Special stain (Modified Giemsa and Toluidine Blue) and Immunohistochemical study using Rabbit monoclonal (clone EP279, Bio SB) antibody and degree of antibody expression were scored in each case. Positive control was taken as a stomach infected with H Pylori.

H&E staining, Modified Giemsa and Toluidine Blue staining : First, the sections were deparaffinized by xylene and rehydrated by descending grades of alcohols. In H&E, the deparaffinized sections were stained with Harris' hematoxylin, then treated with 1% acid alcohol, and finally with 1% eosin. The modified Giemsa and Toluidine Blue staining was performed according to Culling et al.[81]

The H&E-stained, modified Giemsa and Toluidine Blue-stained slides were classified and graded according to the Sydney classification and grading system. Additionally, in H&E, the following histopathological features were examined: i) H. pylori-associated gastritis, ii) chronic gastritis, and iii) chronic active gastritis. The sections were classified based on the following parameters: degree of inflammation (mild, moderate, and severe), presence or absence of H. pylori, presence or absence of active chronic gastritis, and presence of peptic ulcer, lymphoid follicles, atrophy, and intestinal metaplasia (IM). Chronic active gastritis was confirmed based on the density of neutrophilic infiltration in the gastric mucosal crypts. IM was assessed based on the amount of glandular tissue replaced by the intestinal epithelium.

IHC staining : Three serial sections of 4 μm thickness from the formalin fixed, paraffin-embedded gastric biopsy specimens blocks, prepared above, were stained using immunostaining with monoclonal rabbit anti-H. pylori (clone EP279, Bio SB, CA 93111, USA), as primary antibodies. First, the sections were deparaffinized and rehydrated.

Then, treated for 20 min with Antigen retrieval using heat induced epitope retrieval (HIER) using Bio SB ImmunoDNA Retriever with Citrate using microwave.To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in 4% Hydrogen Peroxide Block for 10-15 minutes. Apply Ultra V Block to block nonspecific background staining. Apply primary antibody (clone EP279, Bio SB, CA 93111, USA). Apply Ultra Vision ONE HRP Polymer and incubate for 30 minutes at room temperature. Incubate with DAB for 5 minutes. Counter stain with heamatoxylin. Dehydrate, clear and mount with DPX.

All stained sections were examined. In H&E, modified Giemsa and toluidine blue, sections that showed spiral shaped rods were scored positive for H. pylori, while in IHC stained sections, the results were considered positive when the specimens showed brown staining taking spiral shaped rod or dot-like shapes located on the surface epithelium or within the mucous layer. The results of H&E, modified Giemsa and toluidine blue stains were compared to those of IHC which was considered as the gold standard method.

Statistical analysis : All statistical analyses were performed using the SPSS software version. Based on H. pylori detection, the sensitivity, specificity, positive predictive value, negative predictive value were calculated. Chi square test was used to calculate p value. p value ≤ 0.05 = significant.

Results:

Out of 100 cases studied, 90 cases were non neoplastic and 10 were neoplastic cases. Among 90 non neoplastic cases 74 were gastritis and 16 were other histopathological lesions (9- dysplasia, 4- polyp, 3- no pathological abnormality).

Out of 90 patients of Non neoplastic gastric lesions, highest incidence (47.78%) was seen in the age group of 41 to 60 years.Male: Female sex ratio was 1.72:1. p value was 0.222 which was statistically insignificant. Out of 10 patients with gastric adenocarcinoma highest incidence was seen in the age between 41 to 60 years. Male: Female sex ratio was about 4:1. p value was 0.998 which was statistically insignificant.

The most common chief complaint with which the patient presented in the present study was found out to be abdominal pain (80%) followed by vomiting (31%). Other chief complaints were Abdominal Bloating (18%), loss of appetite (13%), Loss of weight (7%) and severe anemia (6%).

The most common endoscopic finding in the present study was Erosions (25%) followed by erythema (22%) and gastric ulcer (19%). In some cases more than 1 finding was present. Other endoscopic findings were flat, nodular, hemorrhagic, mucosal edema, mucus on gastric mucosa and prominent gastric folds.

The broad spectrum of histopathological lesions were identified. Among these the most common lesion was Chronic inflammation which was present in 74 % of the cases followed by H pylori induced gastritis (24%). Neoplasm was identified in 10 cases and all were Adenocacinoma. In 3 cases however no pathologic abnormality

was identifed. Other lesions identified were activity (22 %), dysplasia (9%), polyp (4%), metaplasia (6%) and atrophy (2%).

In the present study of 74 cases of gastritis, maximum number of cases in all graded variables like activity, chronic inflammation, atrophy, intestinal metaplasia and the positivity of Helicobacter Pylori on H&E stain were seen in the Sydney score of 1 which is illustrated in table 1.

Table 1 : Sydney scoring in gastritis on H & E (N=74)

Sydney score	Activity	Chronic inflammation	Intestinal metaplasia	Atrophy	H pylori
1	20	48	6	2	13
2	2	20	0	0	9
3	0	6	0	0	2

Out of 10 cases of Gastric adenocarcinoma, 1 case was of well differentiation, 8 were moderately differentiated and 1 was poorly differentiated.

Out of 74 cases of gastritis studied for Helicobacter pylori with immunohistochemistry 42 cases showed positivity. The most common age group affected was seen between 41 to 60 years which is shown in table 2.

Table 2 : Age distribution of H. Pylori infection in cases of gastritis on IHC (N =74)

AGE YEARS	TOTAL	NEGATIVE	POSITIVE
0-20	2	1	1
21-40	20	7	13
41-60	35	16	19
>60	17	8	9
TOTAL	74	32	42

Seven cases which were negative by H & E were found to be positive by MG. Out of seven cases, four were males and three were females. Six cases which were negative by H & E were found to be positive by TB. Out of six cases, five were males and one was female. Eighteen cases which were negative by H & E but picked up by IHC.

Thirty two cases were negative by all stains. Sex distribution of positive & negative cases of Helicobacter Pylori in gastritis by different stains shown in table 3.

Table 3 : Sex distribution of positive & negative cases of Helicobacter Pylori in gastritis by different stains (N=74)

Method	H & E		MG		TB		IHC	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
M	16	31	20	27	21	26	31	16
F	8	19	11	16	9	18	11	16
Total	24	50	31	43	30	44	42	32

Out of 10 cases of gastric adenocarcinoma studied for Helicobacter pylori with immunohistochemistry 1 cases showed positivity. The age group affected was between 41 to 60 years and was male.

The detection of Helicobacter pylori using H&E, MG, TB and IHC was identical in cases of grade 2 and grade 3 colonisation of H. pylori. Differences in staining pattern were observed in grade 1 when there is low colonisation of H.pylori. The above table concluded that IHC is the best modality to detect H pylori with least negative rate and better detection as in the present study IHC picked up the 18 new cases of H. pylori which were in Grade 1 and were missed by H & E might be due to low density of the organism. p value calculated using Chi Square method was 0.765 and was statistically significant. The grading of H. Pylori infection in gastritis using sydney scoring system by various staining methods shown in table 4.

Table 4 : Grading of H. Pylori infection in gastritis using sydney scoring system by various staining methods (N=74)

Staining method	Total number of cases	H pylori positive cases (%)	Grade I	Grade II	Grade III	H pylori negative
H&E	**74**	24	13	9	2	50
MODIFIED GIEMSA	**74**	31	20	9	2	43
TOLUIDINE BLUE	**74**	30	19	9	2	44
IHC	**74**	42	31	8	3	32
p value	0.765					
Significance	NS					

The results of the three tested staining methods (H&E, MG, TB) were in reference to that of IHC Shown uin table 5. The results were considered false negative for any of the three tested staining methods (H&E, MG, TB), when the method gave negative result while the gold standard (IHC) gave positive result, on other hand, the false positive results were considered when the staining method gave positive result while the gold standard (IHC) gave negative result. The IHC was taken as a gold standard reference method in present study. It detected H. pylori in 42 (56.76%) of tested gastric biopsies, while the remaining 32 (43.24%) cases were H. pylori-negative. Sensitivity of Modified Giemsa (73.81%) was found out to be better than Toluidine blue (71.43%) and H & E (57.14%). p value was <0.001 for each stain and was found to be statistically significant.

Table 5 : Comparison of H and E, MG and TB in reference to IHC for detection of H. Pylori in case of gastritis (N=74)

		IHC			Sensitivity	Specificity	PPV	NPV	p value
		+VE	-VE	Total					
		(n)	(n)	(n)					
H & E	+vE	24	0	24	57.14%	100.0%	100.0%	64%	<0.001
	-vE	18	32	50					
M G	+ve	31	0	31	73.81%	100.0%	100.0%	74.41%	<0.001
	-ve	11	32	43					
T B	+ve	30	0	30	71.43%	100.0%	100.0%	72.73%	<0.001
	-ve	12	32	44					
Total		42	32	74					

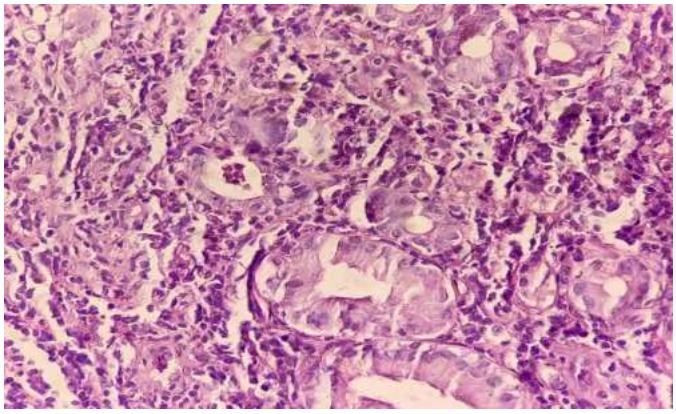

Figure 1 : High power view of gastric biopsy revealing presence of neutrophils in the lumen of glands = Activity (H & E; X400)

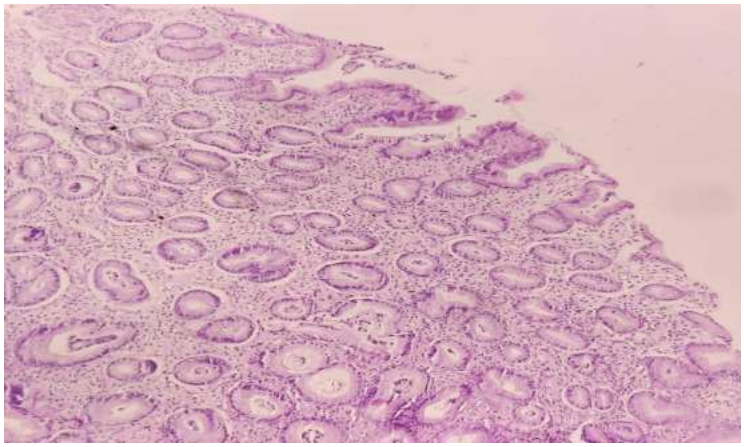

Figure 2 : Scanner view of atrophic gastritis showing loss of gastric pits. (H&E; X40)

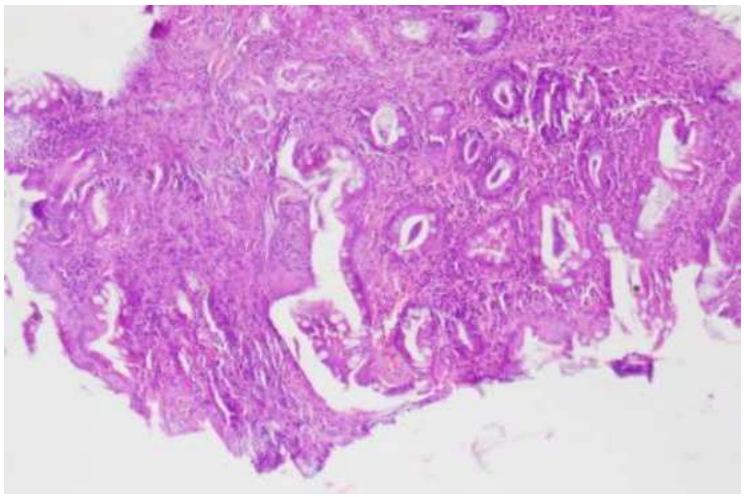

Figure 3 : Low power view of antral biopsy showing intestinal metaplasia along with presence of goblet cells(H & E; X100)

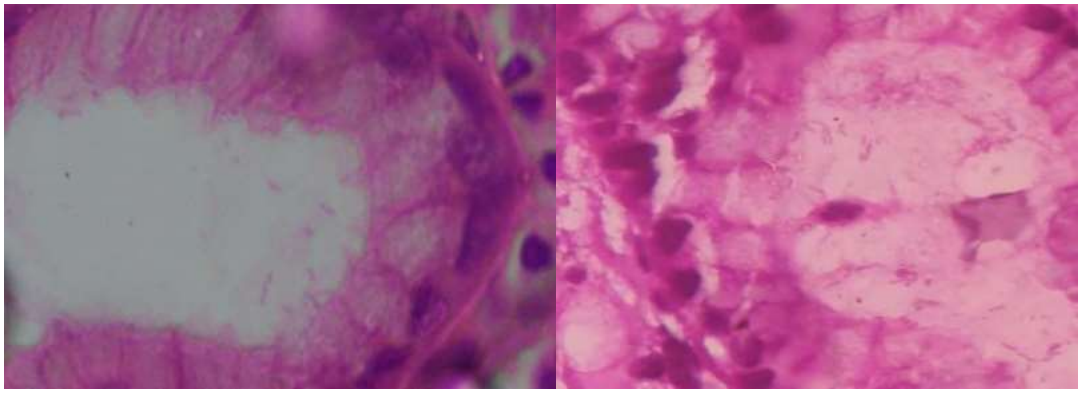

Figure 4: Oil immersion view of antral biopsy showing presence of H. Pylori- curved, slender, spiral shaped bacilli inside the lumen of gland- a) Grade 1 colonisation (H & E ; X1000) b) grade 2 colonisation.

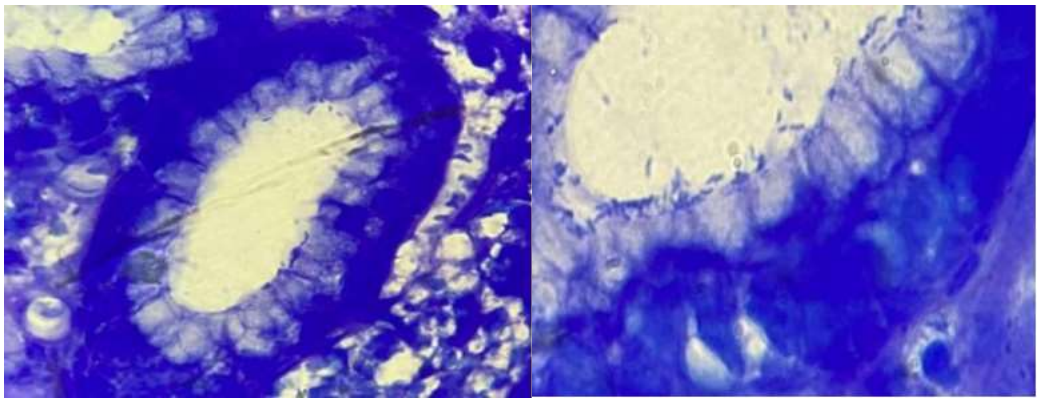

Figure 5: Oil immersion view showing H. Pylori- a) grade 1 colonisation.(MG; X1000) b) grade 2 colonisation (MG; X1000)

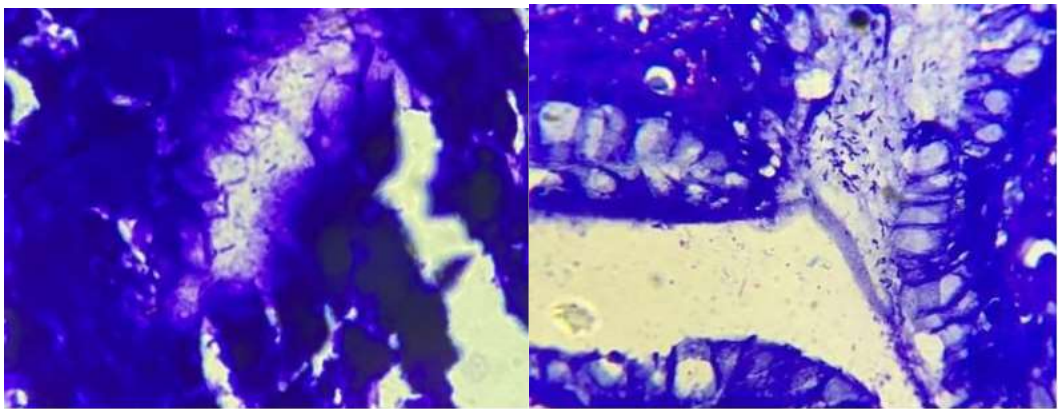

Figure 6: Oil immersion view showing H. Pylori- a) grade 1 colonisation (TB; X1000) b) grade 3 colonisation (TB; X1000)

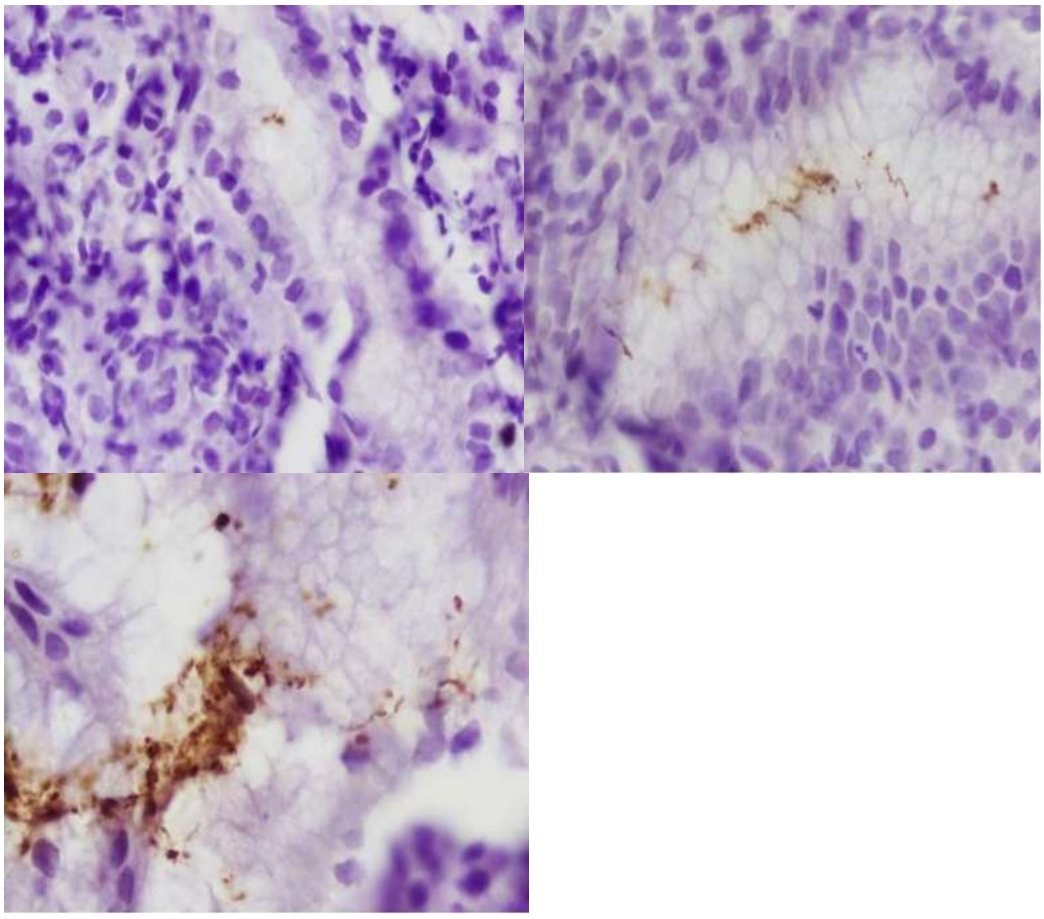

Figure 7 : Oil immersion view showing H. Pylori- a) grade 1 colonisation (IHC; X1000) b) grade 2 colonisation (IHC; X1000) c) grade 3 colonisation (IHC; X1000)

Discussion:

H pylori is a Gram-negative, spiral organism, which colonizes the gastric mucosa. H. pylori infection is found to be seen with gastritis, gastric ulcer, gastric adenocarcinoma, and MALT lymphoma. Therefore, H. pylori documention is a need in a gastric biopsy for giving appropriate patient care. The youngest patient was 10 years old and this was only pediatric case (below 12 year of age) and the oldest patient was 90 years old. This relative variation could be due to different risk factors prevalent among different age groups. Out of 90 patients of non neoplastic lesions, highest incidence seen in the age group of 41 to 60 years. The findings were similar to the study done by Hussain et al[6] and Sharma et al.[7] Out of 10 patients with gastric adenocarcinoma highest incidence was seen in the age group between 41- 60 years. The findings was similar to the study done by Hussain et al.[6]

In the present study, males undergoing upper GI endoscopy outnumbered the female patients. The commonest chief complaint with which the patients presented was abdominal pain which was in concordance with the study done by Narain et al.[8] and Memon et al.[9]

Most of the gastric lesions encountered were non neoplastic (90%) and 10% were neoplastic. Among non neoplastic lesions majority were inflammatory followed by H pylori induced gastritis (42%). The majority of the gastric lesions were benign in present study. These results were comparable to Poudel et al[10] while in contrast to Pailoor et al.[11] Among neoplastic lesions of the stomach, Adenocarcinoma was most commonly seen which was similar to a study conducted by Pesic et al.[12]

In the present study out of 74 cases of gastritis, 42 cases were positive for H. pylori with a prevalence rate of about 56.75 % and out of 10 cases of gastric adenocarcinoma 1 cases showed positivity for H pylori with a prevalence rate of about 10.0 %. The overall prevalence rate was about 51.19%. This was in accordance with the study done by Thappa et al[13], Adisa et al[14] and Keel et al.[15] The most common age group affected with H. pylori was seen between 41-60 years because of the cumulative effect of poor hygiene, lower socioeconomic status, overpopulation, poor sanitation and prolonged use of cigarette smoking, tobacco chewing and alcohol. These findings were in concordance with study done by Adisa et al[14], Dogar et al.[16] In the present

study, among 42 H.Pylori positive cases of gastritis 31 were males and 11 were females. 1 case positive for H.pylori in adenocarcinoma was of male.

As compared to H&E staining, the histopathological changes cannot be determined by IHC staining. However, IHC staining is regarded the most accurate direct histopathological staining method for the detection of H. pylori. The sensitivity and specificity of IHC staining is higher due to the use of specific antibodies, ability to detect atypical bacterial forms, such as coccoid forms, and low false-positive rates. Moreover, IHC is considered when other routine methods fail to detect H. pylori in association with chronic gastritis caused due to minimal infection or atypical distribution of bacteria in affected tissue. The limited use of IHC staining for routine diagnostic application is high cost. Hence, IHC staining is preserved for cases with minimal H. pylori infection, post treatment and to detect the atypical forms

In the present study, the detection of the H. pylori using H & E, MG, TB and IHC was identical in cases of grade 2 and grade 3 colonisation of H. pylori. Difference in the staining pattern were observed in grade 1 when there is low colonisation of H. pylori. As in the present study 13 cases were detected by H & E in grade 1 whereas IHC detected 31 cases of H. pylori in grade 1 leading to the inference that IHC detected the increased number of H. pylori which were in low density in grade 1 and were not picked up by H & E. These results concurred with those of Siddiqui et al.[17] These study correlating between chronic active gastritis and presence of H pylori infection. In this study for detection of H.Pylori, the sensitivity and specificity of various staining methods like H&E, MG, TB and IHC were compared with the study done by Narain A et al[8], Tajalli R et al[18] and Mulimani SB et al[19]. In 74 cases of gastritis H&E showed 57.14% sensitivity and specificity of about 100%, MG showed 73.81 % sensitivity and specificity of about 100%. TB showed 71.43% sensitivity and specificity of about 100%. In 10 cases of gastric adenocarcinoma, only 1 case show positivity for H pylori with IHC.

Conclusion:

IHC is the most sensitive technique but it is not economical to use IHC in all gastric specimens. The cost, reliability and applicability of MG and TB make them as suitable stains for identification of H. pylori in gastric biopsies. In the present study MG stain carries higher level of sensitivity over TB and H&E. MG stain is also a less time consuming procedure when compared with IHC. Hence in the present study MG was more reliable and cost effective stain when compared with H & E, TB and IHC. Though IHC is the gold standard and sensitive technique but its availability in all the health care center is not feasible so in all suspected cases of H. pylori infection Modified Giemsa should be preferred for the early detection and management of H. pylori infection.

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