



Histomorphological Study Of Gastric Biopsies And Gastrectomy Specimens And Their Correlation With *H. Pylori*

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Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Gastritis is defined as inflammation of Gastric mucosa which is a histological diagnosis. Inflammation can be acute with Neutrophilic Infiltration or chronic with lymphocytes and plasma cells and associated with intestinal metaplasia and atrophy. Acute Gastritis result from ingestion of alcohol, salicylates and other anti-inflammatory drugs or by reflux of bile salts. Endoscopic biopsies are taken in these conditions. In chronic Gastritis the two main features are infiltration of lamina propria by inflammatory cells and atrophy of glandular epithelium. Among inflammatory cells, Plasma cells and lymphocytes predominate but eosinophils and Neutrophils may also be present. Two types of metaplastic changes occur in chronic gastritis, pyloric metaplasia in fundic mucosa and intestinal metaplasia.

Aim Of Study: To study the Histomorphological spectrum in endoscopic gastric biopsies and gastrectomy specimens with prevalence of *H.pylori* infection.

Methods : This Cross-sectional observational study was conducted in Pathology and gastroenterology Department of Pathology, Government Tiruvannamalai Medical College, Tiruvannamalai, Tamil Nadu, India .After getting informed consent from all the patients they were subjected to overnight fasting. The upper gastrointestinal endoscopy using a video endoscope under topical anesthesia using 2% xylocaine jelly was done. Using a standard endoscopic forceps gastric mucosal biopsies were taken within 2 cm of the pylorus. The biopsy was fixed in 10% buffered formalin for histopathological examination. Sterilization of biopsy forceps was done using 2% glutaraldehyde solution after each individual patient. Following sterilization the endoscope and biopsy forceps were cleaned with sterile saline as residual glutaraldehyde can also cause suppression of *H.pylori*.

Results: A total number of 100 biopsies were studied, of which 72 were males and 28 were females. The age group varied from 20 years to 88 years with mean age of 54.88 years and standard deviation of 13.2 years. Among the *H.pylori* positive cases, maximum percentage of cases were in the age group of 60 – 69 years (30.7%) and minimum percentage of cases were in the age group of 80 – 89 years (1.0%) Of the total *H. pylori* positive cases 6 (11.1%) had chronic duodenitis, 13 (24.1%) had Gastric ulcer, 26 (48.1%) had chronic gastritis and 9 (16.7%) had Gastric carcinoma. Among the patients diagnosed as chronic duodenitis, 6(11.1%) were

H.pylori positive and 7(13.0%) were *H.pylori* negative. Among the patients diagnosed as gastric ulcer, 13(24.1%) were *H.pylori* positive and 6 (11.1%) were *H.pylori* negative. Among the patients diagnosed as chronic gastritis, 26(48.1%) were *H.pylori* positive and 14(25.9%) were *H.pylori* negative. Among the *H.pylori* positive cases, 10 cases show mild inflammation, 23 cases show moderate inflammation and 14 cases show marked inflammation. p value is found to be significant (< 0.05). Among the *H.pylori* positive cases, 15 cases show mild neutrophilic activity, 20 cases show moderate neutrophilic activity and 0 cases show marked neutrophilic activity. p value is found to be highly significant(< 0.001). Among the *H.pylori* positive cases, 12 cases show mild, 3 cases show moderate and 2 cases show marked intestinal metaplasia. The p value is found to be not significant ($P > 0.05$). Among the biopsies which showed presence of eosinophils, 20 cases were *H.pylori* positive and 15 cases were *H.pylori* negative. P value is found to be not significant ($P > 0.05$). Among the biopsies which showed presence of dysplasia, 7 cases were *H.pylori* positive and 5 cases were *H.pylori* negative. P value is found to be not significant (> 0.05). Among other histopathological parameters gastric carcinoma and lymphoid follicles shows significant P value (< 0.05)

Conclusion : *H.pylori* infection is prevalent world wide but its incidence is more common in developing countries like India. The awareness of histomorphological features like chronic inflammation, Intestinal metaplasia and mucosal atrophy which are typical to *H.pylori* gastritis could help the pathologist in identifying such conditions which can later progress to gastric carcinoma. Thus it is important for early detection of *H.pylori* infection in the high risk population so that treatment strategy can be planned to reduce the menace of *H.pylori* infection and its associated diseases. Our sample size shows significant association with *H.pylori* infection and Gastric carcinoma.

Keywords: laparoscopic sleeve gastrectomy, histopathological findings, active chronic gastritis, Helicobacter pylori.

Introduction

Gastritis is defined as inflammation of Gastric mucosa which is a histological diagnosis. Inflammation can be acute with Neutrophilic Infiltration or chronic with lymphocytes and plasma cells and associated with intestinal metaplasia and atrophy. Acute Gastritis result from ingestion of alcohol, salicylates and other anti-inflammatory drugs or by reflux of bile salts^[1]. Endoscopic biopsies are taken in these conditions. In chronic Gastritis the two main features are infiltration of lamina propria by inflammatory cells and atrophy of glandular epithelium. Among inflammatory cells, Plasma cells and lymphocytes predominate but eosinophils and Neutrophils may also be present. Two types of metaplastic changes occur in chronic gastritis, pyloric metaplasia in fundic mucosa and intestinal metaplasia. Complete [type1] and incomplete[type2] are the two types of intestinal metaplasia. *H.pylori* is absent in foci of type 1 intestinal metaplasia but present in type 2 intestinal metaplasia^[2]. Most of the cases of chronic gastritis are due to infection by *H.pylori*. *H.pylori* are associated with peptic ulcer

disease, gastric cancer and with gastric primary mucosa associated lymphoid tissue lymphoma[MALT]. [3] *H.pylori* organisms were previously called as Campylobacter pylori. *H.pylori* is a non sporing, rod shaped, curvilinear, gram negative organisms measuring $3.5 \times 0.5 \mu\text{m}$. *H.pylori* is part of a genus of bacteria that have adapted to the ecologic niche provided by gastric mucosa. It is mainly present on the mucosal surface with only small percentage of organisms seen intracellularly.[4] *H.pylori* can be recognised in routine H&E stains, and in most instances however if the density of organism is low the detection can be greatly facilitated by performance of special stains which include Giemsa, Warthin- Starry or Steiner silver stains, Alcian yellow- toluidine blue method, Genta stain or by immunohistochemistry^[5]

Methods : This Cross-sectional observational study was conducted in Pathology and gastroenterology, Department of Pathology, Government Tiruvannamalai Medical College, Tiruvannamalai, Tamil Nadu, India After getting informed consent

from all the patients they were subjected to overnight fasting. The upper gastrointestinal endoscopy using a video endoscope under topical anesthesia using 2% xylocaine jelly was done. Using a standard endoscopic forceps gastric mucosal biopsies were taken within 2 cm of the pylorus. The biopsy was fixed in 10% buffered formalin for histopathological examination. Sterilization of biopsy forceps was done using 2% glutaraldehyde solution after each individual patient. Following sterilization the endoscope and biopsy forceps were cleaned with sterile saline as residual glutaraldehyde can also cause suppression of *H.pylori*.
INCLUSION CRITERIA: All gastric biopsies and gastrectomy specimens submitted to pathology department are included.
EXCLUSION CRITERIA: Inadequate biopsies inconclusive for diagnosis.

ENDOSCOPY:After getting informed consent from all the patients they were subjected to overnight fasting. The upper gastrointestinal endoscopy using a video endoscope under topical anaesthesia using 2% xylocaine jelly was done. Using a standard endoscopic forceps gastric mucosal biopsies were taken within 2 cm of the pylorus. The biopsy was fixed in 10% buffered formalin for histopathological examination.

Stastical Analysis

Data was entered in the proforma and was tabulated in Microsoft excel 2013. SPSS (Stasistical package for the social sciences) software was used to analyse the data. Presentations was done using tables, pie charts and bar graphs.

Result

Table 1: Gender-Wise Distribution Of H. Pylori

Gender	H. pylori		Total
	Positive	Negative	
Male	36 (50.0%)	36 (50.0%)	72
Female	11 (39.3%)	17 (60.7%)	28
Total	47	53	100

Table :1 Among the 72 males, 36 (50.0%) were H. pylori positive and 36(50.0%) were H.pylori negative.Among the 28 females 11(39.3%) were H. pylori positive and 17(60.7%) were H.pylori negative.

Table 2: Clinical/ Endoscopic Diagnosis

	H. pylori		Total
	Positive	Negative	
Chronic duodenitis	6 (11.1%)	7 (13.0%)	13
Gastric Ulcer	13 (24.1%)	6 (11.1%)	19
Chronic Gastritis	26 (48.1%)	14 (25.9%)	40
Gastric Carcinoma	9 (16.7%)	27 (50.0%)	36

Of the total H. pylori positive cases 6 (11.1%) had chronic duodenitis, 13 (24.1%) had Gastric ulcer, 26 (48.1%) had chronic gastritis and 9 (16.7%) had Gastric carcinoma

Table 3: Presence Of H.Pylori Among Chronic Duodenitis Cases

	H. pylori	
	Positive	Negative
Chronic duodenitis	6 (11.1%)	7 (13.0%)

Table :3 Among the patients diagnosed as chronic duodenitis, 6(11.1%) were H.pylori positive and 7(13.0%) were H.pylori negative.

Table 4: Presence Of H.Pylori Among Gastric Ulcer Cases

	H. pylori	
	Positive	Negative
Gastric Ulcer	13(24.1%)	6 (11.1%)

Among the patients diagnosed as gastric ulcer, 13(24.1%) were H.pylori positive and 6 (11.1%) were H.pylori negative.

Table 5: Presence Of H.Pylori Among Chronic Gastritis Cases

	H. pylori	
	Positive	Negative
Chronic Gastritis	26 (48.1%)	14 (25.9%)

Among the patients diagnosed as chronic gastritis, 26(48.1%) were H.pylori positive and 14(25.9%) were H.pylori negative.

Table 6: Presence Of H.Pylori Among Gastric Carcinoma Cases

	H. pylori	
	Positive	Negative
Gastric carcinoma	9 (16.7%)	27 (50.0%)

Among the patients diagnosed as Gastric carcinoma 9(16.7%) were H.pylori positive and 27(50%) were H.pylori negative.

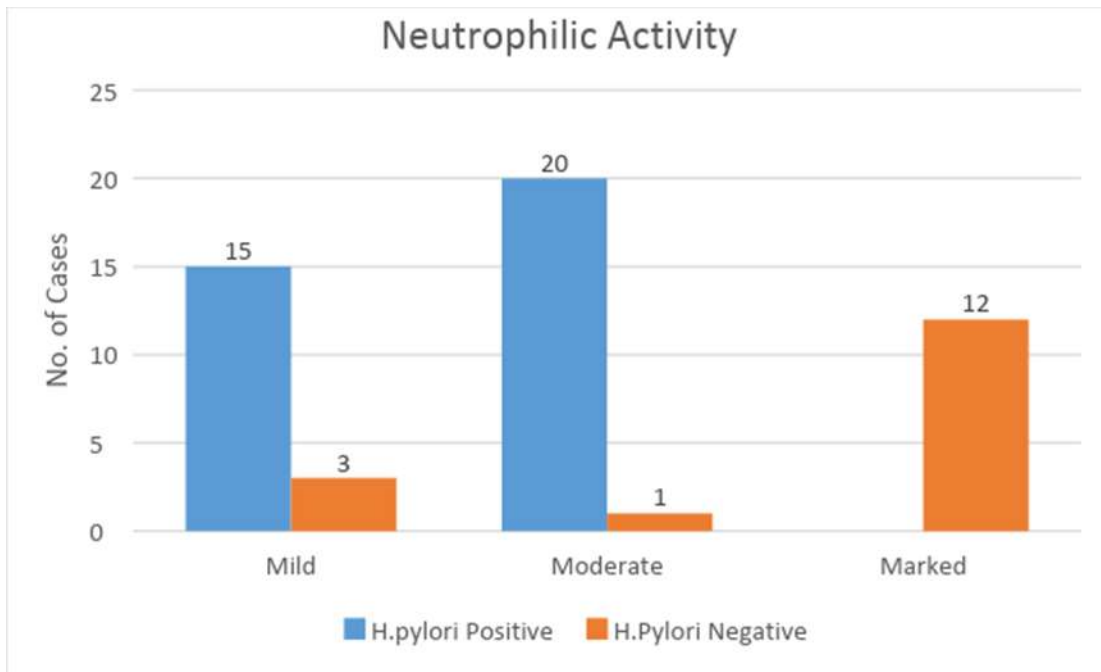
Table 7: Graded Histopathological Parameters

Parameters	H. pylori		Total	p-value
	Positive	Negative		

Chronic Inflammation	Mild	10	25	28	p < 0.05 (Significant)
	Moderate	23	19	42	
	Marked	14	9	30	
Neutrophilic Activity	Mild	15	3	18	P < 0.001 (Highly significant)
	Moderate	20	1	21	
	Marked	0	12	12	
Intestinal Metaplasia	Mild	12	7	19	p > 0.05 (not significant)
	Moderate	3	4	7	
	Marked	2	1	3	
Mucosal atrophy	Mild	4	3	7	p > 0.05 (not significant)
	Moderate	0	0	0	
	Marked	0	0	0	

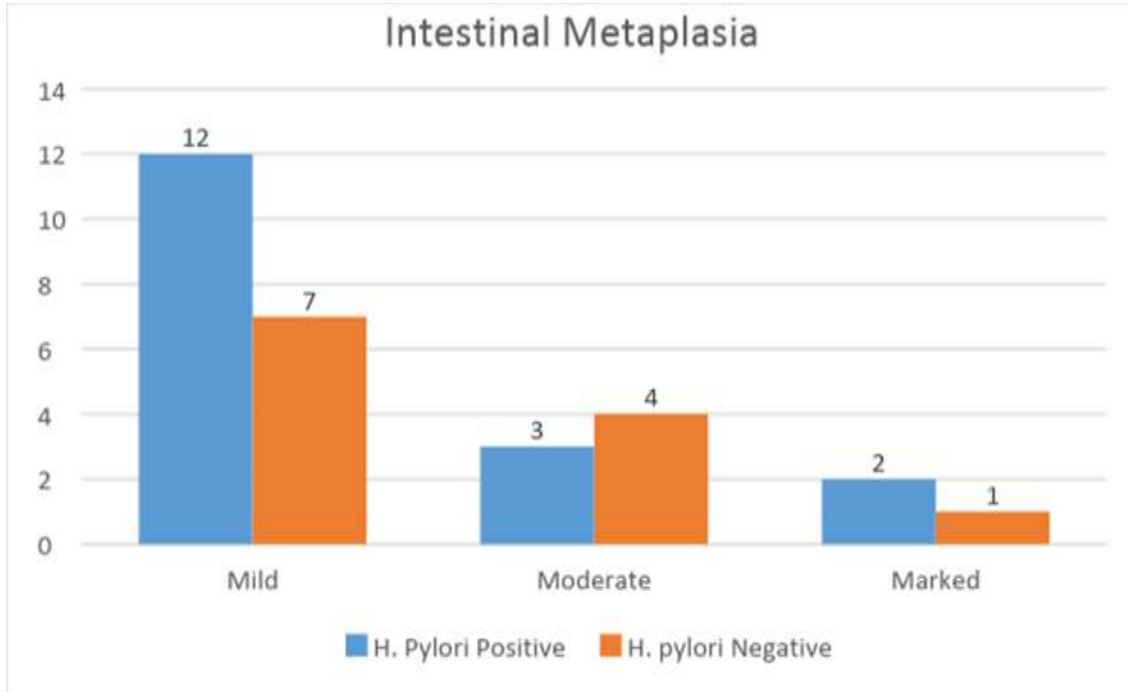
Among the H.pylori positive cases, 10 cases show mild inflammation, 23 cases show moderate inflammation and 14 cases show marked inflammation. p value is found to be significant (< 0.05).

Chart 1: Neutrophilic Activity



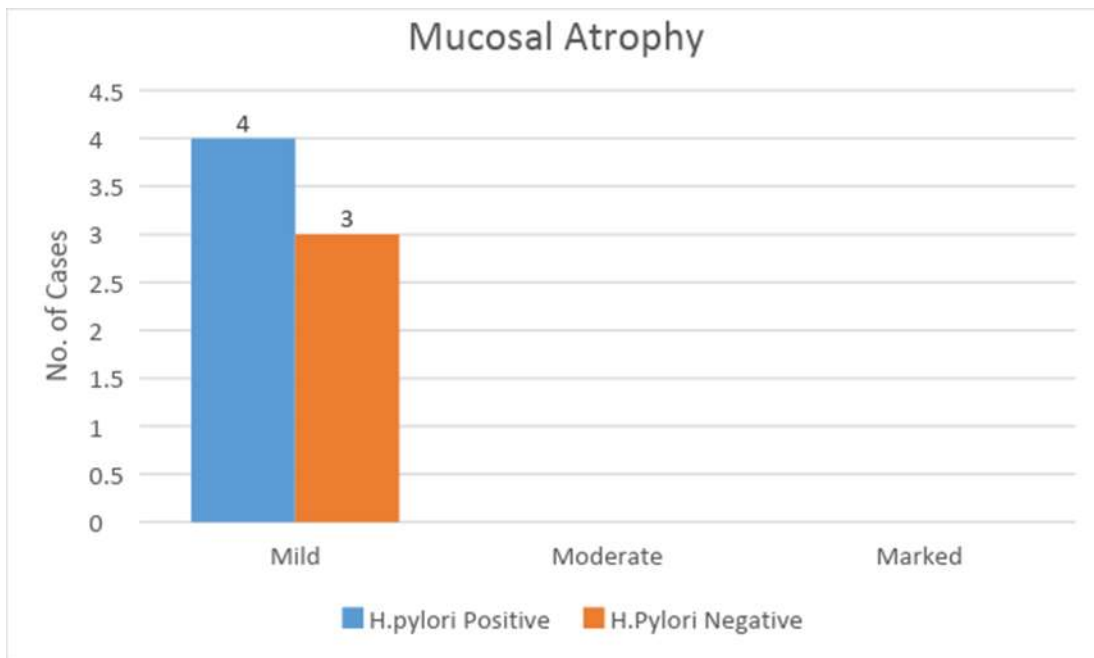
Among the H.pylori positive cases, 15 cases show mild neutrophilic activity, 20 cases show moderate neutrophilic activity and 0 cases show marked neutrophilic activity. p value is found to be highly significant(< 0.001).

Chart 2: Intestinal Metaplasia



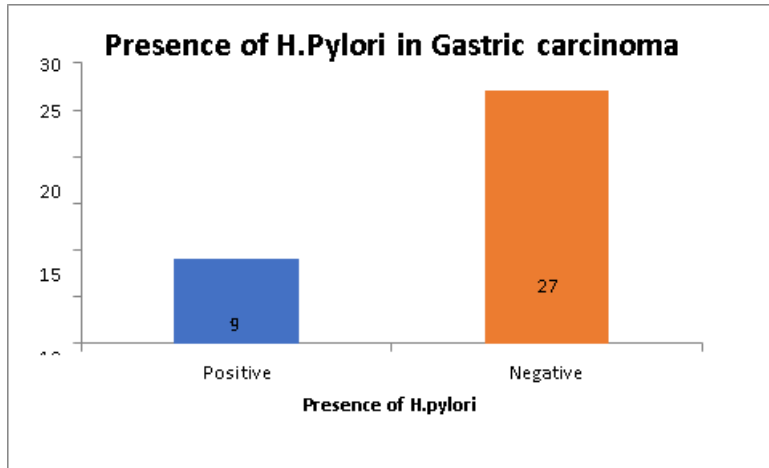
Among the H. pylori positive cases, 12 cases show mild, 3 cases show moderate and 2 cases show marked intestinal metaplasia. The p value is found to be not significant ($P > 0.05$).

Chart 3: Mucosal Atrophy



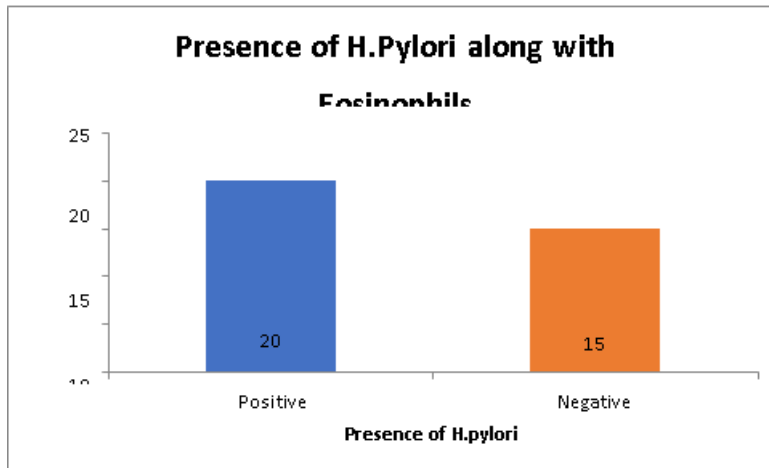
Among the H. pylori positive cases, 4 of them had mucosal atrophy. The p value is found to be not significant ($P > 0.05$).

Chart 4: Presence Of H.Pylori In Gastric Carcinoma



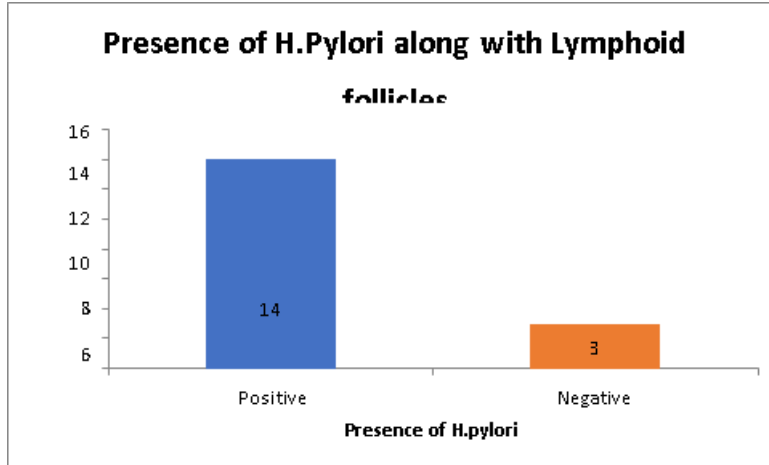
Among the biopsies diagnosed as Gastric carcinoma 9 (16.7%) cases were H.pylori positive and 27 (50%) cases were H.pylori negative. The p value is found to be significant ($P < 0.05$).

Chart 5: Presence Of H.Pylori Along With Eosinophil



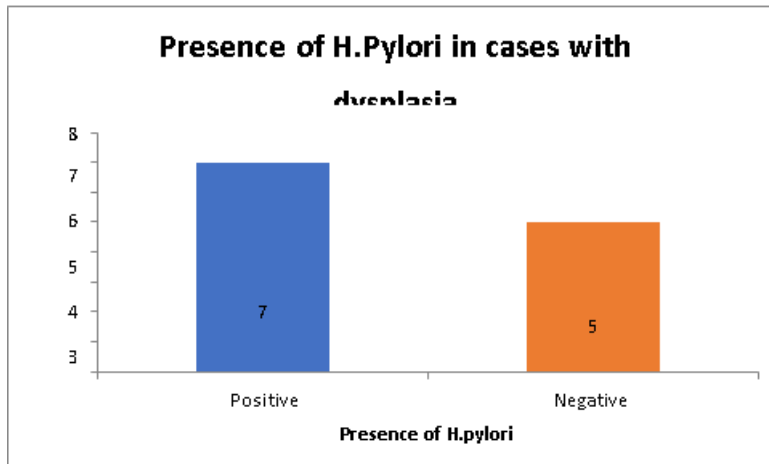
Among the biopsies which showed presence of eosinophils, 20 cases were H.pylori positive and 15 cases were H.pylori negative. P value is found to be not significant ($P > 0.05$).

Chart 6: Presence Of H.Pylori Along With Lymphoid Follicle



Among the biopsies which showed presence of lymphoid follicles, 14 cases were found to be H.pylori positive and 3 cases were found to be H.pylori negative. The p value is found to be significant (< 0.05).

Chart7: Presence Of H.Pylori In Cases With Dysplasia



Among the biopsies which showed presence of dysplasia, 7 cases were H.pylori positive and 5 cases were H.pylori negative. P value is found to be not significant (> 0.05).

Table 8: OTHER HISTOPATHOLOGICAL PARAMETERS

Parameters	H. pylori		P-value
	Positive	Negative	
Gastric carcinoma	9	27	<0.05 (significant)
Eosinophils	20	15	>0.05 (not significant)
Lymphoid follicles	14	3	<0.05 (significant)

Dysplasia	7	5	>0.05 (not significant)
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Among other histopathological parameters gastric carcinoma and lymphoid follicles shows significant P value (< 0.05)

Figure 1 H.PYLORI LYING IN THE MUCOSA(MODIFIED GIEMSA (100 X)

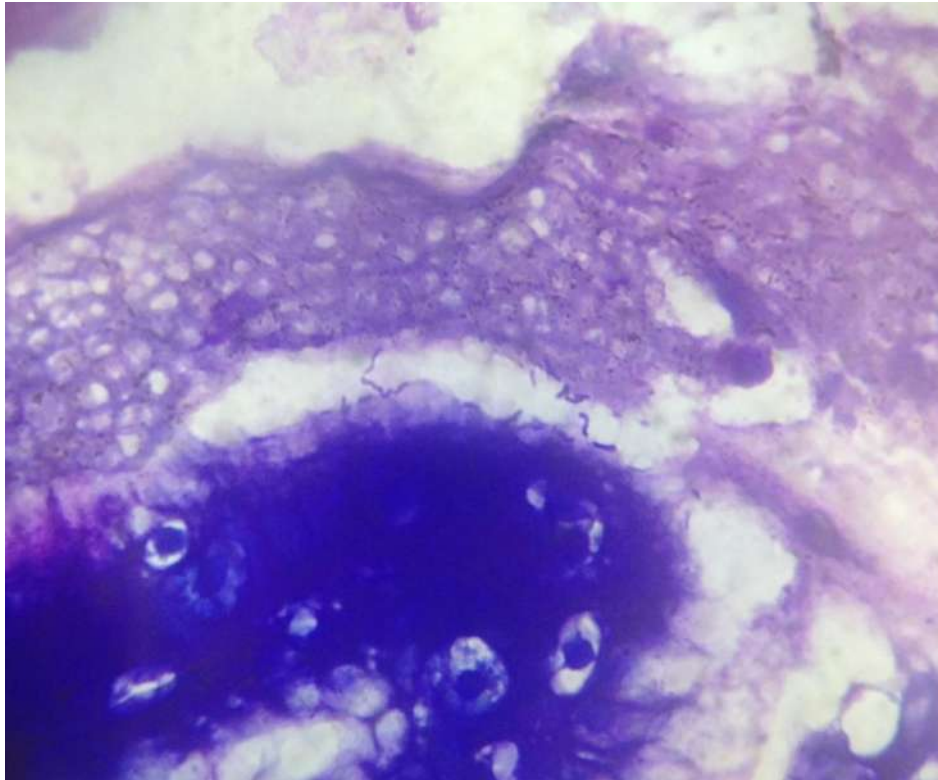


Figure 2 H.PYLORI LYING IN THE CRYPTS (H&E 100X)

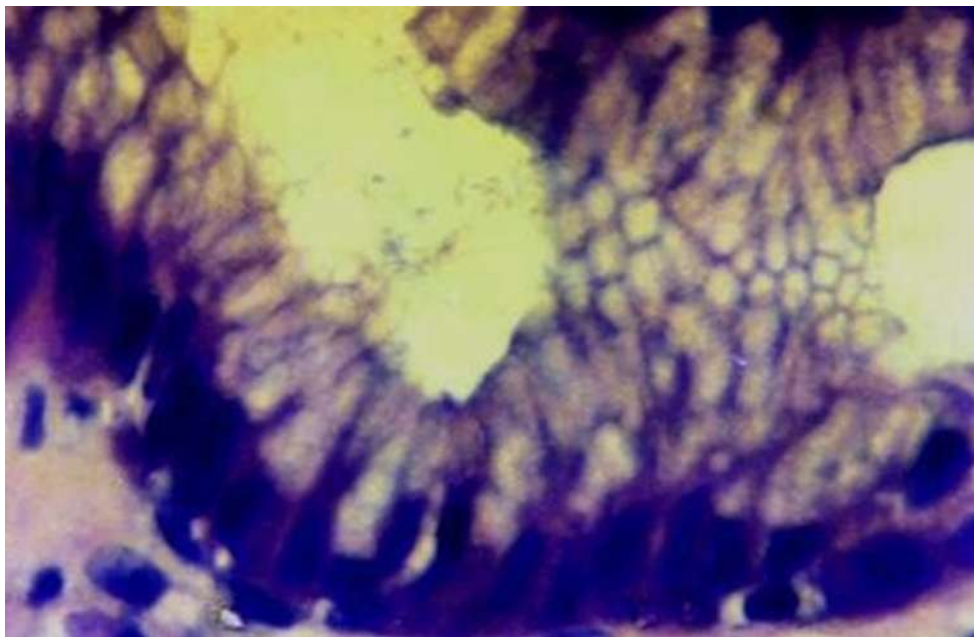


Figure 3. WELL DIFFERENTIATED ADENOCARCINOMA (H&E 10X)

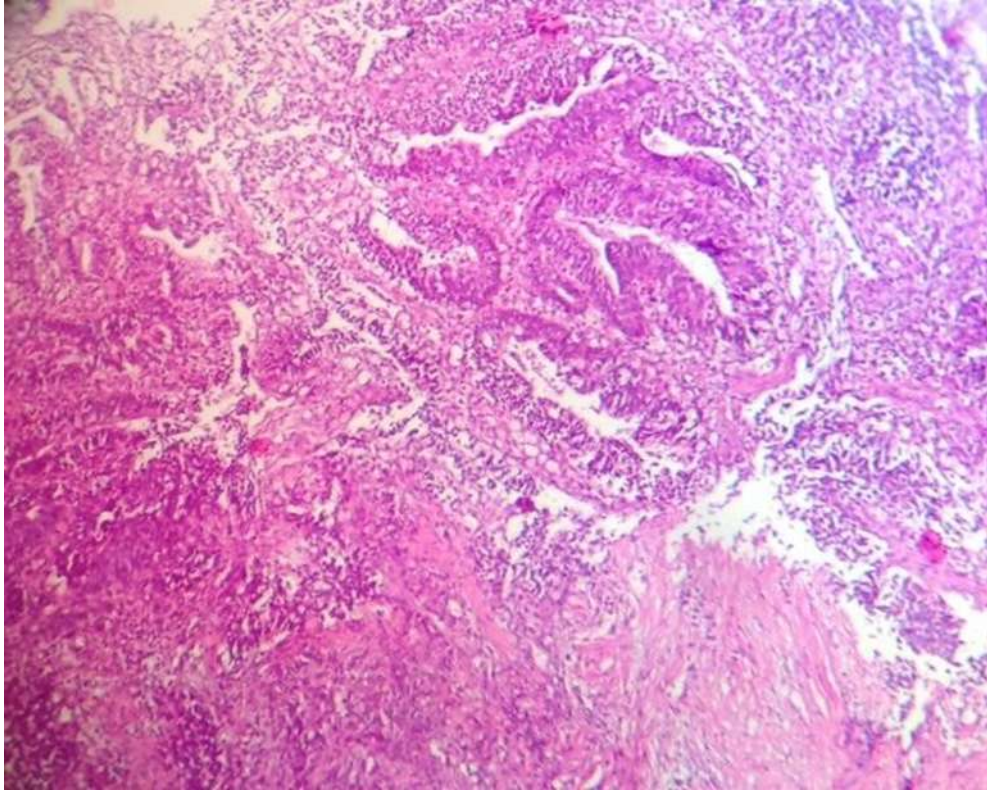


Figure 4 WELL DIFFERENTIATED ADENOCARCINOMA (H&E 40X)

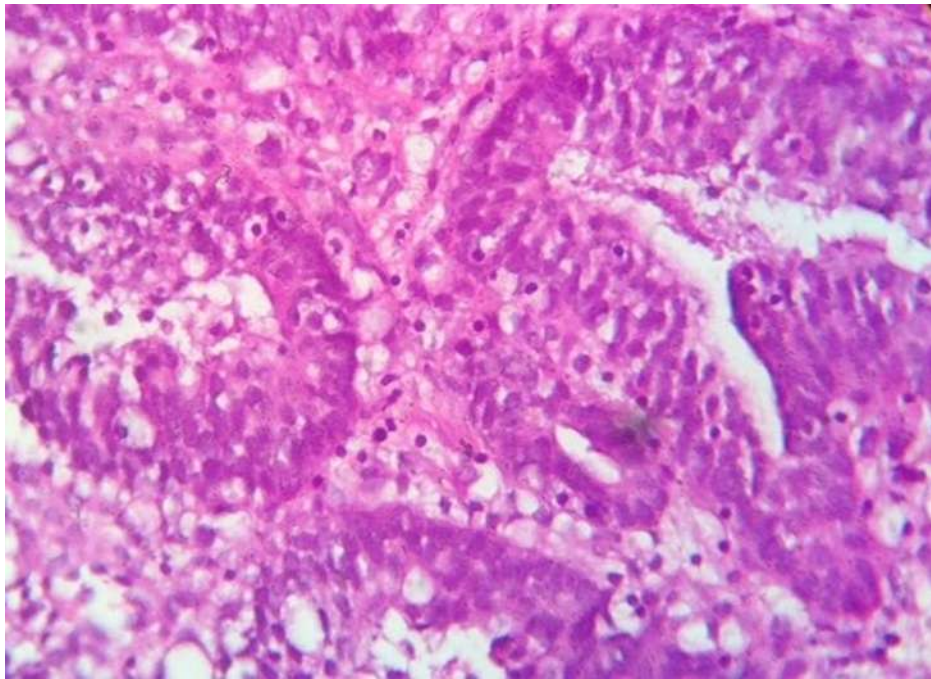


Figure 5 WELL DIFFERENTIATED ADENOCARCINOMA INFILTRATING INTO MUSCULARIS LAYER (H&E 10X)

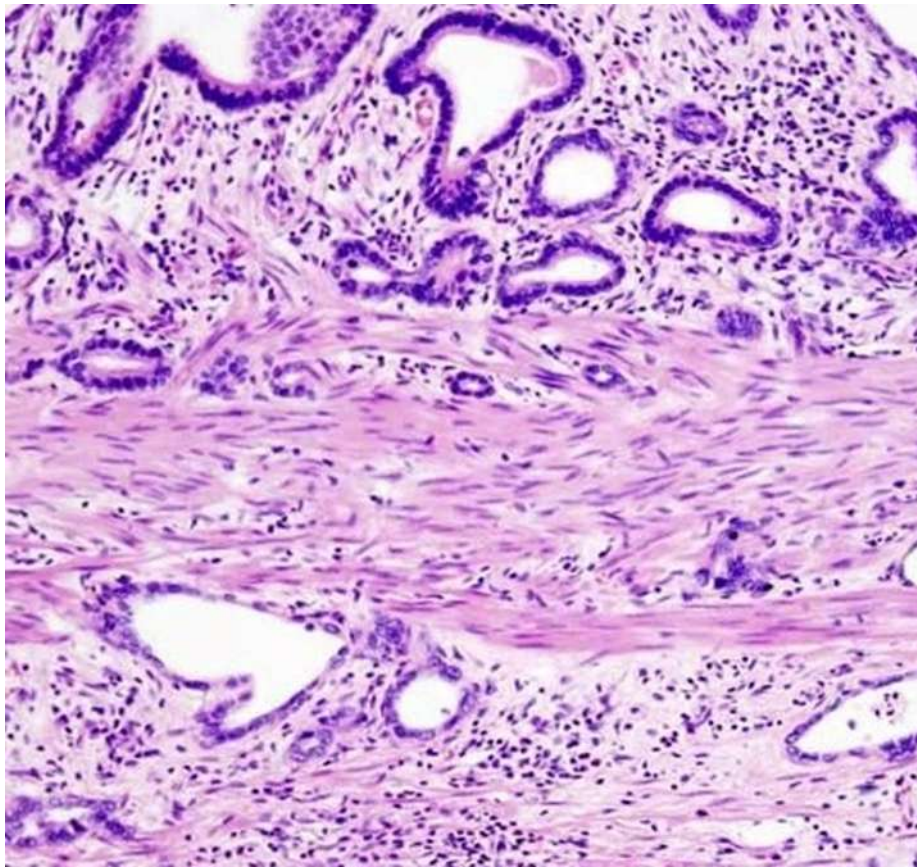


Figure 6 CHRONIC GASTRITIS SHOWING LYMPHOID FOLLICLE FORMATION (H&E 10X)

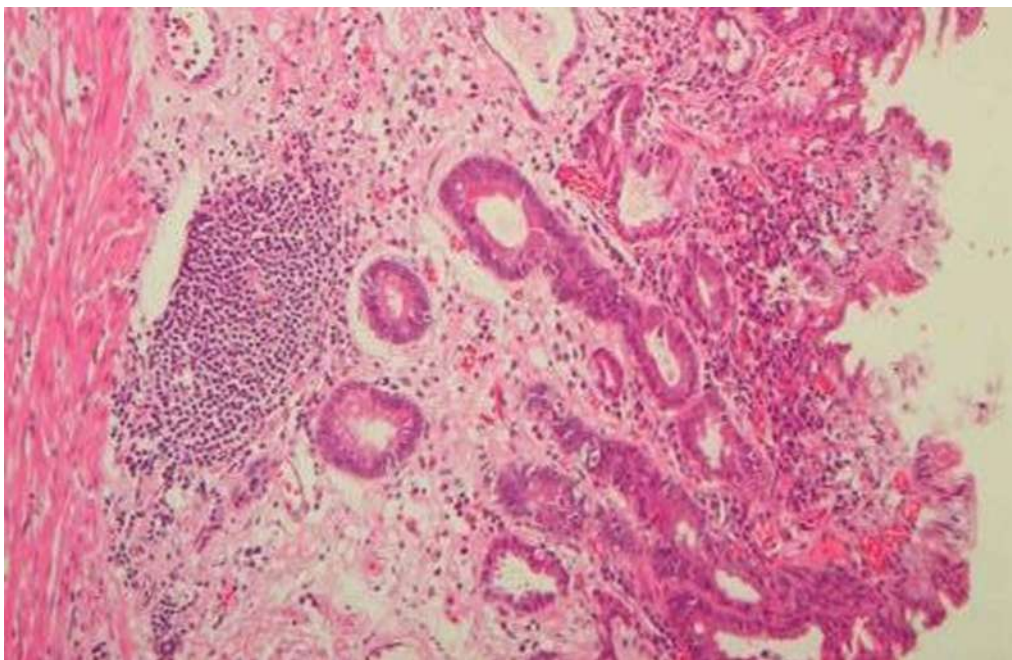


Figure 7 GASTRIC BIOPSY SHOWING ATROPHY AND METAPLASIA (H&E 10X)

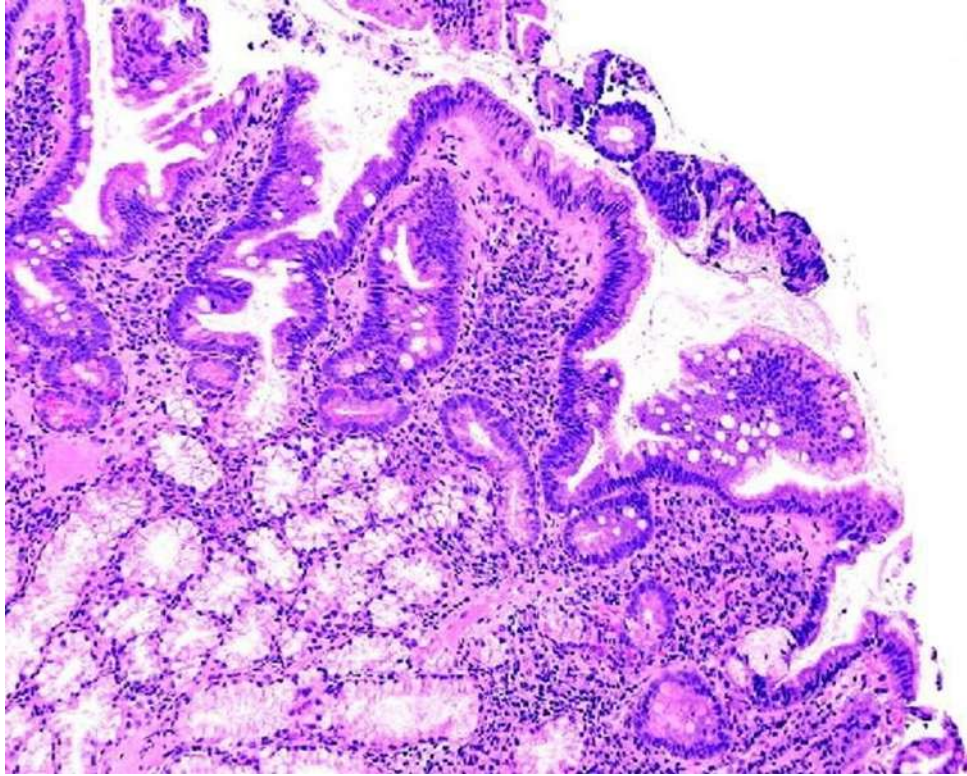


Figure 8 GASTRIC GLANDS SHOWING ATROPHY (H&E 10X)

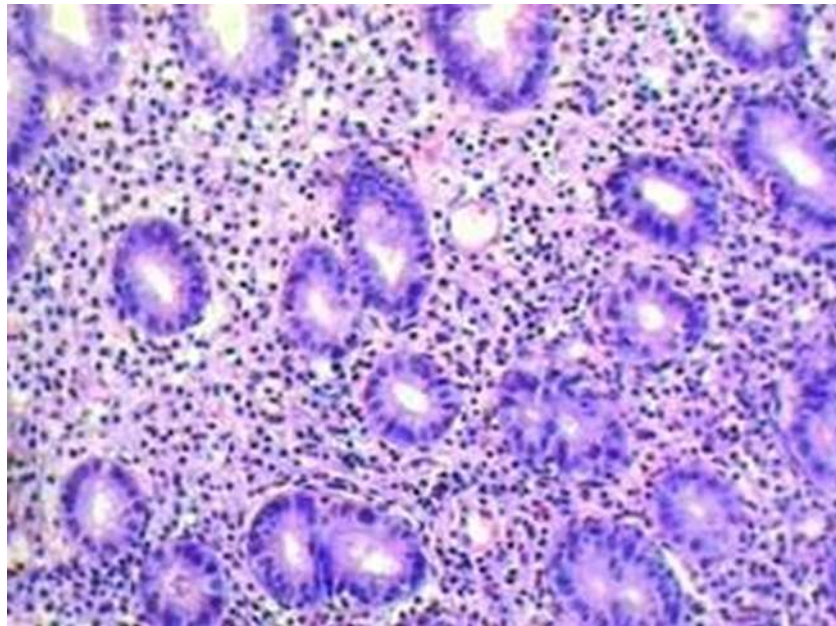


Figure 9 GASTRIC GLANDS SHOWING DYSPLASIA (H&E 40X)

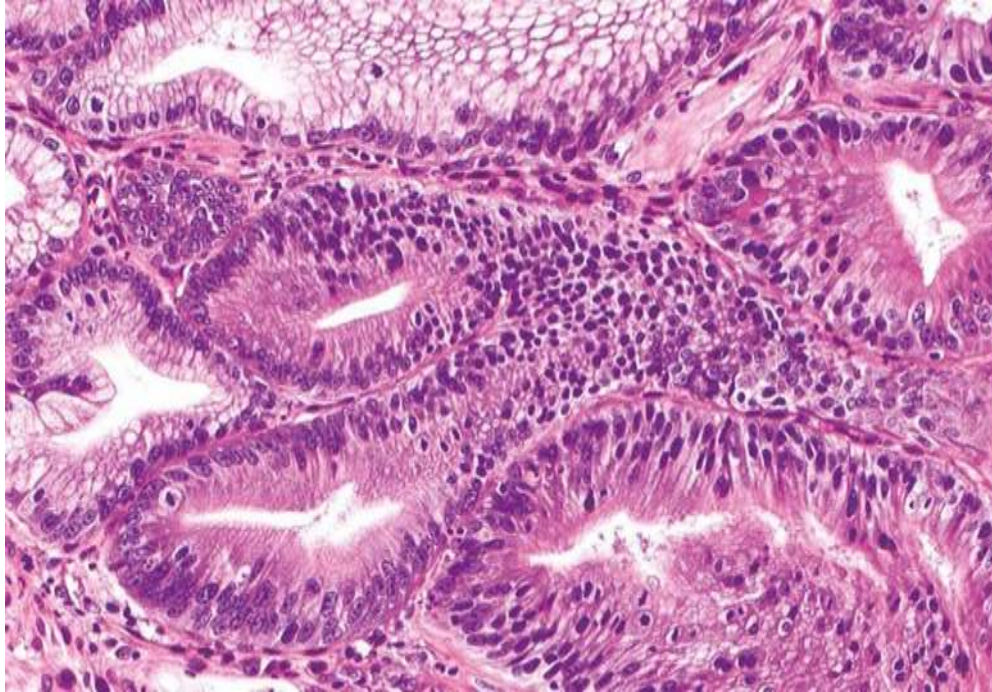


Figure 10 EOSINOPHILIC GASTRITIS (H&E 40x)

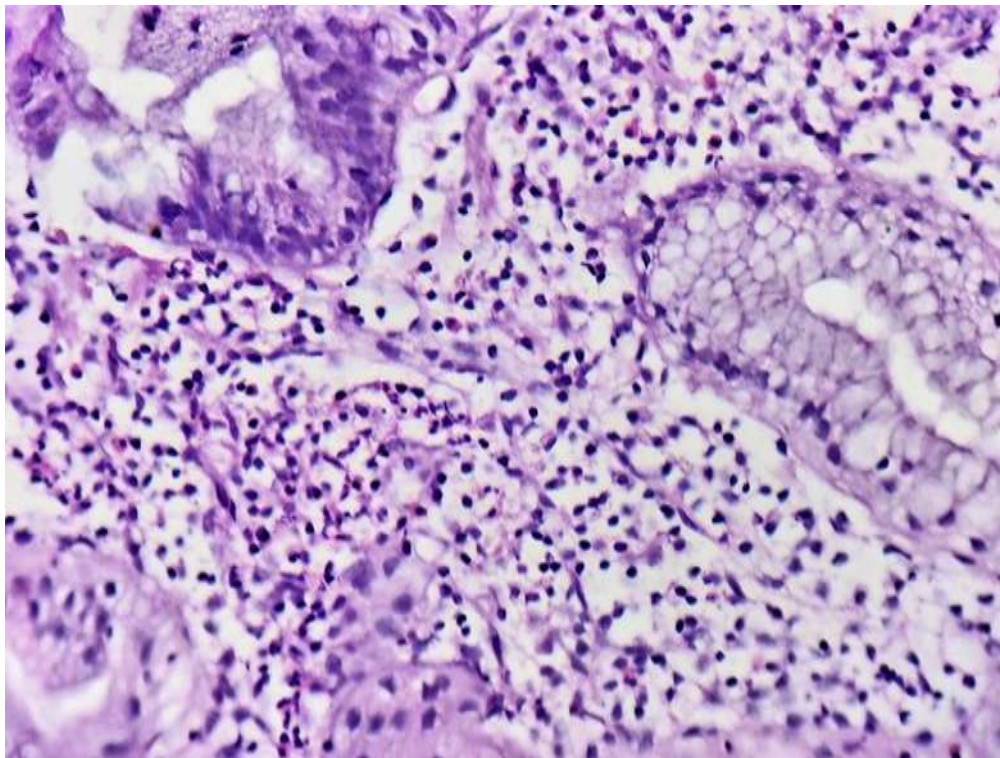


Figure 11 ACUTE GASTRITIS SHOWING NEUTROPHIL INFILTRATION INTO THE GLANDS(H&E 40X)

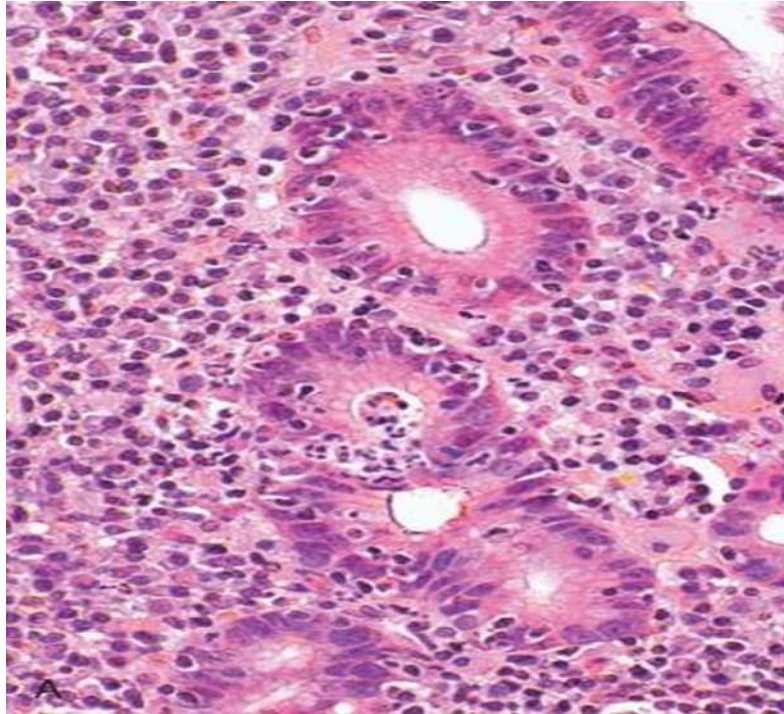


Figure 12 LYMPHOCYTIC INFILTRATION IN CHRONIC GASTRITIS(H&E 40X)

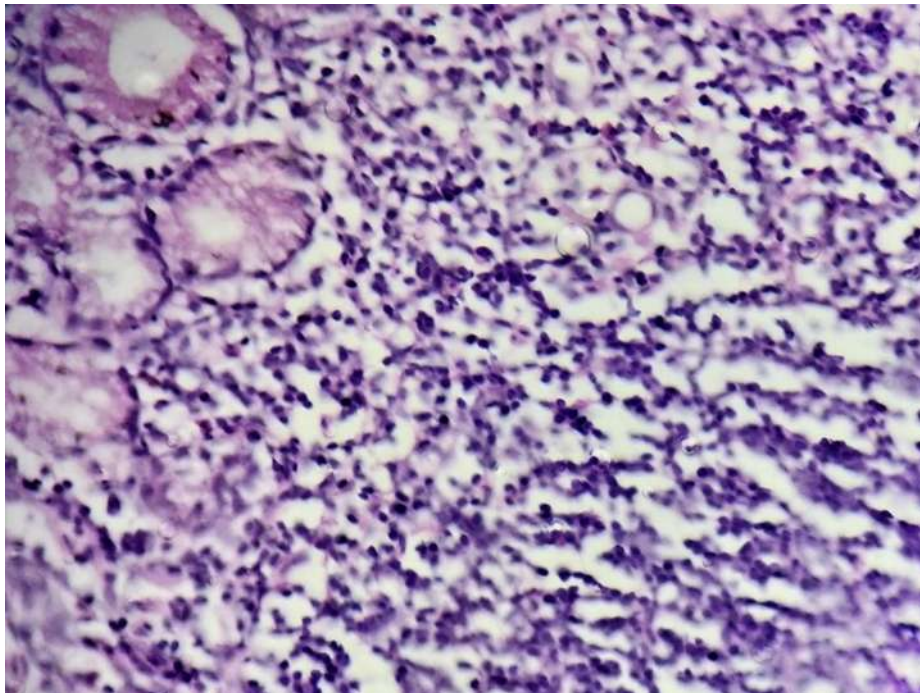


Figure 13 PLASMA CELL INFILTRATION IN CHRONIC GASTRITIS(H&E 40X)

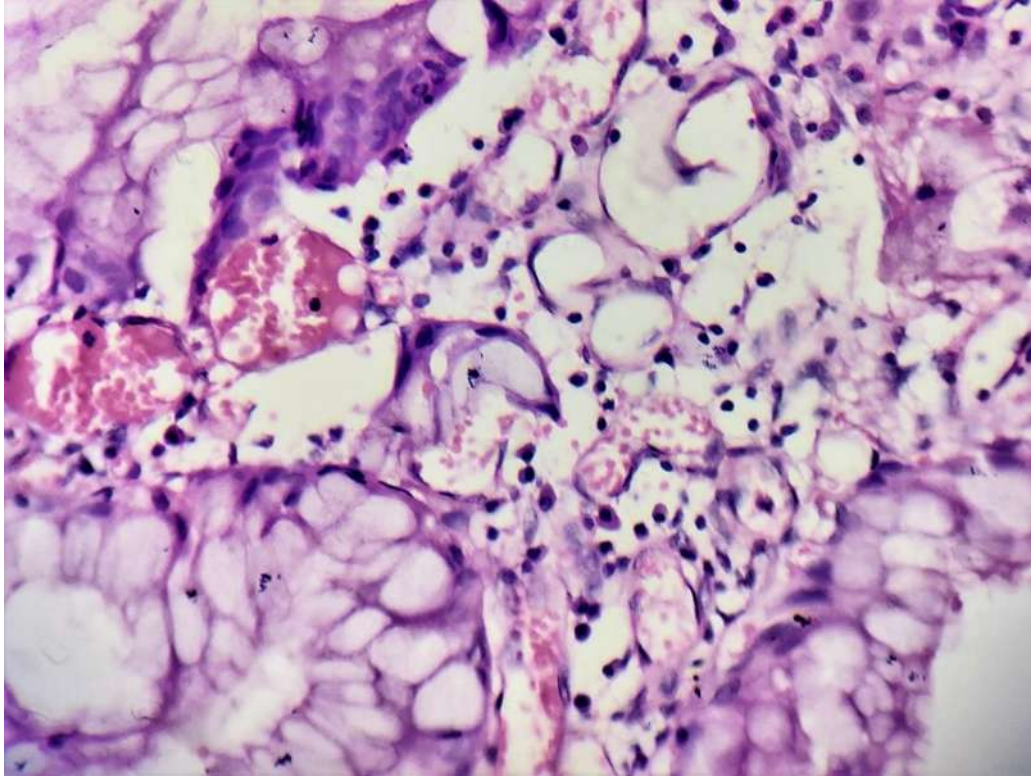
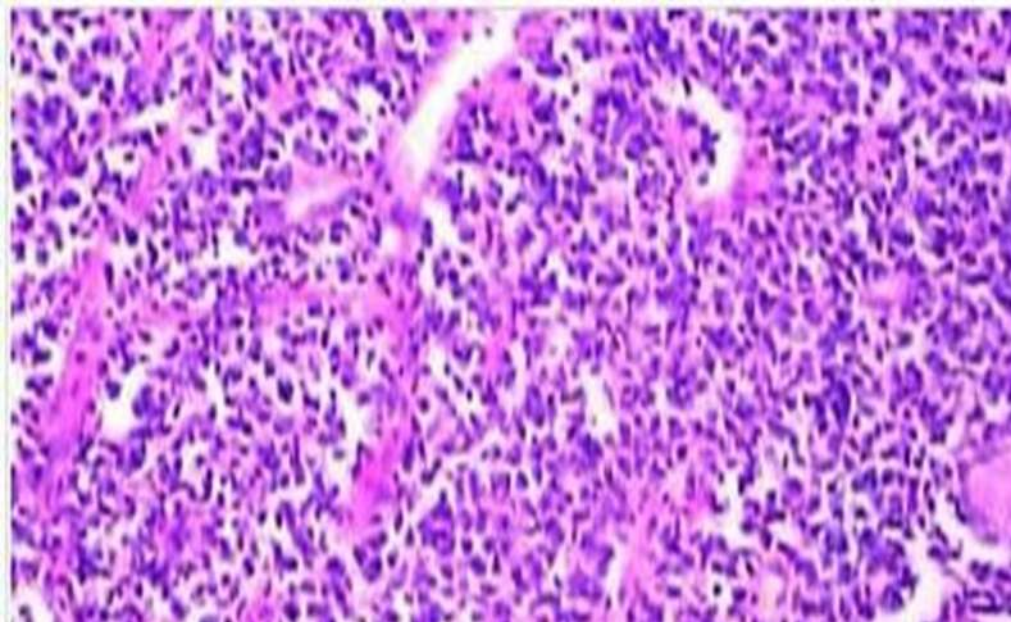


Figure 14 LYMPHOMA



Discussion

A total number of 100 biopsies were studied, out of which 47 were *H.pylori* positive and 53 were *H.pylori* negative. There was no significant

difference in the age and sex distribution of the two groups. The clinical/ endoscopic diagnosis of the two groups were similar except that majority of patients with chronic gastritis (48.1%) and gastric ulcer (24%) showed *H.pylori* positive. The prevalence rate of

H.pylori infection was 47% in this study. [6]The higher incidence of *H.pylori* infection in India is due to lack of good sanitation and overcrowding because *H.pylori* spreads by fecal oral route. 24.1% Of gastric ulcer patients and 11.1% of chronic duodenitis patients showed positivity for *H.pylori* in our study. The distribution of *H.pylori* is patchy in the gastric mucosa. Hence if the appropriate area is not biopsied, the organism may be missed completely. [7]In most of the other studies, multiple antral biopsies were subjected to histopathological examination. The quality of gastric mucus is altered by urease production by *H.pylori*, predisposing gastric mucosa to ulceration. Another cause for decreased mucosal resistance to acid attack is chronic inflammation. Chronic duodenitis occur when high gastric acidity leads to gastric metaplasia in proximal duodenum. The metaplastic mucosa may be colonized by *H.pylori*. Among the antral biopsies 48.1% of patients with chronic gastritis showed *H.pylori* positivity. This compared well with the previous studies.[8] Dysplastic changes in the epithelium was present in 14.89% of *H.pylori* positive cases and the association was not found to be statistically significant. There are studies showing significant relationship between *H.pylori* infection and dysplastic changes. But the sample size being small in the present study, the association was found to be statistically insignificant.[9] The different histopathological parameters were graded according to updated Sydney system, in *H.pylori* positive and negative biopsies and the results were compared. Marked chronic inflammation were present in 29.78% of *H.pylori* positive cases (moderate in 48.93% and mild in 21.27%) compared to only 16.98% of *H.pylori* negative cases. P value < 0.05. Studies have demonstrated a direct relation between the extent of neutrophilic infiltration and severity of mucosal injury in *H.pylori* infection. But intraepithelial neutrophils were rare to find, where as pit abscesses were more common.[10] We found that presence of neutrophils in a background of chronic gastritis (chronic active gastritis) was highly suggestive of *H.pylori* infection. P value < 0.001. Intestinal metaplasia was present in 36.17% of patients with *H.pylori* positivity. This may lead on to dysplastic changes and ultimately constitute the background for development of carcinoma. [11]In the study conducted by Rugge and Colleagues, the

prevalence of intestinal metaplasia among non- ulcer patients with *H.pylori* gastritis was 40.6%. This occurs only in the late stages of *H.pylori* infection. Only 4 of our biopsies showed evidence of mucosal atrophy in *H.pylori* positive cases. Among the graded variables, a comparison between *H.pylori* positive and negative biopsies was done (chi-square test). [12]This showed that presence of chronic inflammation (P value < 0.05) and neutrophils (P value < 0.001) were significantly associated with *H.pylori* positivity. This may be attributed to neutrophil chemotactic factors produced by organisms. The neutrophil activation also thought to play a role in the carcinogenesis by *H.pylori*. The Non graded variables which were significantly associated with *H.pylori* infection were lymphoid follicles (P value < 0.05) and Gastric carcinoma (P value < 0.05).[13] According to Genta, lymphoid follicles were present in all patients with *H.pylori* gastritis. In our study 29.78% of biopsies with lymphoid follicles showed the presence of *H.pylori* infection. When lymphoid follicles were present in the absence of *H.pylori* (5.66% of our patients), it was observed that either the patient had taken antibiotic therapy recently or the organisms might have missed.[14,15] In our study *H.pylori* infection was significantly (P value < 0.05) associated with Gastric carcinoma.

Conclusion

H.pylori infection is prevalent world wide but its incidence is more common in developing countries like India. The awareness of histomorphological features like chronic inflammation, Intestinal metaplasia and mucosal atrophy which are typical to *H.pylori* gastritis could help the pathologist in identifying such conditions which can later progress to gastric carcinoma. Thus it is important for early detection of *H.pylori* infection in the high risk population so that treatment strategy can be planned to reduce the menace of *H.pylori* infection and its associated diseases. Our sample size shows significant association with *H.pylori* infection and Gastric carcinoma.

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