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Role of Immunohistochemistry Versus he Stain in *Helicobacter pylori* Detection in Gastric Lesions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Helicobacter pylori are a spiral campylobacter like bacteria which infects the stomach causing chronic active gastritis. This can result in peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. In 1989, studies involving 16S ribosomal RNA gene sequencing and others revealed that the bacterium does not come under the genus Campylobacter. Hence the bacterium was classified under a unique genus named *Helicobacter*. This term is taken from the Greek language with helix meaning - "spiral" or "coil" and pylori meaning- gatekeeper (pylorus of stomach). This bacterium is said to penetrate the pylori or mucoid lining of the stomach. When infected during the early stages of life, people develop intense inflammation that may be followed by atrophic gastritis which serves as a risk factor for gastric ulcer, gastric cancer or both. Being infected during later stages of life changes the gastric system leading to duodenal ulcer. The present study analyzes the role of Immunohistochemistry versus Hematoxylin and Eosin and special stains in detecting Helicobacter pylori in gastric lesions.

Keywords: Helicobacter pylori; peptic ulcer disease; gatekeeper; gastritis.

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1. INTRODUCTION

Helicobacter pylori, a gram negative, micro aerophilic bacterium usually found in the stomach was previously known as Campylobacter Pylori. Chronic antral gastritis was mainly the result of this bacterium. Studies also show that the development of duodenal ulcer and gastric carcinoma may be caused by *Helicobacter* pylori. Chronic gastritis is a common problem worldwide. Although other causal agents (smoking [1], NSAIDs [2], spicy foods and socioeconomic status [3] were previously implicated in the etiology of chronic gastritis *Helicobacter pylori* has currently been reported as the most common cause [4]. Hence, the determination of its presence in surgical pathology specimens to manage the two common diseases of the upper gastrointestinal tract is significant.

A simple and cost effective means of alternatively diagnosing *Helicobacter pylori* infection is by using the Antral biopsy specimens processed for histology. A topographic study to determine the density, distribution and the comparison of biopsy sites for the histopathological diagnosis of *H.pylori* concludes that two antral biopsy specimens, (from lesser and greater curvature) showed almost 100% sensitivity in detecting *H.Pylori* infection [5]. If intestinal metaplasia is extensively present in the antrum of the biopsy specimens retrieved from the corpus, then the diagnostic yield increases [6]. Although there is a great variation in the sensitivity and specificity, different special stains have been used in detecting *Helicobacter pylori* in these histological sections. The most common stains used in histology are haematoxylin and eosin stain. Additionally, considering its easy usage and availability, the modified version of Giemsa stain is preferred in most laboratories. In spite of a varied range of stains, the identification of the bacterium is based on its morphology.

Development in Immunohistochemical techniques has led to the use of anti *Helicobacter pylori* antibody which reacts with its somatic antigensin correlation with the bacteria's presence. The aim of the current study was to determine the better method in diagnosing *Helicobacter pylori* –the modified Giemsa stain vs Immunohistochemical technique.

This term is taken from the Greek language with helix meaning - "spiral" or "coil" and pylori meaning- gatekeeper (pylorus of stomach) [7]. This bacterium is said to penetrate the pylori or mucoid lining of the stomach [8]. However, among alcoholics, the inflammatory changes seems to be coinciding with Helicobacter pylori infection. Furthermore, continuous alcohol consumption also results in the presence of gastric metaplasia. The present study was aimed to assess *Helicobacter pylori* detection in gastric lesions using stains.

2. MATERIALS AND METHODS

2.1 Source of Data

This prospective study was carried out in the Department of Pathology, Sree Balaji Medical College and Hospital, with the help of Department of Medical Gastroenterology, Sree Balaji Medical College and Hospital, during October 2015 to September 2017. A total of 455antral gastric biopsies were received using Olympus CV – 170 endoscope and out of those 100 biopsy samples with adequate data were selected. Of these, random samples of 60 antral biopsies were taken for this study.

2.2 Inclusion Criteria

All cases of gastritis detected by histopathology irrespective of age were included for study.

2.3 Exclusion Criteria

Those with poor clinical data were excluded from the study.

2.4 Method of Data Collection

Out of the 455 cases, 100 cases had adequate clinical data. Of these 100 cases, 60 cases were selected at random. Those biopsy materials were processed and sections were cut at 5 microns. Hematoxylin and eosin staining of sections was done. Histopathological examinations of these sections were done. Section from gastric biopsy had been categorized using Sydney grading system based on activity, chronic inflammation, intestinal metaplasia, atrophy, Helicobacter pylori colonisation and the results were tabulated.

Special stain (Giemsa stain) and Immunohistochemical study using PathnSitu Rabbit polyclonal antibody for Helicobacter pylori was done on 60 cases and degree of antibody expression was scored in each case. Positive

control was taken as, a stomach infected with H.Pylori.

2.5 Statistical Analysis

The data was statistically analysed using Microsoft Excel 2016 and IBM SPSS ver.23 .The significance of the results was assessed by determining the probability factor 'p-value' using Pearson chi squared test.

P</=0.05 : Significant
P<0.01 : Highly significant
P>0.05 : Not significant

3. RESULTS

Out of 100 gastritis patients, 47% belonged to the age group of 20 – 40 years. After 20 years the occurrence of gastritis was more common. M: F ratio was 1.77:1. The p value was 0.405 which was statistically insignificant.

Out of 100 cases 93% were found to be of lower socio economic status. The socioeconomic status was assessed using Modified Kuppuswamy scale (proposed updating for Jan'17). The scores were given on the basis of factors such as education, occupation, monthly income [9].

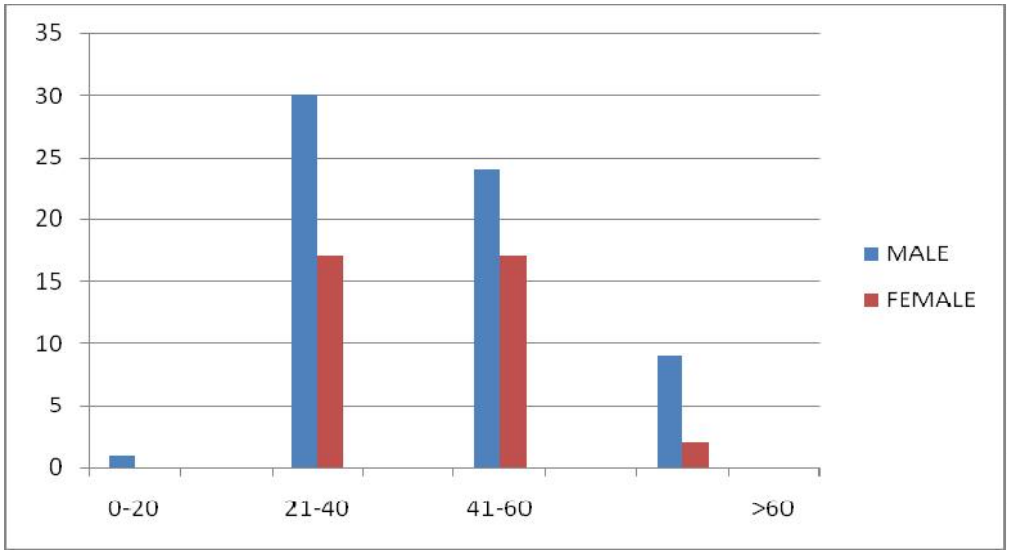


Chart 1. Age and sex distribution in 100 patients with gastritis

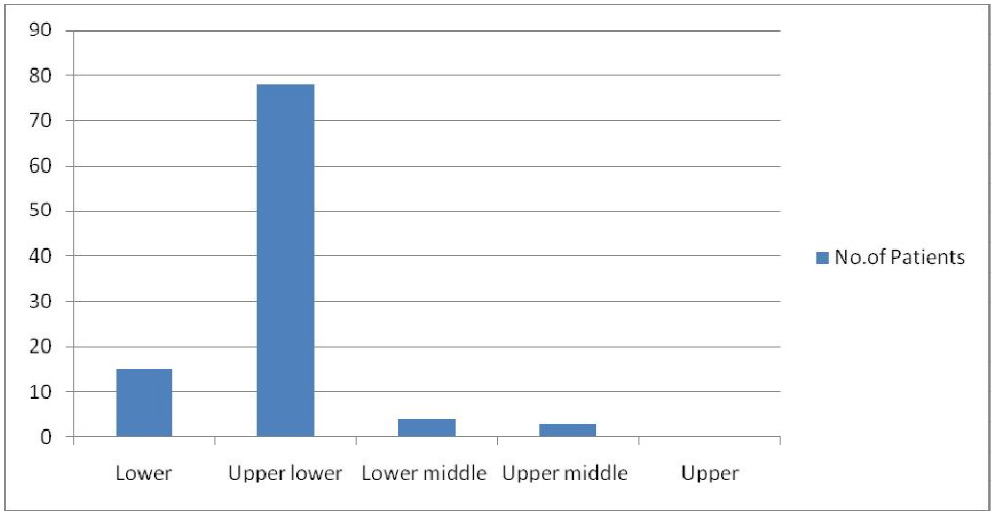


Chart 2. Socioeconomic status of 100 patients with gastritis

The most common symptom that the patients with gastritis exhibited in the present study was found out to be abdominal pain with majority (38%), presenting it.

Amidst the 100 gastritis cases, there were smokers (34 cases), alcoholics (39 cases) and tobacco chewers [10]. A Chi square test for smoking, alcohol, and tobacco gave the following P values - 0.009, 0.006, and 0.025, respectively. It was also observed that prolonged duration of smoking and alcohol increases the risk of *H.pylori* associated gastritis.

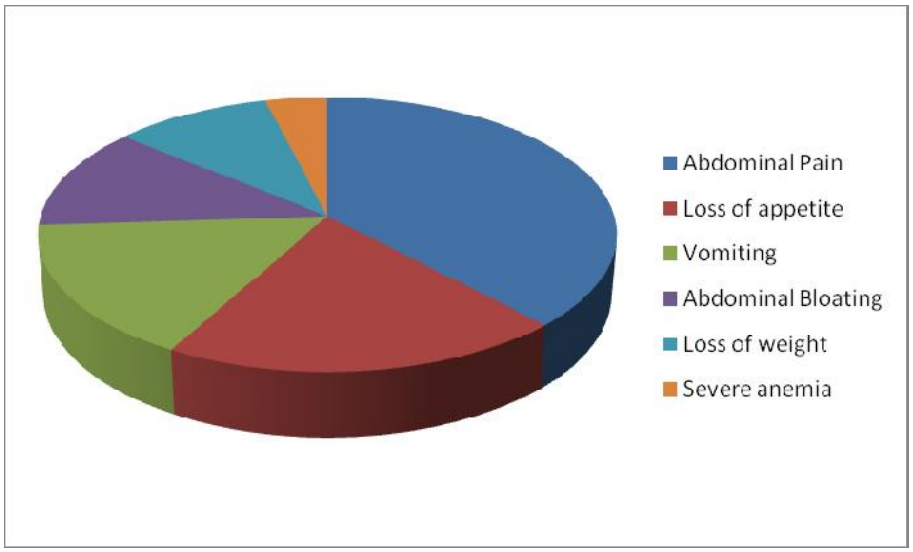


Chart 3. Clinical symptoms of 100 patients associated with Gastritis

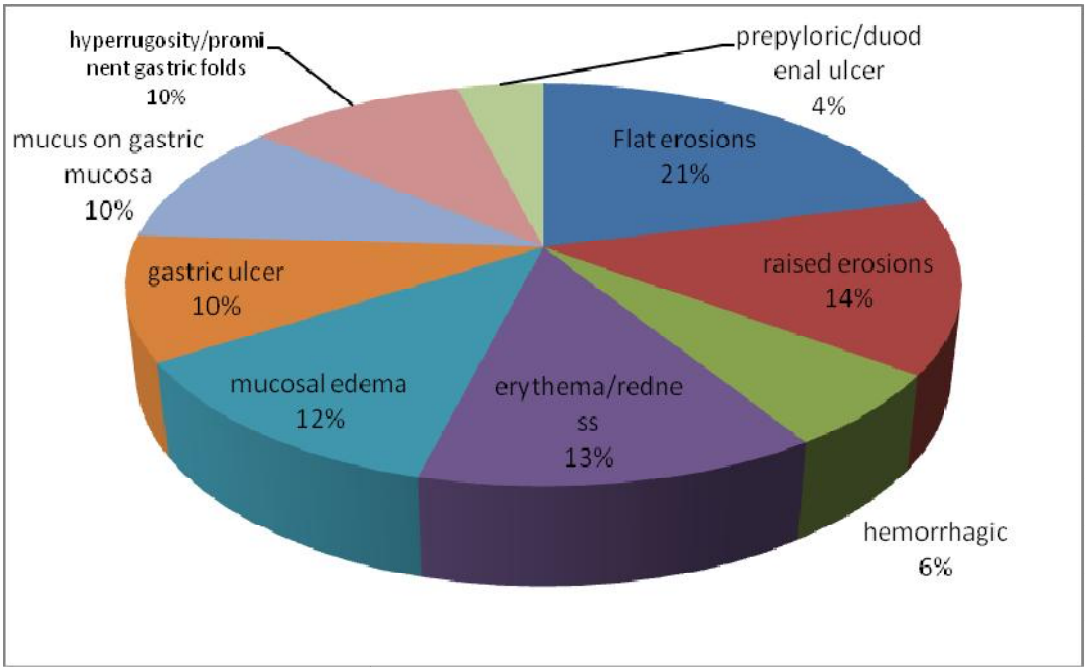


Chart 4. Endoscopic findings amongst 100 patients with gastritis

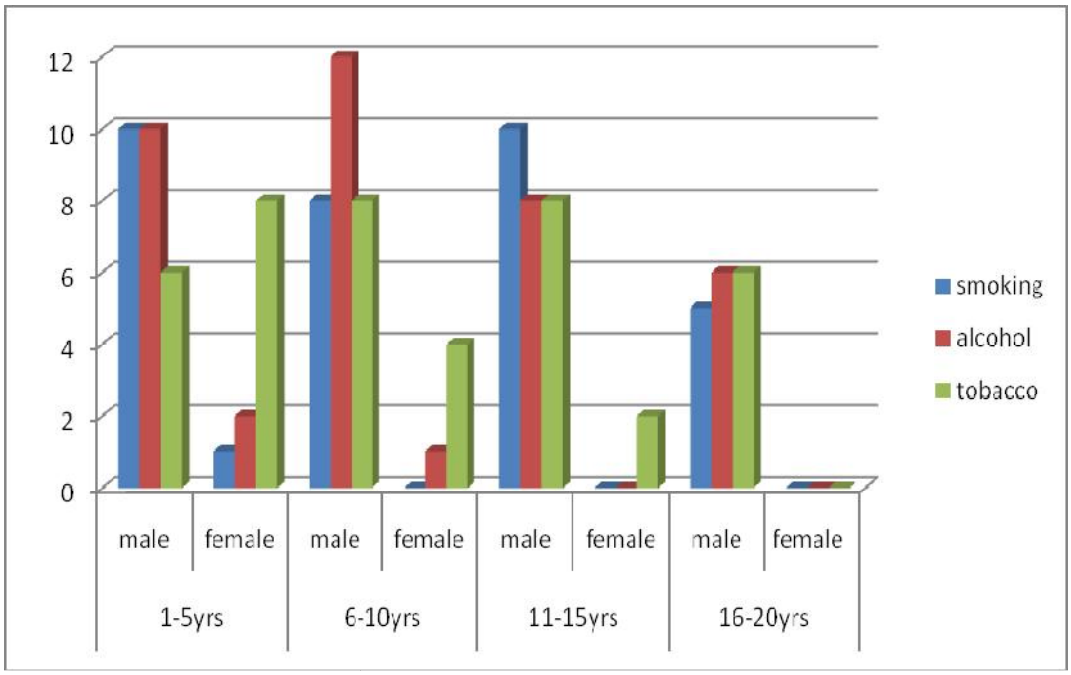


Chart 5. Risk factors such as smoking, intake of alcohol, tobacco in 100 patients with gastritis

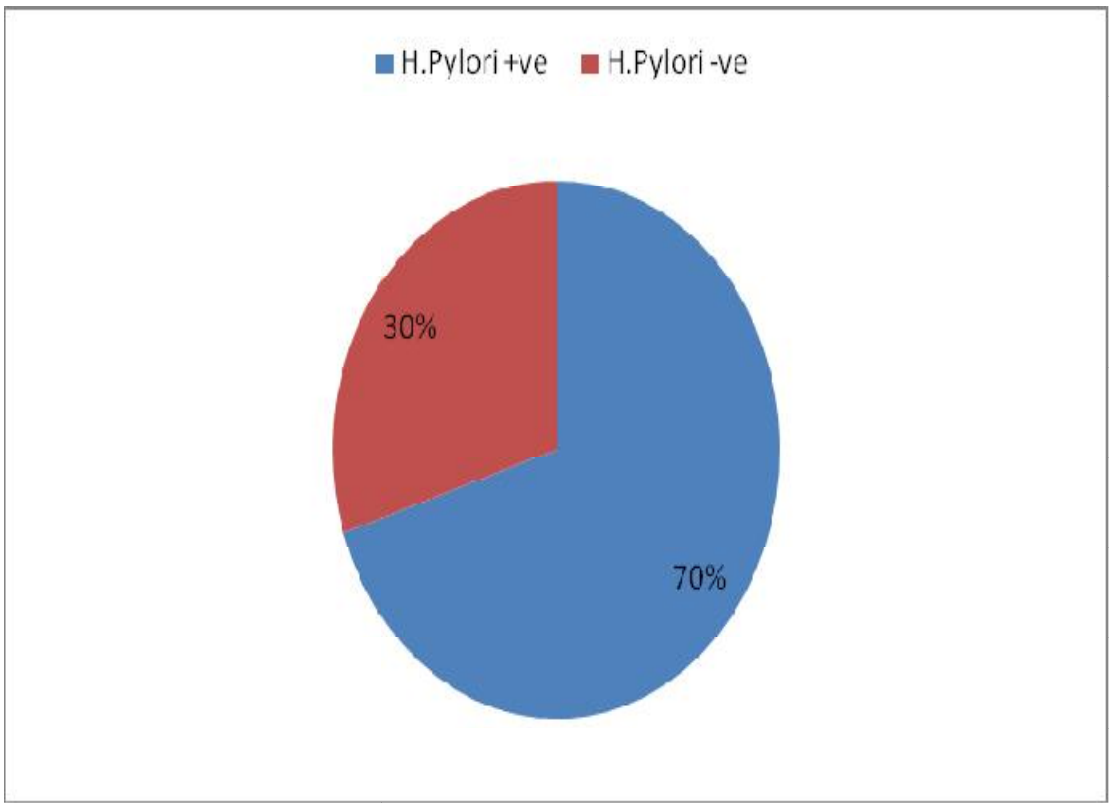


Chart 6. Immunohistochemistry results of 60 cases of antral biopsy for *Helicobacter pylori*

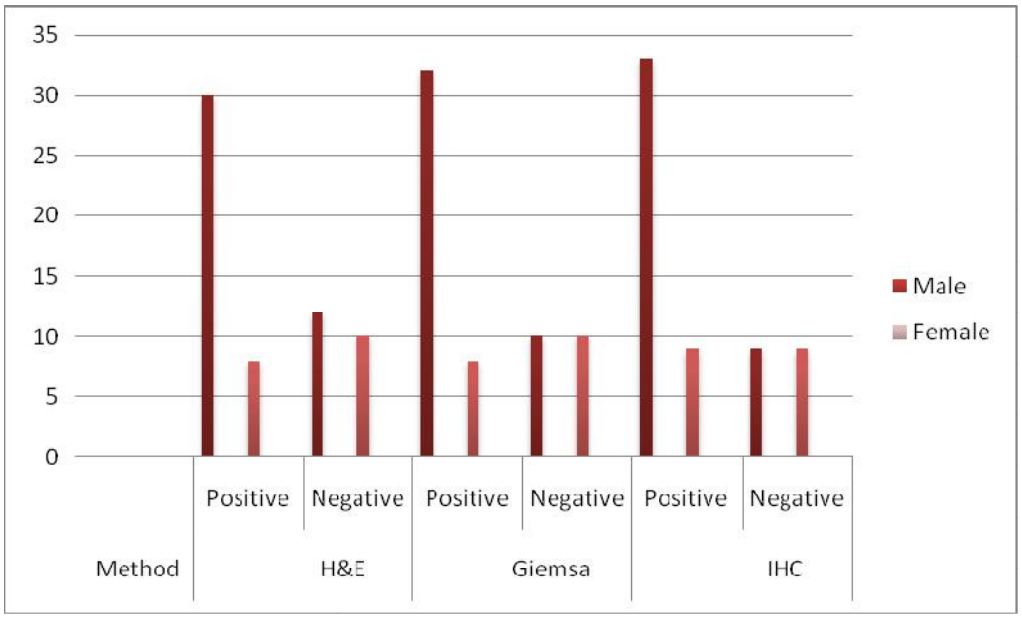


Chart 7. Male and female distribution of positive & negative cases of Helicobacter pylori in H and E, Giemsa, IHC

42 positive and 18 negative cases observed out of the 60 cases studied with IHC for *H.pylori*.

Out of 42 males, 78.5% (33 males) were positive for Helicobacter pylori and out of 18 females, 50% (09 females) were positive for Helicobacter pylori. Hence the Male: female ratio was 2.3:1.

Using IHC technique it was detected in the 60 cases that 31-40 years age group was mostly infested with this bacterium.

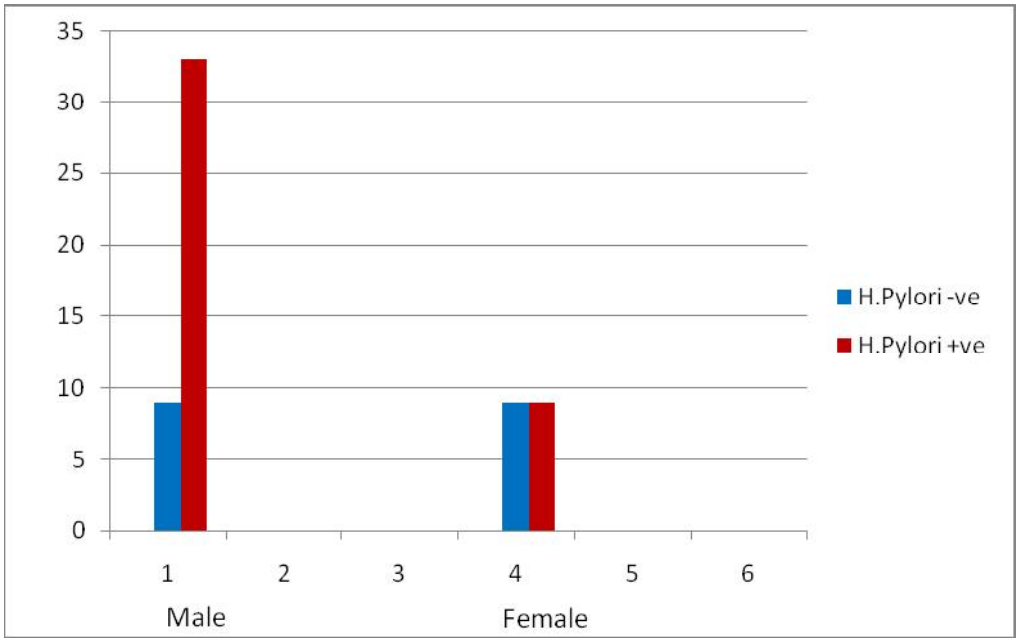


Chart 8. Male: Female ratio of *Helicobacter pylori* infection

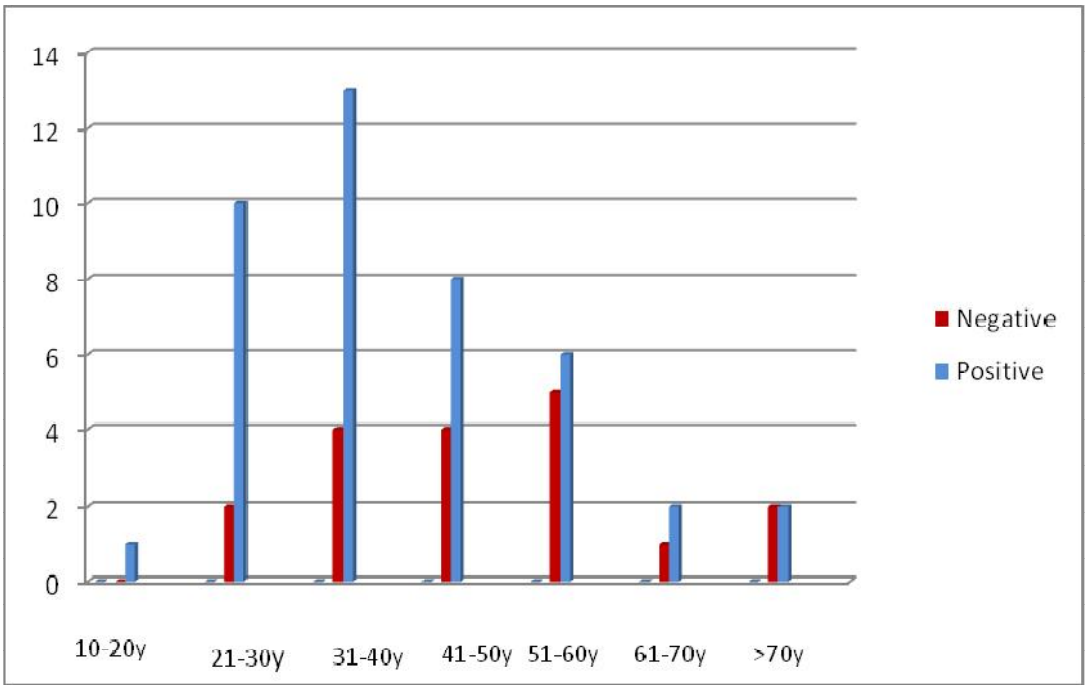


Chart 9. Age distribution of *Helicobacter pylori* infection in 60 cases

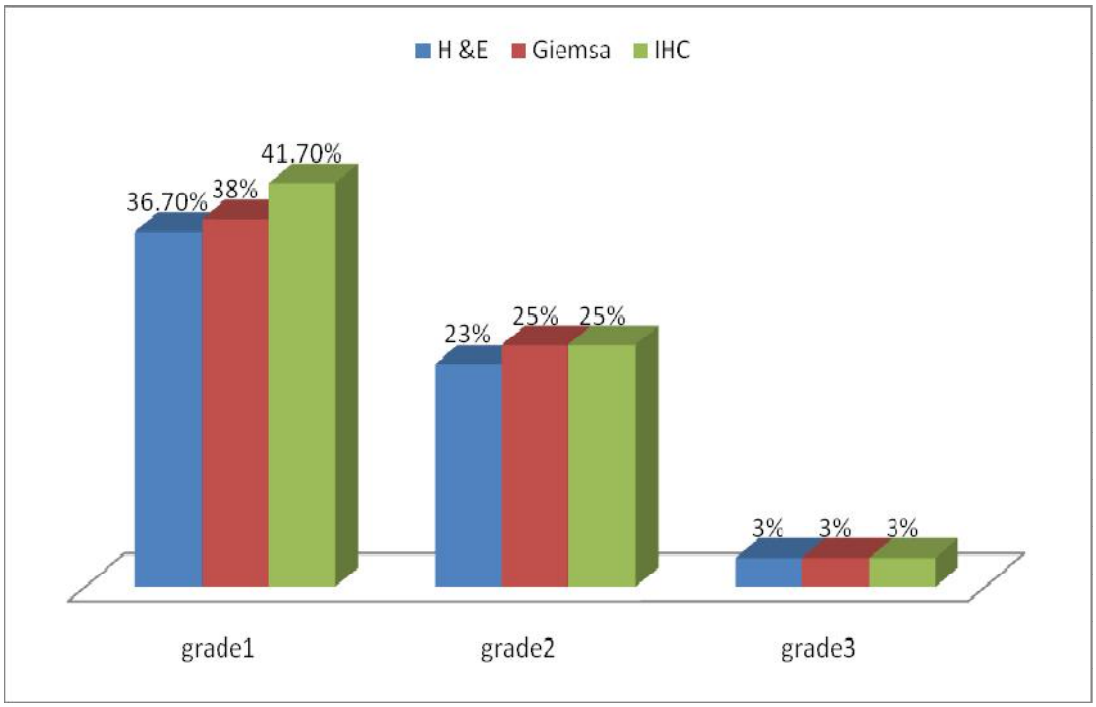


Chart 10. Grading of Helicobacter pylori infection in gastric biopsy using Sydney scoring system in various staining methods like H&E, Giemsa, IHC

From this table it was concluded that 31-40 years is the most common Helicobacter pylori infection group.

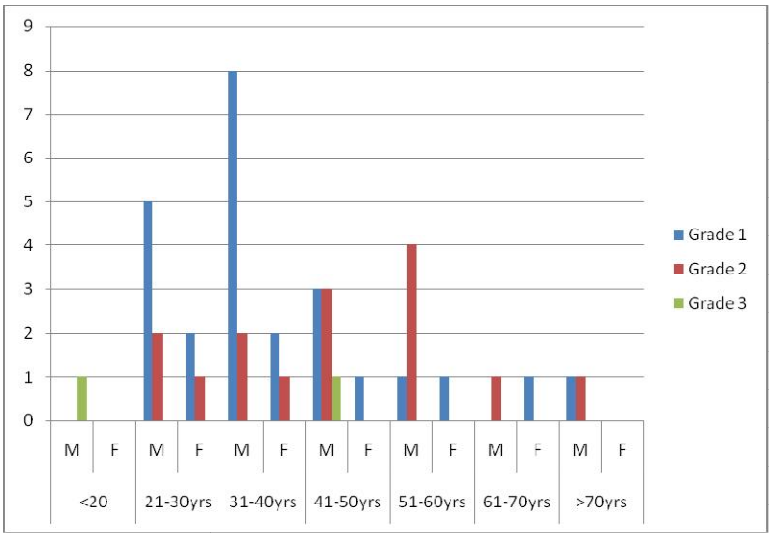


Chart 11. Age and sex distribution among *Helicobacter pylori* positive cases by Immunohistochemistry

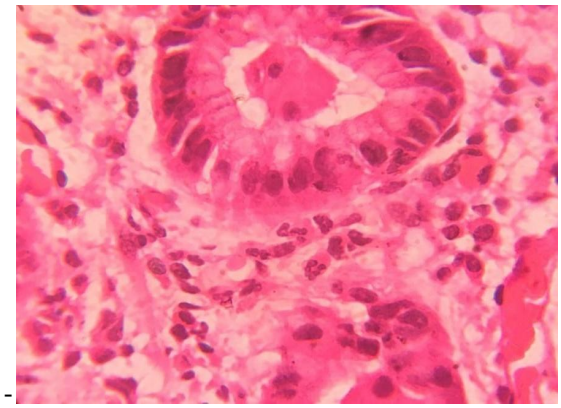


Fig 1. H and E (100x), showing neutrophils in lamina propria

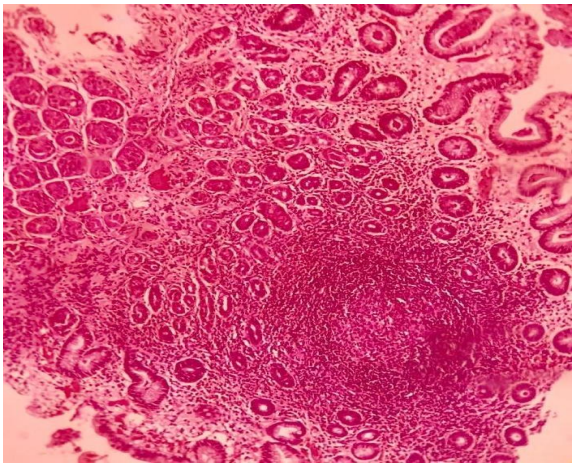
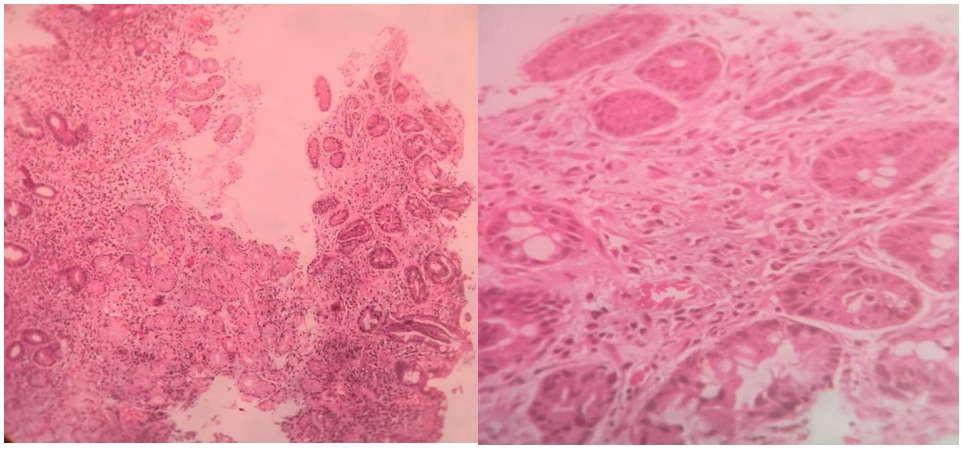


Fig. 2. H and E (40x), showing inflammatory cells forming lymphoid aggregate



(a) (b)

Fig 3. H and E (a)scanner view (b), (40x), showing atrophy of glands

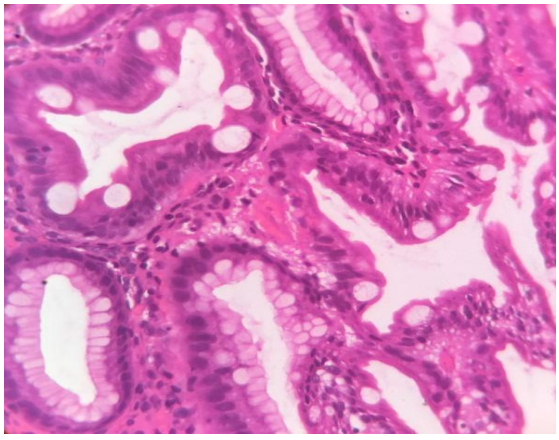


Fig. 4. H and E (100x) showing intestinal metaplasia (grade 2)

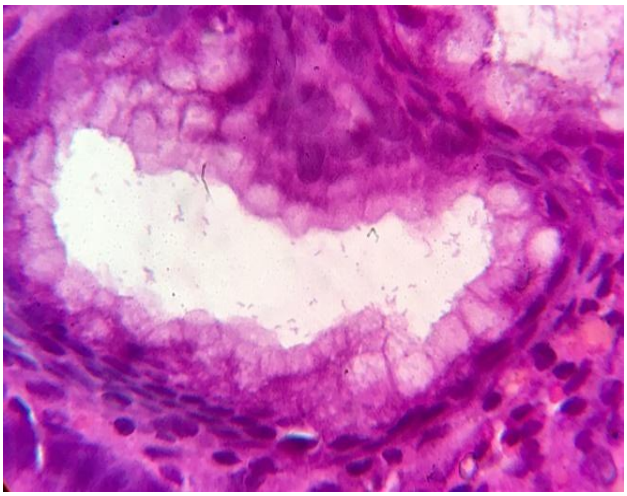


Fig 5. H and E (100x), showing *H.pylori* in luminal surface

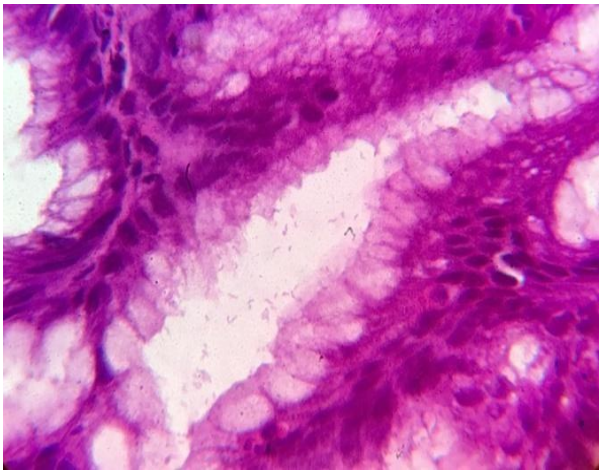


Fig. 6. H and E (100x), showing grade 1 colonisation

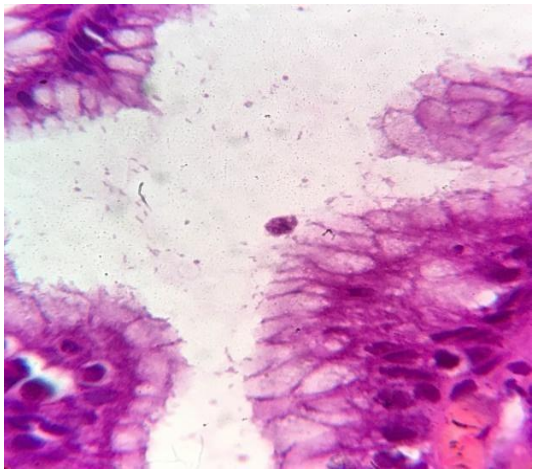


Fig. 7. H and E (100x), showing grade 2 colonisation *H.Pylori*

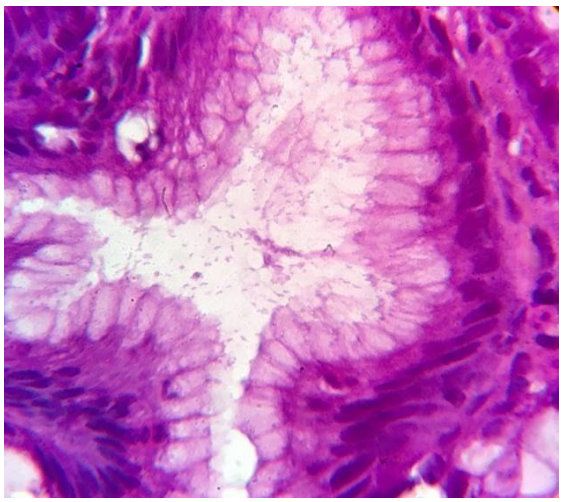


Fig. 8. H and E (100x), showing grade 3 colonisation

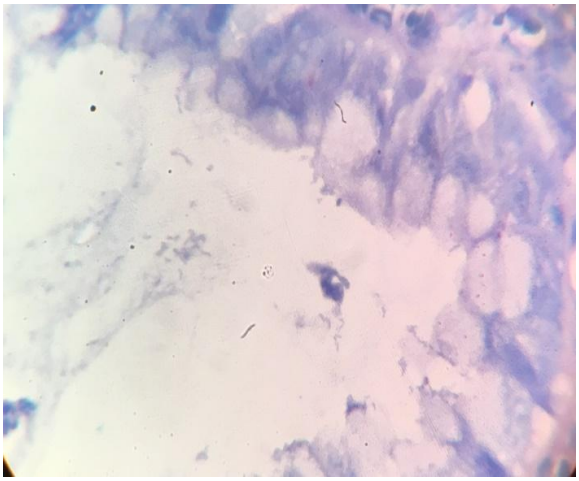


Fig. 9. Giemsa (100x) grade 1 colonisation

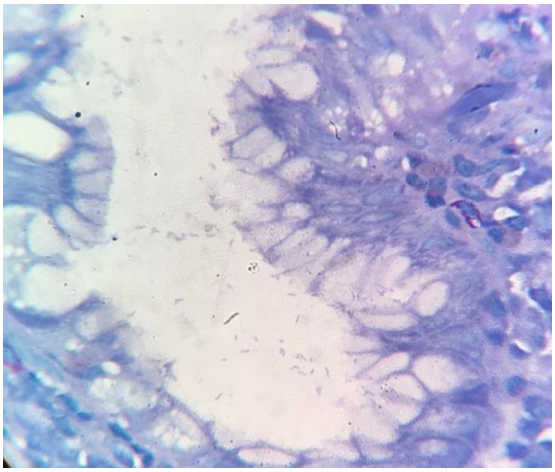


Fig. 10. Giemsa (100x) showing (grade 2) colonisation

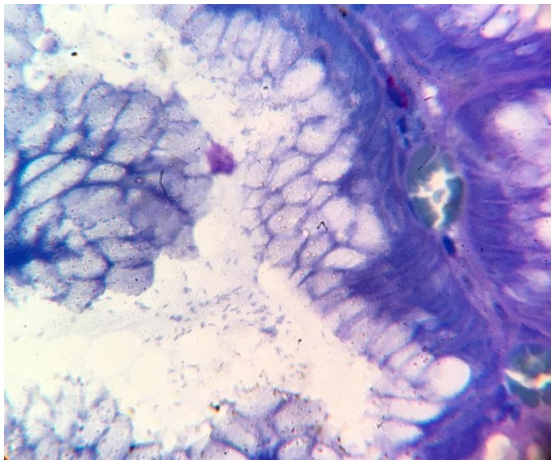


Fig. 11. Giemsa (100x), showing (grade 3) colonisation

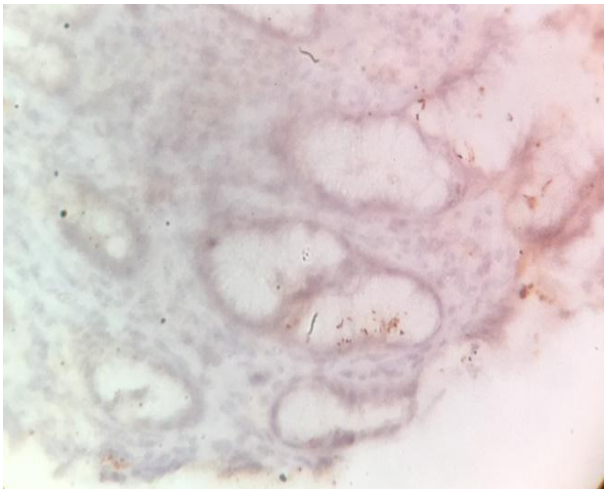


Fig. 12. IHC (100x) , showing grade 1 colonisation

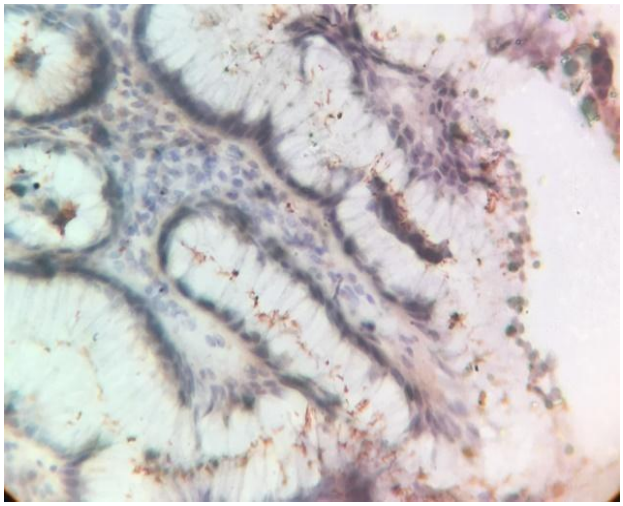


Fig 13. IHC (100x), showing Grade 2 colonisation

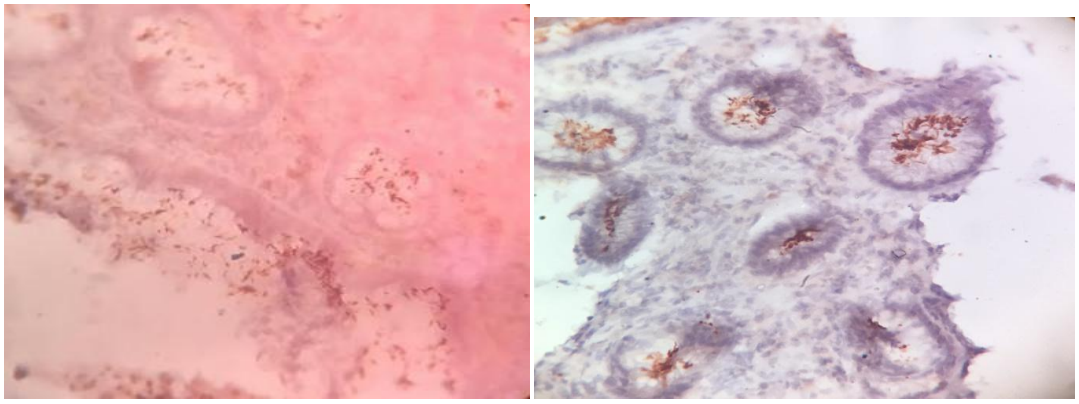


Fig. 14. IHC (100x), showing grade 3 colonisation

4. DISCUSSION

Infecting the stomach, *Helicobacter pylori* causes chronic active gastritis which precedes peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. Totally 100 samples of antral biopsies were collected and studied in the current investigation ruling out clinical data and risk factors related to gastritis. Out of these, random selections of 60 cases were subjected to Giemsa and IHC. Rajesh Kumar et al. [11] in his study reported that 92 cases positive for *Helicobacter pylori* out of the 265 cases studied with a 34.71% overall prevalence. In a similar study, Adisa et al. [12] reported that 345 Out of 603 cases were positive for *Helicobacter pylori* with a 57.2% prevalence. In the present study there were 42 were *Helicobacter pylori* positive out of 100 cases. In spite of differing prevalence among countries and within its population groups, the occurrence of *Helicobacter pylori* infection is found worldwide, with most susceptible groups observed in developing countries, low socioeconomic strata and overcrowded settings. The prevalence of this infection is based on a myriad of criteria such as geographic locality, age, race, ethnicity, and socio-economic ranking. Javed et al. [13] stated that 80% of patients came from a lower and middle class community. In relevance with this, in the present study, the low socio economic group (93%) was observed to be more susceptible to *Helicobacter pylori* infection.

Javed et al. [14] also states that the rate of Helicobacter pylori infection is directly propotional to age with a maximum number seen in the age group of 46-55yrs. In the age group of 41-50 years, the maximum occurrence of *Helicobacter pylori* associated gastritis was noted (Adisa et al. [15]). Similarly, Rajesh kumar et al. [16] showed an occurrence maximum of Helicobacter pylori infection within the age group of 36 – 45 years. In contrast, few studies (Shokrzadeh et al. [17] and Kaore et al. [18]) reported that the *H. pylori* infection increased in age groups of 20-40 years rather than the older age group. In a study conducted by Adlekha et al. [19], highest prevalence of *H pylori* was found between the ages 81 - 90years (75% *H. pylori* positive). In the current investigation, the highest number of positive cases falls in between the age group of 21 – 40 years.

Some studies report that the occurrence of H pylori infection is higher in males (Rajesh kumar et al. [16]) and Kaore et al. [18][). Rajesh kumar et al. [16] in his study showed that the *Helicobacter pylori* infection was 64.13% and 35.87% in males and females, respectively. Contrary to this, few studies state that the occurrence of H pylori infection is higher in females (Adisa et al. [12,15] and Yangchun Zhu et al. [20]). Adisa et al. [12,15] noted an infection rate of 46.8% in males and 53.2% in females. Although the studies determining the male and female ratio of Helicobacter pylori infection are at odds with each other, the results found in the present study, where 42 H pylori positive cases were studied, support the increased percentage of H pylori infection in male (33 cases – 79%) compared to female (9 cases – 21.4%). It was also observed that lack of contrast between the bacteria and the surrounding tissue lowered the sensitivity of the H&E stain. Further, the non-specific staining of non-H*elicobacter pylori* bacteria in the stomach, lowered the specificity of the H&E stain. Easy applicability (performed in 15 minutes), acceptable sensitivity and specificity value and cost effectiveness makes modified Giemsa stain more reliable. Its only disadvantage being lack of contrast , but this can be overcome by careful observation and correct identification of the organisms. In contrast, the alternative technique of *H pylori* identification immunohistochemistry is an expensive and time consuming procedure (1 hour to 24 hours) Inspite of its high Sensitivity and specificity for the detection of *Helicobacter pylori.* Hence, histochemical methods such as H&E, Giemsa and IHC were analysed and compared for its sensitivity and specificity in detecting the *Helicobacter pylori*, in the present study.

Studying the staining ability of Giemsa and IHC in gastric biopsies, HR. Wabinga et al. [21] inferred that the sensitivity of Giemsa stain was 85% (specificity was 89%; positive predictive value - 93% and negative predictive value - 74%). Another study by Hartman and Owens comparing the routine stains and IHC have noted that the sensitivity of special stains and IHC was 62% and 97 -100%, respectively. Comparing the sensitivity of *Helicobacter pylori* detection in gastric biopsies and resected specimens with the help of modified Giemsa and IHC, Babic et al. [16] showed that the sensitivity of Giemsa was 73.3% and was 90% for IHC. In the present study, sensitivity and specificity of (a) Giemsa was 95.2 % and 100% respectively and (b) H&E was 90.5% and 100 % respectively. Consumption of alcohol, cigarette smoking and tobacco chewing are some etiologic factors

positively correlated with acid peptic diseases. Similarly continuous alcohol ingestion is closely associated with chronic active gastritis. However, among alcoholics, the inflammatory changes seems to be coinciding with *Helicobacter pylori* infection. Furthermore, continuous alcohol consumption also results in the presence of gastric metaplasia [15,22-28]. Pandey et al. [17] also reported an association between Helicobacter pylori infection and tobacco chewing.

5. CONCLUSION

In the present study the age groups of patients chosen were in the range of 18 -75 years with peak incidence seen in 21 – 40 years category. Helicobacter pylori was found to be indirectly proportional to the socio economic status. In majority of the patients, the causal agents associated with gastritis in present study were smoking, alcohol intake, tobacco chewing. In the present study,erosions followed by erythema (linear bands/erythematous mucosa) was found to be most commonly associated with gastritis.

In terms of diet, most patients with gastritis were found to intake spicy food. The sensitivity and specificity of Giemsa and H&E was 95.2 % and 100%, and 90.5% and 100%, respectively. When the density of organism was low, IHC proved to obtain best results. Although, the detection rate of Helicobacter pylori using Immunohistochemistry is higher compared to the other two stains, cost-effectiveness, applicability and authenticity of the Giemsa stain make it an ideal stain in detecting Helicobacter pylori infection in gastric biopsies.

CONSENT

It's not applicable.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

ACKNOWLEDGEMENTS

The encouragement and support from Bharath University, Chennai is gratefully acknowledged. For provided the laboratory facilities to carry out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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