A histopathological study of association of *Helicobacter pylori* with gastric malignancies

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KEYWORDS	A B S T R A C T
Gastric malignancy, *Helicobacter pylori,* Special stains	To study the association of Helicobacter pylori with Gastric malignancies and to demonstrate Helicobacter pylori by the use of 4 special stains- Giemsa, Triple Stain, Warthin Starry Stain and 0.1% Acridine Orange. This study was carried out in the Department of Pathology, BMCRI, Bangalore on 100 malignant appearing lesions on upper GI endoscopy from November 2011 to October 2013. A histopathological diagnosis of all the biopsies was done on H & E stained slides and four special stains for the detection on Helicobacter pylori- i.e. Giemsa, Triple stain, Warthin Starry and 0.1 % Acridine Orange were done in all cases. Out of the 100 cases, 50 were diagnosed as malignant and 50 as non-malignant lesions. The most common age range of presentation was 60-70 years-16 cases (32%). The female to male ratio was 1.6:1. Most patients presented with growth in the body of stomach-31 cases (62%). The diagnosis of most of the malignant cases was Adenocarcinoma-48 cases (96%) . *H.pylori* was detected by the 3 special stains-Giemsa, Triple stain and Warthin Starry stain in 29 of the 50 (58%) gastric malignancy cases, in 36 of the 50 (72%) cases stained by Acridine Orange, and in 18/50 (36%) non malignant cases. *H.pylori* can be detected in gastric biopsies by using special stains. Giemsa is cheap and easy to perform. The Alcian blue component of the Triple stain can highlight the areas of intestinal metaplasia and mucin. Warthin Starry stain best detects the curved morphology of *H.pylori*. Detection of *H.pylori* by Acridine Orange is highly sensitive, simple and rapid.

Introduction

Helicobacter pylori, formerly known as Campylobacter pylori, is a microaerophilic, gram-negative, slow growing, spiral-shaped, flagellated spirochaete-like bacterium (3.5x0.5um), of which two major genotypes exist. It is the most common cause of chronic gastritis.

This organism colonizes the gastric mucosa (particularly the antrum and the cardia) in a

variety of ways: free in the mucus, surface adhesion and intercellularly. Helicobacter pylori is known to cause pangastritis which is associated with multifocal mucosal atrophy, reduced acid secretion, intestinal metaplasia, and increased risk of gastric adenocarcinoma and lymphoma (Stricker et al, 2010).

Gastric carcinoma is one of the most common forms of cancer, with approximately 900,000 new cases diagnosed every year and a leading cause of cancer-related deaths in many parts of the world (Parkin et al, 2002)

Significant advances toward the understanding of gastric carcinogenesis have been achieved since the description of Helicobacter pylori by Marshall and Warren, in 1984 (Marshall et al, 1984), and its later classification as a class I carcinogen (by the International Agency for Research on Cancer).

The Center for Disease Control and Prevention (CDC) estimates that approximately two-thirds of the world's population harbors the bacterium, with infection rates much higher in developing countries than in developed nations (Ferlay et al, 2008).

H.pylori acquisition most frequently appears during early childhood, indicating that the decreased prevalence with age is largely due to a birth cohort effect rather than to new infections (Feldman et al, 1998). The prevalence of *H.pylori* is inversely correlated with socioeconomic status, in particular in relation to family income levels, hygiene, and housing conditions. This may be the reason why the prevalence of *H.pylori* in the industrialized world is significantly declining, whereas infection rates in developing countries remain relatively constant. Moreover, the widespread use of antibiotics may have accelerated the progressive decrease of *H.pylori* infection during childhood in developed countries. The elimination of *H.pylori* from the population by improved hygiene, housing conditions, and antibiotic treatment also strongly correlates with a decrease in gastric cancer worldwide (Peek et al, 2002).

Although incidence of gastric carcinoma is on the decline, it remains the second most common cause of death from malignant diseases. Nevertheless, incidence rates differ from one geographical region to another, being rather high in Japan, China, Columbia, and Costa Rica, and comparatively low in the United States (Whelan et al, 1993). However, since there is a geographical association between gastric cancer incidence and *H.pylori* prevalence rates, it has been suggested that gastritis caused by this bacterium may represent an important factor in gastric carcinogenesis (Correa et al, 1990).

This hypothesis was confirmed by prospective case-control studies investigating the association between infection with *H.pylori* and gastric cancer which have calculated an approximately three- to sixfold increase in the risk of gastric cancer developing in patients infected with the organism. (Parsonett et al, 1991; Forman et al, 1991). This odds ratio increases to approximately ninefold if only patients randomized for prospective serological case-control studies with a follow-up period of at least 14 years are considered (Forman, 1995). In addition, a recent study from Japan in which only patients below the age of 40 were investigated reported an odds ratio of 13.3 for developing gastric cancer associated with *H.pylori* infection.

Epidemiologic studies have shown that individuals infected with *H.pylori* have an increased risk of gastric adenocarcinoma (Atherton, 2006). The risk increase appears to be restricted to non-cardia gastric cancer. For example, a 2001 combined analysis of 12 case–control studies of *H.pylori* and gastric cancer estimated that the risk of non-cardia gastric cancer was nearly six times higher for *H.pylori*-infected people than for uninfected people (Helicobacter and Cancer Collaborative Group, 2001). Additional evidence for an association between *H.pylori* infection and the risk of non-cardia gastric cancer comes from prospective cohort studies such as the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study in Finland (The ATBC Cancer Prevention Study Group, 1994). Comparing subjects who developed non-cardia gastric cancer with cancer-free control subjects, the researchers found that *H.pylori*-infected individuals had a nearly eightfold increased risk for non-cardia gastric cancer (Kamangar et al, 2006).

It is not surprising then that several areas in the United States and other parts of the world that are associated with a high risk of gastric carcinoma also have a high prevalence of *H.pylori* infection, even among young children. High rates of infection have also been found in patients with cancer or precancerous conditions, and a case–control study found that patients with gastric carcinoma had a relative risk of *H.pylori* infection of 2.7 relative to healthy control subjects (Hu et al, 1994).

Correa's model of gastric carcinogenesis evolved largely from longitudinal observations of a community-based cohort recruited between 1973 and 1983 from three small towns in the Andean region of Nariño in southern Colombia. In previous studies, natives of this region displayed the highest stomach cancer rates in Colombia. The Nariño cohort revealed an extremely high prevalence of chronic gastritis and more advanced degenerative lesions as well as a high incidence of progression of these lesions and of gastric carcinoma itself. The prevalence of *H.pylori* infection in a random sample of this cohort was over 90 percent (Correa et al, 1989).

In 1998, Watanabe et al established that long-term infection with *H.pylori* generated adenocarcinoma in Mongolian gerbils (Watanabe et al, 1998). A long-term, prospective study in Japan indicated that gastric adenocarcinoma developed in persons infected with *H.pylori* and not in uninfected persons (Uemura et al, 1997). Additionally, the prevalence of *H.pylori* infection is high in several Asian countries, such as India and Bangladesh, but the rates of gastric cancer in these countries are low (Miwa et al, 2002). The African and Asian enigmas reflect the multifactorial etiology of gastric cancer. Therefore, the impacts of *H.pylori* infection on gastric cancer development may differ among various ethnic groups.

Several studies from India failed to show a higher frequency of *H.pylori* infection in patients with gastric cancer than in controls, which could be correlated to the variations of the strains. Diet may play a major role in gastric carcinogenesis. In India, the southern and eastern parts experience a somewhat higher frequency of gastric cancer than the northern parts of the country, which could be due to tobacco smoking, high temperature food intake, spicy food, and rice consumption. Tobacco use and alcohol consumption are the other factors that may influence the variation in the frequency of gastric cancer (Singh et al, 2006). Thus, the actual role of *H.pylori* in relation to the host

genetic makeup and dietary and environmental factors needs to be elucidated in the Indian scenario. *H.pylori* infection is more frequent in less developed Asian countries like India; however, the frequency of gastric cancer is very low in India. The host's genetic makeup and the dietary and environmental factors might explain this mystery. *H.pylori* is present in 90% of patients with chronic gastritis. In the great majority of infected persons, *H.pylori* infection causes no clinical consequences. However, in 20% to 30% of cases the infection leads to gastric ulcers, and in a smaller proportion of cases, gastric carcinomas, and gastric lymphomas may develop. There is much evidence linking gastric infection with the bacterium *H.pylori* to the causation of gastric carcinomas and gastric lymphomas. This relationship, which is particularly strong for gastric lymphomas, has been established by epidemiologic studies as well as by detection of *H.pylori* infection in the great majority of tumors. Furthermore, treatment of *H.pylori* infection with antibiotics results in regression of the lymphoma in most cases.

The mode of transmission of *H.pylori* has not been well defined, although oral-oral transmission, fecal-oral transmission, and environmental spread are among the possible routes (Feldman et al, 1998).

H.pylori is part of a genus of bacteria that have adapted to the ecologic niche provided by gastric mucus, which is lethal to most bacteria. The specialized traits that allow it to flourish include:

1. Motility (via flagella), allowing it to swim through viscous mucus
2. Elaboration of a urease, which produces ammonia and carbon dioxide from endogenous urea, thereby buffering gastric acid in the immediate vicinity of the organism
3. Expression of bacterial adhesins, such as BabA, which binds to the fucosylated Lewis B blood-group antigens, enhances binding to blood group 0 antigen bearing cells.
4. Expression of bacterial toxins, such as cytotoxin associated gene A (CagA) and vacuolating cytotoxin gene A (VacA) (Stricker et al, 2010).

The majority of colonizing *H.pylori* reside within the gastric mucus and do not directly interact with host cells. Although *H.pylori* was long considered to be an extracellular bacterium, recent studies have provided evidence that *H.pylori* occasionally enters epithelial cells via a zipper-like mechanism (Kwok et al, 2002). Despite causing numerous gastric environmental changes and eliciting a host immune response, *H.pylori* can persistently colonize the human stomach for long periods (Algood et al, 2006).

After initial exposure to *H.pylori*, gastritis occurs in two patterns: a predominantly antral-type gastritis with high acid production and elevated risk for duodenal ulcer, and a pangastritis that is followed by multifocal atrophy (multifocal atrophic gastritis) with lower gastric acid secretion and higher risk for adenocarcinoma (Normark et al, 2003).

Particular polymorphisms in the gene encoding the pro-inflammatory cytokine interleukin-1β (IL-1β) correlate with the development of pangastritis after *H.pylori* infection. Polymorphisms in TNF and a variety of other genes associated with the inflammatory response also influence the clinical outcome in *H.pylori* infection Severity of disease may also be influenced by genetic variation among *H.pylori* strains.

For example, the CagA gene, a marker for a pathogenicity island of approximately 20 genes, is present in 50% of *H.pylori* isolates overall but in 90% of *H.pylori* isolates found in populations with elevated gastric cancer risk.

The most common cause of pro-lymphomatous inflammation in the stomach is chronic *H.pylori* infection, which is found in association with most cases of gastric MALToma. The most striking evidence linking *H.pylori* gastritis to MALToma is that eradication of the infection with antibiotics induces durable remissions with low rates of recurrence in most patients.

H.pylori lies in the superficial mucus layer and among the microvilli of epithelial cells. The distribution of organisms can be very patchy and irregular, with areas of heavy colonization adjacent to those with no organisms. In extreme cases, the organisms carpet the luminal surfaces of surface epithelial cells, the mucous neck cells, and the epithelial cells lining the gastric pits; they do not invade the mucosa. Even in heavily colonized stomachs, the organisms are absent from areas with intestinal metaplasia.

Various studies have shown that *H.pylori* may disappear from the stomach along with the progressive changes of gastric milieu from chronic inflammation/atrophic gastritis, intestinal metaplasia, dysplasia to cancer (Huang et al, 1998).

According to the Sydney Classification for evaluation of Gastritis, five biopsy sites should be collected: one specimen each should be obtained from the lesser and the greater curvature of the antrum, both within 2–3 cm form the pylorus; from the lesser curvature of the corpus about 4 cm proximal to the angulus; from the middle portion of the greater curvature of the corpus, approximately 8 cm from the cardia; and one from incisura angularis.

Studies on the most practical biopsy site for diagnosing *H.pylori* infection have conflicting results. Antrum biopsy is recommended by Genta et al (Genta et al, 1994) , while others recommend at least one corpus biopsy (Satoh et al). Hazell et al. and Woo et al. found it necessary to take both antral and corpus biopsies (Hazell at al).

H.pylori can be recognized in routine Hematoxylin and Eosin stains. However, if the density of the organism is low, its detection can be greatly facilitated by the use of special stains like Giemsa, Warthin Starry or Steiner silver stains, the Alcian yellow- toluidine blue method, Genta stain and Triple stain.

With the use of these techniques, *H.pylori* has been found in 90 % of patients with chronic gastritis, 95 % with duodenal ulcer, 70 % with gastric ulcer, and 50 % with gastric malignancies (Rosai J, 2009).

However, the detection of *H.pylori* by the fluorescent stain- Acridine orange is rapid and simple.
Hence this study was done to study the association of Helicobacter pylori with Gastric malignancies and to demonstrate Helicobacter pylori by the use of 4 special stains-Giemsa, Triple Stain, Warthin Starry Stain and Fluorescent Stain-0.1% Acridine Orange.

Materials and methods

A histopathological study of 100 malignant appearing gastric biopsy specimens obtained by Upper GI endoscopy from the Department of Gastroenterology, Victoria Hospital and Bowring and Lady Curzon

Hospital, Bangalore between November 2011 and October 2013 was done.

An informed written consent was obtained from these patients, prior to biopsy.

The clinical diagnosis, site of biopsy and endoscopic findings of the the patients were noted.

The 100 biopsy specimens obtained by upper GI Endoscopy were fixed in 10 % aqueous formalin and Bouin's fixative and subjected for tissue processing. The processed tissue was embedded in paraffin to obtain 3 micron thin serial sections. These sections were stained with routine Hematoxylin and Eosin stains and examined under the microscope for malignant neoplastic lesions. If malignant lesions were present, further evaluation for the presence of Helicobacter pylori was done in routine Hematoxylin and Eosin sections, and also by the use of special stains like Warthin-Starry stain, Giemsa stain, Triple stain, and Fluorescent stain 0.1 % Acridine Orange. The staining procedures were done manually according to the standard protocol.

Interpretation of Acridine Orange stained slides

The sections were studied under fluorescent microscope OLYMPUS-BX51 (Blue filter; Excitation wavelength-502 nm and Emission wavelength-526 nm). The intensity of fluorescent pattern in each specimen was graded as negative or positive. In negative fluorescence, no observable pattern with greater intensity than the background fluorescence was noted. Positive fluorescence was defined as a pattern clearly and consistently above the background intensity and present throughout the specimen.

Statistical analysis

The patient's details were recorded in a standardized format. The data was coded and entered into a Microsoft Excel sheet and analysed. The sensitivity of detection of Helicobacter pylori by each of the special stains was determined (by comparison with the Gold standard-Giemsa stain).

Result and Discussion

The total number of cases studied during the course of present study (from November 2011 to October 2013 was 100. This includes gastric biopsies of all malignant appearing lesions on Upper GI Endoscopy obtained from the Department of Gastroenterology, Victoria and Bowring and Lady Curzon Hospitals, Bangalore.

Out of the 100 cases, 50 were diagnosed as Malignant on routine H & E stained sections.

The age of the patients (malignant cases) ranged from 28-80 years. The most common age range of presentation was 60-70 years-16 cases (32%). Sex distribution of the patients: 31 were females and 19 were males (F: M – 1.6:1).

The results of the Special stains were as follows:

- Helicobacter pylori was detected in 36 of the 50 (72%) gastric malignancy cases.
- In 29 cases (58%), *H.pylori* could be detected by all the 4 special stains (Giemsa, Triple stain, Warthin Starry stain and 0.1% Acridine Orange); whereas the bacillus was detectable only by Acridine Orange in 7 cases.

The density of H pylori colonisation was graded as follows (Updated Sydney System):

- 0- none;
- 1- *H.pylori* found only in one place after a careful search;
- 2- only a few *H.pylori* found;
- 3- scattered *H.pylori* found in separate areas/foci;
- 4- numerous *H.pylori* in separate areas/foci;
- 5- nearly complete gastric surface covered by a layer of *H.pylori*;
- 6- continuous gastric surface coverage by a thick layer of H pylori.

Results of the four special stains are presented in Tables 5, 6, 7 and 8.

Results of the 36 adenocarcinoma cases in which *H.pylori* could be detected are as in Table 7.

H.pylori could be detected in 100% cases of intramucosal and mucinous carcinoma, 89% cases of signet ring cell carcinoma, 80% cases of well differentiated adenocarcinoma, 73.3% cases of moderately differentiated adenocarcinoma, and only in 44.5% cases of poorly differentiated carcinoma.

Special stains were also done for the 50 non malignant cases. *H.pylori* was detected in 18/50 cases. The results are presented in Table 8.

Out of the 36/50 malignant cases in which *H.pylori* could be detected, the bacillus was present in 29 cases (58%) which were stained by Giemsa, Triple stain and Warthin Starry stain; and all in 36 cases (72%) stained with 0.1% Acridine Orange. All the 36 cases were adenocarcinomas. *H.pylori* was not detectable in Non Hodgkin's lymphoma and carcinoid stomach.

And only Acridine Orange could detect *H.pylori* in the remaining 7 cases (14%).The number and density of *H.pylori* detected reduced as the adenocarcinoma progressed from well differentiated to the poorly differentiated state.

Along with detection of *H.pylori*, Triple stain also highlighted the areas of intestinal metaplasia and the mucin in adenocarcinomas.

The curved morphology of *H.pylori* was best made out in the Warthin Starry stain.

Acridine Orange could detect the bacillus in all the cases. Also the fluorescent detection was simple and rapid.

The present study group comprised of 100 patients who were suspected to have gastric malignancy on Upper GI endoscopy and the study was done over a period of 2 years from November 2011 to October 2013. Out of the 100 cases, special stains for the detection of Helicobacter pylori were done in all 50 benign and 50 malignant cases.

The most important advance in the field of chronic gastritis and other gastric diseases (peptic ulcer, carcinoma and lymphoma) has been the awareness of the crucial role played by Helicobacter pylori. This organism colonises the gastric mucosa in a variety of ways, and cases with intercellular colonisation show the greatest degree of epithelial damage. These changes include disintegration and loss of apical mucus with formation of epithelial pits and less frequently-erosions and ulcerations. The presumed main mechanisms for these alterations are motility and urease activity by the organism (Rosai J, 2009).

The association between *H.pylori* and gastric cancer may be explained by two possible mechanisms: one is based on a

carcinogenesis-promoting effect of *H.pylori* itself and the other is based on the establishment of a carcinogenic environment due to long-term infection with *H.pylori*. In the second case, although *H.pylori* may have no carcinogenesis-promoting effect itself, infection causes inflammation of the gastric mucosa and chronic infection causes mucosal atrophy, resulting in intestinal metaplasia. These latter changes are considered precursors of gastric cancer.

Research concerning the association between gastric cancer and *H.pylori* has achieved enormous progress over time, leading to the recognition of this relationship by the WHO. The onset of gastric cancer can be explained as being caused not only by *H.pylori* infection, but also by a combination of various factors such as food and the environment. However, the possibility that the occurrence of gastric cancer, like the recurrence of peptic ulcer disease, can be prevented by eradication of *H.pylori* has also been suggested (Asaka et al, 2001).

Chronic H pylori infection of the stomach, particularly by strains harbouring the Cag-A gene plays a major role in the early stages of the pathogenesis of gastric cancer (except carcinoma of the cardia).

Individuals with *H.pylori* gastritis that is widespread, mainly affects the corpus, and progresses more rapidly to atrophy, appears to be most at risk for developing malignancy. >90% of primary gastric B-cell lymphomas (of MALT type) arise in a background of chronic *H.pylori* associated gastritis (Stricker at al, 2010).

Most gastric cancers occur in elderly males (males-almost twice as females), with increased incidence above 60 years of age

(Rosai J, 2009). In our study, most patients were females (62%) and the female to male ratio was 1.6: 1. The highest numbers of patients were between 60 to 70 years of age-16 cases (32%).

Most of the gastric carcinomas are located in the pylorus and the antrum (50-60%), followed by the cardia (25%) and the body and fundus of stomach (15-25%) (Rosai J, 2009). In our study, most of the carcinomas were located in the body of the stomach-31 cases (62%), followed by the antrum and pylorus-13 cases (26%). This can be explained by the pangastritis caused by *H.pylori* progressing to gastric carcinoma (Stricker et al, 2010).

Most gastric cancers are adenocarcinomas (90-95%), followed by lymphoma (4%) and carcinoid (3%) (Stricker et al, 2010). In our study too we found that most gastric cancers were adenocarcinomas-48 cases (96%), followed by lymphoma-1 case (2%), and carcinoid-1 case (2%).

H.pylori can be recognized in routine hematoxylin–eosin stains, and in most instances that is all that is needed. However, if the density of the organism is low, its detection can be greatly facilitated by the performance of special stains, which include Giemsa, Warthin–Starry or Steiner silver stains, the Alcian yellow–toluidine blue method, Genta stain, or immunohistochemistry. No clear advantage of one over the others has been demonstrated. With the use of these techniques, *H.pylori* has been found in 90% of patients with chronic gastritis, 95% with duodenal ulcer disease, 70% with gastric ulcer, and 50% with gastric carcinoma (Rosai J, 2009).

Various studies have shown that *H.pylori* may disappear from the stomach along with

the progressive changes of gastric milieu from chronic inflammation/atrophic gastritis, intestinal metaplasia, dysplasia to cancer (Huang et al, 1998).

Hence, the main aim of our study was to determine the association of Helicobacter pylori with gastric malignancies by the use of for special stains-Giemsa, Warthin Starry, Triple stain (Carbol fuchsin, Alcian Blue and H & E), 0.1% Acridine Orange to detect the bacilli. We also studied the sensitivity of detection of *H.pylori* by each of these stains.

In our study, *H.pylori* could be detected by the 4 special stains in 36/50 malignant cases-i.e. in 58% of cases by Giemsa,Triple Stain and Warthin Starry stain and in 72% of cases stained by 0.1 % Acridine Orange. All cases in which *H.pylori* could be detected were adenocarcinomas. *H.pylori* could not be detected in NHL and carcinoid of stomach.

The sensitivity of detection of *H.pylori* by Giemsa, Triple Stain and Warthin Starry stain was the same- 29/36 cases-80.5 % sensitivity. Whereas, Acridine Orange showed 100% sensitivity (36/36 cases) in detection of the bacilli (taking Giemsa as gold standard for histopathological detection of *H.pylori*). Also, a higher grade of *H.pylori* colonisation could be detected by the fluorescent stain.

We also determined that the rate and density of *H.pylori* detection reduced as the gastric adenocarcinoma progressed from the well differentiated to the poorly differentiated type.

As we all know, Giemsa is the most widely used special stain and the accepted gold standard for the histopathological detection of *H.pylori* because it is cheap, easily available, easily performed and may be

repeated without excessive cost on subsequent tissue biopsies during follow-up examinations by a gastroenterologist. The only disadvantage is the lack of contrast between the bacilli and the surrounding tissue (Anim et al, 2000).

The modified triple stain using Carbol fuchsin, Alcin Blue and H & E is a recently described one for *H.pylori* detection. Aside from being infected with HP, the other predisposing pathologic condition of gastric cancer is goblet cell intestinal metaplasia. Goblet cell IM is diagnosed by the morphologic changes of the gastric mucosa on H&E stain. However, the pathologist may fail to notice and report this pathology. Goblet cell IM may be accentuated by the use of alcian blue staining to identify acid mucin (such as sialomucin and sulfomucin) which is secreted by goblet cells. This can be highlighted by the Alcian Blue component in the triple stain (Yodayudh et al, 2008). Also the components of the stain are easily available and can be done on an autostainer. Our study too supported these findings.

The silver stain Warthin Starry can also be used for *H.pylori* detection. Studies have shown that the sensitivity is higher than that of Giemsa. The disadvantage of this stain is that it is technically demanding, not reproducible and difficult to interpret because of non specific staining of mucus, debris and water bath contaminants (Ashton et al, 1996).

In our study, the sensitivity of this stain was the same as that of Geimsa and Triple stain. Also we determined that the curved morphology of *H.pylori* could be best made out by this stain. However, the staining procedure was difficult to perform.
The fluorescent stain-Acridine Orange is usually used for detection of

microorganisms like malaria (Quantitative buffy coat), trichomonas vaginalis, cryptococci and some spirochaetes. After 16 years of use, Haqqani stated that the AO staining has proved to be exremely useful in the identification of *H.pylori*, and is inexpensive, quick, and easy to perform and is more sensitive that other stains (Haqqani et al, 1988). In our study too we found similar results. The sensitivity of detection of *H.pylori* was 100% by this stain, and also it was rapid and easy to perform. The only disadvantage was the requirement of a fluorescent microscope.

Table.1 Clinical presentation of the patients (malignant cases)

Clinical Presentation	Number of cases	Percentage
Growth in the body of stomach	31	62
Growth in the antrum/pylorus	13	26
Gastric Ulcer	04	08
Gastric Perforation	02	04
Total	50	100

Most of the patients presented with growth in the body of stomach-31 cases (62%)

Table.2 Site of biopsy in malignant cases

Site of Biopsy	Number of cases	Percentage
Body of stomach	37	74
Antrum	11	22
Pylorus	02	04
Total	**50**	**100**

The site of biopsy in most cases was from the Body of stomach-37 cases (74%)

Table.3 Showing the histopathological diagnosis of the 50 malignant cases on H & E stained sections

Cases	Number	Percentage
Adenocarcinoma	48	96
1. Moderately differentiated adenocarcinoma	15	30
2. Well differentiated adenocarcinoma	10	20
3. Signet ring cell carcinoma	09	18
4. Poorly differentiated carcinoma	09	18
5. Mucinous carcinoma	03	06
6. Intramucosal carcinoma	02	04
Carcinoid	01	02
Non Hodgkin's lymphoma	01	02
Total	50	100

Most of the patients in our study presented predominantly with Adenocarcinoma-48 cases (96%), out of which Moderately differentiated adenocarcinomas were most common-15 cases (30%).

Table.4 Showing diagnosis of non malignant cases on H & E stained sections:

Cases	Number	Percentage
Gastric ulcer (benign)	14	28
Chronic non specific gastritis	12	24
Gastric perforation	12	24
H.pylori induced gastritis	08	16
Mucosal dysplasia	02	04
Gastric polyp	02	04
Total	**50**	**100**

Most of the non malignant cases were diagnosed as Benign gastric ulcer-14 cases (28%).

Table.5 Detection and Grading of *H.pylori* by special stains (36/50 malignant cases)

Special stain & Grading 1. **Giemsa, Triple stain & Warthin Starry stain**	Number of cases	Percentage
Grade 1	10	28
Grade 2	23	64
Grade 3	03	36
2. **0.1 % Acridine Orange**		
Grade 1	11	30.5
Grade 2	16	44.5
Grade 3	09	25

Table.6 Results of the 7 malignant cases where *H.pylori* could be detected only by 0.1% Acridine Orange stain (all Grade 1)

CASES	NUMBER	PERCENTAGE
Moderately differentiated adenocarcinoma	3	43
Poorly differentiated adenocarcinoma	2	28.5
Signet ring cell adenocarcinoma	2	28.5

Table.7 *H.pylori* positive cases in different types of adenocarcinoma

Type of Adenocarcinoma	Total cases	*H.pylori* positive cases
Moderately differentiated adenocarcinoma	15	11
Well differentiated adenocarcinoma	10	08
Signet ring cell carcinoma	09	08
Poorly differentiated carcinoma	09	04
Mucinous carcinoma	03	03
Intramucosal carcinoma	02	02

Table.8 Detection of *H.pylori* in the 18/50 non malignant cases

HPE diagnosis (on H &E)	Total no of cases	*H.pylori* positive cases
Gastric ulcer	14	06
Chronic non specific gastritis	12	02
Gastric perforation	12	00
H.pylori induced gastritis	08	08
Gastric polyp	02	02
Mucosal dysplasia	02	00
Total	**50**	**18**

Figure.1 Mucinous carcinoma stomach showing tumor cells floating in a pool of mucin (x10, H & E)
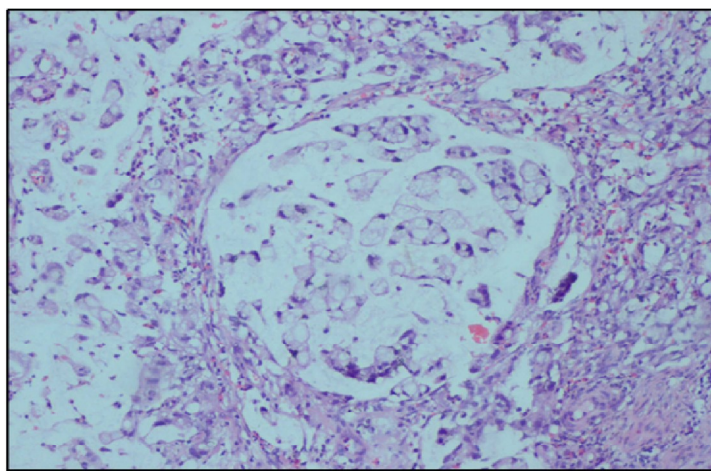
Figure.2 Signet ring cell carcinoma stomach showing signet ring cells (x40, H & E)
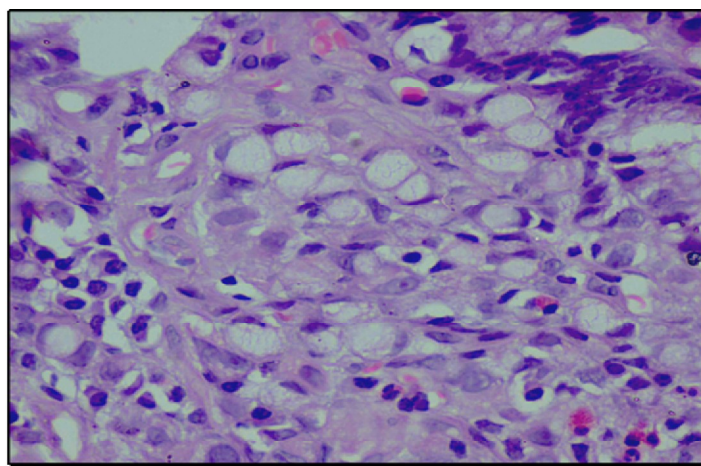
Figure.3 Poorly differentiated carcinoma stomach (x10, H & E)
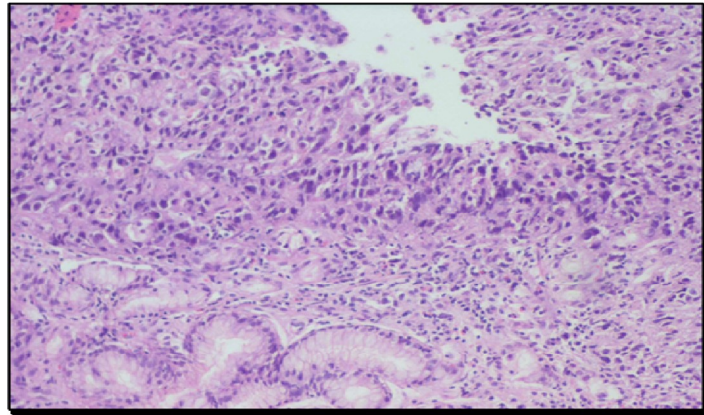
Figure.4 Non Hodgkin's lymphoma stomach (x10 and x40, H & E)
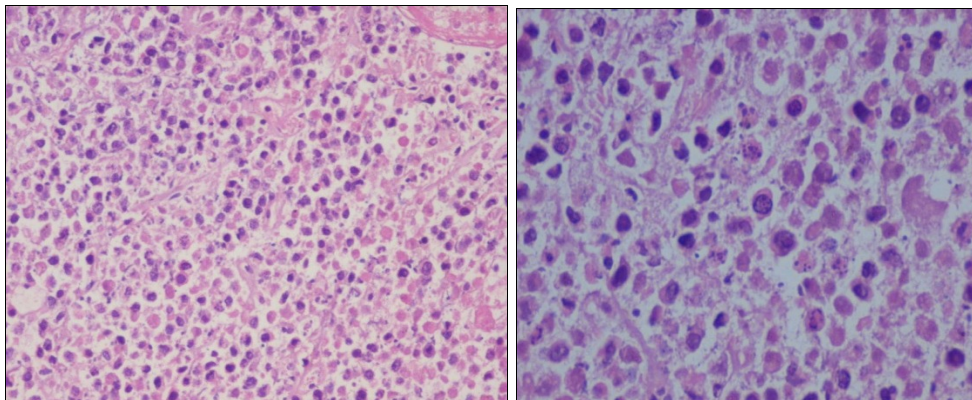
Figure.5 Carcinoid- stomach (x10, H & E)
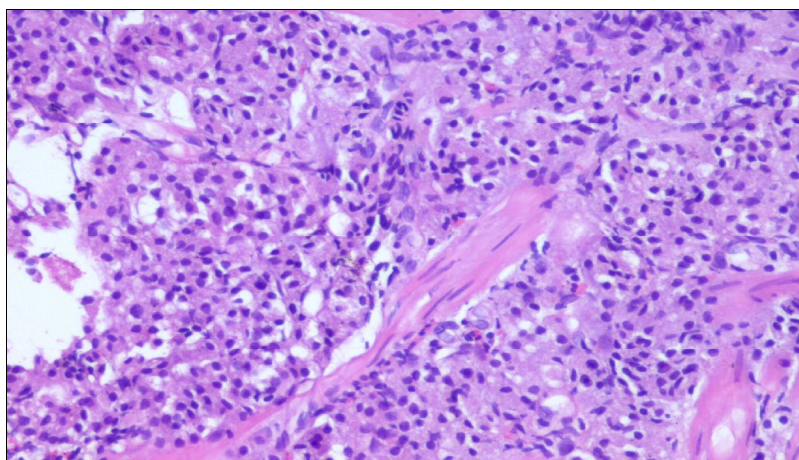
Figure.6 H pylori gastritis showing numerous *H.pylori*-stained blue (x40, Giemsa)
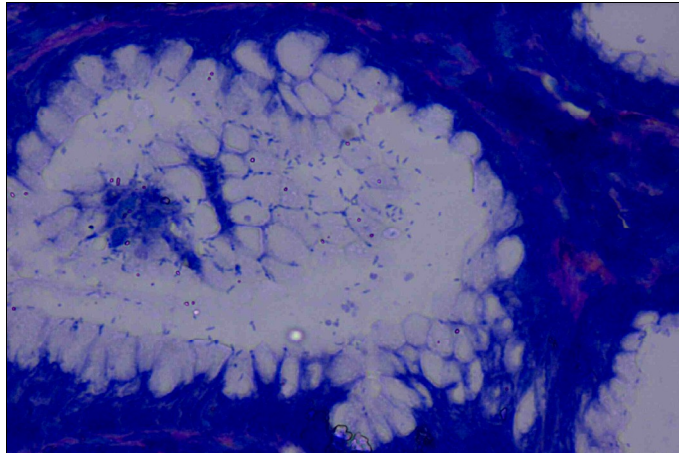
Figure.7 *H. pylori* gastritis showing numerous *H.pylori*-stained pink-red (x40, Triple stain)
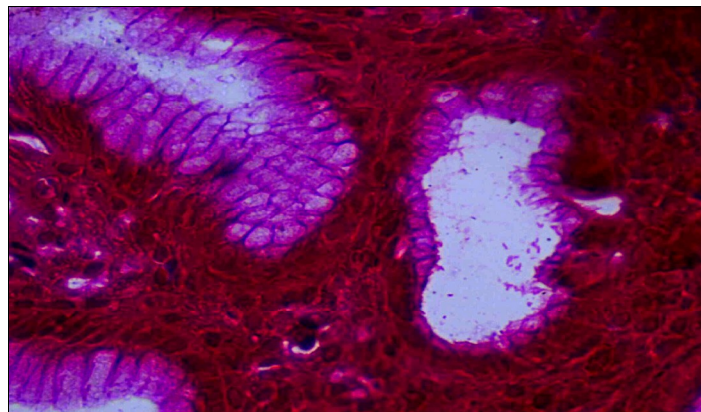
Figure.8 *H. pylori* gastritis showing numerous *H.pylori*-stained black, background is golden brown (x40, Warthin Starry stain)
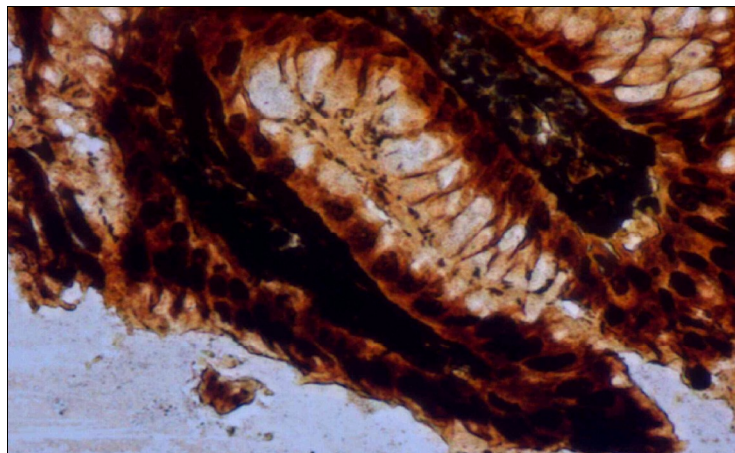
Figure.9 *H. pylori* gastritis showing numerous *H.pylori*-fluorescing green
(x40, 0.1 % Acridine Orange)
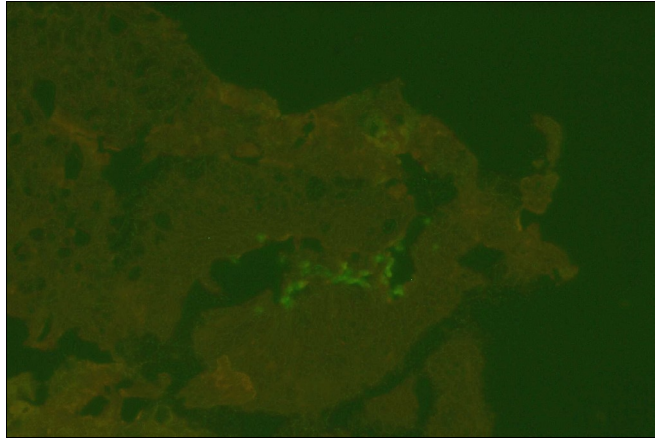
Figure.10 H pylori-Grade 2, in Adenocarcinoma stomach (x40, Giemsa)
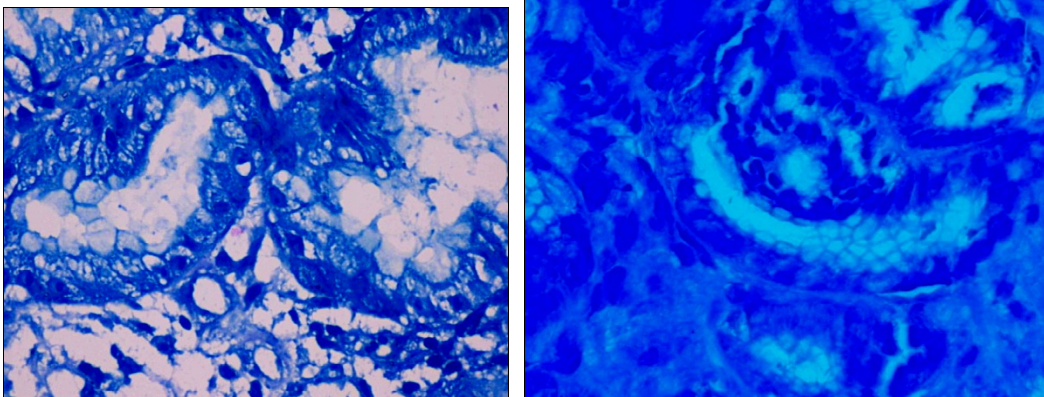
Figure.11 H pylori-Grade 3, in Adenocarcinoma stomach (x40, Triple Stain)
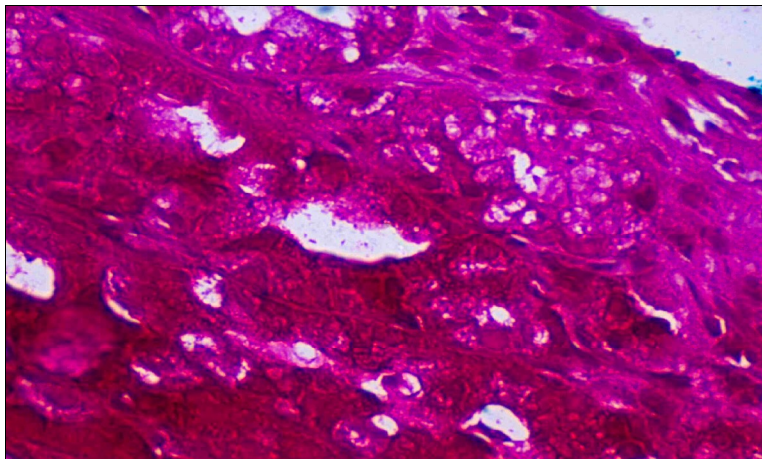
Figure.12 Intestinal metaplasia and mucin highlighted by the Alcian blue component of Triple stain (x40, Triple stain)
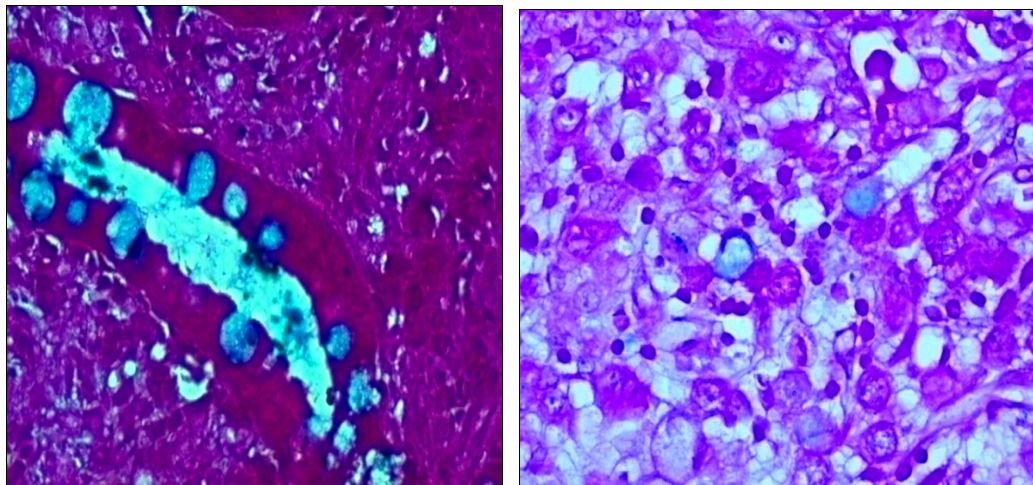
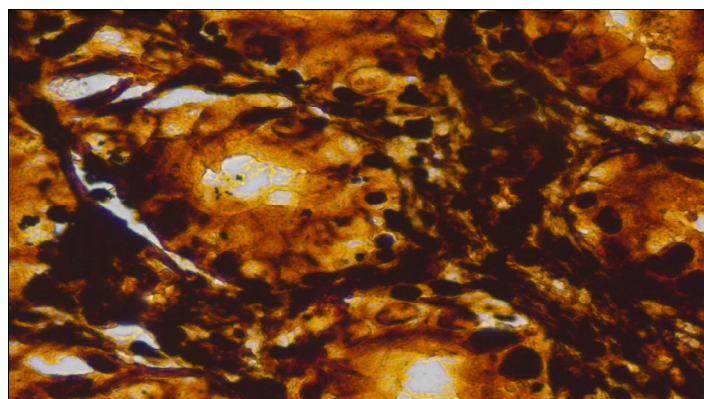**Figure.13** H pylori-Grade 2, in Adenocarcinoma stomach (x40, Warthin Starry Stain)

Figure.14 H pylori in Adenocarcinoma stomach (x40, 0.1 % Acridine Orange Stain)
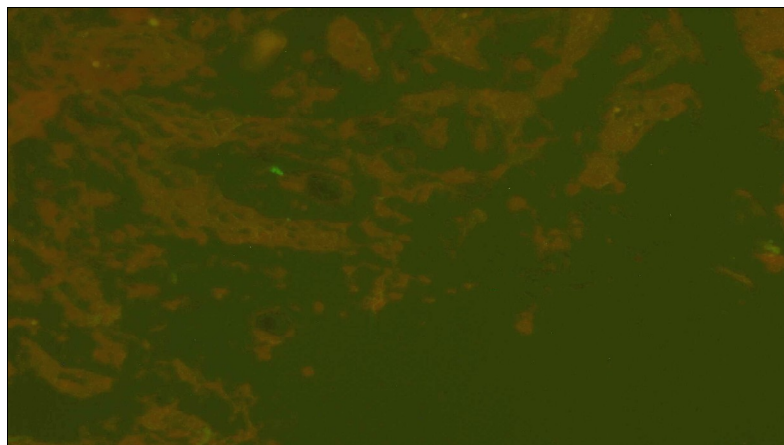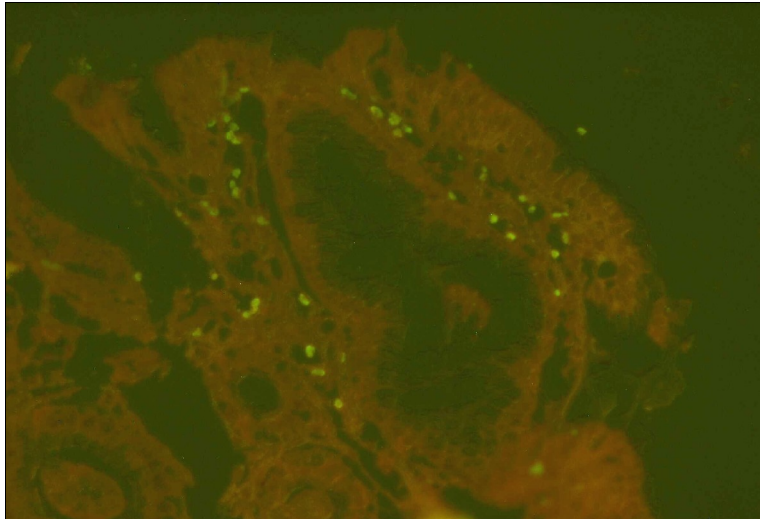
Figure.15 H pylori-Grade 3, in Adenocarcinoma stomach (x40, 0.1 % Acridine Orange Stain)

Conclusion

Gastric carcinoma is one of the most common forms of cancer, with approximately 900,000 new cases diagnosed every year and a leading cause of cancer-related deaths in many parts of the world. It is now well established that *H.pylori* infection is the major risk factor for the development of noncardiac Gastric carcinoma. It is estimated that half of the world's population is infected with *H.pylori*. Malignancy constituted 50% of the cases included in this study.

Adenocarcinoma was the most common type of gastric malignancy in this study. Helicobacter pylori are said to disappear from the stomach as the inflammation progresses to dysplasia and malignancy. Hence, special stains are required to detect the presence of the bacilli in malignant cases. In this study, we could detect *H.pylori* in 58% of malignant cases by using 3 special stains-Giemsa, Triple stain and Warthin Starry stain and in 72% cases using 0.1% Acridine Orange. The sensitivity of detection of the bacilli by Giemsa, Triple stain and Warthin Starry stain are almost same. The number and density of the bacilli reduces as the gastric carcinoma progresses from the well differentiated to the poorly differentiated state. Giemsa is cheap and easy to perform, and is the histopathological gold standard for detection of *H.pylori*. The Alcian blue component of the Triple stain can highlight the areas of intestinal metaplasia in inflammation and can hence detect the predisposing pathological factor in gastric carcinoma. Also it highlights the areas of mucin in gastric adenocarcinomas. Warthin Starry stain best detects the curved morphology of *H.pylori*. Detection of *H.pylori* by the flourescent stain Acridine Orange is highly sensitive (100% in this study), simple and rapid.

Acknowledgements

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