DISSERTATION ON

Role of Immunohistochemistry versus Hematoxylin & Eosin and special stains in Helicobacter pylori detection and analysis of risk factors associated with gastritis

A STUDY OF 100 CASES

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Under the guidance of

Dr. Nalli. R. Sumitra Devi, M.D.,

Associate Professor,

Department of Pathology

Govt. Stanley Medical College

Chennai

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI – TAMIL NADU

CERTIFICATE

This is to certify that this dissertation titled **"Role of Immunohistochemistry versus Hematoxylin & Eosin and special stains in Helicobacter pylori detection and analysis of risk factors associated with gastritis – A Study of 100 cases"** is the original and bonafide work done by **Dr. M.Priyadharshini** under the guidance of **Dr. Nalli. R. Sumitra Devi, M.D.,** Associate Professor, Department of Pathology at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of her course in M.D. Pathology from May-2009 to April-2012 held under the regulation of the Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai - 600032.

PROF. S. MARY LILLY, M.D.,
Professor and Head
Department of Pathology
Government Stanley Medical College
Chennai- 600 001.

Place : Chennai

Date : .12.2011

PROF. R. SELVI, M.D.,
Dean-In-Charge
Government Stanley Medical College
Chennai- 600 001.

Place : Chennai

Date : .12.2011

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CERTIFICATE BY THE GUIDE

This is to certify that this dissertation titled **"Role of Immunohistochemistry versus Hematoxylin & Eosin and special stains in Helicobacter pylori detection and analysis of risk factors associated with gastritis – A Study of 100 cases"** is the original and bonafide work done by **Dr. M.Priyadharshini** under my guidance and supervision at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of her course in M.D. Pathology from May-2009 to April-2012 held under the regulation of the Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai - 600032.

Dr. Nalli. R. Sumitra Devi, M.D.,
Associate Professor
Department of Pathology
Government Stanley Medical College
Chennai- 600 001.

Place : Chennai
Date: .12.2011

DECLARATION BY THE CANDIDATE

I solemnly declare that this dissertation titled **"Role of Immunohistochemistry versus Hematoxylin & Eosin and special stains in Helicobacter pylori detection and analysis of risk factors associated with gastritis – A Study of 100 cases"**is the original and bonafide work done by me under the guidance of **Dr. Nalli. R. Sumitra Devi, M.D.,** Associate Professor, Department of Pathology at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of my course in M.D. Pathology from May-2009 to April-2012 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai - 600032.

Place : Chennai **Signature by the candidate**

Date: .12.2011 **Dr. M.Priyadharshini**

CONTENTS

ABBREVIATIONS

HPE	Histopathological examination
IHC	Immunohistochemistry
H.pylori	Helicobacter pylori
HRP	Horse raddish peroxidase
Cag A	Cytotoxin- associated gene
OMP	Outer Membrane Protein
LPS	Lipopolysaccharides
MUC1	Mucin 1
Oip A	Outer inflammatory protein
IL-8	Interleukin 8
ELISA	Enzyme Linked Immune Sorbent Assay
PCR	Polymerase Chain Reaction
H&E	Heamotoxylin and Eosin
HPSS	Helicobacter pylori silver stain
APAAP	Alkaline Phosphatase Anti-Alkaline Phosphatase
OR	Odds Ratio
CI	Cumulative Incidence

INTRODUCTION

INTRODUCTION

Helicobacter pylori was now accepted as a major cause of chronic active antral gastritis and there is accumulating evidence to incriminate this microbe in the aetiology of duodenal ulcer and gastric carcinoma. It was therefore of paramount importance to determine the presence of the organism in surgical pathology specimens in order to manage these two common diseases of the upper gastrointestinal tract. Antral biopsy specimens processed for histology would therefore provide an easier and more cost-effective alternative means of diagnosing Helicobacter pylori infection. Various special stains have been devised to detect Helicobacter pylori in these histological sections but their specificity and sensitivity vary greatly. The haematoxylin and eosin stain, is the most frequently used stain in histology. Modified Giemsa stain has been favoured because of its easiness to perform and availability in most histopathology laboratories.

However all the above-mentioned stains depend on the morphology of the bacterium for identification and it is possible that there are other microbes in the gastric mucosa, which could resemble and become difficult to differentiate from Helicobacter pylori. It is also known that Helicobacter pylori may demonstrate pleomorphism so that depending on morphology alone may not be reliable for diagnosis. Immunohistochemical techniques have been developed and use of anti Helicobacter pylori antibody which reacts with somatic antigens of the whole bacteria have been found to correlate well with the presence of the bacteria. The aim of this study was therefore to ascertain the reliability of modified Giemsa stain in comparison with Immunohistochemical technique in diagnosing Helicobacter pylori.

HELICOBACTER PYLORI

Helicobacter pylori -Spiral campylobacter like bacteria was observed in close apposition to the gastric mucosa in several cases of gastritis and peptic ulcer, by Warren and Marshall in Australia in 1983.It was originally named Campylobacter pylori. When 16S ribosomal RNA gene sequencing and other research showed in 1989 that the bacterium did not belong in the genus Campylobacter it was placed in its own genus Helicobacter. The genus Helicobacter derived from the Greek word helix means -"spiral" or "coil" and pylori means- gatekeeper.[1] Helicobacter pylori's helix shape is thought to have evolved to penetrate the mucoid lining of the stomach.[2]

EPIDEMIOLOGY

At least half the world's population are infected by the bacterium, making it the most widespread infection in the world.[3] Actual infection rates vary from nation to nation; the developing world has much higher infection rates than the West (Western Europe, North America, Australia), where rates are estimated to be around 25%.[3]. Infections is usually acquired in early childhood in all countries.[4]. However, the infection rate of children in developing nations is higher than in industrialized nations, probably due to poor sanitary conditions.

In developed nations it is currently uncommon to find infected children, but the percentage of infected adults increases with age, with about 50% of patients over the age of 60 years and 10% between 18 and 30 years.[3].The higher prevalence among the elderly reflects higher infection rates during their childhood rather than infection at later age.[4]. The lower rate of infection in the West is largely attributed to higher hygiene standards and

widespread use of antibiotics. Despite high rates of infection in certain areas of the world, the overall frequency of Helicobacter pylori infection is declining.[5].However, antibiotic resistance is appearing in Helicobacter pylori and there are already many metronidazole- and clarithromycin-resistant strains in most parts of the world[6]. The human stomach is the primary reservoir for the organism and it is transmitted via oral-oral route and possibly via a gastric – oral and fecal-oral route[7]

GENERAL CHARACTERISTIC FEATURES

Helicobacter pylori is a helix shaped, gram negative bacterium, about 3 micrometres long with a diameter of about 0.5 micrometres. It is microaerophilic (it requires oxygen, but at lower concentration than is found in the atmosphere) . It contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen (H_2) that is produced by intestinal bacteria[8].It produces oxidase, catalase, and urease. It is capable of forming biofilm[9] and can convert from spiral to a possibly viable but nonculturable coccoid form,[10] both likely to favor its survival in environment. The coccoid form can adhere to gastric epithelial cells in vitro.[11]

PATHOGENESIS :

Features linked to Helicobacter pylori virulence:

Flagella, which allow the bacteria to be motile in viscous mucus

Urease, which generates ammonia from endogenous urea and thereby elevates local gastric pH

Adhesins; that enhance their bacterial adherence to surface foveolar cells.

Toxins; such as cytotoxin- associated gene (cagA), that is involved in ulcer and cancer development

OUTER MEMBRANE PROTEIN :

Helicobacter pylori possess five major outer membrane protein (OMP) families. The largest family includes adhesins. The other four families include porins, iron transporters, flagellum -associated proteins and proteins of unknown function. Like other typical Gram-negative bacteria, the outer membrane of Helicobacter pylori consists of phospholipids and lipopolysaccharides (LPS). The O antigen of LPS may be fucosylated and mimic Lewis blood group antigens found on the gastric epithelium.[12] The outer membrane also contains cholesterol glucosides, which are found in few other bacteria. Helicobacter pylori has 4-6 lophotrichous flagella. All gastric and enterohepatic Helicobacter species were highly motile due to flagella.[13] The characteristic sheathed flagellar filaments of Helicobacter are composed of two copolymerized flagellins, flaA and flaB.

ADHESINS

Outcome of helicobacter pylori infection reflects strain specific, environmental, and host related factors. To colonize the stomach, Helicobacter pylori must survive the acidic pH of the lumen and burrow into the mucus to reach its niche, close to the stomach's epithelial cell layer. The bacterium has flagella and moves through the stomach lumen and drills into the mucoid lining of the stomach[14]. To avoid being carried into the lumen, Helicobacter pylori senses the pH gradient within the mucus layer by chemo taxis and swims away from the acidic contents of the lumen towards the more neutral pH environment of the epithelial cell surface [15]. It produces adhesins which binds to membrane-associated lipids and carbohydrates and help it adhere to epithelial cells. For example, the adhesin BabA which

binds to the Lewis blood group carbohydrate structures are present on the ends of MUC1 carbohydrate side chain as well as on secreted mucins. MUC1 is highly polymorphic and evidence suggests that functional allelic difference affect infection susceptibility [16].

UREASE ENZYME :

Helicobacter pylori produces large amounts of the enzyme urease, molecules of which are localized inside and outside of the bacterium. Urease breaks down urea (which is normally secreted into the stomach) to carbon dioxide and ammonia. The ammonia is converted to ammonium by taking a proton (H^+) from water, which leaves only a hydroxyl ion. Hydroxyl ions then react with carbon dioxide, producing bicarbonate, which neutralizes gastric acid. The survival of Helicobacter pylori in the acidic stomach is dependent on urease. The ammonia is toxic to epithelial cells. Other products of Helicobacter pylori—including proteases, vacuolating cytotoxin A (Vac A), and certain phospholipase damages the epithelial cells[17].

Following attachment of Helicobacter pylori to stomach epithelial cells, the type IV secretion system expressed by the Cag "injects" the inflammation-inducing agent, peptidoglycan, from their own cell wall into the epithelial cells. The injected peptidoglycan is recognized by the cytoplasmic pattern recognition receptor (immune sensor) Nod1, which then stimulates expression of cytokines that promote inflammation[18].Outer inflammatory protein (Oip A) and Cag are necessary for full activation of the IL-8 promoter. IL-8, a potent neutrophil activating chemokine expressed by gastric epithelium plays a central role in the inflammatory response [19].

SIGNS AND SYMPTOMS

Most people (over 80%) infected with Helicobacter pylori show no symptoms. Acute infection may appear as acute gastritis with abdominal pain (stomach ache) or nausea. Where this develops into chronic gastritis, the symptoms, if present, are often those of non-ulcer dyspepsia: abdominal pain, nausea, bloating, belching and sometimes vomiting.

Individuals infected with Helicobacter pylori have a 10 to 20% lifetime risk of developing peptic ulcers and a 1 to 2% risk of acquiring stomach cancer [20]. Inflammation of the pyloric antrum is more likely to lead to duodenal ulcers, while inflammation of the corpus (body of the stomach) is more likely to lead to gastric ulcers and gastric carcinoma[21].

MORPHOLOGY

Gastric biopsy specimens demonstrate Helicobacter pylori in infected individuals. The organism is concentrated within the superficial mucus overlying epithelial cells in the surface and neck region. The distribution can be irregular, with areas of heavy colonisation adjacent to those with few organisms in extreme cases the organisms carpet the luminal surfaces of foveolar and mucous neck cells, and can even extend into the gastric pits. Helicobacter pylori is uncommon in oxyntic mucosa of the fundus and body except in heavy colonisation. Thus an antral biopsy is preferred for evaluation of Helicobacter pylori gastritis. Intraepithelial neutrophils and subepithelial plasma cells are characteristic of Helicobacter pylori [22].

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

1. To analyse the role of Immunohistochemistry versus hematoxylin and eosin and special stains in detection of helicobacter pylori

2. To analyse various risk factors associated with gastritis.

REVIEW OF LITERATURE

REVEIW OF LITERATURE

Helicobacter pylori plays a significant role in the genesis of several gastric diseases like acute and chronic gastritis, follicular gastritis, atrophic gastritis, lymphocytic gastritis, intestinal metaplasia, gastrin cell hyperplasia, giant fold gastritis, gastric adenocarcinoma, gastric mucosa associated lymphoma. Helicobacter pylori result in acute gastritis with cytoplasmic swelling, vacuolization, mucin loss, erosion of juxtaluminal cytoplasm, and desquamation of surface foveolar cells. Marked neutrophilic infiltrates appear in the mucous neck region. Neutrophilic aggregation within the crypt lumen forms pit abscess. Mucosa may appear normal in thickness or slightly expanded with lymphoplasmocytic infiltrate if so it is termed as chronic active gastritis. Regenerative pit bases are characterised by mucin loss, cytoplasmic basophilia, hyperchromatic nuclei and increased mitosis

ACUTE FOVEOLITIS

Acute foveolitis may be associate with an epithelial alteration known as the malgun(clear) cell change. Malgun cells will have enlarged nuclei, abundant cytoplasm, and increased expression of proliferating cell nuclear antigen (PNCA) and cytokeratin 8. Malgun cells may be morphological indicator of genomic damage and repair [23].

QUIESCENT SUPERFICIAL GASTRITIS

Quiescent superficial gastritis is the condition where the signs of acute inflammation such as oedema, and vascular congestion disappear, and epithelium returns to normal. However, the lamina propria contains increased number of mononuclear cells. Chronic superficial gastritis leads to atrophic gastritis.

FOLLICULAR GASTRITIS.

When lymphoid follicle develops with or without germinal centers, then the lesion is termed as follicular gastritis. Lymphocytic gastritis contains prominent intraepithelial lymphocytosis. Helicobacter pylori induced mucosal fold thickening is termed as gaint fold gastritis. About 95% of patients with duodenal ulcer and 70% to 95% of patients with gastric ulcer have Helicobacter pylori infection [24].

Diagnostic tests of helicobacter pylori are of two kinds-

1. Non invasive methods includes - serology (ELISA) and urease breath test.

2. Invasive test includes - endoscopic biopsy of gastric mucosa, for examination by microscopy, culture and urease test.

Bacteriological methods would be the ideal confirmatory tests for Helicobacter pylori diagnosis but are difficult to perform as they require specialised enrichment media with complicated incubation techniques and characterisation of the microbe is time consuming.

ANTRAL BIOPSY:

HISTOPATHOLOGICAL EXAMINATION

Antral biopsy specimens processed for histology would therefore provide an easier and more cost-effective alternative means of diagnosing Helicobacter pylori infection. Various special stains have been devised to detect Helicobacter pylori in these histological sections, but their specificity and sensitivity vary greatly. The haematoxylin and eosin stain, the most frequently used stain in histology, has been found to be the most unreliable [25].

Silver stain, though found to be more superior, is quite complicated to carry out and the granular appearance it gives to the organisms may be confused with silver precipitate [26]. Modified Giemsa stain described by Gray et al (1986)[27] has been favoured by many researchers because of its easiness to perform and availability in most histopathology labs. Other special stains available for detection of Helicobacter pylori were Genta stain, Alcian yellow - Toluidine blue method.

However all the above-mentioned stains depend on the morphology of the bacterium for identification and it is possible that there are other microbes in the gastric mucosa, which could resemble and become difficult to differentiate from Helicobacter pylori. It is also known that Helicobacter pylori may demonstrate pleomorphism, so that depending on morphology alone, may not be reliable for diagnosis. Immunohistochemical techniques have been developed which make use of anti Helicobacter pylori antibody which reacts with somatic antigens of the whole bacteria and have been found to correlate well with the presence of the bacteria.[28]

RAPID UREASE TEST IN ANTRAL BIOPSY

Rapid urease test, also known as the CLO test (Campylobacter-like organism test), is a rapid test for diagnosis of Helicobacter pylori. The basis of the test is the ability of Helicobacter pylori to secrete the urease enzyme, which catalyzes the conversion of urea to ammonia and bicarbonate. The test is performed at the time of gastroscopy. A biopsy of mucosa is taken from the antrum of the stomach, and is placed into a medium containing urea and an indicator such as phenol red. The urease produced by Helicobacter pylori hydrolyzes urea to ammonia, which raises the pH of the medium, and changes the colour of the specimen from yellow (negative) to red (positive).

UREASE BREATH TEST

Patients swallow urea labelled with an uncommon isotope, either radioactive carbon-14 or non-radioactive carbon-13. In the subsequent 10–30 minutes, the detection of isotope-labelled carbon dioxide in exhaled breath indicates that the urea was split; this indicates that urease (the enzyme that Helicobacter pylori uses to metabolize urea) is present in the stomach, and hence proves that Helicobacter pylori bacteria are present.

SEROLOGICAL TEST

IgG antibodies in serum or even whole blood to Helicobacter pylori antigens can be detected using enzyme linked immunosorbent assay. Screening test are used to detect current or past infection. Performance varies with an overall sensitivity and specificity of 91% and 83% respectively.

Reduced sensitivity was seen in HIV infected individuals. Serological results were positive for a very long time, making them less useful for follow-up. Follow-up serology to test for eradication should be done only after 6 months [29].

SYDNEY GRADING SYSTEM FOR GASTRITIS

In general, gastritis is classified into acute and chronic gastritis. Chronic gastritis is divided into non atrophic chronic gastritis, usually caused by Helicobacter pylori infection, and atrophic gastritis composed of autoimmune and multifocal atrophic gastritis caused by Helicobacter pylori or dietary factors, as well as special forms of gastritis composed of reactive (chemical, reflux), radiation, lymphocytic, non-infectious granulomatous, eosinophilic and other infectious gastritis.

The Sydney system is a novel classification and grading of gastritis that was devised by a group of experts at the 9th World Congress of Gastroenterology in Sydney, Australia in 1990. In 1994 in Houston, Texas, experts devised the new updated Sydney system[30]. The histo-pathological variables (Helicobacter pylori density, neutrophil and mononuclear infiltration, atrophy, intestinal metaplasia and dysplasia were graded on a scale of 3 (mild, moderate and severe).

The degree of inflammatory activity was investigated for involvement according to the density of neutrophils in gastric mucosal crypts, from one to all crypts. The degree of mononuclear infiltration was investigated. The degree of intestinal metaplasia was assessed and graded according to the amount of glandular tissue replaced by intestinal type epithelium. Mucosal atrophy was defined as a loss of specialized gastric glands in mucosa, partly replaced by intestinal metaplastic epithelium. It was characterized by architectural changes manifested by variation in the volume and irregularity in the shape, branching, and spacing of the glands.

Sydney grading of gastritis in gastric biopsy

Table No.1

Features	Grade		
Chronic inflammation	Mild	moderate	marked
Activity	<1/3 of pits –mild	1/3 to 2/3 – moderate	>2/3 – marked
Atrophy	Mild	moderate	marked
Intestinal metaplasia	Mild	moderate	marked
Helicobacter pylori colonization	<1/3 of surface –mild	1/3 to 2/3 – moderate	>2/3 – marked

Risk factors associated with gastritis-

- Bacterial infection- vulnerability to the bacterium could be inherited or it could be caused by lifestyle choices, such as smoking and high stress levels.
- Regular use of pain relievers.
- Older age- Older adults have an increased risk of gastritis because the stomach lining tends to thin with age and because older adults are more likely to have Helicobacter pylori infection or autoimmune disorders than younger people are.

- Excessive alcohol use. Alcohol can irritate and erode the stomach lining, which makes the stomach more vulnerable to digestive juices. Excessive alcohol use is more likely to cause acute gastritis.
- Bile reflux disease.
- Spicy foods
- Caffeine

Other less common causes includes radiation injury, mechanical injury, and systemic diseases such as Crohn disease, amyloidosis or graft versus host disease.

Loffeld et al [31] studied antral biopsy specimens of 302 different endoscopic specimens. 200 patients with non-ulcer dyspepsia were investigated for the presence of Helicobacter pylori in order to determine the most sensitive detection method. Part of the biopsy was cultured, and part stained using a modification of the Giemsa stain, and with an Immunoperoxidase technique using a polyclonal rabbit anti-Helicobacter pylori antiserum. Culture was positive in 44 per cent, Giemsa in 78 per cent, and Immunoperoxidase in 89 per cent of these biopsy specimens. Culture results correlated significantly with the bacterial load observed in the Giemsa stain. It is concluded that culture of Helicobacter pylori is the least sensitive detection method, whereas Immunoperoxidase staining is the most sensitive. For daily practice the modified Giemsa stain, however, appears to be sufficient to diagnose the presence of the micro-organism.

Ashton et al[32] compared the sensitivity of detecting Helicobacter pylori in gastric biopsy and resection specimens using tinctorial and silver impregnation stains, Immunohistochemistry and the polymerase chain reaction (PCR).Helicobacter pylori was

detected in 14 (37%) sections stained with haematoxylin and eosin, 21 (55%) with Giemsa, 23 (61%) with Warthin-Starry, and 25 (66%) that was stained with the antibody. Seventeen (45%) cases were positive on PCR. Immunohistochemistry was positive in all cases in which Helicobacter pylori was detected by other methods .Immunohistochemistry using an Immunoperoxidase technique following heat induced antigen retrieval for detecting Helicobacter pylori in gastric biopsy and resection specimens is highly sensitive and easy to use.

Laine et al [33] studied biopsies taken from the gastric antrum and the body for which H&E, Genta, and Giemsa stains were done in 101 patients. Four separate biopsy specimens were also taken from the antrum and the body for culture, rapid urease test, and 13C-urea breath tests. Sensitivities were comparable for the three stains (H&E, 92%; Giemsa, 88%; Genta, 91%), while H&E specificity (89%) was significantly lower than that of the special stains (98%). The Giemsa stain appears to be the preferred stain for Helicobacter pylori diagnosis on the basis of its good sensitivity, excellent specificity, and lack of technical difficulty in preparation. However, H&E provides excellent accuracy when more than minimal (grade 1) Helicobacter pylori density is present.

Casazza et al[34] studied 201 gastric biopsies. These samples were studied for the detection of the presence of Helicobacter Pylori by histological staining (HE/Giemsa), Immunohistochemistry and PCR by using a primer pair derived from the nucleotide sequence of the Urease A gene of Helicobacter Pylori. Specific amplification of a 411 base pair DNA fragment from all strains of Helicobacter Pylori was tested. Of the 201 gastric biopsy analyzed, 63 (31%) were infected with Helicobacter Pylori on the basis of both histological and Immunohistochemical staining, and 81 (41%) were positive with PCR ($P < 0.001$).

Results conclude that PCR was rapid, highly sensitive and specific for identification of Helicobacter Pylori in gastric biopsy specimens.

Maher Toulaymat et al[35] studied 100 cases of gastritis which was identified positive for Helicobacter pylori, by Genta stain and 100 cases was considered negative by the same technique and also stained using an anti-Helicobacter pylori–specific polyclonal antibody. Laboratory reagent and labour costs for the 2 methods were compared. Chronic active gastritis with lymphoid follicles was significantly associated with Helicobacter pylori infection ($P < .0001$). The Immunohistochemical method had a sensitivity of 97% and a specificity of 98% compared with the Genta stain, with strong agreement for grading density of organisms ($P < .001$). Reagent costs were similar for both methods, but Immunohistochemistry using an autoimmunostainer required less dedicated technical time and hence was less expensive than the Genta stain. Immunohistochemistry using a specific antibody is an accurate and cost-effective method for Helicobacter pylori detection in gastric biopsies.

Jehoram et al[36] compared 5 staining methods, namely, haematoxylin and eosin (H&E), Immunohistochemistry (IHC), the silver staining , the Alcian yellow-Toluidine blue method and Genta staining, for the demonstration of the organism in gastric biopsies taken from antrum, body and fundus of 118 patients who presented to hospital with upper gastrointestinal symptoms. He found that there was no significant difference in the efficacy of H&E, IHC, silver stain and the Alcian yellow-Toluidine blue in the demonstration of Helicobacter pylori in all 3 gastric sites. The least reproducible stain in our hands was the Genta stain. We conclude that H&E is adequate for the initial assessment of gastric biopsies in symptomatic upper gastrointestinal patients. This is because it is a well-tested, cheap and easy staining method, requiring a relatively short period of time to perform, with highly

reproducible results. It has an added advantage of enabling simultaneous assessment of morphological changes accompanying Helicobacter pylori infection. When the density of the organism is expected to be low, we recommend addition of silver stain staining because of its high sensitivity and low cost.

John K. Eshun et al[37] conducted a retrospective study of 37 patients whose gastric antral biopsies were negative for the rapid urease test but positive for lymphocytic infiltration. Specimens had been subjected to a rapid urease test, hematoxylin and eosin staining, silver staining and Immunohistochemical staining specific for Helicobacter pylori. Although both stains H&E and silver stains yielded comparable results with Helicobacter pylori–positive biopsies, silver staining was potentially confusing because of nonspecific staining of other organisms. It was therefore recommend that the use of Immunohistochemical staining rather than silver staining was better for evaluation of urease-negative gastric biopsies in children.

Rotimi et al[38] compared staining methods such as the modified Mcmullen's and the Helicobacter pylori silver stain (hpss) methods with two established techniques (the modified Giemsa and anti-Helicobacter pylori antibody immunostain) in terms of availability, reproducibility, rapidity, sensitivity, and cost. Histological sections from 63 paired gastric biopsies from adult patients previously investigated for dyspepsia were stained with the four methods and these were assessed blindly and independently by two observers. When Helicobacter pylori were present, careful examination reveal them, whichever of these stain was used. However, the modified Giemsa stain is the method of choice because it is sensitive, cheap, easy to perform, and reproducible.

Jehoram et al[39] compared 5 staining methods, namely, haematoxylin and eosin (H&E), immunohistochemistry (IHC), the silver staining hpss, the alcian yellow-toluidine blue method and Genta staining, for the demonstration of the organism in gastric biopsies taken from antrum, body and fundus of 118 patients who presented to hospital with upper gastrointestinal symptoms. No significant differences was observed in the efficacy of H&E, IHC, Hpss and the Alcian yellow-Toluidine blue in the demonstration of Helicobacter pylori in all 3 gastric sites. The least reproducible stain was Genta stain. He concluded that H&E is adequate for the initial assessment of gastric biopsies in symptomatic upper gastrointestinal patients. This is because it is a well-tested, cheap and easy staining method, requiring a relatively short period of time to perform, with highly reproducible results. It has an added advantage of enabling simultaneous assessment of morphological changes accompanying Helicobacter pylori infection. When the density of the organism is expected to be low, he recommended addition of hpss staining because of its high sensitivity and low cost.

Wright et al[40] studied Helicobacter pylori and intestinal metaplasia in hematoxylin and eosin-stained slides of, 613 gastric and/or oesophageal biopsies from 494 patients. The slides were stained with hematoxylin and eosin, Toluidine blue for Helicobacter pylori, and Alcian blue for intestinal metaplasia. The hematoxylin and eosin slide was classed as positive or negative for Helicobacter pylori and intestinal metaplasia. Then it was determined whether that case needed a Toluidine Blue or Alcian Blue stain. It was concluded that routine special stains are not required for all gastric and oesophageal biopsies, and hematoxylin and eosin assessment combined with selective ordering of special stains will identify virtually all cases of Helicobacter pylori gastritis and intestinal metaplasia.

Basic et al[41] reviewed thirty gastric antral biopsies showing chronic gastritis together with gastrectomy specimens done for duodenal ulcer were reviewed . The paraffin

sections were stained with modified Giemsa and immunoenzymatic by alkaline phosphatase anti-alkaline phosphatase (APAAP) method for the identification of Helicobacter pylori. Similarly, in modified Giemsa treated sections, coccoid forms, which were particularly seen in sections from resection specimens, caused some uncertainty. These coccoid Helicobacter pylori were obvious in immunostained preparations. Imunoenzymatic staining can be performed on cryostat and paraffin sections, but it was found that reaction was more intense and diffuse in cryostat sections. Helicobacter pylori was identified in 71.8% sections stained with modified Giemsa, but it could be identified with greater frequency in sections stained with APAAP (90.6%). In all cases the bacteria were more prominent and easier to detect in the immunostained sections than with other stains.

Kacar et al[42] studied 60 cases of helicobacter pylori positive and 10 Helicobacter pylori negative cases were selected based on the results of urease test, urease breath test and histopathologic examination of the tissue sections. Histopathologic examination was performed by Hematoxylin-Eosin (H&E), Toluidine Blue, modified Giemsa and Helicobacter pylori Immunohistochemistry. The sections were evaluated by pathologists using double blinding method. The interobserver agreements of the two pathologists were analyzed by Kappa statistics. It was concluded that Helicobacter pylori can be detected on tissue sections regardless of the stain performed and the best results are obtained by the Immunohistochemical stains and the modified Giemsa stain. The costs, applicability and the reliability of the Giemsa stain make it a perfect candidate as an adjunct for diagnosis of Helicobacter pylori on gastric biopsies.

Wang et al[43] studied a total of 224 cases which includes chronic active gastritis (68), chronic gastritis (76), biopsy specimen with no pathologic abnormalities(50), reactive gastropathy (24), and polyps (6). Fifty-four cases were positive for Helicobacter pylori on

IHC, including 50 cases of chronic active gastritis and 4 cases of chronic gastritis. The IHC positive rate was 73.5% (50/68) in chronic active gastritis, 5.3% (4/76) in chronic gastritis, and 0% (0/80) in other diagnoses. The sensitivity/specificity of finding Helicobacter pylori by blindly reviewing hematoxylin and eosin slides was 100%/100%, 100%/100%, 95%/100%, and 100%/100% from the 4 authors. Our results showed that many gastric biopsies (35.7%, 80/224) had no pathologic abnormalities or reactive gastropathy and did not need a routine IHC for Helicobacter pylori. It was concluded that, IHC for Helicobacter pylori should not be routinely used, especially during economically challenging times. Immunohistochemistry should be reserved for unexplained gastritis and previously treated patients with likely low organism density.

Riba et al[44] and colleges with expertise in gastrointestinal pathology examined 300 biopsies that previously demonstrated Helicobacter pylori gastritis using the monoclonal and polyclonal antibody Immunohistochemical method. The sensitivity of the 2 methods (IHC monoclonal and polyclonal antibody) was compared. 96.2% of the cases were identified by the monoclonal antibody method and 98.5% were identified by the polyclonal antibody method. The pathologists scored the 2 methods for quality of organism morphology and background staining. The new Helicobacter pylori monoclonal antibody(Novocastra monoclonal antibody) showed improved quality of organism morphology and reduced background staining compared to the polyclonal antibody.

Kato et al[45] studied the effects of environmental exposures on the development of gastric and duodenal ulcers were investigated in a prospective study of 7,624 American men of Japanese ancestry in Hawaii. The risk of both gastric and duodenal ulcers progressively increased with increasing pack-years of cigarette smoking. In contrast, alcohol intake was not associated with either type of ulcer. The risk of gastric ulcer was positively associated

with the use of table salt/soy sauce, but there was no association with the consumption of other oriental foods. The risk of duodenal ulcer was inversely associated with western style diet around 1940 and with bread intake of two or more servings per day. The authors did not find any protective or adverse effect of milk and fruit consumption on peptic ulcer risk.

Kurata et al [46] studied the risk factors for gastritis in 100 cases including general population, males and females separately, and the elderly. Risk percents were as follows: 24%, NSAIDs; 48%, Helicobacter pylori; and 23%, cigarette smoking. Based on these results 95% of total peptic ulcer related risk is attributable to those factors which have been mentioned in this study. The "interaction" model attributes 89% of cases to these risk factors: 24%, NSAIDs alone; 31%, Helicobacter pylori alone; 34%, Helicobacter pylori/smoking combined. Between 89% and 95% of peptic ulcer-related serious upper GI events may be attributed to NSAID use, Helicobacter pylori infection, and cigarette smoking.

Li Zhang et al [47] studied a total of 139 patients with functional dyspepsia out of which 38 patients were Helicobacter pylori positive which was confirmed by CLO test and histology on at least two biopsies. Active chronic gastritis was diagnosed using the updated Sydney system. In addition to gender and age, information on drinking and smoking habits was collected using a standard questionnaire. Both age and gender were not significantly associated with Helicobacter pylori infection. A multiple logistic model found that alcohol consumption (OR = 9.05, 95% CI: 1.05–77.98) and pathology (active gastritis) (OR = 595.39, 95% CI: 81.43–4353.33) were associated with Helicobacter pylori infection. Active gastritis was associated with alcohol consumption (OR = 2.89, 95% CI: 1.03–8.02), smoking (OR = 2.72, 95% CI: 1.22–6.05) and age (OR = 1.03, 95% CI: 1.01–1.06). In patients with functional dyspepsia, there is no significant association between active

Helicobacter pylori infection and smoking. However, alcohol consumption appears to be associated with Helicobacter pylori infection.

Masood Javed et al[48] studied 50 patients (40 males, 10 females) with upper gastrointestinal symptoms of acid peptic disease and patients with endoscopy proved duodenal ulcer were subjected to gastric antral mucosal biopsies for evaluation of the Helicobacter pylori status with the help of urease test and histological examination of biopsy specimen was done. Epigastric pain was the most frequent symptom in 90%of patients, 92 %(46 out of 50 patients) showed evidence of Helicobacter pylori infection. Maximum incidence of Helicobacter pylori was recorded in the age group of 46 - 55 years. Maximum number of patients was skilled workers (35 out of 50) 70%. 80 % of the patients belonged to lower and middle class.

A retrospective study by Adisa et al[49] of 603 antral biopsies already processed into paraffin wax was undertaken. Each biopsy was stained by Haematoxylin and Eosin method, Giemsa method and the Grocott's modification of Hexamine Silver method. Peak incidence (24.8%) was in the age group of 31-40years. Gastritis in general was recorded in 572 (94.9%)of patients while Helicobacter pylori associated gastritis was recorded in 345(57.2%). The age group of patients with the highest prevalence (26%) of Helicobacter pylori associated gastritis was 41-50years. Specific diagnosis of Helicobacter pylori associated gastritis is crucial in the prevention of cancer.

Rajesh kumar et al [50] studied 265 patients of which 92 patients were Helicobacter pylori positive (by biopsy urease and histopathological test) giving a prevalence of 34.71%. Out of total 92 Helicobacter pylori positive patients assigned for study, 59 were males and 33 were females. The minimum age of Helicobacter pylori positive patient was 18 years and

maximum age was 74 years. The maximum numbers of patients were in the age group of 36-45 years. Upper abdominal pain was the most frequent symptom seen in 49 of Helicobacter pylori positive patients with epigastric fullness and retrosternal burning accounting for the second and third most common complaints. Rest of the clinical features like belching, vomiting and anorexia were almost of equal frequency in both Helicobacter pylori positive as well as negative patients. Multiple complaints were recorded in same patient. Regarding endoscopic and histopathological features, chronic superficial gastritis was the most common feature seen in 87 patients. Duodenitis and oesophagitis were the other common findings documented in 11 and 8 patients, respectively. A single chronic gastric ulcer was noted in 2 and acute duodenal ulcer in 4 patients. Multiple endoscopic and histopathological changes were recorded in the same patient. Overall, inadequate sanitation practices, low social class (32/92), and crowded or high-density living conditions seem to be related to a higher prevalence of Helicobacter pylori infection. All the positive patients were given anti-Helicobacter pylori treatment.

Hoda M. et al[51] and colleagues attempted to determine whether there was a difference in prevalence of Helicobacter pylori infection in Korean children of different socioeconomic classes, despite the high prevalence of infection in childbearing adults. The authors also attempted to identify the factors responsible for the different patterns of transmission by estimating the age-specific prevalence of Helicobacter pylori infection in 413 healthy 1- to 75-year-old asymptomatic volunteers who resided in Seoul. Helicobacter pylori status was evaluated using an enzyme-linked immunosorbent assay for anti-Helicobacter pylori immunoglobulin G. Demographic data were obtained from each individual. Socioeconomic class was assessed by the education level of the adults, parents and family income. Helicobacter pylori infection was present in 75% of adults and 22% of children, and its prevalence increased with age ($p < 0.001$). In adults, the rate of infection was high and

independent of socioeconomic class. In children, it was inversely related to the socioeconomic class of the child's family: 12% among upper socioeconomic class, 25% among the middle class, and 4 1% among the lowest class ($p = 0.016$). No associations were found between prevalence of Helicobacter pylori infection and any factors tested including sex, smoking, and alcohol consumption.

Sibel Öztürk et al evaluated [52] 50 gastric biopsy specimens with Hematoxylin and eosin & Giemsa stains. These were full thickness mucosa of at least one corpus and one antral biopsy, diagnosed as chronic gastritis during routine histopathological examination were taken up for the study. Each observer graded chronic inflammation, activity, atrophy, intestinal metaplasia and Helicobacter pylori density in the corpus and antrum on a scale 0-3 using Sydney's grading of gastritis. The measurement of agreement among observers and the pair wise agreement on the histopathological grades were examined by "measures of agreement among k ratters" and kappa statistics respectively. While the proportion of overall agreement on the various features by the observers ranged from 22% for atrophy in the corpus to 96% for intestinal metaplasia in the corpus. Pair wise agreement ranged from 34% for atrophy in the corpus to 98% for antral intestinal metaplasia. Although the results of this study showed poor to moderate inter-observer agreement, it was observed that the Sydney's grading of gastritis, has a potential value in routine practice.

Chow JY et al [53] reviewed some of the mechanisms involved in cigarette smoking-related gastric ulceration and healing. Experimental findings suggest that cigarette smoking increases xanthine oxidase activity, leukotrienes, and nitric oxide production and also neutrophil infiltration in the gastric mucosa. On the other hand, it reduces blood flow, prostaglandin production, epithelial cell proliferation, and formation of blood vessels in the tissue. These actions are important for ulcer formation and healing. The evidence thus

available strengthens the hypothesis that cigarette smoke is indeed harmful to gastric mucosa through defined mechanisms.

Ko JK et al [54] studied the relationship between Alcohol consumption and cigarette smoking with peptic ulcer disease. Chronic active gastritis is reportedly associated with chronic alcohol ingestion. Nonetheless, the inflammatory changes are likely to be related to concurrent Helicobacter pylori infection that is common among alcoholics. Moreover, chronic alcoholism is also correlated with the presence of gastric metaplasia. Both clinically and experimentally, alcohol has been shown to affect the mucosal barrier and histology. These ulcerogenic effects play a crucial role in altering gastric mucosal defence mechanisms. Cigarette smoking is coupled with the initiation and prolongation of gastric ulcers. Epidemiologic data showed that cigarette smoking increases both the incidence and relapse rate of peptic ulcer disease and also delays ulcer healing in humans. Cigarette smoking is a key factor in inducing ulcer diseases rather than a linked behaviour. The general detrimental effects of cigarette smoking in the gastric mucosa include reduction of circulating epidermal growth factor, increase in tissue free radical production and the presence of free radicals in smoke, together with reduction of mucosal constitutive nitric oxide synthase activity. Furthermore, the alteration of normal gastric mucosal blood flow and angiogenesis and the suppression of cell proliferation contribute largely to the delay in ulcer healing in cigarette smokers. Concurrent consumption of alcohol and cigarette smoking significantly increases the risk of gastric ulcers. The reduction of mucus secretion, increase in leukotriene B4 level, increased activities of inducible nitric oxide synthase, xanthine oxidase and myeloperoxidase, and the expression of adhesion molecules in the gastric mucosa accompanied such potentiating effects. Substances other than nicotine in cigarette smoke may also contribute to the above effects.

IMMUNO HISTOCHEMISTRY

IMMUNOHISTOCHEMISTRY

Immunohistochemistry involves two disciplines – immunology and histology. Immunohistochemistry is used to determine expression of particular antigen and its micro anatomic location in the tissue. IHC uses antibodies to distinguish the antigenic differences between the cells. These differences can specifically identify the lineage of cell population and define biologically distinct populations of cells within the same lineage.

Immunohistochemistry was started in 1940, when Coons developed an immunofluorescence technique to detect corresponding antigen in frozen sections.

Taylor and colleagues in 1974 showed it was possible to demonstrate antigens in routinely processed tissues. Antigen retrieval technique was introduced by Shi and associates in 1991. Antigen retrieval technique is a simple method that involves heating paraffin processed sections at high temperature before IHC staining.

The use of antibody in IHC depends on the sensitivity and specificity of the antigen – antibody reaction and the Hybridoma technique provides limitless source of highly specific antibodies.

Blocking non – specific background staining

Background staining is due to either non specific binding or presence of endogenous enzymes. Non specific binding with polyclonal primary antibody is minimized by pre incubating sections with serum from same species on optimal working dilution.

Endogenous enzymes such as peroxidase seen in normal and neoplastic tissues are abolished by peroxidase blocking or by using alternate systems such as Immunogold technique.

Methods suggested to overcome endogenous activity include incubation in methanol containing 0.5% hydrogen peroxide for 10 minutes at room temperature (almost complete abolition of endogenous peroxidase activity). Endogenous alkaline phosphatase is blocked by addition of 0.1 M concentration of levamisole to the enzyme substrate solution.

Detection systems

Antibodies are labelled or flagged by some method to permit visualization – these include fluorescent substances, enzymes forming colored reaction with suitable substrate (light microscopy) or heavy metals (electron microscopy).

Methods of IHC

Direct labelling method

Antibody is attached with a label by chemical means and directly applied to tissue sections. It is a rapid and easy procedure and carries the disadvantage of multiple antigens which require separate incubation with respective antibodies.

Indirect labelling method

Enzymes are labelled with the secondary antibody, which is produced against primary antibody. This method is more sensitive and easy to handle. The advantages also include increased versality, higher working dilution of primary antibody, secondary antibodies against primary antibodies of different species and easy to prepare.

Avidin biotin techniques

High affinity binding between biotin and avidin is used in this procedure. Biotin is chemically linked to primary antibody and avidin is conjugated chemically to enzyme. The avidin binds to biotinylated antibody thus localizing the peroxidase moiety at the site of antigen.

Disadvantage of this technique is that the endogenous biotin produces non specific background staining.

Avidin biotin conjugate procedure

In this technique primary antibody is added followed by biotinylated secondary antibody and next by preformed complexes of avidin and biotin horse radish peroxidase conjugate. This is a more sensitive method.

Biotin streptavidin system

Streptavidin is used in place of avidin. Streptavidin complexes are more stable.

Immunogold silver staining technique

This is used in ultrastructural immunolocalization. Gold particles are enhanced by the addition of several layers of metallic silver. The fine silver deposits in the background create confusion when small amounts of antigen are identified.

Polymeric method

This technique permits binding of large number of enzyme molecules to a secondary antibody via the dextran backbone. Advantages of this technique are increased sensitivity, minimized non specific background staining and a reduction in the total number of assay steps.

Tissue fixation, processing and antigen retrieval techniques

Tissues for IHC undergo fixation, dehydration and paraffin embedding.

Fixation

This is a critical step as the preservation of morphology is essential for interpretation of IHC. 10% buffered neutral formalin is commonly used because of the following advantages.

1. Good morphological preservation
2. Cheap
3. Sterilizes tissues
4. Carbohydrate antigens are better preserved.

The disadvantage of masking of antigens during fixation can be overcome by antigen retrieval techniques.

Antigen retrieval

This procedure involves unmasking of the antigens. Following techniques can be used.

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval
3. Microwave and trypsin antigen retrieval technique
4. Pressure cooker antigen retrieval

MATERIALS AND METHODS

MATERIALS AND METHODS

SOURCES OF DATA:

The study was carried out in the Department of Pathology, Govt. Stanley Medical College, with the help of Department of Medical Gastroenterology, Govt. Stanley Medical College Hospital, during 2009 to 2011. A total of 120 antral gastric biopsies were received and out of this, random samples of 50 antral biopsies were taken for this study.

INCLUSION CRITERIA:

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

EXCLUSION CRITERIA:

Those with poor clinical data were excluded from the study.

METHOD OF DATA COLLECTION:

Out of the 120 cases, 100 cases had adequate clinical data. Of these 100 cases 50 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 examination of these sections was done. Necessary microphotographs were taken.

Section from gastric biopsy had been categorized using Sydney grading system based on activity, chronic inflammation, metaplasia, atrophy, Helicobacter pylori colinisation and the results were tabulated. Special stain(Giemsa stain) and Immunohistochemical study using Helicobacter pylori polyclonal antibody was done in 50 cases and degree of antibody expression was scored in each case.

GIEMSA STAINING TECHNIQUE

Giemsa stock solution:

Giemsa stain powder - 4gm

Glycerol - 250ml

Methanol - 250ml

The powder is dissolved in glycerol at 60^{o}C with regular shaking. Methanol is added; the mixture is well shaken and then allowed to stand for 7 days. Filter before use.

Working Giemsa stain:

Giemsa stock solution - 4ml

Acetate buffered distilled water - 96ml

METHOD:

1. Dewax in Xylol, hydrate through graded alcohol water
2. Rinse in buffered distilled water (pH 6.8)
3. Stain in working Giemsa stain overnight.
4. Rinse in distilled water.
5. Rinse in 0.5 aqueous acetic acid until the section is pink.
6. Dehydrate, clear in xylene and mount in DPX.

Result: Microorganism – dark blue

Background- pink pale blue

METHODS OF TISSUE PREPARATION FOR IHC

10% buffered formalin was used for fixing the specimens, the tissues were processed in various grades of alcohol and xylol using automated histokinette. Paraffin blocks were prepared and sections of 5 microns thickness were cut in semiautomatic microtome using disposable blades and stained with hematoxylin and eosin. Suitable blocks were chosen for IHC.

Sections for immunohistochemistry were also cut in semiautomatic microtome using disposable blades. Slides were subjected to antigen retrieval using the microwave technique using TRIS EDTA (pH 9.2) buffer solution and then treated by HRP (Horse radish peroxidase) polymer technique.

HRP POLYMER TECHNIQUE

The coated slides were taken through the following stages

1. Treatment with peroxidase block – for inhibiting endogenous peroxidase in the tissue for 20 minutes.
2. Wash in TRIS buffer for 5 minutes.
3. Application of power block – blocks non specific antigen antibody reaction – 20 minutes.
4. Blot dry the excess power block.
5. Application of primary antibody for 60 minutes.
6. Wash in TRIS buffer for 5 minutes thrice.
7. Application of super enhancer for 30 minutes which enhances the final reaction product by increasing the sensitivity of antigen antibody reaction.

8. Application of SS label – secondary antibody from goat with the tagged horse radish peroxidase enzyme for 30 minutes.
9. Wash thrice in TRIS buffer.
10. Application of DAB (Diamino benzidine) chromogen for 5 minutes – this is cleaved by the enzyme to give the coloured product at the antigen sites.
11. Wash in distilled water for 5 minutes.
12. The slides are counterstained with hematoxylin.
13. Air dried and mounted with DPX (Distrene dibutyl pthalide in xylol).

OBSERVATION AND RESULTS

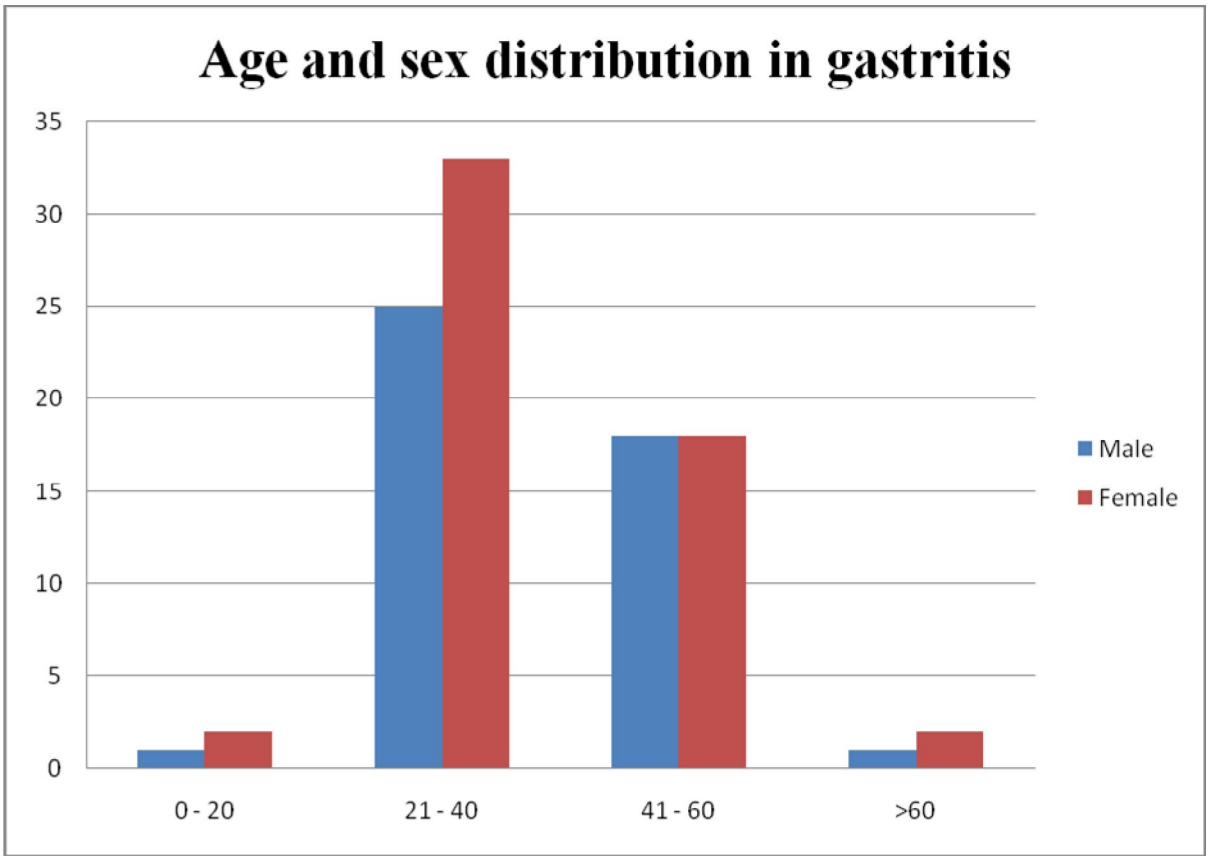
OBSERVATION AND RESULTS

Age &sex distribution of 100 patients presented with gastritis

Table No.2

Age	Male	Female	Total
0 - 20	1	2	3
21 - 40	25	33	58
41 - 60	18	18	36
>60	1	2	3
Total number	45	55	100

Out of 100 patients who presented with gastritis, 58% were in the age group of 20 – 40 and gastritis was found to be more common after 20 years. The P value was0.000 and it was statistically significant. Incidence of gastritis was found in 55 females and 45 males with Male: Female sex ratio account to 1: 1.22. The P value was 0.317 which was not statistically significant.

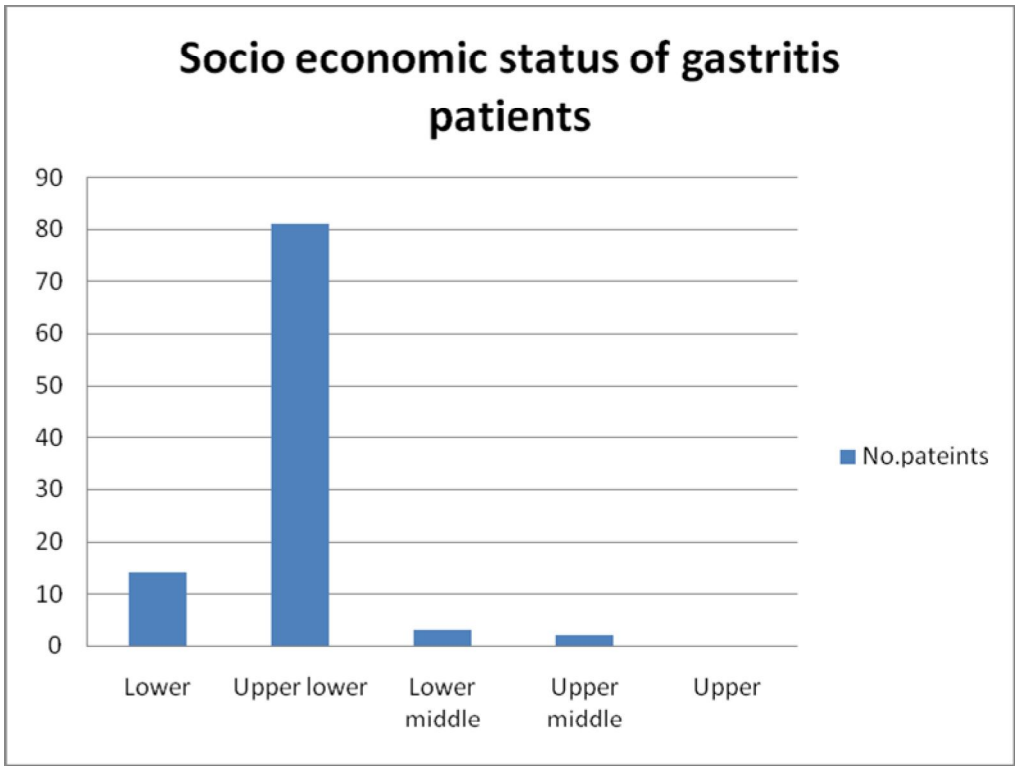

Age and sex distribution in gastritis
35
30
25
20
15
10
5
0
0 - 20
21 - 40
41 - 60
>60
Male
Female

Socio economic status of 100 patients presented with gastritis

Table No.3

Socioeconomic status	No.pateints
Lower	14
Upper lower	81
Lower middle	3
Upper middle	2
upper	0
Total	100

Out of the 100 cases 94% of patients belonged to lower socio economic status. P value for this was 0.000 and it was statistically significant.

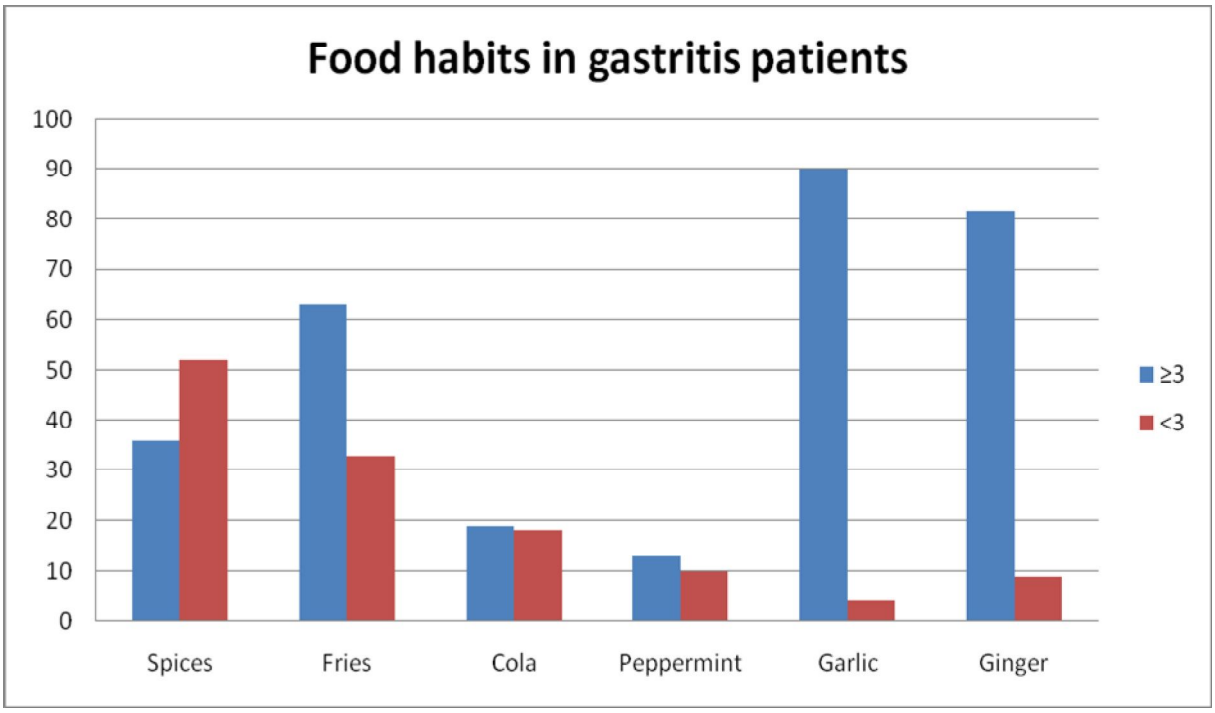

Socio economic status of gastritis patients
90
80
70
60
50
40
30
20
10
0
Lower
Upper lower
Lower middle
Upper middle
Upper
No.pateints

Dietary habits such as intake of spicy foods, fries, cola, peppermint, garlic & ginger in patients presented with gastritis

Table No.4

Food habits	Absent	present	≥3 days per week	<3 days per week
Spices	12	88	36	52
Fries	4	96	63	33
Cola	63	37	19	18
Pepermint	77	23	13	10
Garlic	6	94	90	4
Ginger	9	91	82	9

Most common dietary habits associated with gastritis were intake of spices, fries, garlic, and ginger. Of these P value obtained for intake of spices by using chi square test was found to be 0.005, which was statistically significant.

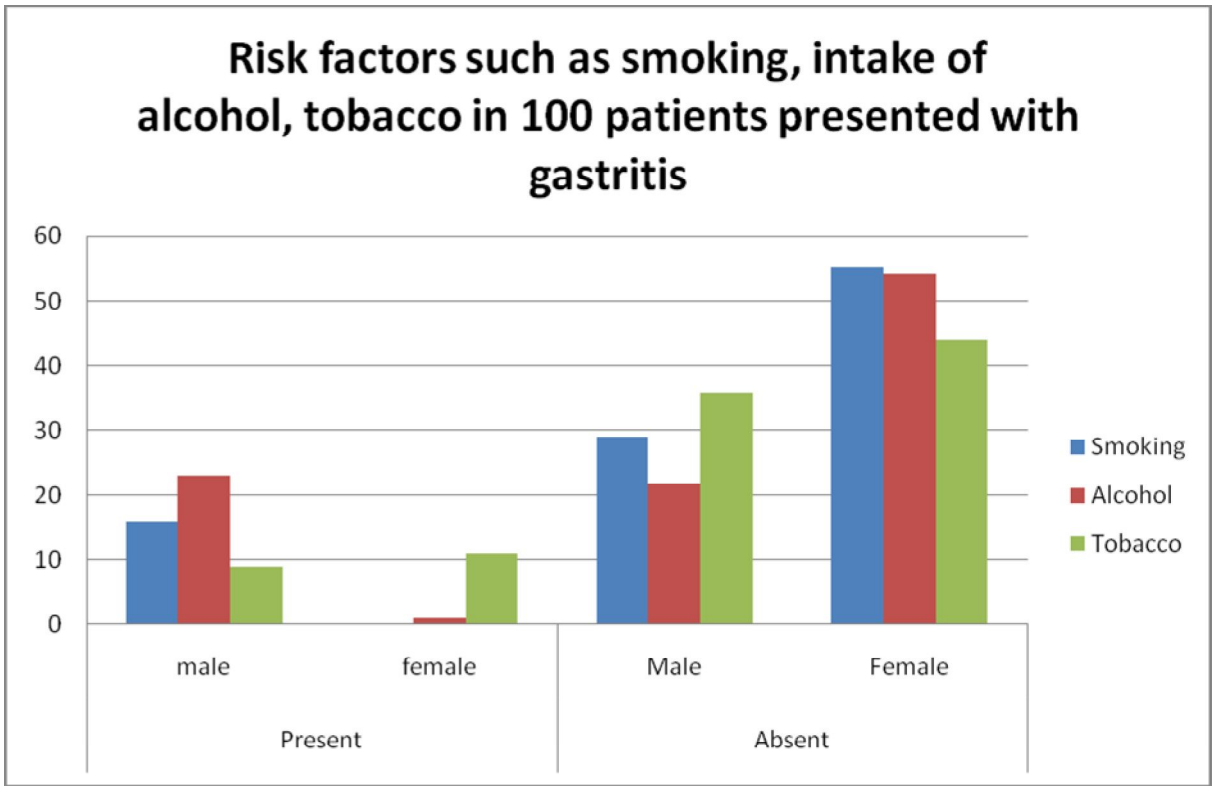

Food habits in gastritis patients
100
90
80
70
60
50
40
30
20
10
0
Spices
Fries
Cola
Peppermint
Garlic
Ginger
≥3
<3

Risk factors such as smoking, intake of alcohol, tobacco in 100 patients presented with gastritis

Table No.5

Risk Factors	Present		Absent		1- 5		6-10		11-15		16-20	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Smoking	16	0	29	55	3	0	7	0	3	0	3	0
Alcohol	23	1	22	54	9	0	11	1	1	0	2	0
Tobacco	9	11	36	44	5	8	0	2	2	0	2	1

Out of 100 cases of gastritis, 16 cases were smokers, 24 were alcoholics and 20 were tobacco chewers. P value obtained for alcohol intake, smoking, tobacco chewing was 0.000, which was statistically significant.


Risk factors such as smoking, intake of alcohol, tobacco in 100 patients presented with gastritis
60
50
40
30
20
10
0
male
female
Male
Female
Present
Absent
Smoking
Alcohol
Tobacco

Sydney scoring in gastric biopsy of 100 cases presented with symptoms

Table No.6

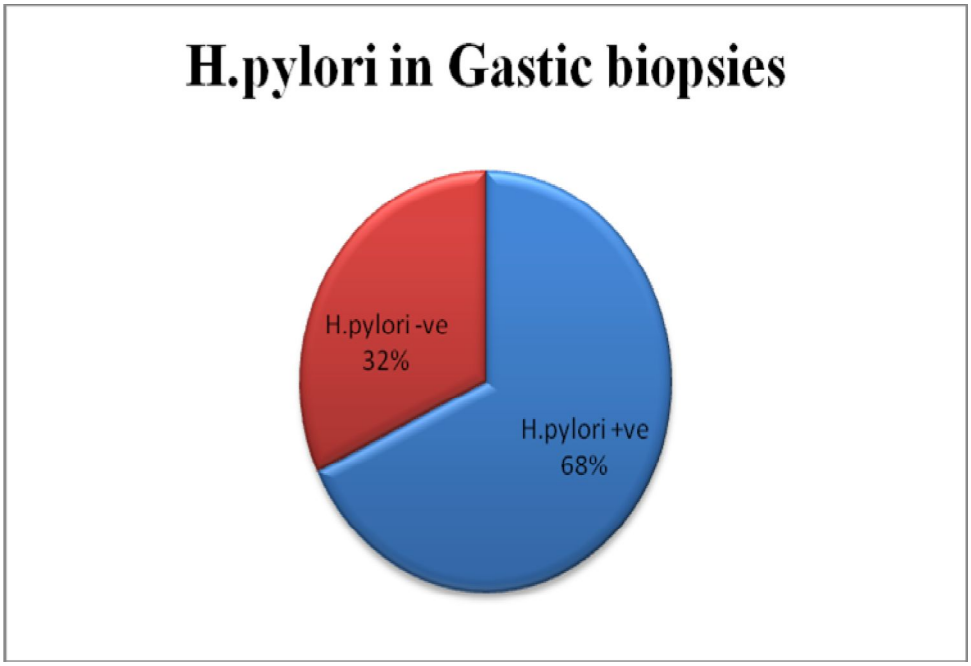
Sydney score	Activity	Chronic inflamation	Intestinal metaplasia	Atrophy	Helicobacter pylori
1	65	48	1	25	18
2	8	47	1	2	12
3	0	5	0	0	5

Immunohistochemistry results of 50 cases of antral biopsy for Helicobacter pylori

Table No. 7

Total No. of patients	Helicobacter pylori +ve	Helicobacter pylori -ve
50	34(68%)	16(32%)

Out of 50 cases studied with IHC for H.pylori, 34 cases were positive and 16 were negative.


H.pylori in Gastic biopsies
H.pylori -ve
32%
H.pylori +ve
68%

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

Table No. 8

Method	IHC		GIEMSA		H&E	
	Positive	Negative	Positive	Negative	Positive	Negative
Male	13	9	13	9	9	13
Female	21	7	19	9	15	13
Total no cases	34	16	32	18	24	26

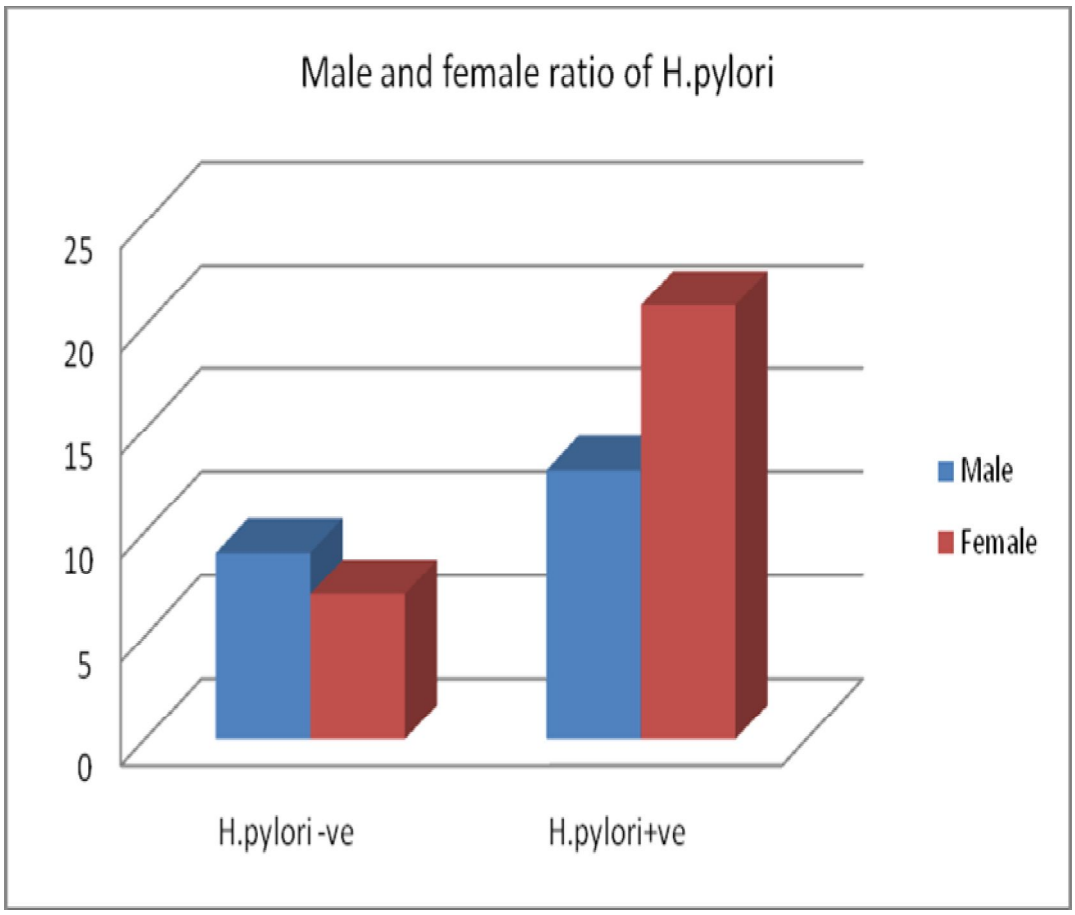
Out of the 50 cases, Helicobacter pylori was positive in 34 cases (68%) of which 21 were female 13 were male. Helicobacter pylori was negative in 16 cases.

Male: Female ratio of Helicobacter pylori infection

Table No.9

Sex	No. of patients	Helicobacter pylori -ve	Helicobacter pylori +ve
Male	22	9(40.9%)	13(59.1%)
Female	28	7(25%)	21(75%)
Total	50	16(32%)	34(68%)

Out of 22 males, 13(59.1%) were Helicobacter pylori positive and out of 28 females, 21(75%) were Helicobacter pylori positive. Male: female ratio was 1:1.27.


Male and female ratio of H.pylori
25
20
15
10
5
0
H.pylori -ve
H.pylori+ve
Male
Female

Age distribution of Helicobacter pylori infection in 50 cases

Table No. 10

AGE	Total No. cases	Negative	Positive
10 - 20	1(2%)	1(2%)	0(0%)
21 - 30	10(20%)	3(6%)	7(14%)
31 - 40	14(28%)	4(8%)	10(20%)
41 - 50	14(28%)	5(10%)	9(18%)
51 - 60	9(18%)	3(6%)	6(12%)
61 - 70	2(4%)	0(0%)	2(4%)
Total	50(100%)	16(32%)	34(68%)

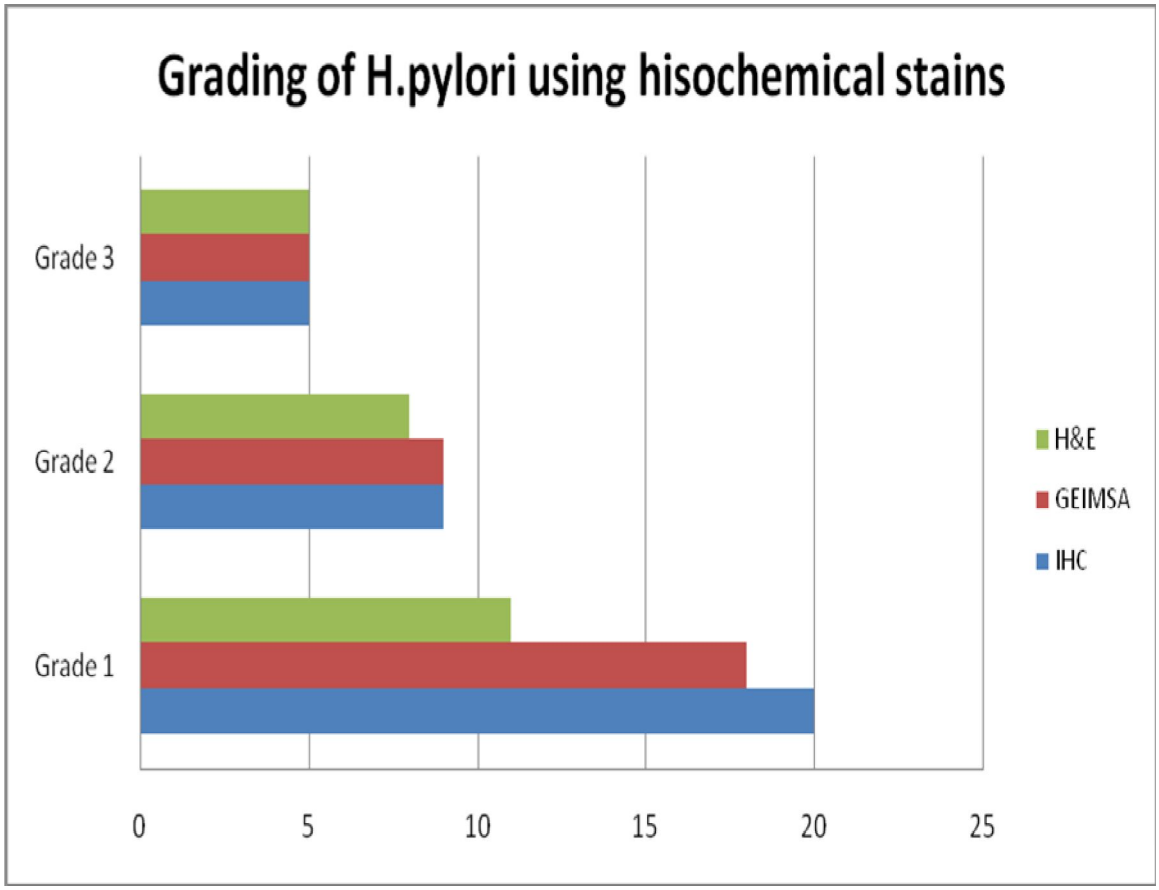
The most common age group affected was in between 31 – 40 years out of the 50 cases in whom Helicobacter pylori was detected using Immunohistochemical method.

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

Table No. 11

Staining method	Total No. cases	Helicobacter pylori Positive	Grade 1	Grade 2	Grade 3	Helicobacter pylori negative
IHC	50	34(68%)	20(40%)	9(18%)	5(10%)	16(32%)
GIEMSA	50	32(64%)	18(36%)	9(18%)	5(10%)	18(36%)
H&E	50	24(48%)	11(22%)	8(16%)	5(10%)	26(52%)

From this table it was evident that Helicobacter pylori detection using H&E, Giemsa and IHC were similar in grade 2 & grade 3 Helicobacter pylori colonisation. Discrepancies were noted in staining pattern when Helicobacter pylori colonisation was low (grade 1).

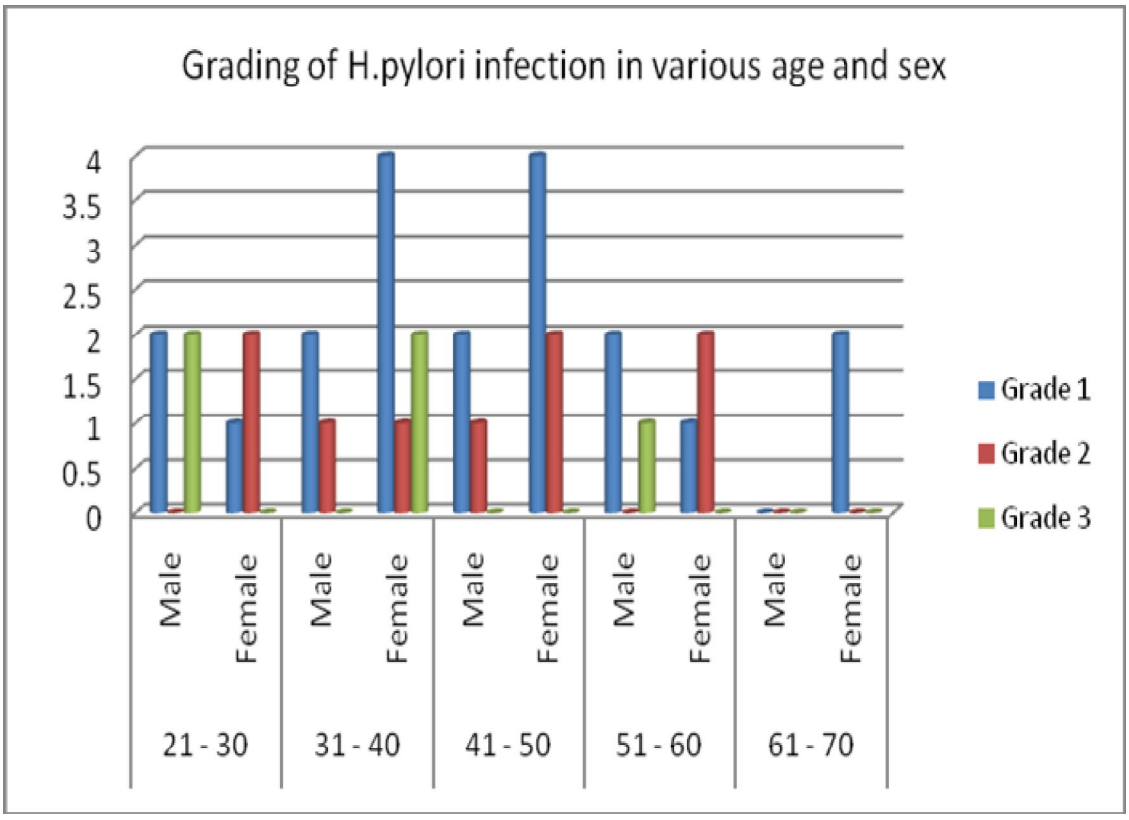

Grading of H.pylori using hisochemical stains
Grade 3
Grade 2
Grade 1
0
5
10
15
20
25
H&E
GEIMSA
IHC

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

Table No.12

Grade	21 - 30		31 - 40		41 - 50		51 - 60		61 - 70		Total Cases
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
Grade 1	2	1	2	4	2	4	2	1	0	2	20
Grade 2	0	2	1	1	1	2	0	2	0	0	9
Grade 3	2	0	0	2	0	0	1	0	0	0	5
Total	4	3	3	7	3	6	3	3	0	2	34

From this table it was inferred that Helicobacter pylori infection was most common in the age group of 31-50


Grading of H.pylori infection in various age and sex
4
3.5
3
2.5
2
1.5
1
0.5
0
Male
Female
Male
Female
Male
Female
Male
Female
Male
Female
21 - 30
31 - 40
41 - 50
51 - 60
61 - 70
Grade 1
Grade 2
Grade 3

Comparison between IHC and Giemsa.

Table No.13

Giemsa	IHC	
	Positive	Negative
Positive	32	0
Negative	2	16

Out of 50 cases, IHC detected Helicobacter pylori in 34 cases with a positivity of 68%. 2 cases which were negative by Giemsa stain was found to be positive on using IHC. 16 cases were negative for both Giemsa and IHC.

Sensitivity-94.1%

Specificity – 100%

Positive predictive value- 100%

Negative predictive value- 88.89%

% of false negative- 5.9

% of false positive- 0

Comparison of H&E and IHC :

Table No.14

H&E	IHC	
	Positive	Negative
Positive	24	0
Negative	10	16

Out of 50 cases, IHC detected Helicobacter pylori in 34 cases, whereas H&E detected Helicobacter pylori in 24 cases only, in 16 cases both H&E and IHC were negative.

Sensitivity- 70.59%

Specificity- 100%

Positive predictive value- 100%

Negative predictive value- 61.54%

% of false negative – 29.41

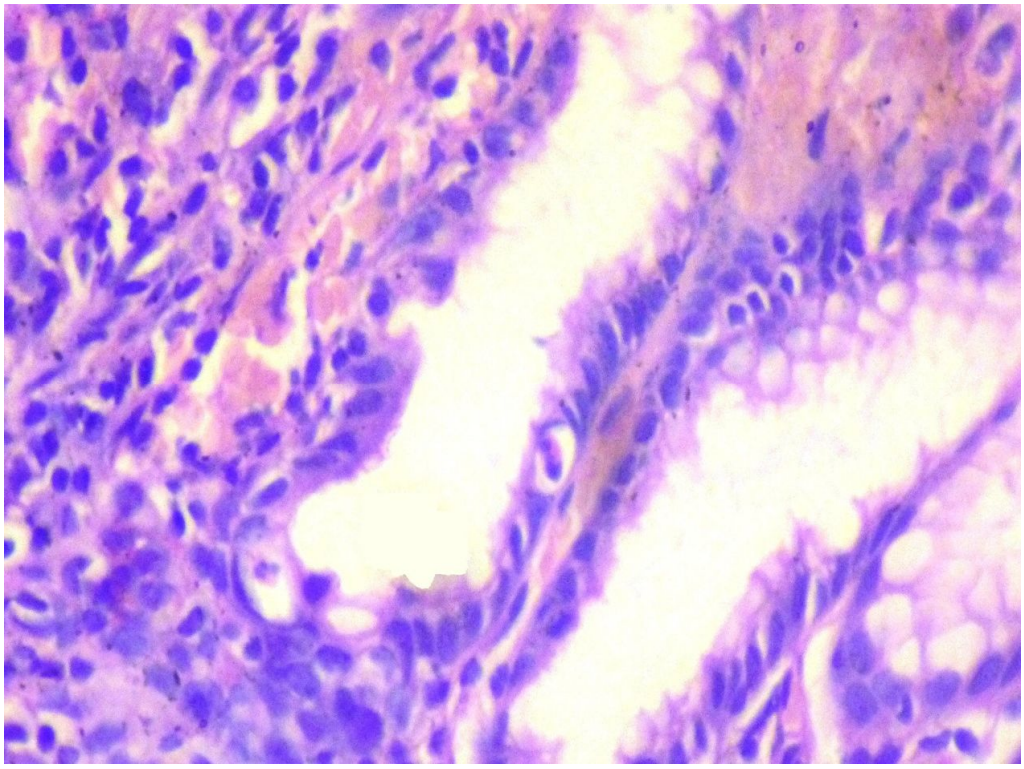
% of false positive – 0

Results of H&E ,Giemsa, & IHC of 50 cases of antral biopsies

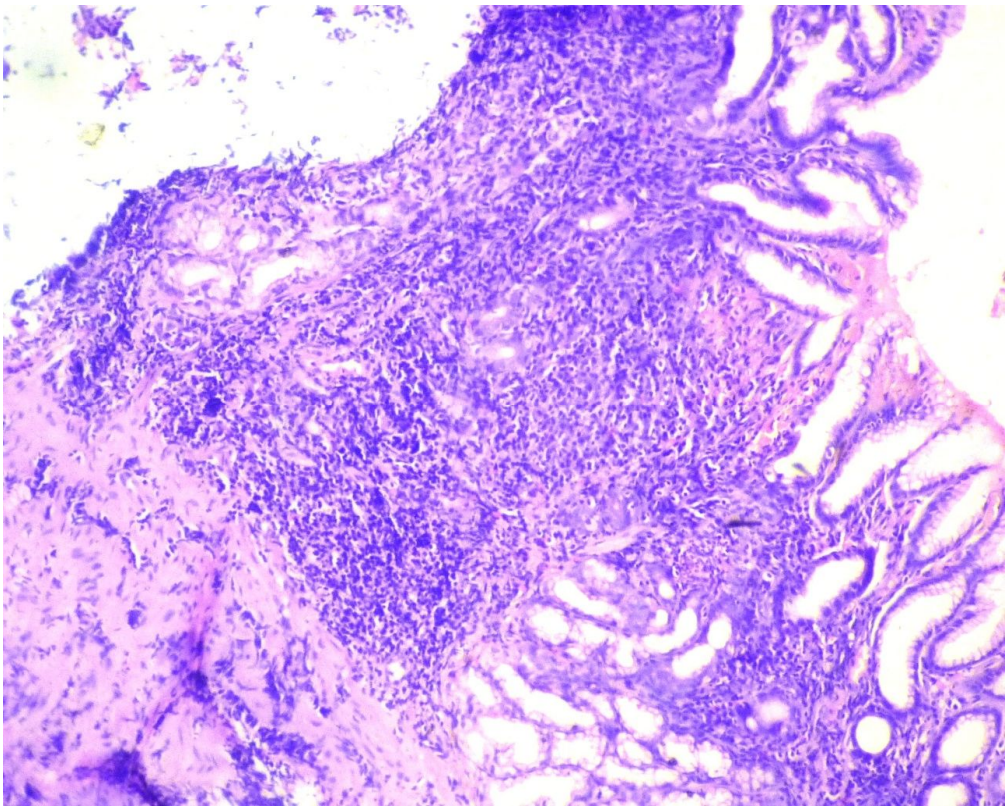
Table no: 14

S. No	Biopsy no	Activity	Chronic inflammation	Intestinal metaplasia	Atrophy	Helicobacter pylori	Giemsa	IHC
1	3016/10	_	1	_	_	_	1	1
2	3018/10	_	1	_	_	_	_	_
3	3019/10	1	1	_	_	_	_	1
4	3020/10	_	1	_	_	_	1	1
5	3021/10	_	2	_	_	_	2	2
6	3024/10	1	1	_	_	1	1	1
7	3025/10	1	1	_	_	_		1
8	3026/10	1	1	_	_	1	1	1
9	3028/10	_	1	_	_	_	1	1
10	3030/10	_	1	_	_	_	1	1
11	3032/10	_	2	_	_	2	2	2
12	5332/09	1	2	_	_	2	2	2
13	3228/09	_	2	_	_	2	2	2
14	3253/09	_	1	_	_	_	_	_
15	3461/09	1	2	_	1	_	_	_
16	3530/09	_	2	_	_	_	_	_
17	3717/09	_	1	_	_	_	_	_
18	3309/09	_	1	_	_	_	_	_
19	3334/09	_	1	_	_	1	1	1
20	3771/09	_	2	_	1	3	3	3
21	3994/09	1	2	_	1	_	_	_
22	4092/09	1	2	_	1	2	2	2
23	4343/09	1	2	_	1	3	3	3
24	4345/09	2	2	_	1	_	_	_
25	4545/09	1	2	_	1	3	3	3

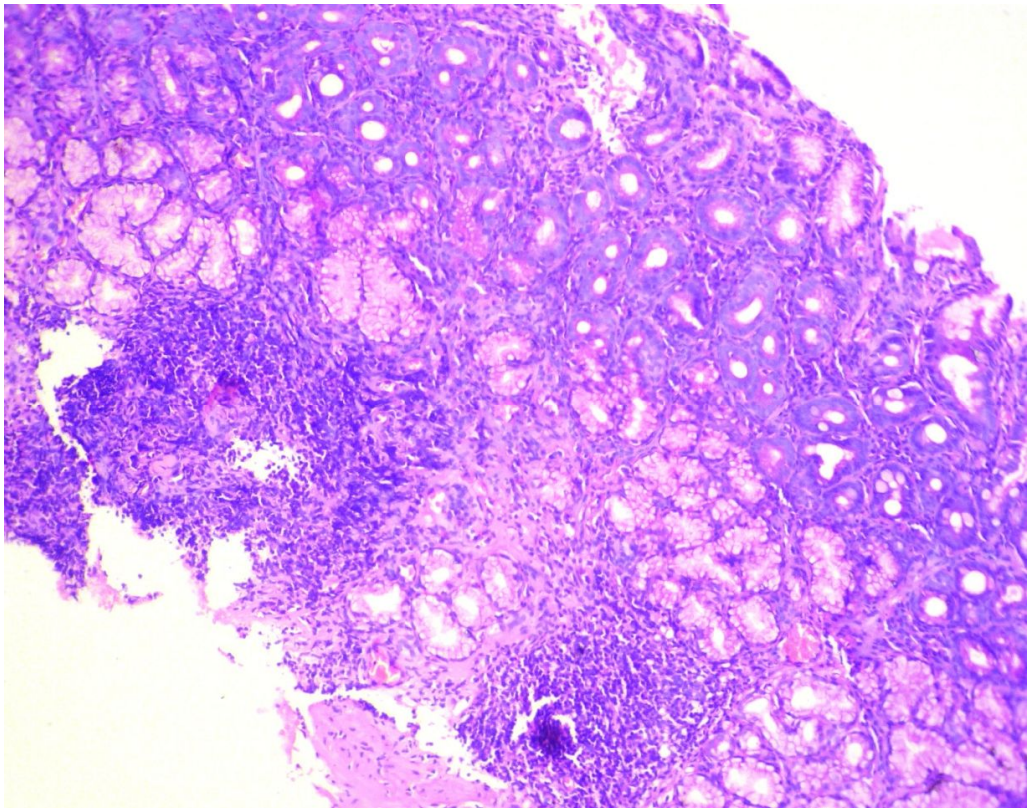
S. No	Biopsy no	Activity	Chronic inflammation	Intestinal metaplasia	Atrophy	Helicobacter pylori	Giemsa	IHC
26	4811/09	1	2	_	1	1	1	1
27	4794/09	1	2	_	1	1	1	1
28	3284/09	_	1	_	_	1	1	1
29	3528/09	1	2	_	1	_	_	_
30	3752/09	_	2	_	1	_	1	1
31	4371/09	1	3	_	1	3	3	3
32	4428/09	1	1	_	_	_	_	_
33	4642/09	_	2	_	_	1	1	1
34	4644/09	1	2	_	1	3	3	3
35	4795/09	_	1	_	_	_	1	1
36	3331/09	_	1	1	1	_	_	_
37	3370/09	_	1	_	_	_	_	_
38	4546/09	_	1	_	_	1	1	1
39	4678/09	_	1	_	_	_	_	_
40	4757/09	1	2	_	1	2	2	2
41	4758/09	_	2	_	1	_	1	1
42	4760/09	1	2	_	_	2	2	2
43	5725/09	1	2	_	_	_	_	_
44	3419/09	1	2	_	_	2	2	2
45	5524/09	1	1	_	_	1	1	1
46	4344/09	1	2	_	_	2	2	2
47	4756/09	_	1	_	_	_	_	_
48	4564/09	1	1	2	1	1	1	1
49	3460/09	_	1	_	_	_	_	_
50	3931/09	1	2	_	1	1	1	1



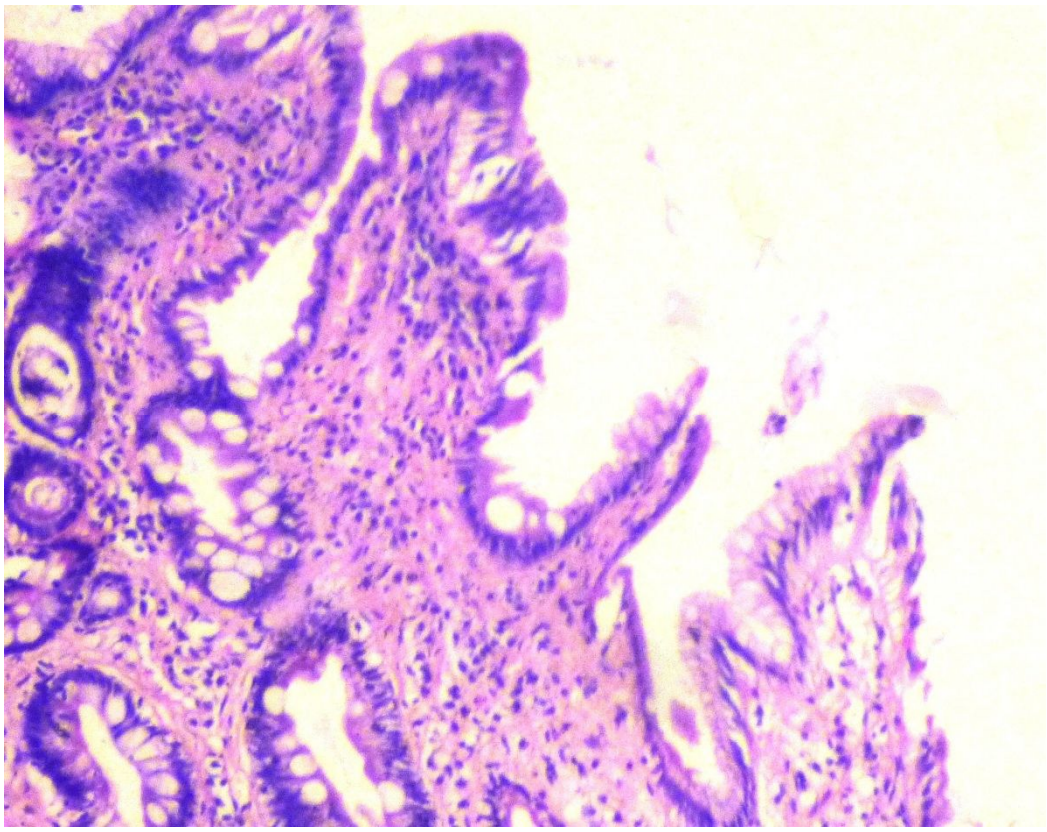
H&E(45X)- Intraepithelial neutrophils present in antral mucosal surface



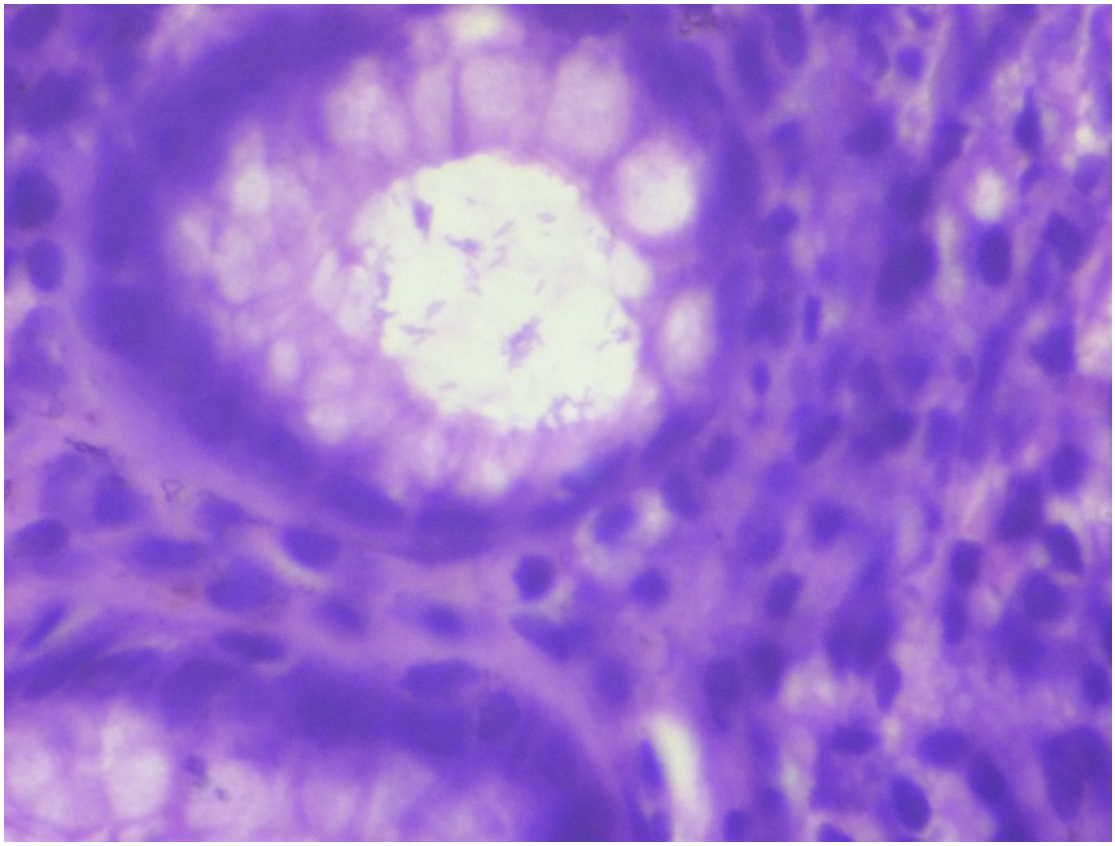
H&E (10X) - Lamina propria showing dense lymphocytic infiltration, atrophy of glands and intestinal metaplasia



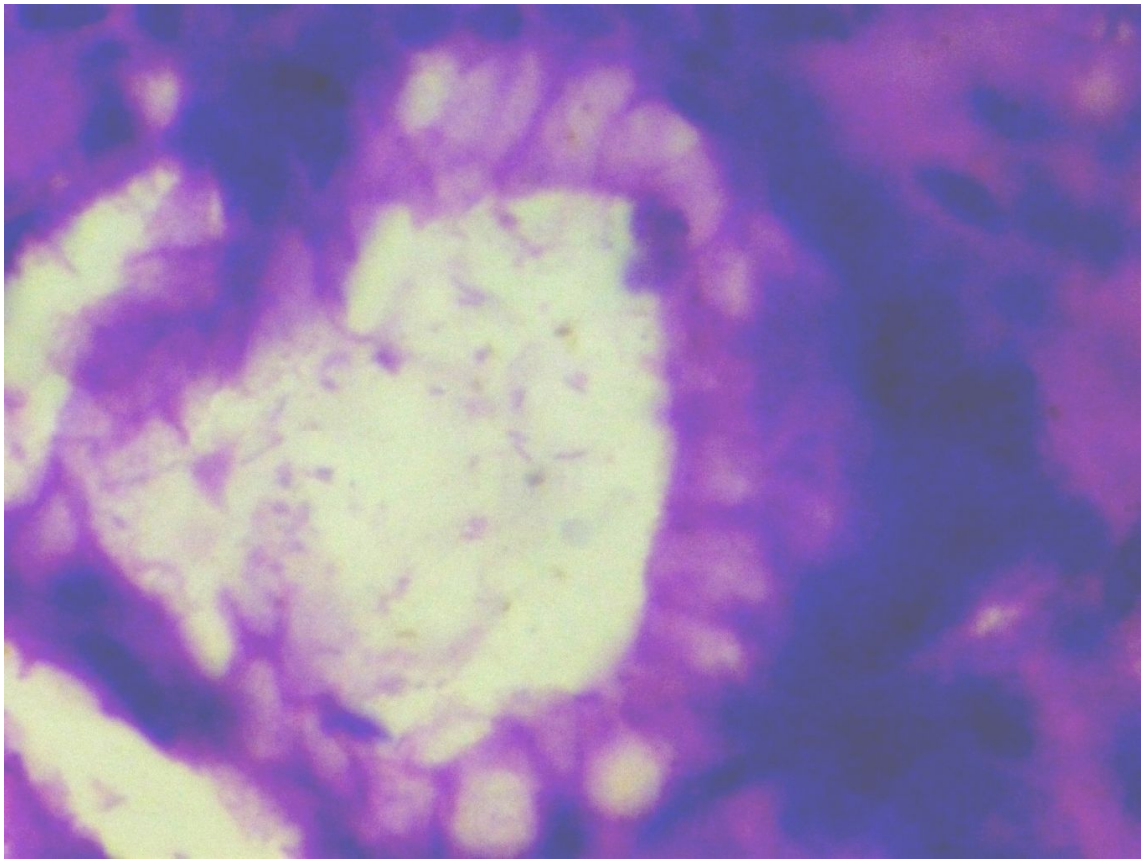
H&E(10X)- Lymphoid follicle present in the lamina propria and atrophy of glands



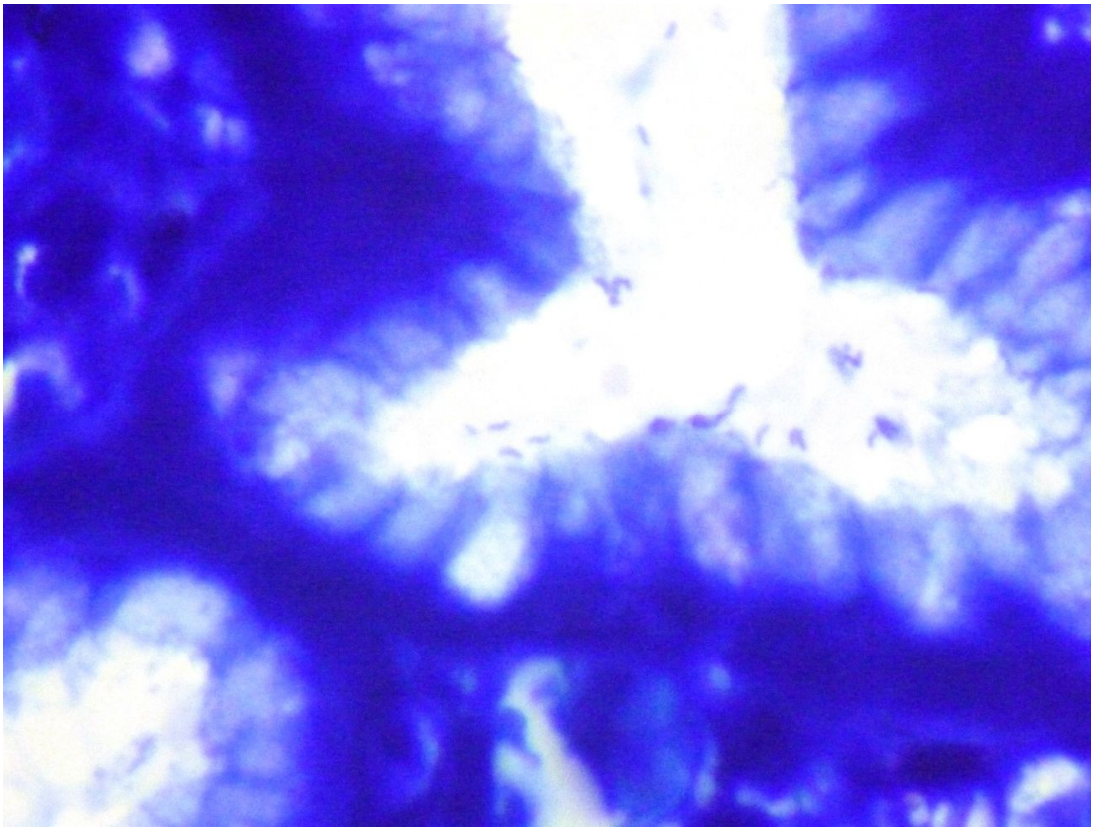
H&E(45X)-Antral mucosa showing Intestinal metaplasia (grade 3)



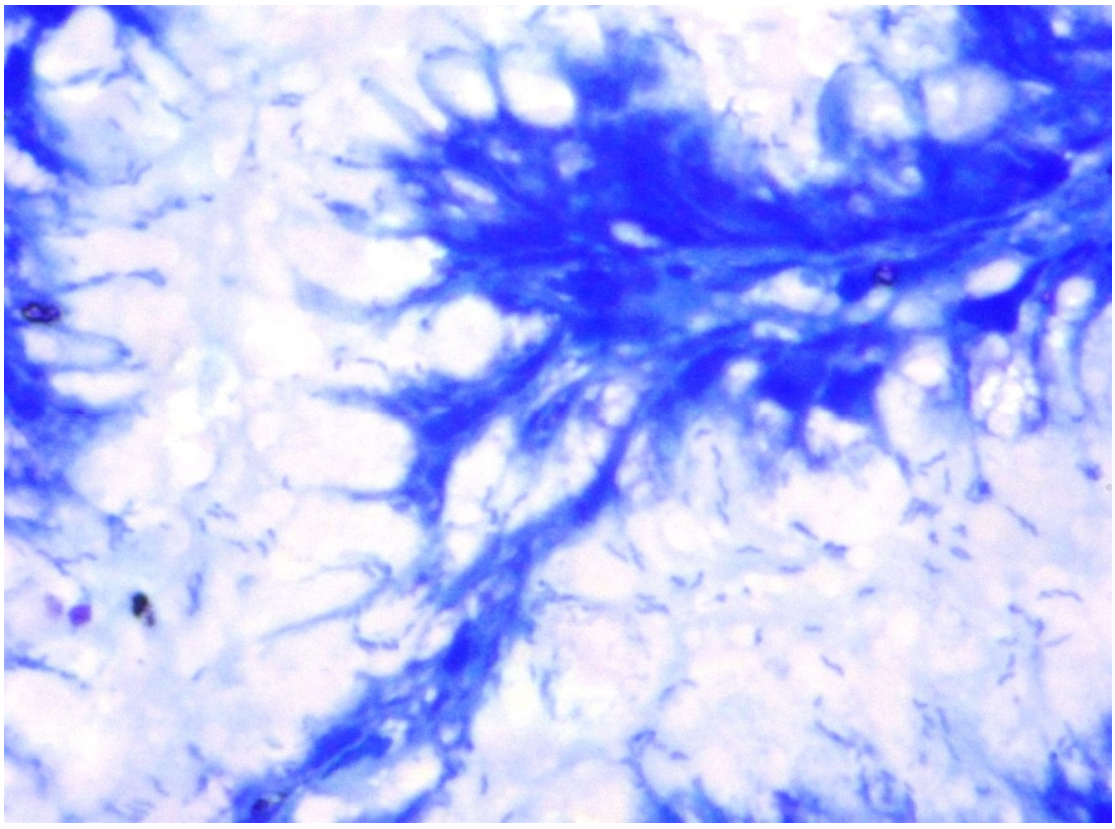
H&E(100X)- Helicobacter pylori present in luminal surface



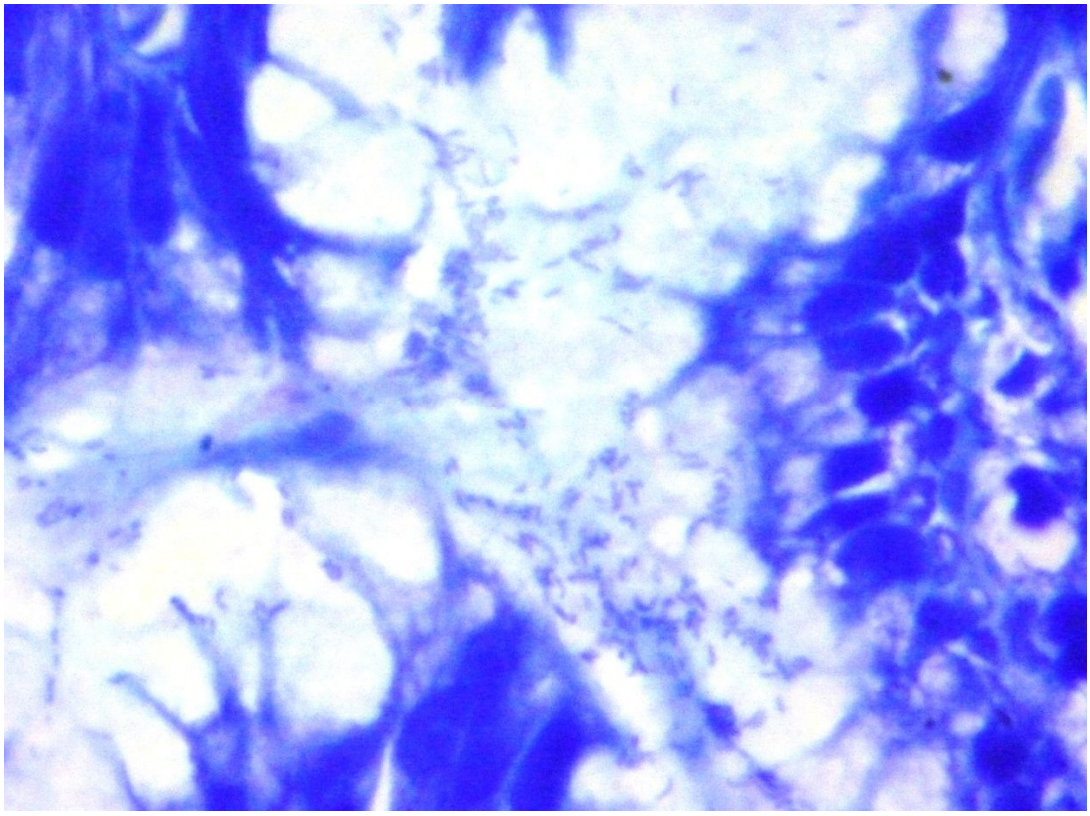
H&E(100X)- Helicobacter pylori in antral surface epithelium



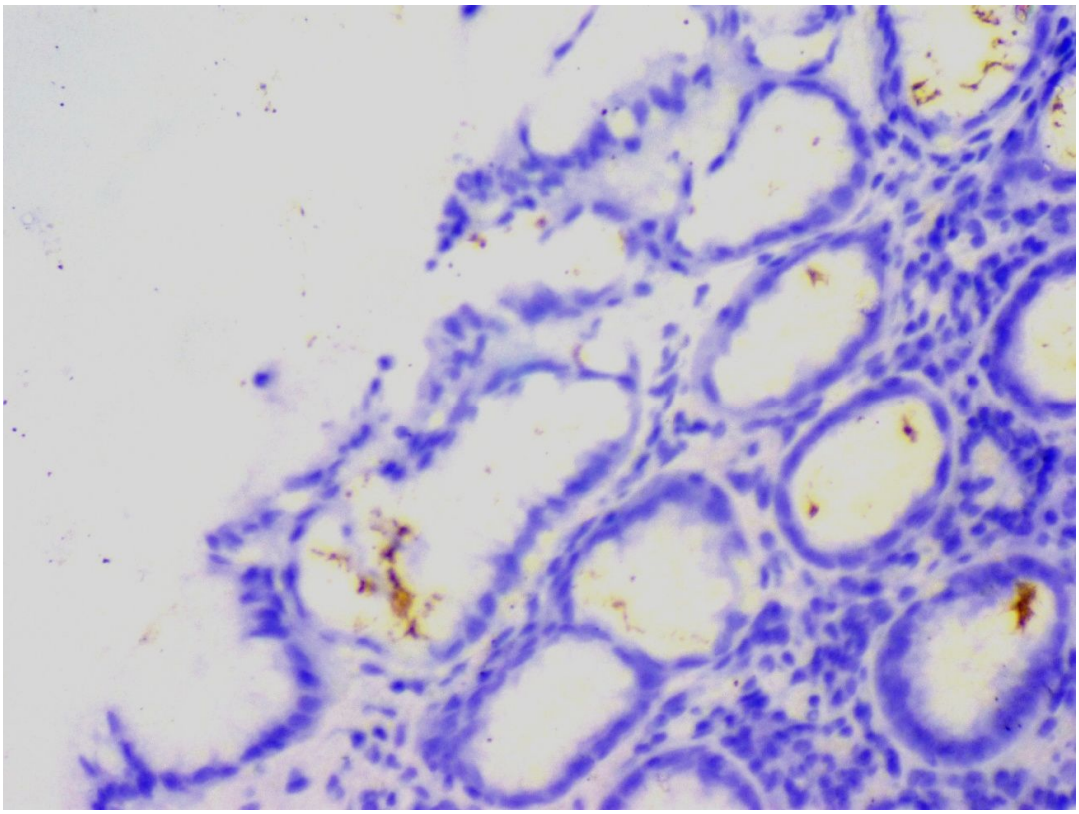
Geimsa (100X) – H.pylori (grade 1) infestation present in the epithelial surface



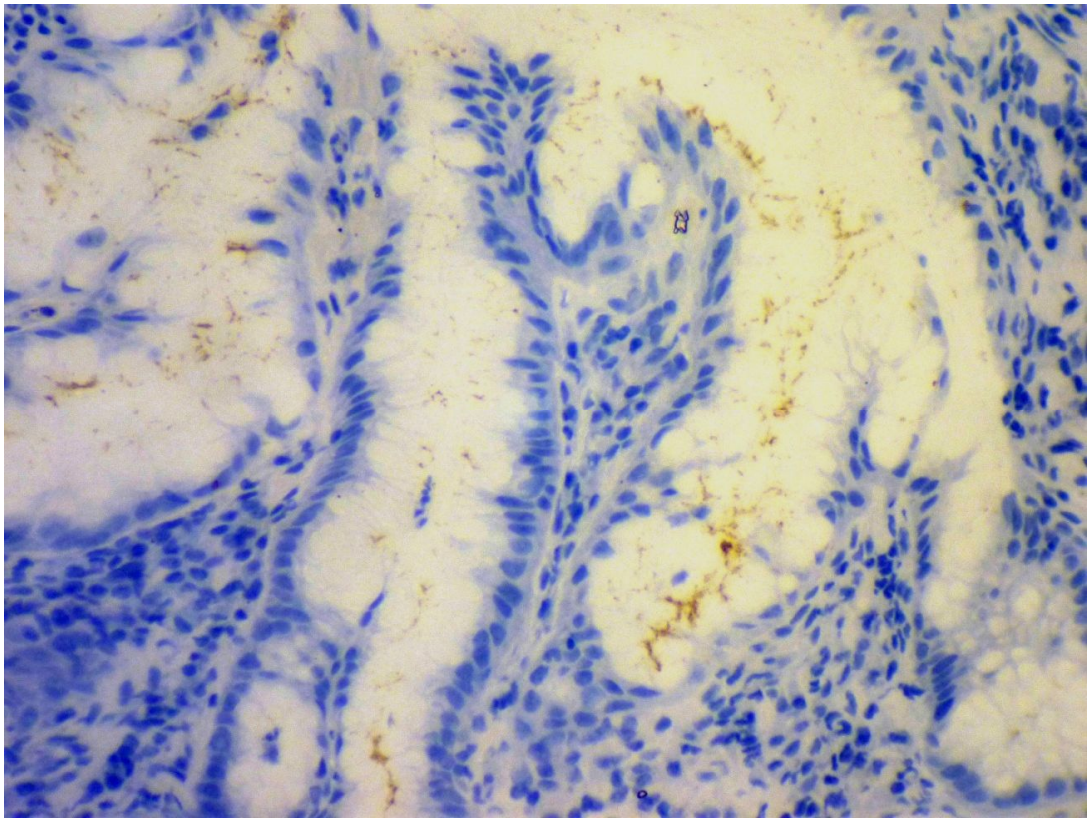
Geimsa (100X) - Mucosal surface epithelium showing grade 2 H.pylori colonisation



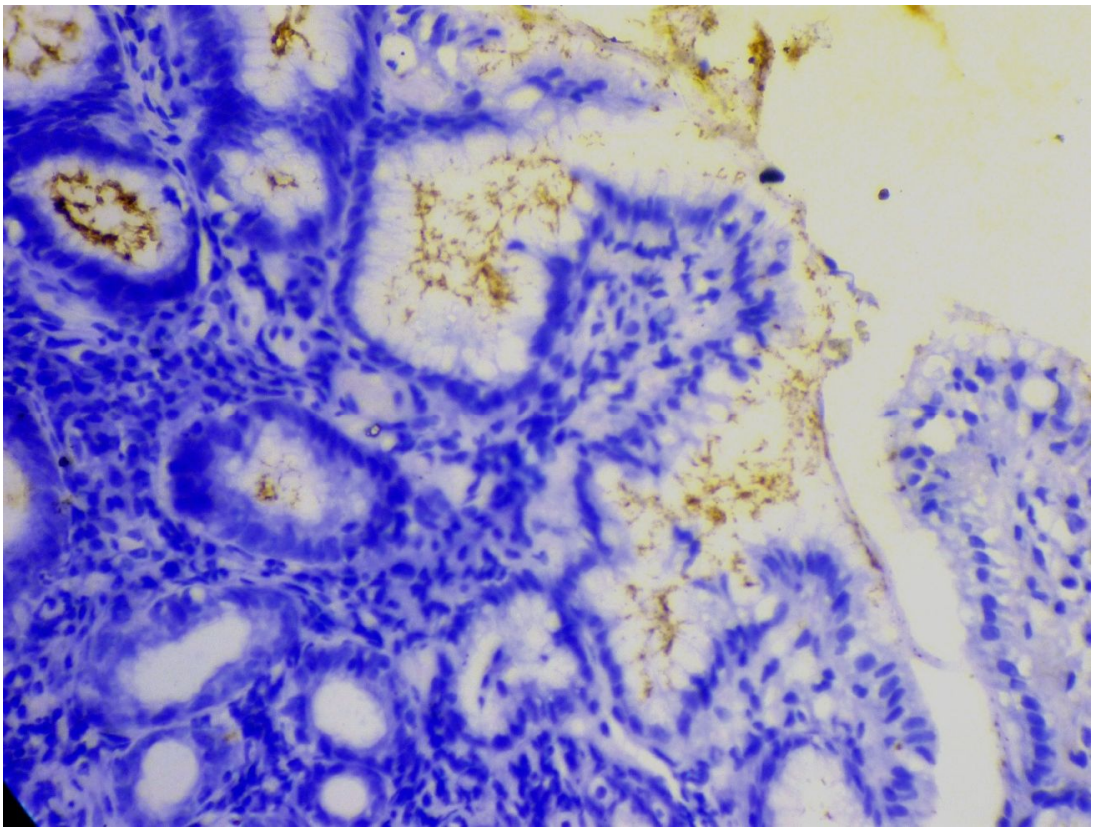
Geimsa (100X)- Helicobacter pylori(grade 3)



IHC-(10X)- Grade 1 H.pylori colonisation



IHC(10X)- Helicobacter pylori (grade 2) colonisation



IHC(10X)- Helicobacter pylori (grade 3) colonisation

DISCUSSION

DISCUSSION

Helicobacter pylori infects the stomach and causes chronic active gastritis, which can lead to peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. Around half of the human population worldwide have been infected by Helicobacter pylori.

In this present study a total of 100 samples of antral biopsies were studied. For all these cases, clinical data and risk factors related to gastritis were collected. Out of 100 cases 50 cases were randomly selected for Giemsa and IHC.

In a study conducted by Rajeshkumar et al [50] 92 out of 265 cases were found to be positive for Helicobacter pylori with over all prevalence of 34.71%.

Out of 603 cases, Helicobacter pylori was positive in 345 cases with prevalence of about 57.2% in the study conducted by Adisa et al[49].

In the present study out of 50 cases 34 cases (68%) were positive for Helicobacter pylori

Comparison of prevalence of Helicobacter pylori with other studies

Table no: 15

Study	Prevalence(in percentage)
Rajesh kumar et al.	34.71%
Adisa et al	57.2%
Present study	68%

Infection with Helicobacter pylori occurs worldwide, but the prevalence varies greatly among countries and among population groups in the same country. It is more common in developing countries where the prevalence is generally over 80% in middle aged adults as compared to 20-50% in industrialized countries.

According to Javed et al[48] prevalence of Helicobacter pylori infection increases with age. Maximum number of Helicobacter pylori infection was seen in the age group of 30-50yrs.

Adisa et al[49] in his retrospective study observed that the prevalence of Helicobacter pylori associated gastritis was maximum between the age group of 41 to 50 years.

A prospective study done by Rajesh kumar et al.[50] showed maximum prevalence of Helicobacter pylori infection in the age group of 36 – 45 years.

In the present study the number of positive cases fall between the age group of 31 – 50 years.

Comparison of Helicobacter pylori infected age groups with other studies

Table no: 16

Study	Age in years
Javed et al	30 - 50
Adisa et al	41- 50
Rajesh kumar et al	36-45
Present study	31-50

The prevalence of Helicobacter pylori infection varies widely by geographic area, age,race, ethnicity, and socio-economic status.

Rajesh kumar et al[50] in his study showed that among the Helicobacter pylori positive patient 64.13% were males and 35.87% were females.

The prevalence of Helicobacter pylori gastritis among males was 46.8% and females was 53.2%. in the study conducted by Adisa et al[49]

In the present study out of 34 Helicobacter pylori cases 13 cases (38.2%) were males and 21 (61.8%) were females.

Male and female distribution of Helicobacter pylori infection.

Table no: 17

Study	Male(in percentage)	Female(in percentage)
Rajesh kumar et al	64.13%	35.87%
Adisa et al	46.8%	53.2%
Present study	38.2%	61.8%

According to Javed et al[48] 80% of Helicobacter pylori infection was found in the low and middle class.

In our present study Helicobacter pylori infection was found to be more prevalent in low socio economic group.

The recent implication of Helicobacter pylori in the pathogenesis of gastritis-peptic ulcer syndrome and its relevance for the development of upper gastro intestinal malignancy warrant efficient methods for detection and demonstration of the organism in the biopsy specimens.

Sensitivity of H&E stain is low due to lack of contrast between the bacteria and the surrounding tissue. The specificity of H&E is also low due to its non specific staining of non Helicobacter pylori bacteria in the stomach.

Modified Giemsa is a cheap, easily applicable stain that can be performed in 15minutes. The results are reliable. The sensitivity and specificity value are acceptable. Lack of contrast is the disadvantage of the Giemsa technique but careful observation allows identification of the organisms correctly.

Helicobacter pylori immunohistochemistry is an expensive and time consuming technique with procedure length, ranging from 1 hour to 24 hours. Sensitivity and specificity are high for the detection of Helicobacter pylori using IHC.

In the current study, the histochemical methods such as H&E, Giemsa and IHC were analysed and compared for the sensitivity and specificity in detecting the Helicobacter pylori.

Kacar N et al. observed that the sensitivity and specificity for H&E, Giemsa and IHC in detection of Helicobacter pylori was 97%/80%; 97%/90%;100%/100% respectively.

HR. Wabinga et al in his study evaluated the staining ability of Giemsa and IHC in gastric biopsies and inferred that the sensitivity of Giemsa stain was 85%, specificity was 89%, positive predictive value was 93% and negative predictive value was 74%.

Sensitivity of detection of Helicobacter pylori in gastric biopsies and resected specimens using modified Giemsa and IHC were compared by Babic et al. which revealed the sensitivity of Giemsa to be 73.3% and 90% for IHC.

In the present study sensitivity and specificity of Giemsa was 94% and 100% respectively. Sensitivity and specificity of H&E was 70.59% and 100% respectively

Comparison of specificity and sensitivity of histochemical stains in various studies.

Table no: 17

Study	sensitivity		specificity		positive predictive		negative predective	
	H&E	Giemsa	H&E	Giemsa	H&E	Giemsa	H&E	Giemsa
Kacar N et al	97%	97%	80%	90%	-	-	-	-
Wabinga et al	-	85%	-	89%	-	93%	-	74%
Babic et al	-	73.30%	-	-	-	-	-	-
Present study	70.59%	94.10%	100%	100%	100%	100%	61.54%	88.89%

Alcohol consumption and cigarette smoking are two etiologic factors that have a close relationship with acid peptic diseases. Chronic active gastritis is reportedly associated with chronic alcohol ingestion. Nonetheless, the inflammatory changes are likely to be related to concurrent Helicobacter pylori infection that is common among alcoholics. Moreover, chronic alcoholism is also correlated with the presence of gastric metaplasia[54].

Javed et al[48] in his study of 50 cases of patients with gastritis observed 28% of patients were smokers, 12% of them were tobacco chewers and 2% were alcoholics.

In the present study of 50 cases we observe that 16% were smokers, 20% were tobacco chewers and 24% were alcoholics.

Study	Smoking	Tobacco chewing	Alcoholism
Javed et al	28 %	12%	2%
Present study	16%	20%	24%

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

A total of 100 gastric biopsy specimens were received in the Department of Pathology, Stanley Medical College during the year 2009 July to 2011 August. Of these 50 cases were randomly selected and analysed for Helicobacter pylori infection using H&E, Giemsa and IHC.

In the present study the age group of patients were in the range of 17 -73 years with peak incidence occurred between 31 – 50 years.

It was observed that Helicobacter pylori infection was more common in female.

Helicobacter pylori was found to be more prevalent in the low socio economic status.

Risk factors associated with gastritis were smoking, alcohol intake, tobacco chewing. Dietary habits associated with were spicy food intake. The most common risk factor for gastritis was alcoholism in our study which accounted to 24%

Sensitivity and specificity of Giemsa was 94% and 100% respectively.

Sensitivity and specificity of H&E was found to be 70.59% and 100% respectively.

Helicobacter pylori can be detected on tissue section regardless of the stains performed. However the best results were obtained by Immunohistochemistry, especially when the density of organism is low Immunohistochemistry is recommended for detection of Helicobacter pylori where the other two stains have low detection rate.

The cost, applicability and the reliability of the Giemsa stain make it an ideal stain in detecting Helicobacter pylori infection in gastric biopsies.

Giemsa stain is also less time consuming and readily available when compared to Immunohistochemistry technique. Hence in the present study Giemsa was more reliable and cost effective stain when compared with H&E and IHC.

MASTER CHART

S.No	Biopsy No	Name	Age	Sex	Occupation		Literacy		Percapita		Score	Class
					Occ	score	Literacy	Score	Income	Score		
1	3016/10	Ramalingam	50	m	Labourer	2	High	4	1400	2	8	UpperLower
2	3018/10	Shakeela	43	f	None	1	Mid	3	1500	2	6	UpperLower
3	3019/10	Renuga	48	f	None	1	Mid	3	1500	2	6	UpperLower
4	3020/10	Babu	40	m	Labourer	2	Primary	2	289	1	5	UpperLower
5	3021/10	Shanthi	30	f	None	1	College	5	3000	4	10	UpperLower
6	3024/10	Mohan	51	m	Labourer	2	Primary	2	600	1	5	UpperLower
7	3025/10	Thulasi	65	f	None	1	Mid	3	1500	2	6	UpperLower
8	3026/10	Kamala	35	f	Labourer	2	Mid	3	1600	2	7	UpperLower
9	3028/10	Jayaraman	27	m	officer	5	College	5	8888	6	16	UpperMiddle
10	3030/10	Ramesh	39	m	Labourer	2	Mid	3	1000	2	7	UpperLower
11	3032/10	Nagammal	48	f	pettywork	3	Illiterate	1	300	1	5	UpperLower
12	5332/09	Ranganathan	41	m	Labourer	2	Primary	2	1250	2	6	UpperLower
13	3228/09	Nagammal	55	f	None	1	Illiterate	1	2000	2	4	Lower
14	3253/09	Kalidas	44	m	Labourer	2	High	4	1750	2	8	UpperLower
15	3461/09	Nagammal	45	f	None	1	Primary	2	1500	2	5	UpperLower
16	3530/09	Ravi	36	m	Labourer	2	Mid	3	1500	2	7	UpperLower
17	3717/09	Panchavarnam	32	f	Labourer	2	Mid	3	1000	2	7	UpperLower
18	3309/09	Kumar	41	m	Labourer	2	Primary	2	1250	2	6	UpperLower
19	3334/09	Kumar	30	m	Labourer	2	Mid	3	750	1	6	UpperLower
20	3771/09	Elumalai	26	m	Labourer	2	Mid	3	1200	2	7	UpperLower
21	3994/09	Kamola	26	f	None	1	Mid	3	500	1	5	UpperLower
22	4092/09	Kuppammal	52	f	None	1	Mid	3	1250	2	6	UpperLower
23	4343/09	Shankariah	57	m	Labourer	2	Mid	3	1333	2	7	UpperLower
24	4345/09	Rajammal	35	f	pettywork	3	Illiterate	1	500	1	5	UpperLower
25	4545/09	Gunasekar	22	m	Labourer	2	College	5	1500	2	9	UpperLower

S.No	Biopsy No	Name	Age	Sex	Occupation		Literacy		Percapita		Score	Class
					Occ	score	Literacy	Score	Income	Score		
26	4811/09	Kalpana	34	f	None	1	College	5	1250	2	8	UpperLower
27	4794/09	Shanthi	30	f	pettywork	3	Illiterate	1	325	1	5	UpperLower
28	3284/09	Krishnaveni	48	f	pettywork	3	Illiterate	1	150	1	5	UpperLower
29	3528/09	Suriya	18	f	None	1	Mid	3	1500	2	6	UpperLower
30	3752/09	Sumithra	32	f	officer	5	College	5	13333	10	20	UpperMiddle
31	4371/09	selvi	35	f	None	1	Illiterate	1	1000	2	4	Lower
32	4428/09	Mohana	57	m	Labourer	2	Mid	3	1300	2	7	UpperLower
33	4642/09	Mala	37	f	None	1	Mid	3	2500	2	6	UpperLower
34	4644/09	Kasthuri	36	f	Labourer	2	Mid	3	1600	2	7	UpperLower
35	4795/09	Thulasi	65	f	None	1	Mid	3	2500	2	6	UpperLower
36	3331/09	Arogadas	25	m	Labourer	2	Primary	2	600	1	5	UpperLower
37	3370/09	Ahamed	40	m	Labourer	2	Mid	3	600	1	6	UpperLower
38	4546/09	Sumathi	45	f	None	1	Illiterate	1	750	1	3	Lower
39	4678/09	Lakshmi	60	f	None	1	Illiterate	1	570	1	3	Lower
40	4757/09	Ponni	40	f	Labourer	2	Primary	2	1000	2	6	UpperLower
41	4758/09	Zeenath	52	f	None	1	Mid	3	800	1	5	UpperLower
42	4760/09	Veera	47	f	officer	5	College	5	2500	2	12	LowerMiddle
43	5725/09	Murugan	27	m	Labourer	2	Mid	3	1500	2	7	UpperLower
44	3419/09	Dakshinamorthy	38	m	Farmer	2	Primary	2	600	1	5	UpperLower
45	5524/09	Prema	44	f	Labourer	2	Mid	3	1300	2	7	UpperLower
46	4344/09	Rajeswari	26	f	None	1	Illiterate	1	650	1	3	Lower
47	4756/09	Ramesh	41	m	Labourer	2	Mid	3	1000	2	7	UpperLower
48	4564/09	Alamelu	54	m	Labourer	2	Mid	3	1000	2	7	UpperLower
49	3460/09	Mohan	57	m	Labourer	2	Primary	2	625	1	5	UpperLower
50	3931/09	Ramesh	41	m	Labourer	2	Mid	3	1300	2	7	UpperLower

S.No	Biopsy No	Name	Age	Sex	Occupation		Literacy		Percapita		Score	Class
					Occ	score	Literacy	Score	Income	Score		
51	4196/09	Tamilarasi	35	f	None	1	Illiterate	1	1400	2	4	Lower
52	4594/09	Patrik	40	m	officer	5	College	5	2250	2	12	LowerMiddle
53	4504/09	Mary	33	f	None	1	Primary	2	800	1	4	Lower
54	4425/09	Vijaya	28	f	None	1	Mid	3	2000	2	6	UpperLower
55	4426/09	Raja	40	m	Labourer	2	Primary	2	500	1	5	UpperLower
56	4507/09	Muralikrishnan	33	m	Labourer	2	Mid	3	1500	2	7	UpperLower
57	4482/09	Abdulrazaq	42	m	Labourer	2	Primary	2	1250	2	6	UpperLower
58	4595/09	Srinivasan	27	m	Labourer	2	Illiterate	1	1000	2	5	UpperLower
59	4715/09	Ashok	32	m	Labourer	2	Illiterate	1	1600	2	5	UpperLower
60	4917/09	Jamuna	29	f	None	1	Mid	3	1300	2	6	UpperLower
61	4430/09	MaNohar	42	m	Labourer	2	High	4	1160	2	8	UpperLower
62	3205/09	Vimala	26	f	None	1	Primary	2	1250	2	5	UpperLower
63	3155/09	Queenmary	40	f	None	1	Primary	2	666	1	4	Lower
64	3283/09	Umamaheswari	29	f	None	1	Primary	2	500	1	4	Lower
65	3682/09	Sumathi	45	f	None	1	Mid	3	333	1	5	UpperLower
66	3254/09	Sankaravel	35	m	pettywork	3	Primary	2	333	1	6	UpperLower
67	3531/09	Muniammal	32	f	Labourer	2	Primary	2	1650	2	6	UpperLower
68	4243/09	Bhavani	24	f	None	1	Mid	3	600	1	5	UpperLower
69	4269/09	Saravanan	20	m	Labourer	2	College	5	5000	4	11	LowerMiddle
70	4330/09	Kuppan	52	m	Labourer	2	Mid	3	500	1	6	UpperLower
71	4480/09	Babu	47	m	Labourer	2	Mid	3	750	1	6	UpperLower
72	4562/09	Arumugathammal	53	f	None	1	Mid	3	1700	2	6	UpperLower
73	4681/09	Jayanthi	35	f	None	1	Mid	3	1000	2	6	UpperLower
74	4815/09	Bhuvaneswari	27	f	Farmer	2	Mid	3	444	1	6	UpperLower
75	4836/09	Indumathi	29	f	None	1	Mid	3	750	1	5	UpperLower

S.No	Biopsy No	Name	Age	Sex	Occupation		Literacy		Percapita		Score	Class
					Occ	score	Literacy	Score	Income	Score		
76	4918/09	Alli	48	f	None	1	Primary	2	500	1	4	Lower
77	4952/09	Sarangapani	40	m	Labourer	2	Primary	2	1250	2	6	UpperLower
78	4481/09	Valarmathi	29	f	Labourer	2	College	5	999	2	9	UpperLower
79	3332/09	Sivakumar	30	m	Labourer	2	College	5	1000	2	9	UpperLower
80	3204/09	Gopal	45	m	Labourer	2	Primary	2	300	1	5	UpperLower
81	3231/09	Prakash	30	m	Labourer	2	Mid	3	700	1	6	UpperLower
82	3308/09	Murugan	23	m	Labourer	2	Mid	3	800	1	6	UpperLower
83	3418/09	Vadivu	40	f	Labourer	2	Primary	2	500	1	5	UpperLower
84	3528/09	Muniammal	60	f	None	1	Illiterate	1	800	1	3	Lower
85	3767/09	Arokiyasamy	41	m	Labourer	2	Mid	3	1000	2	7	UpperLower
86	3858/09	Mohamedmeran	73	m	None	1	Primary	2	660	1	4	Lower
87	4091/09	Saraswathi	40	f	None	1	Illiterate	1	750	1	3	Lower
88	4331/09	Anbunathan	38	m	Labourer	2	Mid	3	2000	2	7	UpperLower
89	4914/09	Elumalai	42	m	Labourer	2	Mid	3	2300	2	7	UpperLower
90	4915/09	Devi	30	f	Labourer	2	Primary	2	800	1	5	UpperLower
91	4505/09	Velayutham	34	m	Labourer	2	Mid	3	3000	3	8	UpperLower
92	4483/09	Kandasamy	40	m	Labourer	2	Mid	3	600	1	6	UpperLower
93	4969/09	Usha	30	f	None	1	Primary	2	1300	2	5	UpperLower
94	3529/09	Muniammal	60	f	None	1	Illiterate	1	570	1	3	Lower
95	3957/09	Kala	34	f	Labourer	2	High	4	1000	2	8	UpperLower
96	4563/09	Sridevi	24	f	None	1	High	4	1500	2	7	UpperLower
97	4561/09	Kavinnilavu	17	f	None	1	College	5	3000	3	9	UpperLower
98	4598/09	Vempuli	39	f	Labourer	2	Mid	3	1000	2	7	UpperLower
99	4919/09	Ganesh	28	m	Labourer	2	Mid	3	1000	2	7	UpperLower
100	4790/09	Ponnammal	49	f	None	1	Mid	3	1250	2	6	UpperLower

S.No	Biopsy No	Smoking		Alcohol		Tobacco		Food	Spices	Fries	Cola	PepM	Garlic	Ginger
		Yes/No	Years	Yes/ No	Years	Yes/No	Years							
1	3016/10	No	NA	No	NA	No	NA	NV	Daily	Daily	No	No	Daily	Daily
2	3018/10	No	NA	No	NA	No	NA	NV	1\7	Daily	1\7	No	Daily	3\7
3	3019/10	No	NA	No	NA	Yes	5	NV	3\7	1\7	Daily	No	No	Daily
4	3020/10	Yes	20	Yes	10	No	NA	NV	1\7	3\7	No	No	3\7	1\7
5	3021/10	No	NA	No	NA	No	NA	NV	No	3\7	No	No	Daily	Daily
6	3024/10	Yes	15	Yes	10	No	NA	NV	Daily	Daily	3\7	No	5\7	5\7
7	3025/10	No	NA	No	NA	No	NA	NV	Daily	Daily	1\7	No	Daily	Daily
8	3026/10	No	NA	No	NA	No	NA	NV	1\7	3\7	No	No	Daily	Daily
9	3028/10	No	NA	No	NA	No	NA	NV	1\7	3\7	No	No	Daily	No
10	3030/10	No	NA	Yes	10	Yes	5	NV	Daily	1\7	No	No	1\7	3\7
11	3032/10	No	NA	No	NA	No	NA	NV	No	1\7	No	No	Daily	Daily
12	5332/09	No	NA	Yes	5	No	NA	NV	1\7	1\7	Daily	No	Daily	Daily
13	3228/09	No	NA	No	NA	Yes	1	NV	3\7	No	1\7	No	Daily	Daily
14	3253/09	No	NA	Yes	10	No	NA	NV	Daily	5\7	No	No	No	No
15	3461/09	No	NA	No	NA	No	NA	NV	1\7	3\7	No	No	Daily	Daily
16	3530/09	Yes	15	Yes	3	No	NA	NV	1\7	5\7	No	No	Daily	Daily
17	3717/09	No	NA	No	NA	Yes	1	NV	1\7	1\7	1\7	No	1\7	1\7
18	3309/09	No	NA	Yes	5	No	NA	NV	1\7	1\7	Daily	No	Daily	Daily
19	3334/09	No	NA	No	NA	No	NA	NV	1\7	1\7	1\7	3\7	Daily	Daily
20	3771/09	No	NA	No	NA	No	NA	NV	3\7	3\7	5\7	No	Daily	No
21	3994/09	No	NA	No	NA	No	NA	NV	Daily	3\7	1\7	1\7	Daily	Daily
22	4092/09	No	NA	No	NA	Yes	1	NV	Daily	Daily	No	No	Daily	Daily
23	4343/09	No	NA	No	NA	No	NA	NV	1\7	1\7	No	No	Daily	Daily
24	4345/09	No	NA	No	NA	No	NA	NV	1\7	3\7	No	No	Daily	Daily
25	4545/09	No	NA	No	NA	No	NA	NV	Daily	Daily	3\7	1\7	Daily	Daily

S.No	Biopsy No	Smoking		Alcohol		Tobacco		Food	Spices	Fries	Cola	PepM	Garlic	Ginger
		Yes/No	Years	Yes/ No	Years	Yes/No	Years							
26	4811/09	No	NA	No	NA	No	NA	NV	1\7	Daily	No	No	Daily	Daily
27	4794/09	No	NA	No	NA	No	NA	NV	No	Daily	No	No	Daily	Daily
28	3284/09	No	NA	No	NA	No	NA	NV	1\7	No	No	No	No	1\7
29	3528/09	No	NA	No	NA	No	NA	NV	Daily	Daily	No	No	Daily	Daily
30	3752/09	No	NA	No	NA	No	NA	NV	1\7	1\7	No	No	Daily	Daily
31	4371/09	No	NA	No	NA	No	NA	NV	Daily	3\7	Daily	No	Daily	Daily
32	4428/09	Yes	10	Yes	10	No	NA	NV	1\7	5\7	No	No	Daily	Daily
33	4642/09	No	NA	No	NA	No	NA	NV	1\7	3\7	No	3\7	Daily	Daily
34	4644/09	No	NA	No	NA	No	NA	NV	1\7	Daily	No	No	Daily	Daily
35	4795/09	No	NA	No	NA	No	NA	NV	Daily	Daily	1\7	No	Daily	Daily
36	3331/09	No	NA	No	NA	No	NA	NV	1\7	3\7	No	No	Daily	No
37	3370/09	No	NA	No	NA	No	NA	NV	1\7	Daily	No	No	Daily	Daily
38	4546/09	No	NA	No	NA	No	NA	NV	1\7	3\7	No	No	5\7	Daily
39	4678/09	No	NA	No	NA	No	NA	veg	5\7	1\7	No	No	No	No
40	4757/09	No	NA	No	NA	Yes	5	NV	1\7	Daily	No	No	Daily	Daily
41	4758/09	No	NA	No	NA	No	NA	NV	1\7	Daily	No	No	Daily	Daily
42	4760/09	No	NA	No	NA	No	NA	NV	1\7	Daily	No	Daily	Daily	Daily
43	5725/09	No	NA	No	NA	No	NA	NV	1\7	1\7	No	No	Daily	Daily
44	3419/09	No	NA	Yes	8	No	NA	NV	1\7	Daily	1\7	No	Daily	1\7
45	5524/09	No	NA	No	NA	No	NA	NV	1\7	Daily	No	No	Daily	Daily
46	4344/09	No	NA	No	NA	No	NA	NV	No	3\7	No	No	Daily	Daily
47	4756/09	Yes	5	Yes	5	Yes	5	NV	1\7	Daily	No	No	Daily	Daily
48	4564/09	No	NA	No	NA	No	NA	NV	5\7	5\7	1\7	3\7	Daily	Daily
49	3460/09	Yes	10	Yes	10	Yes	20	NV	1\7	3\7	No	No	Daily	Daily
50	3931/09	Yes	10	No	NA	No	NA	NV	1\7	5\7	No	No	Daily	Daily

S.No	Biopsy No	Smoking		Alcohol		Tobacco		Food	Spices	Fries	Cola	PepM	Garlic	Ginger
		Yes/No	Years	Yes/ No	Years	Yes/No	Years							
51	4196/09	No	NA	No	NA	Yes	5	NV	1\7	5\7	1\7	No	Daily	Daily
52	4594/09	No	NA	Yes	15	Yes	15	NV	1\7	5\7	No	No	Daily	Daily
53	4504/09	No	NA	No	NA	No	NA	NV	1\7	1\7	No	3\7	Daily	Daily
54	4425/09	No	NA	No	NA	No	NA	NV	Daily	1\7	No	No	5\7	3\7
55	4426/09	No	NA	Yes	20	Yes	20	NV	1\7	Daily	1\7	Daily	Daily	Daily
56	4507/09	No	NA	No	NA	No	NA	NV	1\7	5\7	1\7	Daily	Daily	Daily
57	4482/09	No	NA	No	NA	No	NA	NV	1\7	Daily	No	No	Daily	Daily
58	4595/09	No	NA	No	NA	No	NA	NV	3\7	Daily	3\7	Daily	Daily	Daily
59	4715/09	No	NA	No	NA	No	NA	NV	1\7	Daily	5\7	Daily	Daily	Daily
60	4917/09	No	NA	No	NA	No	NA	NV	1\7	3\7	No	No	Daily	Daily
61	4430/09	Yes	20	No	NA	No	NA	NV	1\7	1\7	No	No	Daily	Daily
62	3205/09	No	NA	No	NA	No	NA	NV	3\7	Daily	No	No	Daily	Daily
63	3155/09	No	NA	No	NA	No	NA	NV	5\7	5\7	No	No	Daily	Daily
64	3283/09	No	NA	No	NA	No	NA	NV	Daily	3\7	3\7	1\7	Daily	1\7
65	3682/09	No	NA	No	NA	No	NA	veg	No	1\7	No	1\7	No	No
66	3254/09	Yes	5	Yes	5	No	NA	NV	Daily	3\7	3\7	No	Daily	Daily
67	3531/09	No	NA	No	NA	No	NA	NV	No	1\7	1\7	No	Daily	1\7
68	4243/09	No	NA	No	NA	No	NA	NV	No	1\7	No	No	Daily	No
69	4269/09	No	NA	No	NA	No	NA	NV	1\7	Daily	3\7	No	Daily	Daily
70	4330/09	No	NA	No	NA	Yes	5	NV	1\7	Daily	1\7	1\7	Daily	1\7
71	4480/09	Yes	10	No	NA	No	NA	NV	1\7	Daily	Daily	No	Daily	Daily
72	4562/09	No	NA	No	NA	No	NA	NV	3\7	1\7	1\7	No	Daily	Daily
73	4681/09	No	NA	No	NA	No	NA	NV	Daily	Daily	No	No	Daily	Daily
74	4815/09	No	NA	No	NA	No	NA	NV	No	No	3\7	No	Daily	No
75	4836/09	No	NA	No	NA	No	NA	NV	No	1\7	No	No	Daily	Daily

S.No	Biopsy No	Smoking		Alcohol		Tobacco		Food	Spices	Fries	Cola	PepM	Garlic	Ginger
		Yes/No	Years	Yes/ No	Years	Yes/No	Years							
76	4918/09	No	NA	No	NA	Yes	5	NV	Daily	Daily	No	No	Daily	Daily
77	4952/09	Yes	10	Yes	10	Yes	12	NV	Daily	Daily	No	No	Daily	Daily
78	4481/09	No	NA	No	NA	No	NA	NV	No	1\7	No	No	Daily	Daily
79	3332/09	No	NA	Yes	5	No	NA	NV	No	1\7	5\7	1\7	Daily	Daily
80	3204/09	Yes	15	No	NA	No	NA	NV	Daily	1\7	3\7	1\7	Daily	Daily
81	3231/09	No	NA	No	NA	No	NA	NV	Daily	3\7	Daily	No	Daily	Daily
82	3308/09	No	NA	Yes	10	No	NA	NV	1\7	1\7	1\7	No	Daily	Daily
83	3418/09	No	NA	No	NA	No	NA	NV	3\7	5\7	No	1\7	Daily	Daily
84	3528/09	No	NA	No	NA	No	NA	NV	1\7	Daily	No	3\7	Daily	Daily
85	3767/09	No	NA	Yes	10	No	NA	NV	1\7	No	No	Daily	Daily	Daily
86	3858/09	No	NA	Yes	5	No	NA	NV	3\7	1\7	No	3\7	Daily	5\7
87	4091/09	No	NA	No	NA	No	NA	NV	1\7	1\7	No	1\7	Daily	Daily
88	4331/09	No	NA	Yes	3	Yes	3	NV	Daily	1\7	Daily	Daily	Daily	Daily
89	4914/09	Yes	10	Yes	10	No	NA	NV	Daily	Daily	No	No	Daily	Daily
90	4915/09	No	NA	No	NA	No	NA	NV	3\7	1\7	1\7	No	5\7	Daily
91	4505/09	Yes	10	No	NA	No	NA	NV	1\7	1\7	No	1\7	3\7	Daily
92	4483/09	Yes	20	Yes	20	No	NA	NV	1\7	1\7	3\7	No	Daily	Daily
93	4969/09	No	NA	No	NA	Yes	7	NV	1\7	1\7	No	No	Daily	Daily
94	3529/09	No	NA	No	NA	No	NA	veg	5\7	1\7	No	No	No	No
95	3957/09	No	NA	No	NA	Yes	1	NV	1\7	1\7	1\7	No	1\7	1\7
96	4563/09	No	NA	No	NA	No	NA	NV	Daily	Daily	No	No	Daily	Daily
97	4561/09	No	NA	No	NA	No	NA	NV	No	3\7	No	No	Daily	Daily
98	4598/09	No	NA	Yes	10	Yes	20	NV	Daily	1\7	No	No	1\7	1\7
99	4919/09	Yes	5	Yes	5	Yes	5	NV	1\7	Daily	No	No	Daily	Daily
100	4790/09	No	NA	No	NA	Yes	10	NV	1\7	Daily	No	No	Daily	Daily

S.No	Biopsy No	Sydney scoring of gastritis - H & E						Geimsa		IHC	
		Activity	Chronic inflamation	Intestinal metaplasia	Atrophy	H.pylori		Pos/ Neg	Grade	Pos/ Neg	Grade
						Pos/ Neg	Grade				
1	3016/10	0	1	0	0	Neg	0	Pos	1	Pos	1
2	3018/10	0	1	0	0	Neg	0	Neg	0	Neg	0
3	3019/10	1	1	0	0	Neg	0	Neg	0	Pos	1
4	3020/10	0	1	0	0	Neg	0	Pos	1	Pos	1
5	3021/10	0	2	0	0	Neg	0	Pos	2	Pos	2
6	3024/10	1	1	0	0	Pos	1	Pos	1	Pos	1
7	3025/10	1	1	0	0	Neg	0	Neg	0	Pos	1
8	3026/10	1	1	0	0	Pos	1	Pos	1	Pos	1
9	3028/10	0	1	0	0	Neg	0	Pos	1	Pos	1
10	3030/10	0	1	0	0	Neg	0	Pos	1	Pos	1
11	3032/10	0	2	0	0	Pos	2	Pos	2	Pos	2
12	5332/09	1	2	0	0	Pos	2	Pos	2	Pos	2
13	3228/09	0	2	0	0	Pos	2	Pos	2	Pos	2
14	3253/09	0	1	0	0	Neg	0	Neg	0	Neg	0
15	3461/09	1	2	0	1	Neg	0	Neg	0	Neg	0
16	3530/09	0	2	0	0	Neg	0	Neg	0	Neg	0
17	3717/09	0	1	0	0	Neg	0	Neg	0	Neg	0
18	3309/09	0	1	0	0	Neg	0	Neg	0	Neg	0
19	3334/09	0	1	0	0	Pos	1	Pos	1	Pos	1
20	3771/09	0	2	0	1	Pos	3	Pos	3	Pos	3
21	3994/09	1	2	0	1	Neg	0	Neg	0	Neg	0
22	4092/09	1	2	0	1	Pos	2	Pos	2	Pos	2
23	4343/09	1	2	0	1	Pos	3	Pos	3	Pos	3
24	4345/09	2	2	0	1	Neg	0	Neg	0	Neg	0
25	4545/09	1	2	0	1	Pos	3	Pos	3	Pos	3

S.No	Biopsy No	Sydney scoring of gastritis - H & E						Geimsa		IHC	
		Activity	Chronic inflamation	Intestinal metaplasia	Atrophy	H.pylori		Pos/ Neg	Grade	Pos/ Neg	Grade
						Pos/ Neg	Grade				
26	4811/09	1	2	0	1	pos	1	Pos	1	Pos	1
27	4794/09	1	2	0	1	Pos	1	Pos	1	Pos	1
28	3284/09	0	1	0	0	Pos	1	Pos	1	Pos	1
29	3528/09	1	2	0	1	Neg	0	Neg	0	Neg	0
30	3752/09	0	2	0	1	Neg	0	Pos	1	Pos	1
31	4371/09	1	3	0	1	Pos	3	Pos	3	Pos	3
32	4428/09	1	1	0	0	Neg	0	Neg	0	Neg	0
33	4642/09	0	2	0	0	Pos	1	Pos	1	Pos	1
34	4644/09	1	2	0	1	Pos	3	Pos	3	Pos	3
35	4795/09	0	1	0	0	Neg	0	Pos	1	Pos	1
36	3331/09	0	1	1	1	Neg	0	Neg	0	Neg	0
37	3370/09	0	1	0	0	Neg	0	Neg	0	Neg	0
38	4546/09	0	1	0	0	Pos	1	Pos	1	Pos	1
39	4678/09	0	1	0	0	Neg	0	Neg	0	Neg	0
40	4757/09	1	2	0	1	Pos	2	Pos	2	Pos	2
41	4758/09	0	2	0	1	Neg	0	Pos	1	Pos	1
42	4760/09	1	2	0	0	Pos	2	Pos	2	Pos	2
43	5725/09	1	2	0	0	Neg	0	Neg	0	Neg	0
44	3419/09	1	2	0	0	Pos	2	Pos	2	Pos	2
45	5524/09	1	1	0	0	Pos	1	Pos	1	Pos	1
46	4344/09	1	2	0	0	Pos	2	Pos	2	Pos	2
47	4756/09	0	1	0	0	Neg	0	Neg	0	Neg	0
48	4564/09	1	1	2	1	Pos	1	Pos	1	Pos	1
49	3460/09	0	1	0	0	Neg	0	Neg	0	Neg	0
50	3931/09	1	2	0	1	Pos	1	Pos	1	Pos	1

S.No	Biopsy No	Sydney scoring of gastritis - H & E						Geimsa		IHC	
		Activity	Chronic inflamation	Intestinal metaplasia	Atrophy	H.pylori		Pos/ Neg	Grade	Pos/ Neg	Grade
						Pos/ Neg	Grade				
51	4196/09	1	1	0	0	Neg	0	ND	0	ND	0
52	4594/09	1	2	0	0	Neg	0	ND	0	ND	0
53	4504/09	1	1	0	0	Neg	0	ND	0	ND	0
54	4425/09	1	2	0	0	Neg	0	ND	0	ND	0
55	4426/09	1	1	0	0	Neg	0	ND	0	ND	0
56	4507/09	1	2	0	0	Neg	0	ND	0	ND	0
57	4482/09	2	2	0	1	Pos	1	ND	0	ND	0
58	4595/09	2	1	0	0	Pos	2	ND	0	ND	0
59	4715/09	1	1	0	0	Neg	0	ND	0	ND	0
60	4917/09	1	1	0	0	Neg	0	ND	0	ND	0
61	4430/09	1	1	0	0	Neg	0	ND	0	ND	0
62	3205/09	0	1	0	1	Pos	1	ND	0	ND	0
63	3155/09	1	2	0	0	Neg	0	ND	0	ND	0
64	3283/09	1	1	0	0	Neg	0	ND	0	ND	0
65	3682/09	1	2	0	0	Pos	1	ND	0	ND	0
66	3254/09	1	1	0	0	Neg	0	ND	0	ND	0
67	3531/09	1	1	0	1	Neg	0	ND	0	ND	0
68	4243/09	1	1	0	0	Neg	0	ND	0	ND	0
69	4269/09	1	1	0	0	Neg	0	ND	0	ND	0
70	4330/09	1	1	0	0	Neg	0	ND	0	ND	0
71	4480/09	2	2	0	2	Neg	0	ND	0	ND	0
72	4562/09	1	3	0	0	Neg	0	ND	0	ND	0
73	4681/09	1	2	0	0	Neg	0	ND	0	ND	0
74	4815/09	2	3	0	1	Neg	0	ND	0	ND	0
75	4836/09	1	2	0	0	Neg	0	ND	0	ND	0

S.No	Biopsy No	Sydney scoring of gastritis - H & E						Geimsa		IHC	
		Activity	Chronic inflamation	Intestinal metaplasia	Atrophy	H.pylori		Pos/ Neg	Grade	Pos/ Neg	Grade
						Pos/ Neg	Grade				
76	4918/09	1	1	0	0	Neg	0	ND	0	ND	0
77	4952/09	1	2	0	0	Neg	0	ND	0	ND	0
78	4481/09	1	2	0	0	Neg	0	ND	0	ND	0
79	3332/09	1	2	0	0	Neg	0	ND	0	ND	0
80	3204/09	1	2	0	0	Neg	0	ND	0	ND	0
81	3231/09	0	2	0	0	Neg	0	ND	0	ND	0
82	3308/09	1	1	0	0	Neg	0	ND	0	ND	0
83	3418/09	1	3	0	1	Neg	0	ND	0	ND	0
84	3528/09	1	2	0	2	Neg	0	ND	0	ND	0
85	3767/09	1	2	0	0	Pos	2	ND	0	ND	0
86	3858/09	1	2	0	0	Neg	0	ND	0	ND	0
87	4091/09	1	1	0	0	Neg	0	ND	0	ND	0
88	4331/09	1	1	0	0	Neg	0	ND	0	ND	0
89	4914/09	1	1	0	0	Pos	2	ND	0	ND	0
90	4915/09	1	1	0	0	Pos	1	ND	0	ND	0
91	4505/09	1	1	0	0	Pos	2	ND	0	ND	0
92	4483/09	1	2	0	0	Pos	1	ND	0	ND	0
93	4969/09	2	2	0	1	Pos	1	ND	0	ND	0
94	3529/09	1	2	0	1	Neg	0	ND	0	ND	0
95	3957/09	1	1	0	0	Neg	0	ND	0	ND	0
96	4563/09	2	2	0	0	Neg	0	ND	0	ND	0
97	4561/09	1	3	0	0	Neg	0	ND	0	ND	0
98	4598/09	1	2	0	0	Neg	0	ND	0	ND	0
99	4919/09	1	1	0	0	Neg	0	ND	0	ND	0
100	4790/09	2	1	0	0	Pos	1	ND	0	ND	0

Neg - Negative
Pos - Positive
ND - Not Done
NA - Not Applicable
NV - Non Vegetarian
Veg -Vegetarian
PepM - Pepper Mint

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INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the work	:	Role of Immunohistochemistry versus hematoxylin & eosin and special stain in Helicobacter pylori detection and analysis of risk factors associated with gastritis – A study of 100 cases.
Principal Investigator	:	Dr. M.Priyadharshini
Designation	:	PG in MD (Path)
Department	:	Pathology

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 28.06.2010 at the Modernised Seminar Hall, Stanley Medical College, Chennai-1 at 2 PM

The members of the committee, the secretary and the chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work
6. You should submit the summary of the work to the ethical committee on completion of the work.

07/12/10

MEMBER SECRETARY
IEC, SMC, CHENNAI

Role of Immunohistochemistry versus Hematoxylin & Eosin and special stains in Helicobacter pylori detection and analysis of risk factors associated with gastritis – A Study of 100 cases

Abstract:

The recent implication of Helicobacter pylori in pathogenesis of gastritis – peptic ulcer syndrome and development of upper gastrointestinal malignancy warrant efficient method for the detection and demonstration of the organism in biopsy specimen. Three staining methods, Hematoxylin & Eosin, Giemsa and Immunohistochemistry were compared for the detection of Helicobacter pylori. The risk factors associated with gastritis were also analysed.

A total of 100 cases with gastritis were taken, out of this 50 cases were selected randomly and all the three stains were applied. When compared with Immunohistochemistry, sensitivity and specificity of Hematoxylin & Eosin was of 70.59% and 100% respectively and Giemsa was 94.1% and 100% respectively. Risk factors associated with gastritis includes intake of spicy foods and alcohol intake.

It was concluded that Immunohistochemistry is recommended when the density of organism is low. In terms of cost, applicability and reliability, Giemsa is considered as an ideal technique for detection of Helicobacter pylori.

KEY WORDS-

Helicobacter pylori, Hematoxylin & Eosin, Giemsa, Immunohistochemistry, risk factors of gastritis.