

**A HISTOCHEMICAL & IMMUNOHISTOCHEMICAL STUDY
ON HELICOBACTER PYLORI ASSOCIATED CHRONIC
GASTRITIS**

DISSERTATION

SUBMITTED FOR

M.D. IN PATHOLOGY

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY



DEPARTMENT OF PATHOLOGY

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CERTIFICATE

This is to certify that the dissertation work entitled “**A HISTOCHEMICAL & IMMUNOHISTOCHEMICAL STUDY ON HELICOBACTER PYLORI ASSOCIATED CHRONIC GASTRITIS**” submitted by Dr.Karthick Prabhu R, is work done by him during the period of study in this department from June 2010 to April 2013. This work was done under the guidance of Dr. Alamelu Jayaraman, Professor and Head, Department of Pathology, PSG IMS & R.

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LIST OF ABBREVIATIONS

- HPE – HISTOPATHOLOGY
- H.PYLORI – HELICOBACTER PYLORI
- IHC – IMMUNO HISTOCHEMISTRY
- H&E – HAEMATOXYLIN AND EOSIN
- MOD.GIEMSA – MODIFIED GIEMSA
- IL – INTERLEUKINS
- COX – CYCLO OXYGENASE
- MMP – MATRIX METALLOPROTEINASE
- IM – INTESTINAL METAPLASIA
- AB – ALCIAN BLUE
- PAS – PERIODIC ACID SCHIFF

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Introduction

H.pylori infection is chronic and common throughout the world with higher prevalence in developing than in developed countries. H.pylori infection causes gastritis and is the most prime risk factor for peptic ulcer disease (gastric & duodenal). It also contributes to the onset of gastric cancer and primary B-cell lymphomas.

H.pylori is seen in significant number of dyspeptic patients with endoscopically normal stomach. A strong relationship is also documented between H.pylori and peptic ulcer disease. It has been classified recently by WHO as a CLASS I carcinogen because it is involved in the development of Atrophic gastritis with Intestinal metaplasia (pre-neoplastic gastric lesion). This bacterium induces a characteristic inflammatory response with an initial development of acute gastritis, which when prolonged leads to chronic inflammation.

Inflammation with this micro aerobic, gram negative, spiral, motile bacterium has become major prime cause for chronic active gastritis. It resides in the gastric pits and the overlying mucus. H.pylori colonization of stomach is associated with a spectrum of gastro duodenal disease. H.pylori can receive signals from gastric epithelium, allowing the host and bacteria to

Introduction

participate in a dynamic equilibrium. However, there are biological effects to this long term relationship.

Chronic inflammation induced by bacteria causes the loss of normal architecture of gastric mucosa with destruction of glandular gastric cells and their replacement by the intestinal type epithelium , pyloric type glands , & fibrous tissue (Atrophic gastritis). There is an increased risk of developing gastric cancer with ATROPHY – METAPLASIA – DYSPLASIA sequence. H.pylori is localised in the apical portion of the gastric foveolar glands and does not penetrate the gland cytoplasm. H.pylori colonisation usually triggers an inflammatory reaction in the *lamina propria* .Mucosal neutrophils are a distinctive histological feature of H.pylori infection. The grading of activity in gastritis is done according to *MODIFIED SYDNEY SYSTEM*.

There are a number of methods for detection of H.pylori, including the Breath test, Urease test & Culture. But the histological detection in gastric biopsy is the commonest and more sensitive of all methods. This spiral shaped organism can be seen in routine H&E stains, but are more easily detected with special stains such as *Giemsa &, Warthin – Starry silver stain* .None of these are more specific for the organism and the *cocoid forms and indolent forms* are missed out easily in routine stains. More recently, *IMMUNO HISTOCHEMISTRY, IN SITU HYBRIDISATION & PCR has*

Introduction

been proposed as alternative & more specific modalities in detection.

Few studies have been done towards this, and found out that *I H C* against H.pylori is more specific (66% positivity) followed by *Warthin Starry silver* (61% positivity). Hence, *I H C* is more reliable and accurate in the cases of *cocoid* forms and those negative in Genta stains. To extend further , this study is focussed , to *compare* the efficacy of basic staining techniques , *I H C* & the histological tissue changes in H.pylori associated Chronic gastritis.

AIMS & OBJECTIVES

Aims & Objectives

1. To study the *histopathological changes* in the gastric mucosa in patients with H.pylori associated chronic gastritis.
2. To identify the H.pylori organisms in biopsies on routine *H & E* and *special stains*.
3. Use of anti H.pylori *antibody* to identify the H.pylori in gastric mucosa.
4. To *compare* the efficacy of I H C & Special stains

MATERIALS & METHODS

Materials & Methods

In this study, we included the gastric biopsies taken from the patients presenting with complaints of dyspepsia, or clinically suspected *Helicobacter pylori* infection. These biopsies once received in our laboratory were given a unique reference number to identify them later from the archives. We excluded the biopsy samples taken for conditions other than simple peptic ulcer disease such as suspected malignancy, GERD, etc.

Sample size is 55, for a period of 1 year duration (2010-2011). Overall there were around 700 endoscopy guided biopsy samples received, of which about 250 were from the *stomach*, followed by small intestine and oesophagus.

It is a retrospective study, where we included the cases that were clinically and histopathologically diagnosed positive for *H.pylori* infection. We maintained a routine protocol and proper procedures to access the ***ARCHIVES OF DEPARTMENT OF PATHOLOGY, PSG IMS&R***. The cases complying with our inclusion criteria were sorted out and the data were collected regarding initial report. In most cases it was reported as “*H.pylori* like organisms “on H&E stain confirmed by Modified Giemsa which we use in our routine practice. The tissue blocks were taken out accordingly.

Materials & Methods

The samples were taken out randomly and numbered in an order so as to maintain the data for this study. The H&E and Modified Giemsa slides were reviewed again .As this study includes the use of *Antibody to Detect the H.pylori*, the tissues were also processed separately over Poly – L – Lysine coated slides.

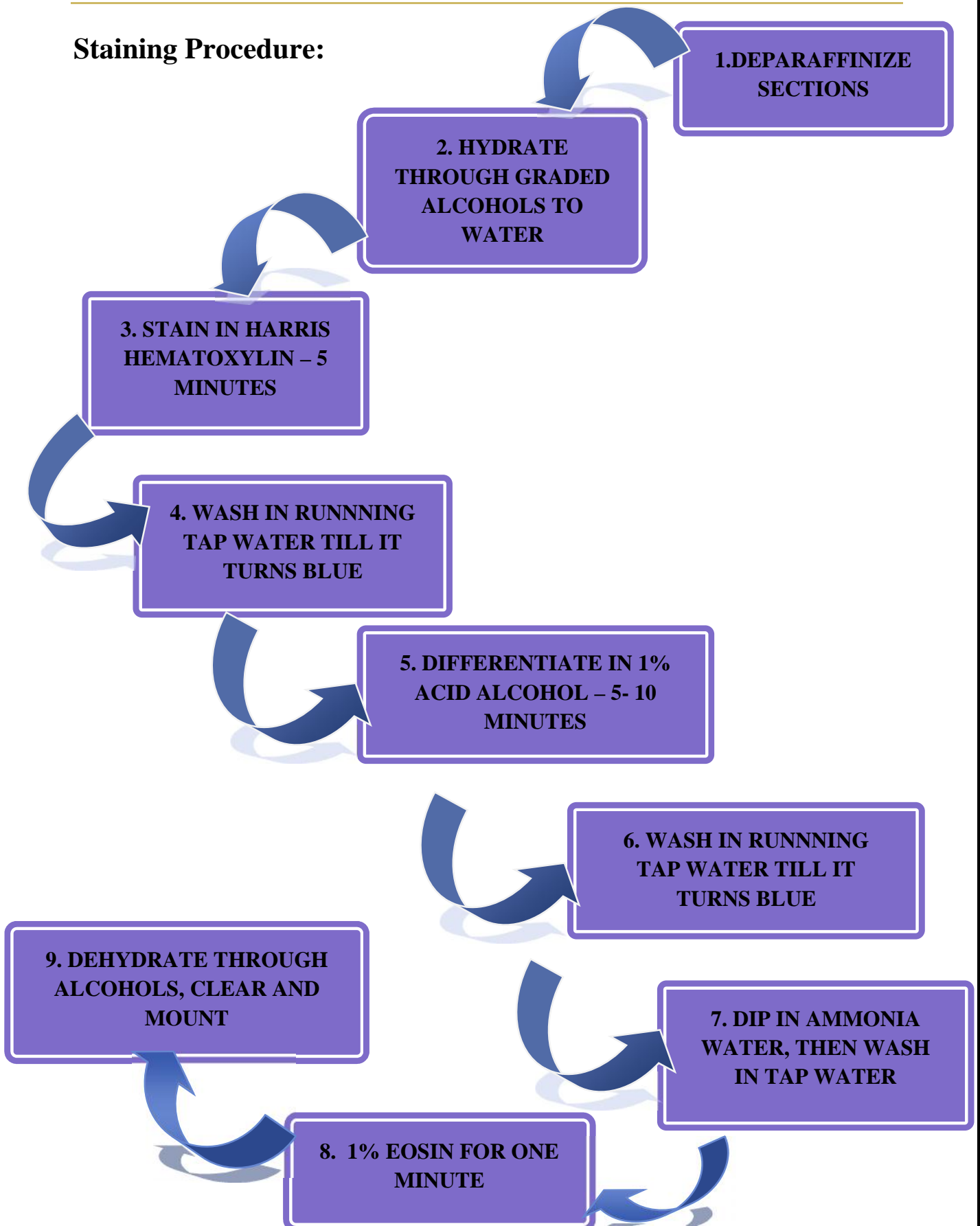
I. Haematoxylin and Eosin Staining:

Materials required:

- Harris Haematoxylin
- Eosin
- Xylene
- 1% Acetic acid
- Ammonia water
- 1% Eosin
- Graded Alcohols

Materials & Methods

Staining Procedure:



Materials & Methods

Used as a staining procedure in routine paraffin sections.

RESULTS:

Nuclei – Blue

Cytoplasm – Pink

Red Cells – Orange / Red

Fibrin – Deep pink

H.pylori – pink rods

Materials & Methods

II. MODIFIED GIEMSA:

Purpose: To demonstrate the H.pylori organisms

PREPARATION OF SOLUTION:

Giemsa stock solution

Giemsa stain powder – 4gm

Glycerol – 250 ml

Pure methanol – 250 ml

The powder is dissolved in the glycerol at 60° c with regular shaking. Add methanol .Mix well. Allow to stand for 7 days. Filter before use.

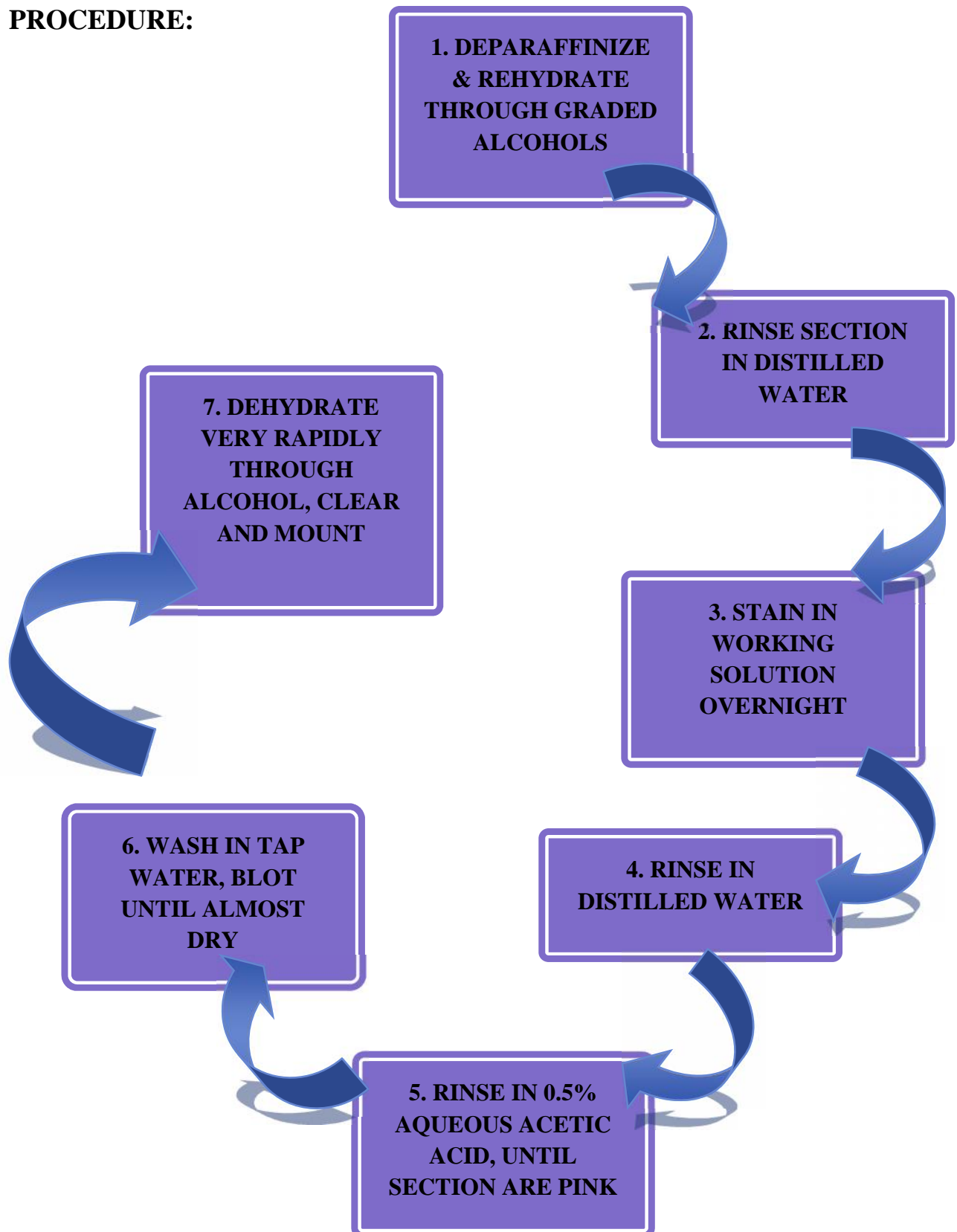
Working solution

Giemsa stock – 4 ml

Acetate buffered distilled water, pH 6.8 – 96 ml

Materials & Methods

PROCEDURE:



Materials & Methods

RESULTS:

Organisms – bluish

Background – pink to blue

Nuclei – Blue




Materials & Methods

III. STAINING USING ANTIBODY:

i). Sections are cut at approximately 4 μ m , floated on to Poly – L – Lysine coated slides and incubated at 37 $^{\circ}$ c for one day and further incubated at 58 $^{\circ}$ c for overnight.

Note:




- Do not allow sections to dry at any stage of the procedure
- Carry out the steps of incubation with antibody at 37 $^{\circ}$ c
- Use appropriate controls for each antibody tested

ii). Xylene – I	- 15 min		Deparaffinization
Xylene – II	- 15 min		
iii). Absolute Alcohol – I	- 1 min		Dexylenization
Absolute Alcohol – II	- 1 min		
iv). 90% Alcohol	- 1 min		Dealcoholisation
70% Alcohol	- 1 min		

Materials & Methods

- v). Tap water - 10 min Rehydration
- vi). Distilled water - 5 min Rinsing
- vii). Pressure cooked in Citrate buffer (pH- 6) } 10 min – Heat induced Antigen retrieval
- viii). Leave the pressure cooker in a sink with water } 20 min – Cooled at room temperature
- ix). Distilled water - 5 min Rinsing
- x). Transfer to Trisodium phosphate buffer (pH 7.6) – 5 min × 2 times (wash)
- xi). Peroxidase block – 10 to 15 min } to block endogenous Peroxidase Enzyme
- xii). TBS buffer - 5 min × 3 times – washing
- xiii). Power Block – 15 min } to block non specific reaction with Other tissue antigen
- xiv). Drain & cover sections with markers } 1 hour } To identify Tumour concerned **Primary Antibody** Ag – Ab reaction

Materials & Methods

- xv). TBS buffer - 5 min × 3 times – to wash unbound Ab's
- xvi). Super enhancer - 30 min  to enhance the reaction
between 1° & 2° antibody
- xvii). TBS buffer - 5 min × 3 times – to wash unbound Ab
- xviii). Super sensitive poly – HRP – 30 min  to elongate chain and also
to label the enzyme
- xix). TBS buffer - 5 min × 3 times – to wash unbound Ab
- xx). Colour development with working  5-8 min – to give colour to the
colour development solution Antigen
- xxi). TBS buffer - 5 min × 3 times – to wash
- xxii). Tap water - 5 min – to wash
- xxiii). Haematoxylin - 1 min – Counterstain
- xxiv). Tap water - 5 min – to wash excess stain
- xxv). Air Dry, clear in Xylene, Mount with DPX
- xxvi). **RESULTS:** Organism /Tumour markers → Brown
Nucleus → Blue

Materials & Methods

Once the slides were prepared, we placed them separately segregating them into three stains for each case. They were accompanied by positive controls for the special stain (Modified Giemsa) and for H.pylori Ab. The controls were used to compare the morphology of bacterium to term them as “positive “. The slides were reviewed in the following order: *H&E, Modified Giemsa and H.pylori Antibody.*

In H&E we looked for the features suggesting the H.pylori infection, inflammation grade (Modified Sydney System) and other reactive changes of the gastric mucosa. Whereas Modified Giemsa was used to correlate the diagnosis along with the supplementation by the use of immunohistochemistry...

The slides were observed using the LABOMED VISION 2000 microscope.

The representative photomicrographs were taken using the microscope (LEICA) and Leica Application Suite.

REVIEW OF LITERATURE

Review of literature

EMBRYOLOGY OF STOMACH:

The stomach in its embryonal stage is seen distal to the oesophagus. Present as a fusiform dilatation of the *foregut*¹. Have two borders dorsal and ventral. Dorsal being attached to the posterior abdominal wall by a fold of peritoneum called the *dorsal* mesogastrium. Ventral border being attached to *the septum transversum* by another fold of peritoneum called the *ventral Mesogastrium*. The part of the ventral mesogastrium between the liver and stomach becomes *lesser omentum*. The stomach undergoes differential growth resulting in alteration in shape & orientation. The ventral border faces upwards and then left to form *lesser curvature*. The dorsal border points downwards and then left, to form *greater curvature*. Enzyme and acid production first occur at the *fourth month* of foetal life & are well established by the time of birth¹. The new born stomach is fully developed and is similar to that of the adults. Stomach develops from the *endoderm*, and the early glandular differentiation *of* the mucosal lining occurs first at the *80 mm stage* of foetal development.

Review of literature

ANATOMY OF STOMACH:

The stomach is a sac like structure², reservoir for swallowed food. With a capacity of one litre, it is the dilated part of the alimentary canal. It is capable of varying its shape and size depending on the amount of food content. Anatomically described to have two ends, the *cranial* & *caudal*². The former being continuous with the oesophagus and located towards the heart apex, it is also termed as “cardiac *end* “. The caudal end continues downwards distally into the duodenum, also termed as “ *pyloric end* / *pylorus* “. The anterior and posterior surfaces of the stomach meet at a concave upper border & a convex lower border. These are termed as lesser *curvature* & *greater curvature* respectively.²

The stomach is lined by peritoneum, covering both the surfaces and coalesces at the lesser curvature to continue with lesser omentum. At the greater curvature, the anterior and posterior layers of peritoneum become continuous with gastro-splenic ligament and with greater omentum.

Review of literature

Anatomically, stomach is divided into many parts as follows:

A. The left margin of oesophagus and greater curvature junction → *Cardiac notch*

B. *Fundus*

C. *Body*

D. *Pyloric antrum*

Review of literature

HISTOLOGY OF STOMACH:

Microscopically, the mucosa has a similar pattern throughout the stomach ³. It is composed of superficial layer containing *foveolae (pits)*. The deep layer comprises of *coiled glands* that empty into the base of the foveolae. Adjacent to the gastro -oesophageal junction of the cardiac mucosa the glands are mucus *secreting*.³

Surface epithelium consists of tall, columnar, mucus secreting cells with intervening foveolar epithelium. The cells are tall, columnar with basal nuclei and superficial cytoplasm filled with mucus. Histochemically, the foveolar mucus is all *Neutral*, Periodic Acid Schiff (*P A S*) positive ⁹, but Alcian Blue negative at pH 2.5 and lower.

In the *Cardiac & Pyloric zone*, the foveolae occupy approximately one half of mucosal thickness. The glands secrete mucus , loosely packed within the lamina propria ³ .The cells of the mucus glands have ill defined cell borders , bubbly cytoplasm that is different from foveolar and surface epithelium . The pyloric glands secrete

Review of literature

neutral mucin only. The cardiac glands secrete predominantly neutral mucin with small amount of *sialomucin*⁹.

The fundic gland or oxyntic gland mucosa has foveolae that occupy less than a quarter of the mucosal thickness³. In contrast to pyloric and cardiac mucosa, the glands are tightly packed and are straight, rather than being coiled. These are divided into 3 portions. The *basal* portion consists mainly of zymogenic cells. These are cuboidal & have basal nucleus and moderate amount of cytoplasm. The *isthmic* portion of glands contains predominantly parietal cells (acid and intrinsic factor secreting). These cells are roughly triangular⁴, with their base along the basement membrane. The nucleus is centrally placed with abundant eosinophilic cytoplasm. The *Neck* portion of fundic gland contains mixture of zymogen and parietal cells along with mucus neck cells. These cells produce neutral and acidic mucin, especially sialomucin which stains Alcian Blue, positivity at pH 2.5⁹.

ENDOCRINE CELLS:

In antrum, 50 % of the population are G cells (*gastrin producing*), 30 % are enterochromaffin cells (*serotonin producing*) & 15 % are D cells (*somatostatin producing*). In the fundic mucosa, major portion of

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endocrine cells are enterochromaffin – like cells (*ECL*) and secrete *histamine*.

Small numbers of X cells and EC cells are also present ³.

LAMINA PROPRIA:

The epithelial cells of the surface, foveolae and glands are supported by a fine meshwork of reticulin, collagen and elastic fibres suspended beneath the basement membrane. These give structural support to the overlying epithelium. Lamina propria contains fibroblasts, histiocytes, plasma cells, lymphocytes. There are capillaries, arterioles & non myelinated nerve fibres. Intraepithelial lymphocytes are also seen and are of T cell origin. Normally there may be *lymphoid aggregates* ^{5, 32}. However, secondary lymphoid follicles with germinal centre are seen in gastritis, usually secondary to H.pylori infection ⁷.

SUB MUCOSA:

It is located between the muscularis mucosa and lamina propria; it consists of loose connective tissue, autonomic nerve plexus, veins arteries & lymphatics ⁷.

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MUSCULAR LAYER:

The *muscularis mucosa* consists of inner circular and outer longitudinal layers. The muscularis propria has three layers outer longitudinal, inner circular & innermost oblique.

HELICOBACTER PYLORI

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HISTORICAL ASPECTS:

It was described in 1983; the spiral shaped organisms resembling *Campylobacteria* were isolated from the stomach and were named as, *C.pylori*⁸. In 1989 it was renamed as *Helicobacter pylori*. In 1947, in the outset of gastroscopy, *Rudolf Schindler* termed the gastritis as “one of the most debated diseases of the human body”. The discussion on terming gastritis was started since 1800’s and in early 20th century after mixed views and discussions, the concept of gastritis as a disease entity was detained. Later after many decades, the term gastritis was finally recognized, and the search for cause, classification and complications began to bloom.

Since 1870, the presence of tiny curved bacterium within gastric mucosa had been described in both humans and in animals. But they were thought to be irrelevant contaminants and were ruled out⁶. Apart from the bacterium, various etiologic factors were described, such as nicotine, improper mastication, spices, drugs, etc. Subsequently several morphological data were obtained by pathologists on autopsy specimens. As a result, distinct types and patterns of gastritis were recognised and many classification systems were outlined¹¹.

Review of literature

Later in 1984, *Warren & Marshall* proposed that chronic idiopathic gastritis had a bacterial cause (i.e. *H.pylori*)³⁷. This hypothesis led to further research, analysis and proposals and later in few years, the association between *H.pylori* gastritis, peptic ulcer and gastric cancer became recognised and ultimately accepted. In 1990, guidelines for gastritis were described by a group of investigators in Sydney, Australia⁵. This system for grading and classification of gastritis is accepted universally as “***SYDNEY SYSTEM*** “. Subsequently, this system is updated periodically and modifications were added in an idea to improve the criteria for evaluation of atrophy. Sydney system combines topographic, morphologic and etiological factors for relevant possible diagnoses.

EPIDEMIOLOGY:

The prevalence of *H.pylori* infection in adults reaches 90% in many developing countries²⁶. These bacteria are regarded as the main etiological factor for the asymptomatic non specific gastritis. In developing countries, due to other co- morbid conditions, dietary variations, etc. The *H.pylori* infection is on rise worldwide⁵. Cross sectional studies have revealed a higher prevalence rate of infection in *children*. In industrialized parts of the world (Western Europe, Canada, US, Australia), the exposure tends to occur in adulthood¹¹.

Review of literature

An average of 20% to 30% adults is infected by the age of 50. In Eastern Asia due to proper implementation of sanitation methods, there is a decline in the incidence of H.pylori infection.

The prevalence of H.pylori infection has been steadily declining in industrialized and emerging countries. This is mainly due to proper sanitation and wide use of prophylactic antibiotics ⁸.

Despite the declining rates of H.pylori infection in general, the prevalence rate of H.pylori in patients undergoing endoscopy remains significant. Therefore, H.pylori should be considered in all gastric biopsy specimens examined, regardless of patient's age.

MORPHOLOGIC CHARACTERISTICS:

Helix shaped, Curved, micro aerophilic, flagellated, gram negative rods measuring 3micrometres long & 0.5 micrometres diameter ¹⁸.

These organisms produce oxidase; catalase & urease¹⁶ .These organisms show urease positivity and are found in the gastric biopsy specimens, most common in antrum¹⁹. It is eminent in forming bio film & can convert itself from spiral into a possibly viable but non-culturable coccoid forms ²¹.

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H. pylori possesses 5 major outer membrane protein (OMP) families. Largest family include putative adhesins. The other four include porins, iron transferrin, flagellum associated protein. Outer membrane consists of phospholipids & lipopolysaccharides (LPS). The O Ag of Lipopolysaccharides may be fucosylated and mimic Lewis blood group Ag found in gastric epithelium²². These organisms are highly motile, fastidious due to flagella which are 4 to 6. The flagellar filaments contain flagellins. (FlaA & FlaB)²⁵

GENETICS:

H. pylori, possess large variations of strains. The genomes of three have been completely sequenced. Genome of strain 26695 consists of about 1.7 million base pairs with around 1550 genes. The 2 sequenced genes show large genetic differences with up to 6 % of nucleotides differing in them.

The studies on *H. pylori* genes approached on an aim to describe the pathogenesis of *H. pylori* associated diseases, common being gastric ulcer. Two of the sequenced strains have 40kb long *Cag* pathogenicity island with over 40 genes. This island is absent in chronic carriers of *H. pylori* infection and remain asymptomatic. The *Cag A* codes for one major virulence protein³⁰. Bacterial strains that have *Cag A* gene are associated with an ability to cause *Ulcer*³⁰.

Review of literature

Thus by the studies performed we find that virulent cytotoxin Vac A and Cag A producing strains are more common among patients with peptic ulcer and gastric cancer. These toxins act as the virulence factors in enhancing the disease progression³⁰.

PATHOPHYSIOLOGY:

When the pathogen is ingested, the spiral organism enters the gastro – intestinal tract by counteracting the host defence mechanism by the motility inducing flagella³⁰. The host defence mechanism prevents the bacterium from infection. The defence mechanisms include the acidic pH, bactericidal agents and the columnar cells secreting the sticky mucus which blocks the motile bacterium. After entering the gastric lumen, the bacterium evades through the host defence by its “cork *screw like* bacterial movement” and *enzyme* production (urease, lipase, protease)³². It progresses down to the epithelial surface from mucus and attach to the apical membranes of foveolar cells, with the help of bacterial adhesin such as *BabA*. H.pylori in addition binds to *MUC 5A*¹⁸. Then it secretes the vacuolating cytotoxin VacA. By inducing the vacuolating action, it possess the *Cag* pathogenicity island facilitating transfer of *CagA* protein into foveolar epithelium causing cell response, cytokine production & cell proliferation¹⁵.

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BabA adhesion causes binding to the fucosylated Lewis B blood group antigen

Vac A forms channels which deliver nutrition to H.pylori

Thus by initiating the action of cytokines, the inflammatory response occurs. To surpass the effect of inflammatory mediators secreted by the host, the H.pylori releases IL-10 which exhibits the anti inflammatory effect, preventing the cytokine to secrete the mediators of inflammation²⁴. Thus it survives the target action exerted by the body. It stays indolent for long, causing chronic effects on the gastric mucosa and associated diseases. These post effects are much deleterious than the acute inflammatory reaction. They vary from simple chronic gastritis to a malignant lymphoma or carcinoma. Early detection of these organisms could pave way for the treatment strategy and eradication of disease in endemic areas³¹.

H.PYLORI – RELATED Changes in Gastric mucosa

1. ACUTE GASTRITIS
2. **CHRONIC GASTRITIS**
3. CHRONIC **ACTIVE** GASTRITIS
4. FOLLICULAR GASTRITIS
5. **ATROPHIC GASTRITIS**
6. LYMHOCYTIC GASTRITIS
7. GRANULOMATOUS GASTRITIS
8. **GASTRIC & DUODENAL ULCERS**
9. SOME FORMS OF AUTOIMMUNE GASTRITIS
10. HYPERPLASTIC POLYPS
11. **INTESTINAL METAPLASIA**
12. G- CELL HYPERPLASIA
13. **GASTRIC ADENO CARCINOMA**
14. **MALT oma**
15. MENETRIER DISEASE

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CLINICAL OUTCOMES ASSOCIATED WITH H. PYLORI

INFECTION:

The helicobacter pylori, is one of the highly devastating organism which causes initial, acute inflammatory reaction followed by chronic irreversible damage on the underlying surface. The outcomes are variable from the benign ulceration predisposing to carcinoma and the reactive lymphoid hyperplasia transforming into gastric lymphomas¹¹.

PEPTIC ULCER:

By definition, a peptic ulcer results from the effects of acid on the mucosa⁶. This may be acute or chronic. An ulcer is termed as “chronic”, when it is long standing over months and does not heal. Microscopically, the ulcer site shows inflammatory infiltrates predominantly eosinophils surrounded by the area of fibrosis. An acute ulcer is merely a reaction to an aggressive pathogen causing local effects. It's usually large erosion that has penetrated through the muscularis mucosa deep into the gastric wall⁵. Sectioning through the ulcer site reveals the *three* distinct zones⁶. A *Superficial zone* of necrotic slough, with fibrin and neutrophils; a *Middle zone* composed of chronic inflammatory infiltrates and granulation tissue; the *Deep zone* consisting of fibrous tissue with vessels showing endarteritis obliterans.

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The gastric ulcers which are benign are accompanied by the areas of acute and chronic inflammation. The pattern of gastritis differs depending on the site of involvement. In the pyloric canal & pre pyloric region, there is diffuse antral gastritis with the presence of helicobacter pylori like organisms. More proximally, the ulcers in the region of incisura angularis are accompanied by multifocal atrophic gastritis & intestinal metaplasia. The morphology and clinical features are common in both the gastric and intestinal ulcers.

At the edge of all ulcers, it's universal that in all types there can be reactive changes in the overlying epithelium. The changes include high N: C ratio, hyperchromasia. This may be confused with the dysplastic epithelium or malignancy. A focus of intestinal metaplasia may show foveolae with basal nuclear crowding and increased mitosis similar to normal small bowel. However towards the surface, the epithelial maturation is identified. Inflammatory epithelial changes vary depending on severity and there is gradual transition from normal to atypical; whereas the transition between the dysplastic and normal epithelium tends to be abrupt. The reactive epithelium shows nuclei which are vesicular & rounded with one or two enlarged nucleoli³¹. Dysplastic nuclei are enlarged, crowded, compressed and tend to have hyperchromicity. Adjacent to site of peptic ulcer, atypia is noted more severe in areas of severe inflammation. The dysplasia may also show

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focal inflammation, but the less inflamed areas are also equally atypical. When a clear cut diagnoses can't be made out, it is termed as *indefinite for dysplasia*. The biopsy is done again after treatment with anti inflammatory therapy.

GASTRIC CARCINOMA:

Almost 1 million cases of gastric cancer are diagnosed each year, elaborating this disease as the 4th most common cancer worldwide irrespective of the gender²⁹. This is the second leading cause of cancer deaths worldwide. Though the virulence, etiology and social factors vary according to the region, the majority of patients succumb due to malignancy. The most common being the gastric adeno carcinoma. In some regions of the world, gastric carcinoma is the most common malignancy, & in Japan the incidence is 10- fold higher than the rates observed in United States³⁴. Typically, the diagnosis is delayed due to the lack of early specific symptoms, and most patients are diagnosed only after the cancer has invaded the muscularis propria³³.

Histologically, 2 distinct variants of gastric carcinoma have been identified: *Diffuse –type* gastric cancer which consists of neoplastic cells that are individually scattered & do not form glandular structures; and *Intestinal-type* adenocarcinoma which involutes through a series of well defined histological subtypes. Intestinal type adenocarcinomas are initiated by the transition from normal mucosa to chronic superficial gastritis; followed by the

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atrophic gastritis and intestinal metaplasia. This finally leads to the dysplasia and adenocarcinomas of the gastric epithelium. The histological features seen in this aspect of malignancy are synonymous to that of the intestinal epithelium counterpart. They are classified histologically into various subtypes depending on the pattern of growth and aggressiveness. The pathogenesis is variable and involves the host response which may turn fatal and also the virulence factors of the h.pylori, environmental carcinogenesis and other factors, which will be discussed further.

This form of gastric cancer affects men twice as commonly as women with a mean age of 50 yrs and 46 yrs respectively²⁶. The corpus predominant gastritis predisposes individuals towards gastric cancer; whereas the antrum related infection causes Peptic ulcer disease which shows fewer predispositions to carcinomas²⁷.

FACTORS DETERMINING THE PROGRESSION TO GASTRIC

CARCINOMA:

There are various factors that mediate the progression of disease from benign, simple superficial gastritis gradually transforming into an invasive high grade gastric carcinoma. There is an inter link between the

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factors and it is termed a “*multi factorial pathway* “–explaining the carcinogenesis³⁷.

ENVIRONMENTAL FACTORS:

These include the *H. pylori*, *helminths*, *high salt consumption*, *cigarette smoking and anti oxidants*. Of all these, the *H. pylori* infection forms a schematic format, starting from the superficial gastritis and later predisposing to malignancy. Due to the genetic variations, present within *H. pylori* genomes, the bacterial virulence factors often play an important role in determining the outcome of *H. pylori* infection. The genome is composed of *cag PAI*, *cagA*, *vac A* which cause the cytotoxic effects.

The *adhesins & Outer Membrane Proteins* help in the adhesion of *H. pylori* to the gastric epithelium¹¹. Thereby it helps in the initial colonization, persistence of infection and delivery of virulence factors to host epithelial cells. Along with Outer Membrane Proteins and adhesins, there is *BabA*, *DupA* & *FlaA*. These help in causing adhesions, duodenal ulcer promotion and flagellar movements against the host mucous secretions respectively. By promoting these mechanisms, the environmental factors cause initial surface gastritis, leading to the chronic gastritis. There is increased secretion of acid from G cells and exhibiting reactive changes microscopically. Then later it progresses to the atrophy of gastric glands and metaplasia. The

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common being intestinal metaplasia, there can be pyloric metaplasia in pre-pyloric and gastric antral region. This predisposes to the dysplastic epithelium and later into an invasive gastric carcinoma¹⁹.

HOST FACTORS:

The host exhibits the reactive polymorphisms to counteract the pathogen. H. pylori induced carcinogenesis includes gastric inflammation and a reduction in acid secretion. *IL-1* a pleiotropic pro inflammatory molecule is increased in H. pylori infection. This molecule, a potent inhibitor of acid secretion, is profoundly pro inflammatory, and is up regulated by the H. pylori. It is regulated by the promoters with informative polymorphisms and thereby promotes the carcinogenesis.

TNF- is a pro inflammatory acid – suppressive cytokine that is increased within H. pylori colonised human gastric mucosa³³. Polymorphisms that enhance the effect of this cytokine expression have now been associated with an increased risk of gastric cancer and precursor lesions. Also important are the other cytokines, IL – 10 and IL - 8 which helps in the expression of carcinogenesis. In addition to the stimulation of cytokine production, H. pylori also activates pro inflammatory Cyclo oxygenase (*COX*) enzymes. They catalyze the key steps in conversion of arachidonic acid to endoperoxidase, a substrate for a variety of prostaglandin synthase that catalyze the formation of

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prostaglandins and eicosanoids. While COX -2 expressions is inducible and can be stimulated by a variety of growth factors and pro inflammatory cytokines, the COX-2 expression is further increased within gastric pre malignant and malignant lesions. COX inhibitors such as aspirin and NSAIDS decrease the relative risk of gastric carcinomas. The capacity of COX- 2 generated products to promote neoplasia is well established, and specific mechanisms utilised by these molecules include stimulation of proliferation with inhibition of apoptosis. There is promotion of cellular adhesion, stimulation of angiogenesis and cellular transformation.

ACID SECRETION:

Acetylcholine, gastrin, & histamine are major stimulants of gastric acid secretion³⁴. In the gastric *corpus*, gastrin acts directly on parietal cells and indirectly acts through release of histamine from ECL cells, which in turn activates H₂ receptors on parietal cells to elaborate the release of acid. Acetylcholine directly acts on M₃ receptors on parietal cells and helps in secretion of the acid. H.pylori can either inhibit or stimulate the acid secretion, depending on the context of infection and location. Acute infection is usually associated with hypochlorhydria which is a result of increased production of the pro inflammatory cytokines IL- and inhibition of H⁺ K⁺ ATP ase pump. In case of chronic inflammation, there may be hypochlorhydria or

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hyperchlorhydria depending on the severity and distribution of gastritis. Most patients with long term infection develop pan gastritis associated with hypochlorhydria, which may progress to ulceration, dysplasia and cancer³⁴.

A potential contributing factor in the inflammation – to – carcinoma sequence is the generation of oxidative stress. Oxidative DNA damage induced by H .pylori infection has been well explained for gastritis. DNA damage has been demonstrated in the tissues exposed directly to H.pylori³⁸.

H .pylori induces both the *humoral* and *cellular* immune responses. Local and systemic antibody responses have been demonstrated that include IgA, IgM & IgG isotopes. Macrophages are essential as innate responders to H.pylori derived products and signals from epithelial cells that are in direct contact with bacterium on the surface of the mucosa. The effect of **MACROPHAGES** is suppressed by the deleterious effect of the pathogen, thereby preventing the further action of the host¹². The organism *escapes* from the engulfment by macrophages, inactivates the *T cell* signals stimulated by them. In the end the *macrophages* undergo *apoptosis*, as a result there is impairment of chronic inflammatory response and hence predispose to cancer risk¹³.

The maintenance of tissue integrity requires that enhanced cell proliferation along with increased rates of cell loss. In chronic H.pylori infection,

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there is lack of epithelial necrosis, suggesting that other forms of cellular demise may be affected.

Chronic stimulation of MMP-7 by H.pylori in vivo may be seen in a selective population, who possess hyper proliferating cells with reduced sensitivity to apoptosis, thereby contributing to increased carcinogenic risk. Hence, H.pylori is categorised as *Class I carcinogen* by WHO⁵.

To summarise, Gastric cancer is a highly lethal disease, and establishment of H.pylori as a risk factor for this malignancy allows an approach to identify persons at high risk; however infection with this organism is extremely common, and most colonized persons never develop cancer. It is apparent from recent studies that cancer risk is due to cumulative effects of the *polymorphic nature* of the bacterial population in the host, *the host genotype* and *environmental* exposures, each affecting the level of long term *interactions* between H.pylori and humans²².

GASTRIC LYMPHOMA:

The lymphomas are the more common primary to occur in the extra nodal site. The common location being the stomach, in body followed by antrum. Usually it is present as circumscribed nodular lesions over the gastric mucosa endoscopically. They appear similar to a sessile polyp and gives a

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differential diagnoses clinically. The biopsy reveals the proliferation of lymphoid precursors with an active germinal center. It is usually a reactive process and is seen in almost all cases of *H.pylori* associated chronic gastritis. The lesion occurs in the lamina propria where there are increased lymphoid follicles. On prolonged duration, there could be transformation into malignant lymphoma of B cell type. There can be presence of several patterns and variants which could be Diffuse Large B cell lymphoma, lympho-epithelial lesion most commonly MALT lymphoma¹⁷.

GASTRIC PATHOLOGY ASSOCIATED WITH H.PYLORI:

Insult to the gastric mucosa, provokes a variety of inflammatory & reactive responses that depend on the type, location & duration of the injury. The correct diagnoses of gastritis rely on the pathologic recognition of various types of tissue responses, their intensity and location. The common pathologic tissue reactions of gastric mucosa include¹⁹:

- 1. Neutrophilic infiltration***
- 2. Mononuclear infiltrates***
- 3. Lymphoid aggregates and follicles***
- 4. Eosinophils***
- 5. Hyperemia and edema***
- 6. Surface erosion***
- 7. Foveolar hyperplasia***
- 8. Intestinal metaplasia***
- 9. Atrophy***
- 10. Endocrine cell / parietal cell hyperplasia***

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Neutrophilic infiltration:

The normal gastric mucosa may contain a few neutrophils in the lamina propria. However, infiltration of the surface epithelium by neutrophils represents a pathologic response & is considered as an *active* component of gastritis¹⁹. The term active is used to indicate that there is a continuous release of inflammatory mediators. This is most commonly due to H.pylori infection, though many other infections & inflammatory conditions can also cause this. Active phase of H.pylori induced gastritis may reveal moderate to severe levels of neutrophils.

In chronic H.pylori gastritis, due to prolonged contact of organism to the surface, there is irregularity in the epithelial cells exhibiting cuboidal shape with decrease in mucin, these changes are characteristic and is due to *VacA & cagA, urease, ammonia*, etc. There is infiltration of the neutrophils over the altered surface epithelium and it is the distinctive histological feature. Neutrophils are more abundant in antrum and cardia, than in corpus; may fill the lamina propria, could form pit abscesses and surface exudates. The grading of activity is based on the amount of neutrophils being colonised²¹. It is termed as “*mild*” when the neutrophils are seen limited within the lamina propria. As “*moderate*” activity when the neutrophils enter the gastric pits with or

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without abscess formation; and “*severe* “only when there is ulceration with associated atrophy of the pit epithelium²⁴.

Mononuclear infiltration:

Normally, the gastric antrum contains few mononuclear inflammatory cells, whereas normal body mucosa contains virtually none. The presence of scattered lymphocytes and plasma cells in lamina propria is usually normal. In contrast, increased cellularity of lamina propria composed of lymphocytes, plasma cells, variable number of eosinophils and mast cells is characteristic of chronic H.pylori gastritis¹¹.

Lymphoid aggregates and follicles:

Normal gastric mucosa, particularly the body may contain occasional small lymphoid aggregates that are separated by delicate septae. They are usually situated in close proximity to the muscularis mucosa at the base of lamina propria. In studies performed, the lymphoid aggregates and follicles have been detected virtually in all subjects with H.pylori gastritis. The presence of lymphoid follicles is highly specific for H.pylori infection³⁶. In patients who are children and young adults, lymphoid follicles may be numerous and are seen endoscopically as nodular lesions¹⁴.

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Atrophy:

Gastric atrophy is defined as, “a *loss of gastric glands*”⁷. Whenever gastric mucosa is influenced by prolonged insults, regardless of aetiology, it may either regenerate to normal or undergo protective metaplastic change.

When chronic pathology persists, the injured glands fail to regenerate and lose its function. This dysfunctional surface is replaced by the fibroblasts and extracellular matrix. The native glands may be replaced by metaplastic epithelium. The common being pyloric or intestinal metaplasia, which is composed of goblet cells. Several pathology workshops have developed a method for grading the atrophy in mucosal biopsies. If present, it is recommended to describe the atrophy, according to its two sub types –

Non metaplastic or Metaplastic.

Metaplasia:

The transformation of one epithelium into other as a protective mechanism. Two major types are seen in stomach, 1.) **Intestinal** metaplasia (IM), 2.) **Pyloric** metaplasia (pseudo pyloric) 18

PYLORIC metaplasia – there is replacement of the acid and enzyme secreting cells of fundic glands by mucus secreting glands of pyloric mucosa²⁹. This change in epithelium occurs in the zone of fundic mucosa, adjacent to the

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fundo - pyloric junction. **INTESTINAL** metaplasia – there is a change in the cells of surface and pit epithelium, so that morphologically and histochemically they resemble the cells of intestinal epithelium¹⁷.

There are two types of intestinal metaplasia that are observed. They include ***Complete IM*** and ***Incomplete IM***; termed as Type I and Type II respectively.

In **Type I**, the gastric mucosa resembles normal small bowel epithelium with fully developed goblet cells & enterocytes with brush borders. In advanced cases, there may be development of villi and crypts.

In **Type II**, there are no absorptive cells seen. The epithelium consists of a mixture of intestinal type goblet cells and columnar mucus secreting cells, resembling normal gastric epithelium.

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Type of metaplasia	Type of mucin	Special stains used
NORMAL STOMACH	NEUTRAL MUCIN	PAS + / AB -
COMPLETE INTESTINAL METAPLASIA	ACIDIC MUCIN (SIALO / SULFO MUCIN)	PAS + / AB +ve ** AB - ve
INCOMPLETE INTESTINAL METAPLASIA	SIALO MUCIN	
INCOMPLETE INTESTINAL METAPLASIA (LARGE BOWEL)	SULFO MUCIN	HIGH IRON DIAMINE

**** Alcian Blue** is positive at pH 2.5 and negative at pH 0.5 for SIALOMUCIN.

Whereas in case of SULFOMUCIN, Alcian Blue is positive at both pH 0.5 & 2.5. **Alcian blue** stains blue colour and is seen in normal intestinal mucosa. The **PAS** stains magenta colour and is seen in normal gastric mucosa. **High Iron Diamine** stains the sulfomucin present in the colonic type of epithelium and is stained black²⁴.

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The inflammation of the gastric mucosa can be acute and chronic with varying morphological and histochemical features depending on the duration, aetiology and environmental factors.

THE UPDATED SYDNEY SYSTEM⁹:

The system provides guidelines for providing systematic, uniform diagnostic reports. It is recommended that at least 5 biopsy specimens should be evaluated and the findings summarized. This system classifies gastritis on the basis of *topography, morphology and aetiology* into 3 broad categories.

1. Acute 2. Chronic and 3. Special

Chronic gastritis is further divided into ATROPHIC & NON ATROPHIC gastritis.

Each relevant pathologic feature (density of H.pylori, intensity of neutrophilic and mononuclear infiltration, atrophy and intestinal metaplasia) should be graded separately. Each feature is assigned either a numeric or descriptive value.

0 – for absent

1—for mild

2—for moderate

3—for marked (or severe)

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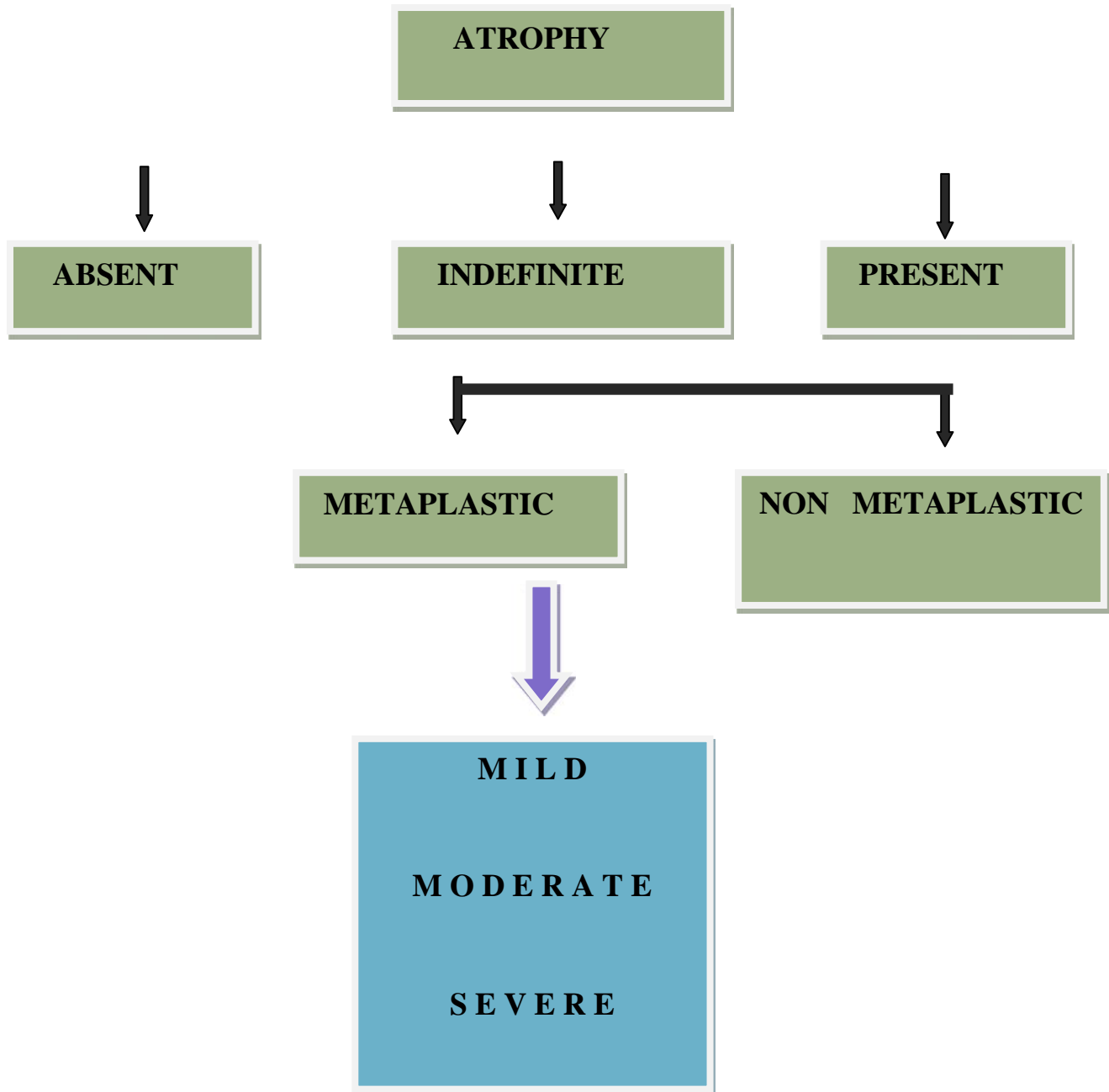
The values of each feature are then averaged separately for each anatomic compartment (antrum and corpus)

Both the 1990 original Sydney System and its updated 1994 version (also known as *Houston – Updated Sydney System*) provided a structure for proper description and to substantiate the primitive lesions , that is , the inflammatory cell populations and the accompanying changes of the epithelium ¹⁷ . There were inter observer variability among the pathologists, with regards to atrophy. *Atrophy Club 2000* included the sequence of gastric atrophy and intestinal metaplasia ²¹ .

Peyalo Correa demonstrated that patchy areas of atrophic – metaplastic changes in antrum and oxyntic mucosa co-exist with gastric ulcer and in carcinoma sequence. Sydney system also recommended 5 main sites for the biopsy sample.

1. greater and lesser curvature of distal antrum
2. greater and lesser curvature of proximal corpus and
3. lesser curvature at the incisura angularis

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The above classification of atrophy is based on the recommendations of the ATROPHY CLUB.

SYDNEY SYSTEM CLASSIFICATION OF GASTRITIS

NON ATROPHIC	ATROPHIC	SPECIAL FORMS
H. Pylori	Auto immune	Chemical
? other factors	? H.Pylori	Radiation
	Multifocal atrophic gastritis (MAG)	Lymphocytic
		Non infectious Granulomatous
		Eosinophilic
		Others

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Identification of the Organism:

H.pylori is a gram negative, spiral, motile bacterium that resides in the gastric pits and the overlying mucus¹¹. The early phase of H.pylori infection exhibits an acute inflammatory response. The patient may be symptomatic or asymptomatic with nausea and vomiting³¹. The findings which alert the pathologist to look for this tiny pathogen include the acute inflammatory response with lymphoid proliferation, villiform transformation, regenerative changes and metaplasia⁴⁸.

These spiral bacilli are picked up in the routine H&E as pink comma shaped structures that reside over the mucus and pits. Due to increased colonization of the bacterium there is exaggerated local inflammatory response with pit abscesses and inflammatory debris. Sometimes, these organisms lie deep inside the mucus covered by inflammatory debris and polymorphonuclear cells. To further confirm the presence of these bacilli, various special stains are used. The most commonly used are Modified Giemsa, Toluidene blue, Warthin Starry stain, Diff- Quick and Silver based "*triple stain*", Genta stain³⁸.

Silver based triple stain: additionally intestinal metaplasia can be detected due to alcian blue application at acidic pH. These include Acridine orange, Gram stain, Brown Hopps³⁸.

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In Modified Giemsa, these bacteria are seen as *dark blue* comma shaped structure present over the site suspicious for its presence, in routine H&E stain. This special stain could be counter stained with other dyes to enhance the visualization³².

In Warthin Starry, the organisms are seen as *black* spiral structures in a yellow background¹⁶. These special stains are more sensitive in identifying the organisms compared to that of routine H & E. Though it is accurate in diagnosing, there are certain conditions in which the diagnosis could be missed out. These are discussed further...

Diagnostic limitations^{17, 21}:

- 1. Indolent forms – such as coccoid**
- 2. More deeper location within mucus**
- 3. Severe inflammatory response**
- 4. Dirty background filled with nuclear debris**
- 5. Pre- treated patients with scanty bacilli.**

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DIAGNOSTIC MODALITIES FOR H.PYLORI:

There are various methods for detection of H.pylori which are both invasive and non invasive procedures¹¹. The *non invasive* methods include – serologic testing, faecal antigen detection and urea breathe testing.

The *invasive* methods require endoscopy evaluation supplemented with bacteriologic culture, histopathology studies, smear examination, rapid urease test and molecular studies³².

Of all the tests described, *histopathology* examination is now termed *gold standard* and more sensitive test for detection of H.pylori¹⁹.

Microbiology – Urease Broth / Urease agar kits, Urease Breath test, PCR / ELISA

Culture – gastric antral biopsies, cultured in Chocolate agar / Brucella agar with 5% sheep blood / Skirrow's and Modified Thayer- Martin medium was considered once as a gold standard.

With numerous studies and updates done frequently, the idea of provoking the antigens for H.PYLORI which could be more specific in diagnoses came into existence.

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IMMUNOHISTOCHEMISTRY – for bacterial detection have been available since 1988, and is reported as *more reliable* for the semi- quantitative diagnoses of H.pylori infection and for the detection of indolent forms of these bacilli⁹.

This method could overcome certain limitations, though there could be some inter - observer variations in morphology and lack of experience in gastrointestinal pathology. The immuno histochemical methods are more easy¹⁸, reliable and less time consuming⁷. The staining is such that , the organism stands out well , with well elaborated tissue morphology .The relationship between H.pylori and gastric cancer had been established (Watanabe et al , 1998) .Thus the invasiveness of H.pylori aroused the interest among pathologists. They found that H.pylori antigens progressively migrate from the cell membrane to the inner cytoplasm with progression of chronic superficial gastritis → pre cancerous lesions → gastric cancer.

H.pylori was highlighted by immunohistochemistry; seem to invade the cytoplasm of epithelium ¹⁷. H.pylori changes its shape from spiral to coccoid forms and may appear indolent leading to malignancy.

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Helicobacter pylori Antibody:

This is concentrated and pre diluted Polyclonal Antibody ²⁸.The antigen detection, in tissues and cells is a multi step immunohistochemical reaction. The initial step is binding of the primary antibody to its specific epitope. After labelling the antigen with a primary antibody at a dilution of 200 µg / ml for 20 minutes at room temperature; a universal affinity purified secondary antibody is added to bind with the primary antibody forming a primary antibody – secondary antibody complex. An enzyme label is then added to bind to the secondary antibody¹⁷. This detection of bound antibody is seen by a calorimetric reaction¹⁸.

H.pylori is spiral – curved, gram – negative bacteria that are present on the surface epithelium of mucous layer of stomach. There is evidence that these bacteria play an important role in development of peptic ulcer disease ¹⁷. Immunohistochemistry can distinguish H.pylori from other types of curved bacteria. These small spiral organisms can be seen clearly using high power (40 x) as clusters and on oil immersion (100x) clear morphology is noted and thus confirms the diagnosis. Both Monoclonal and Polyclonal antibodies are in existence now, with a slight variation in sensitivity / specificity. Of the two, monoclonal antibodies seem to be better than the polyclonal antibody as described by a study ²⁸.

RESULTS & ANALYSIS

Results & Analysis

This study is done *retrospectively* over a period of one year duration. A total of **55 cases** which are clinically suspected and histochemically positive H.pylori associated chronic gastritis were selected. The slides were taken from the department archives and the paraffin blocks of the same, were obtained from our histopathology laboratory with proper procedures.

The tissue blocks were processed again on plain glass slides and stained with routine *H&E, Modified Giemsa*. Combined with the routine stains used for H.pylori, *Antibody* against H.pylori was made to assess the *density* of H.pylori, the *efficacy* and *sensitivity* of the Immunohistochemical staining. Along with the identification, other suggestive *pathological features* were also observed and the results are summarized as follows.

Incidence:

Among the 55 cases studied, there was a *MALE* predominance with the age group being *4th and 5th decades* [21cases] as depicted in Table (1_{a, b})

Observations:

The biopsy samples were endoscopy guided, and the tissue is taken from the gastric antrum & the body with predominant site being *Antrum* [47 cases] in Table (7). The slides were stained using H&E and Modified Giemsa. We found that, initial finding was gastritis, showing *neutrophilic infiltration*

Results & Analysis

indicates the activity of inflammation. The gastritis was graded based on *Modified Sydney System* as mild, moderate & marked.

Majority of the cases studied under H&E showed *MILD* activity [31 cases] (Table 2)

We were also interested to look upon the features suggesting the presence of H.pylori infection. Hence we searched for the colonization of H.pylori under H&E and special stains. In our institution, for our routine practice we use Modified Giemsa as special stain with a positive control for each case. We looked for the H.pylori colonization in H&E and Modified Giemsa and graded as mild , moderate and marked depending on the density of the organism [Table-3] Most of the cases showed only *MILD* colonization of H.pylori under routine stains (H&E and Modified Giemsa).

There were marked changes in the mucosa at the site of inflammation, associated with H.pylori manifestation. The changes include Intestinal Metaplasia, Lymphoid follicles, villiform transformation. Out of all the above mentioned features, there were increased *lymphoid follicles* with active germinal centre, in a dominant **41** cases, followed by Intestinal Metaplasia (33 cases) and Villiform transformation.

Results & Analysis

There was no finding of atrophy in the cases studied, as atrophy is considered, a pre dysplastic lesion. [Table 6]. When compared with the site, the majority being *antrum*, the commonest site for H.pylori colonization. There were combined features in many cases having both villiform transformation and intestinal metaplasia; lymphoid follicle formation is seen in many cases irrespective of other co-existent pathological features. Thereby making this as the diagnostic feature for chronic H.pylori gastritis.

Helicobacter pylori Antibody was used to compare the sensitivity of routine H&E and Modified Giemsa. We looked for all those features mentioned above and compared with the results against the routine stains.

Using H.pylori antibody, there was **MARKED** intensity in staining and it was easy to pick up these spiral organisms compared to routine stains [Table 4]. The sensitivity and specificity was much higher than the routine H&E and histochemical studies. The time consumption was less and diagnosis was more accurate than the routine stains .Thereby it reduces the risk of reporting false positive cases. This antibody also elicits the organisms residing in deeper mucus glands, situated within the debris and in scant colonization. There are some cases in patients who are on treatment with PPI, the spiral organism, takes up a different shape and structure to hide from the host response; the common being *coccioid* forms. The IHC, provokes an **Antigen –**

Results & Analysis

Antibody reaction and lightens up all the H.pylori organisms residing over the gastric mucosa irrespective of the clinical status of the patient.

To concise, 55 clinically suspected and histopathologically diagnosed cases were taken for this study.

We found there is **MALE** predominance, with the age group being 4th to 5th decade, and common site being **GASTRIC ANTRUM**.

In the sections studied, on H&E, there was **MILD** activity in 31 cases out of 55 followed by Moderate activity in around 20 cases.

When H.pylori like organisms was visualized on H&E, Modified Giemsa and Immunohistochemistry we graded the colonization of bacilli on each stain separately.

The Routine stains (H&E and Mod. Giemsa) showed **MILD** colonization in around 37 cases. Whereas there was **MARKED** colonization in majority of cases stained with **antibody**. (34 cases).

The other pathological features were noted, and we found there was an increased lymphoid follicle in around 41 cases, followed by Intestinal metaplasia. There were also mixed overlap in features, many lesions having lymphoid follicles in common with other features in permutations.

DISCUSSION

Discussion

H. pylori infection is one of the major aetiology for asymptomatic chronic gastritis in the developing countries². This is mainly due to varying etiologic factors and diverse environmental parameters. The most common age group for this disease is children and young adults as described by literature⁴. But this data varies accordingly depending on different parts of the world. As a whole, many patients presented with vague abdominal discomfort, dyspepsia and GERD like symptoms¹⁷. It is described by WHO as **Grade I** carcinogen and its susceptibility to cause malignant transformation¹⁴. Early diagnosis and treatment would play a vital role in the prevention of disease progression.

Endoscopically, the gastric mucosa usually shows intestinal metaplasia like areas with or without nodular fleshy lesions suspicious for *H. pylori* infection²⁴. These areas exhibit an irregular squamo columnar junction with a salmon pink tongue extending into the tubular esophagus²⁴. Usually *H. pylori* infection is clinically assumed as one of the prime differentials in a patient with mild symptomatic or asymptomatic chronic gastritis. Hence, the patient is given a prophylactic *H. pylori* eradication therapy composed of triple drug “**O T C kit**” [Omeprazole, Tinidazole & Clarithromycin]. The therapy is for 2 weeks initial triple drug followed by 4 weeks of oral Proton Pump Inhibitors³¹. The PPI's itself cause effects on the gastrin secreting cells thereby causing altered secretion of gastric acid. This also takes toll on *H. pylori* making

Discussion

the organism pleomorphic by changing its morphology into a *Cocoid* form¹⁷. This form of H.pylori is difficult to identify as it is too small and may lie with the inflammatory cells. When there is recurrence of symptoms or progression, the endoscopy biopsy are taken, common sites being Antrum and Body. The histopathology diagnosis is more specific and is supplemented by other biochemical tests. In histopathology, routinely the sections are examined on H&E and Modified Giemsa over years. But now, in the era of modern medicine and broader scope for research, new inventions are on periodically. Due to increased risk of carcinogenesis by H.pylori these is an urge to find a method to increase the specificity on detection.

A few studies done before (Lu Xin et al; Intisar S.Pity et al; Ali k. Riba et al) stated the importance of the Antigen – Antibody reaction on the H.pylori and the application of polyclonal antibody in current practice²⁶. They also stated that the antibodies are more advanced, easy to interpret, high sensitivity and specificity than histochemical stains. Though there are more positive aspects, no studies have suggested the necessity and the need to include this in routine diagnosis of H.pylori. This provoked the interest to do this study and add our information on comparing the IHC with special stains (Modified Giemsa) and H&E. We also observed the *histopathological* changes that are seen in H.pylori associated chronic gastritis. The common changes

Discussion

include gastritis, oedema, hyperaemia, congestion, *metaplasia*, *lymphoid* activity and *dysplasia*¹¹. These features occur as a sequence of events. In this study, we explain the incidence of each finding, their associated features, intensity/ severity of the organism and various etiologic factors we observed in our set up or locality.

Inflammation:

On routine H&E, we found the inflamed gastric mucosa infiltrated by neutrophils due to release of interleukins. All the cases showed inflammation with slight variation in severity, and we graded them according to Modified Sydney System. Out of 55 cases studied, most of the cases showed MILD inflammation, followed by moderate inflammation. The severe or marked inflammation was seen only in about 4 cases. There was dense inflammatory response over the surface. Some studies say that there could be an overlying ulceration covered with slough. But in this study we did not find any case showing active ulceration. This variation may be due to prophylactic use of PPI, or environmental variation.

Discussion

Metaplasia:

Due to chronic irritation of gastric mucosa, the host tries to respond against the pathogen by stimulating the chemokines, and mucus secretion. As this process prolongs, the normal surface epithelium changes itself into other viable forms which are capable of surviving the prolonged insult. This process is termed as “metaplasia”. The common types of metaplasia are: *Intestinal* and *pyloric*. The intestinal metaplasia is more common of the two.

Normally, the gastric mucosa contains *Neutral mucin* which is positive for **PAS**²⁷. When there is transformation into intestinal type of epithelium, there is increased *goblet cell* formation which is similar to that of intestine.

The goblet cells contain acidic mucin (sialo / sulfo) depending on the pH. The goblet cell metaplasia is confirmed using **AB / PAS** stain. The gastric mucosa shows positive for PAS (magenta colour) and the adjacent goblet cells exhibit Alcian Blue positivity (blue colour).

In our study, there were only intestinal metaplasia and no case of pyloric metaplasia was seen. Intestinal metaplasia was seen in 33 cases marking about 60 % of the total 55 cases studied. This is further termed separately as mild, moderate and marked according to Sydney System.

Discussion

Atrophy -- It is defined as, “*the loss of glands* “.

Atrophy is usually termed as an end result of chronic gastritis, common aetiology being H.pylori infection. It stands intermediate between metaplasia and dysplasia.

There are many studies quoting on the importance to look for atrophy. They point out that atrophic gastritis could turn malignant.

In our study, no case showed glandular atrophy.

Lymphoid aggregates and follicles:

Lymphoid aggregates are seen scattered in normal gastric mucosa. In studies, they stated that virtually many lymphoid follicles are seen in all cases of H.pylori gastritis. This feature is highly specific for H.pylori infection.

In our study, we found majority of cases showing active lymphoid follicles consistent with the literature ³⁶ . Out of 55 cases, 41 cases (74.5 %) showed lymphoid follicles, which were consistent with nodules, endoscopically.

We also noted the **Villiform transformation** in around 9 cases (16.3 %) out of 55 cases studied.

Discussion

Interpretation:

To correlate with the H&E findings suspicious for H.pylori aetiology, we used Histo & Immunohistochemistry. The Modified Giemsa and H.pylori antibody were used.

We looked for the staining of organism in both the stains and compared the sensitivity / specificity between both stains.

To start with, we used the routine H&E , the site suspicious for H.pylori infiltration were noted , examined further using the special stain and IHC . The need of the study is fulfilled at this stage, when we analysed the intensity of the bacilli in both Modified Giemsa and IHC.

In this study, we implement the simultaneous use of Monoclonal Ab for H.pylori along with Modified giemsa. When compared, the Antibody was much better than the routine stains. The intensity was more superior, the colonization of the bacilli was seen clearly, especially in the areas of thick mucus and near the pits¹⁶.

Out of 55 cases studied, Modified Giemsa showed MILD bacterial colonization in majority of cases (37), whereas H.pylori antibody proved that there were intensely MARKED colonization in most cases (34). Thereby, it helps in the exact staging of disease and proper grading of

Discussion

inflammation. Immunohistochemistry acts as a specific diagnostic tool in cases of H.pylori induced chronic gastritis.

We meticulously searched for the bacilli and other associated histological features. The findings were classified accordingly and separate grading was used in each pathological feature as suggested by Modified Sydney classification.

SUMMARY

Summary

To summarize,

- ❖ In this study on 55 cases of H.pylori associated chronic gastritis, spanning 1 year duration there was MALE predominance with age group being 4th to 5th decade.
- ❖ The common site being GASTRIC ANTRUM.
- ❖ Comparing with routine H&E and Modified Giemsa, Antibody against H.pylori showed higher sensitivity exhibiting MARKED colonization in majority of cases, which were previously thought as MODERATE in Modified Giemsa.
- ❖ On observing the pathological changes, we found predominantly LYMPHOID FOLLICLES in almost all cases.
- ❖ Other features indicating H.pylori associated chronic gastritis such as Metaplasia (commonly Intestinal), villiform transformation were also noted.

Summary

Though there are number of methods for detection of H.pylori, Histopathology is always the gold standard. To add it on, use of Immunohistochemistry would increase the specificity and also reduces the false negative results.

Thus by using the H.pylori antibody in routine practice along with Modified Giemsa, the diagnosis could be made easily, more specifically and quickly.

In the *developing countries*, though the feasibility is low due to cost, we would suggest that implementation of *IHC* as a routine in near future would help in providing an *early diagnosis* and appropriate treatment for H.pylori associated chronic gastritis.

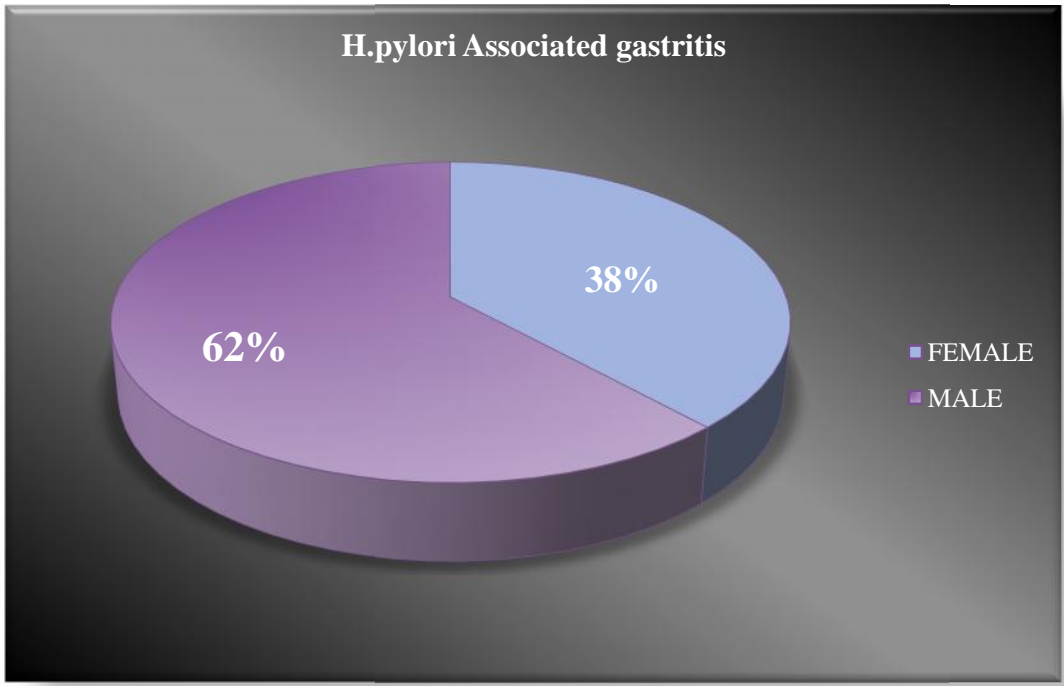


Table 1a

TOTAL CASES = 55

MALE PATIENTS = **34** (62 %)

FEMALE PATIENTS = **21** (38 %)

In the cases studied, there is MALE predominance.

AGE (RANGE)	No. of cases clinically / endoscopically suspected	Positive for H.pylori associated gastritis
10 – 20 yrs	04	04
21 – 30 yrs	03	03
31 – 40 yrs	11	11
41 – 50 yrs	21	21
51 – 60 yrs	14	14
61 – 70 yrs	02	02

Table 1 b

Average predominance of age group is **4th to 5th Decade** and it is about 21 out of 55 cases studied.

Total=55cases

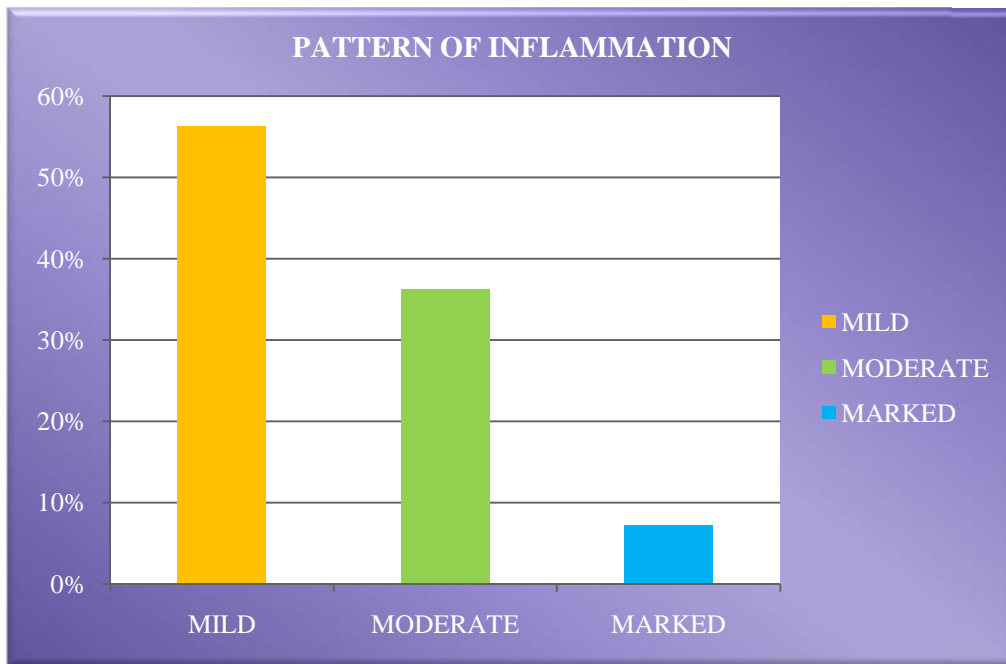


Table 2a

Inflammation	No. of cases	Percentage
MILD	31	56.3 %
MODERATE	20	36.3%
MARKED	04	7.2 %

Table 2b

Of total 55 cases studied, majority of the cases showed MILD inflammation (31 cases) contributing around 56 %.

The Pattern of inflammation was studied based on MODIFIED SYDNEY GRADING, which emphasise the use of MILD, MODERATE & MARKED on each parameter considered.

In the chart above, these terminologies were used to grade the severity of inflammation.

In the context described below, the colonization of H.pylori organism is categorised, again using the **three** grades.

H.Pylori colonization	No. of Cases	Percentage (%)
MILD (++)	37	67.27%
MODERATE (+++)	13	23.63%
MARKED (++++)	05	9.09 %

Table 3

In the Table 3a described above, out of the 55 cases studied, majority of them showed MILD H.pylori colonization when studied under routine H&E and Modified Giemsa stains.

H.pylori density:

(+ /++) Mild – Scattered spiral organism

(+++) Moderate – Moderate colonization covering 1/3rd to 2/3rd of mucosal surface

(++++) Marked – Dense colonization > 2/3rd of mucosal surface

Routine Stains (H&E and Modified Giemsa)

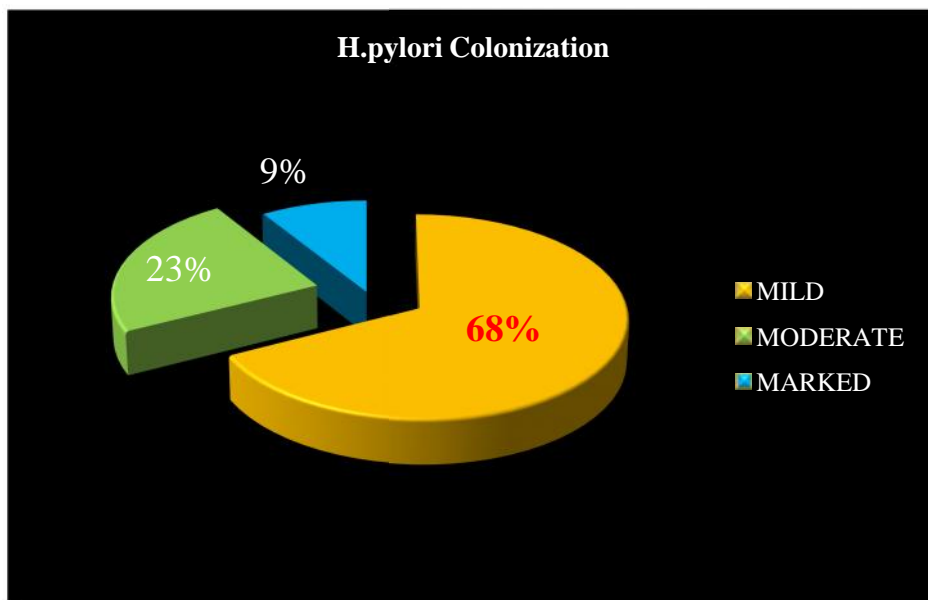


Table 4

USE OF H.PYLORI ANTIBODY

H.PYLORI ANTIBODY	No. Of Cases	Percentage (%)
MILD	03	5.45%
MODERATE	18	32.72%
MARKED	34	61.81%

Table 5a

In the above table, we looked for the H.pylori colonization using the H.pylori Antibody. This is to compare the intensity with the routine stains explained in Table 3. The grading is done again into **Mild, Moderate and Marked**.

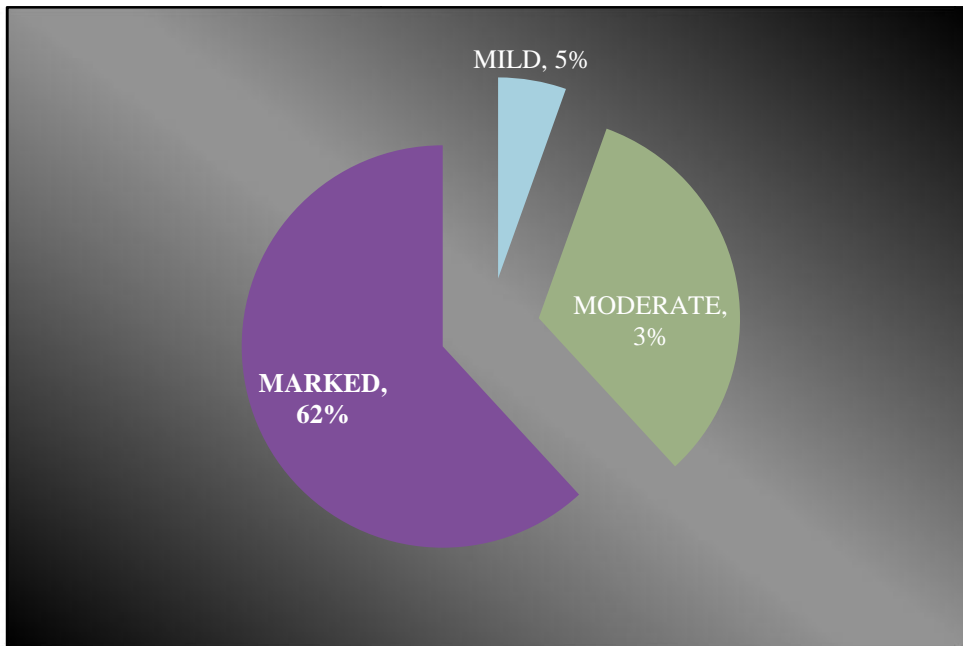


Table 5b

COMPARISON OF INTENSITY ON H&E, MODIFIED GIEMSA AND H.PYLORI ANTIBODY

BACTERIAL COLONIZATION	H & E	MODIFIED GIEMSA	H.PYLORI ANTIBODY
MILD	42 (76.3%)	37 (67.2 %)	03 (5.4%)
MODERATE	09 (16.3%)	13 (23.6 %)	18 (32%)
MARKED	04 (7.27%)	05 (9.09%)	34 (61.8%)

Table 6a

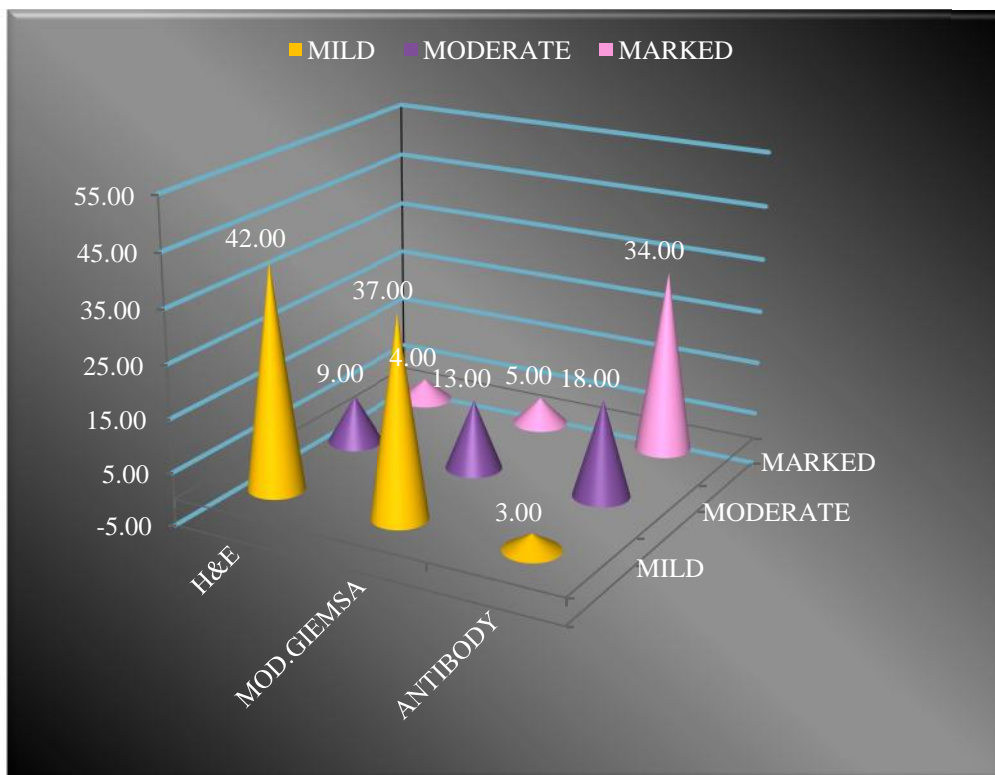


Table 6b

Histopathological Changes associated with H.pylori infection

Pathology of Gastritis	No. of Cases	Percentage (%)
Lymphoid follicles	41	74.5%
Intestinal metaplasia	33	60%
Villiform transformation	09	16.3%
Atrophy	nil	nil

Table 7a

Out of 55 cases studied, around 74.5 % showed increased *lymphoid follicles* in lamina propria some with active germinal centres.

Followed by *Intestinal metaplasia*, this is seen in many cases and probably acts as a clue to the diagnosis of H.pylori associated gastritis (60%).

Villiform transformation, one of the late reactions in the chronic gastritis was observed in around (16.3%).

No evidence of atrophy was seen in the study.

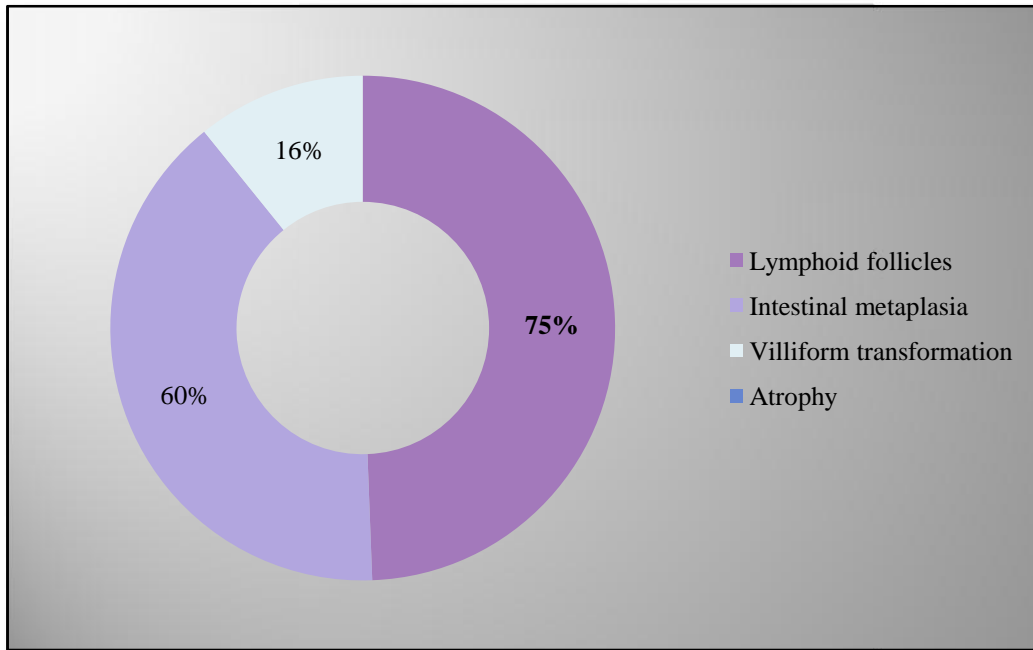


Table 7b

Site of Biopsy	H.pylori / chronic gastritis				Intestinal Metaplasia	Lymphoid aggregates/ follicles	Villiform transformation
	No (0)	Mild (1)	Moderate (2)	Marked (3)			
Antrum (47)	0	12	32	03	32	36	07
Body (8)	0	01	06	01	01	05	02

Table 8a

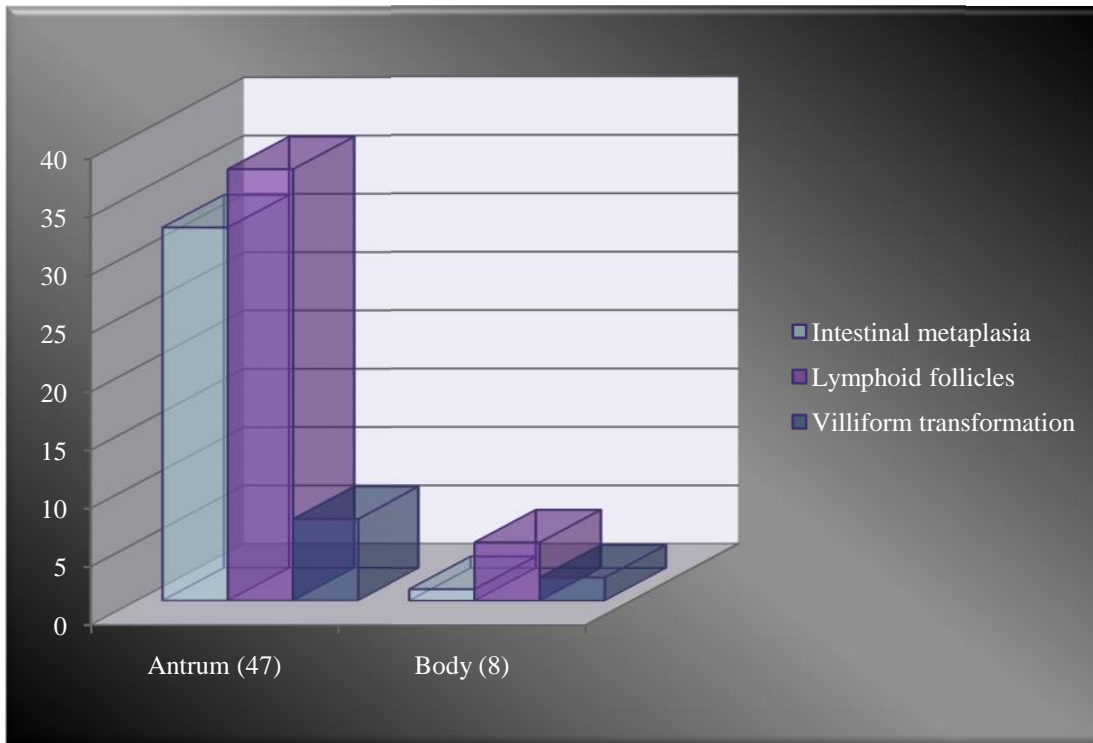


Table 8b

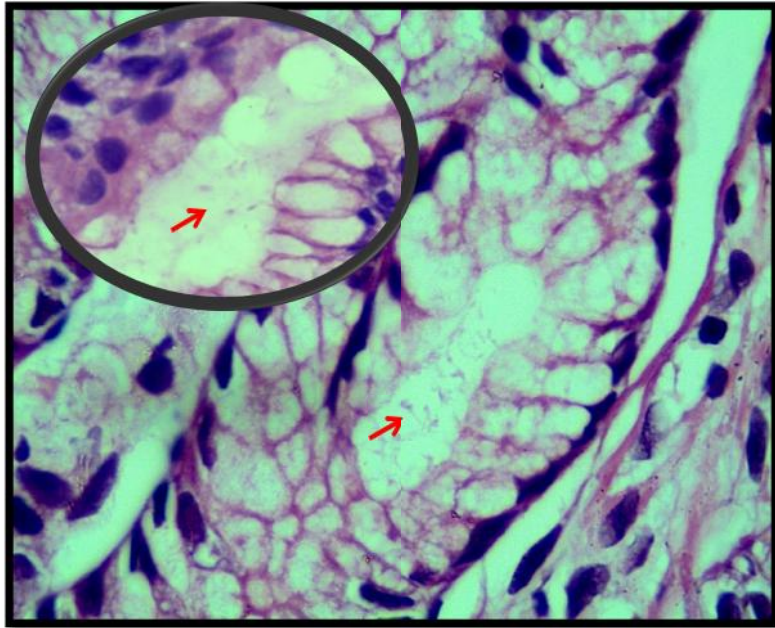


Figure 1 H&E 40 x comma shaped *H.pylori* like organisms (arrow)

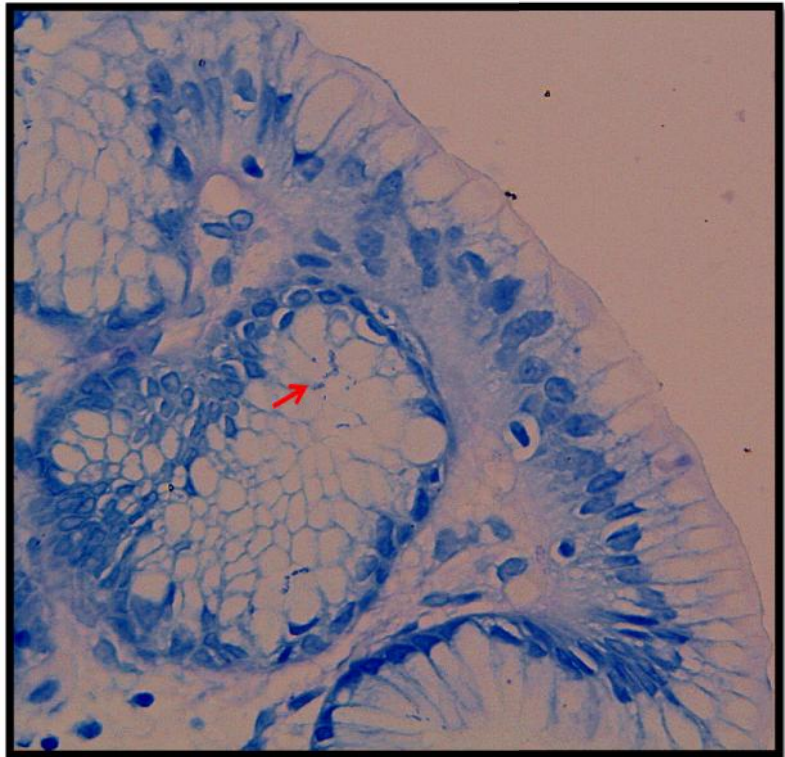


Figure 2. Modified Giemsa (40x).Blue comma shaped organisms (arrow)

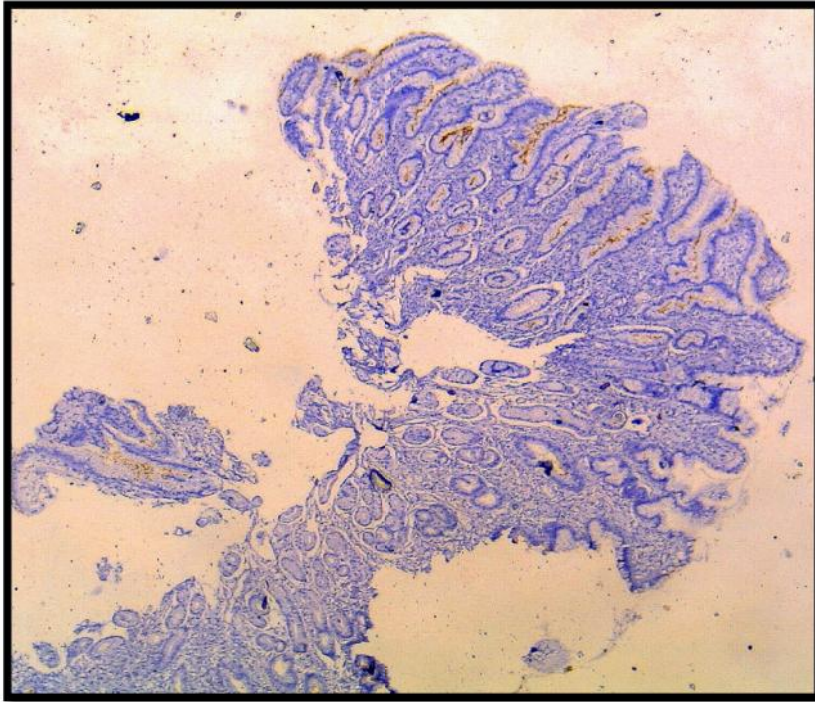


Figure 3a. H.py;ori Ab (5 x) Shows positivity for H.pylori

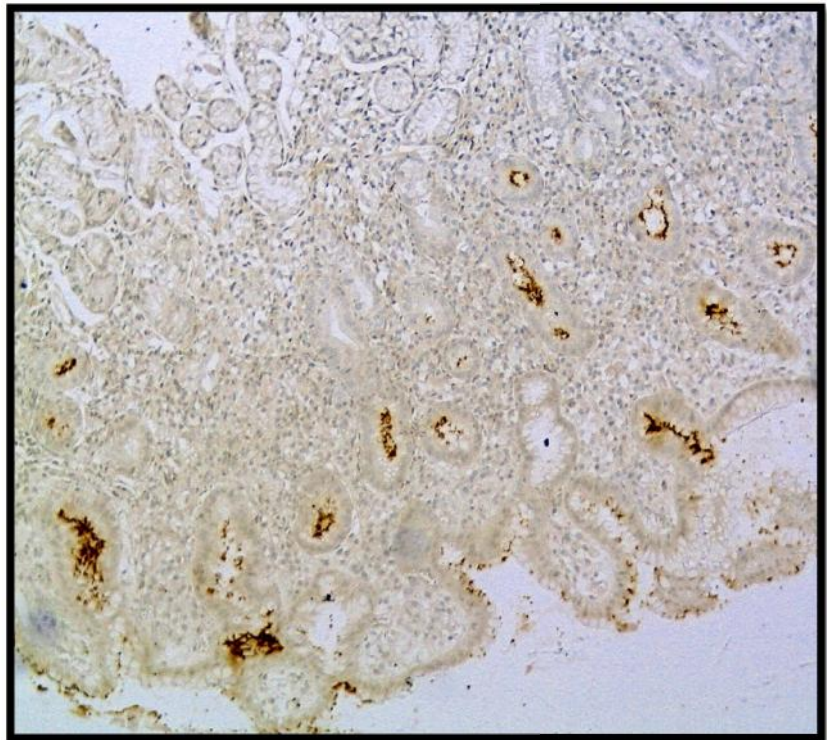


Figure 3b.H.pylori Ab (10 x) strong, marked (+++++) positivity

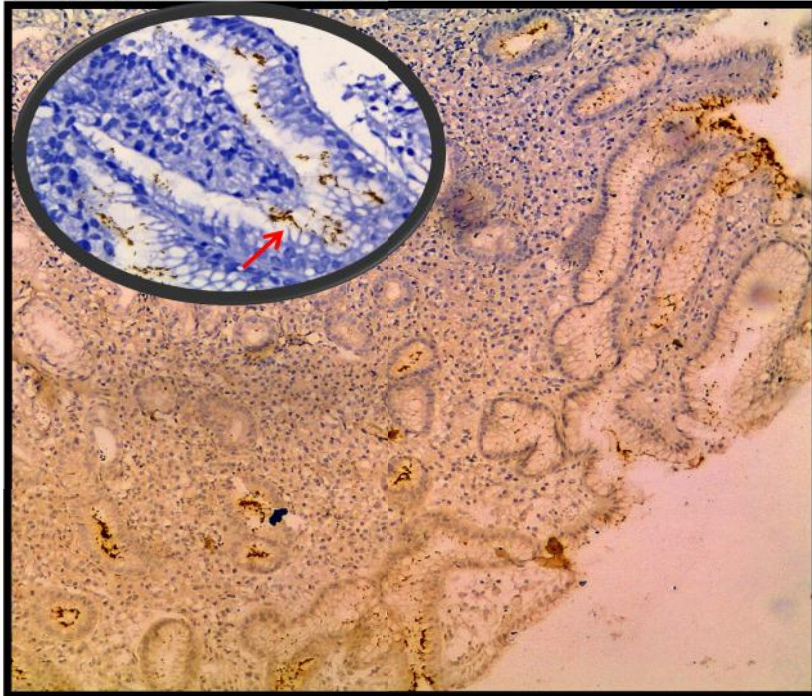


Figure 3c. H.pylori Ab. Marked colonization. Inset – higher magnification

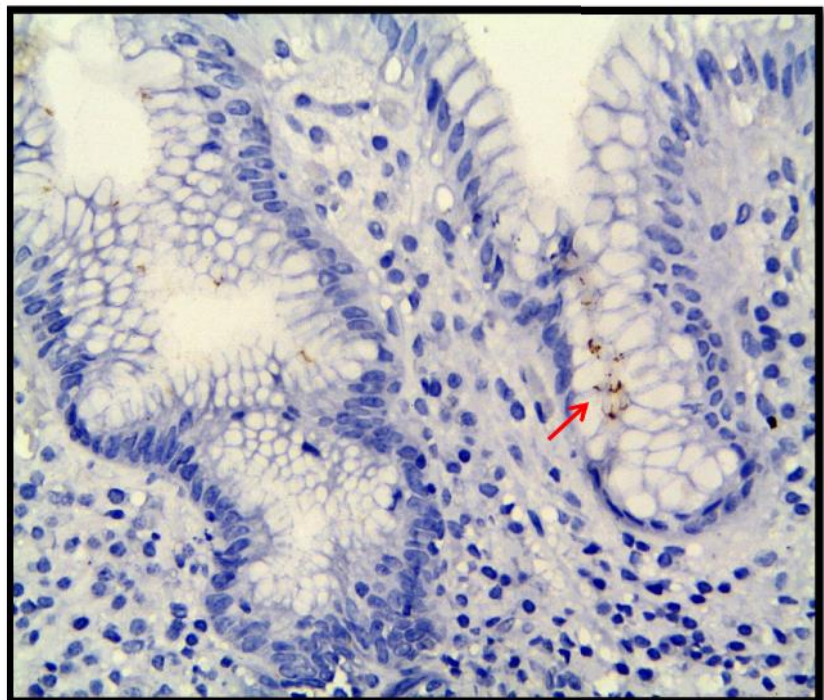


Figure 3d. H.pylori Ab (40x). Mild colonization (arrow)

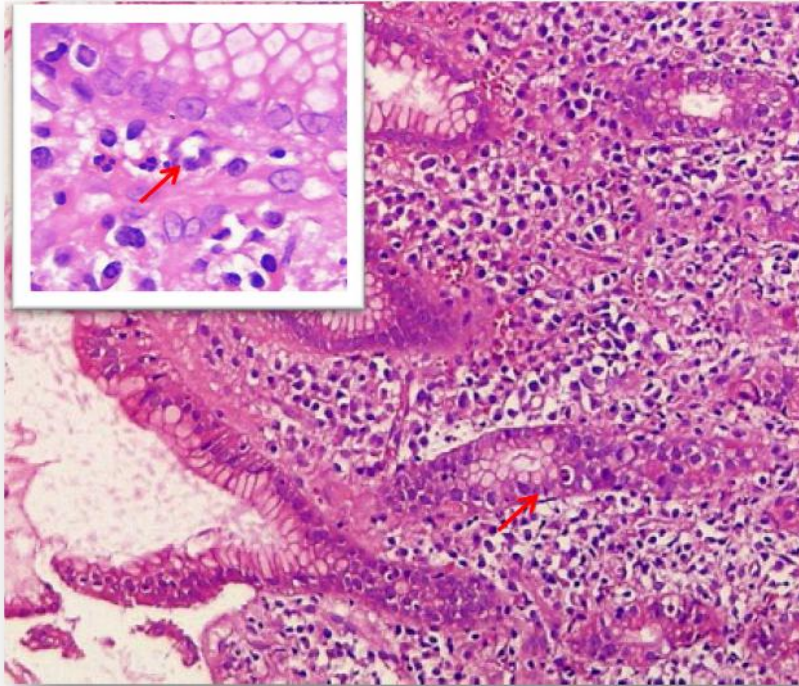


Figure 4. H&E (40x) . Moderate activity (arrow). Inset- Neutrophils entering the crypt

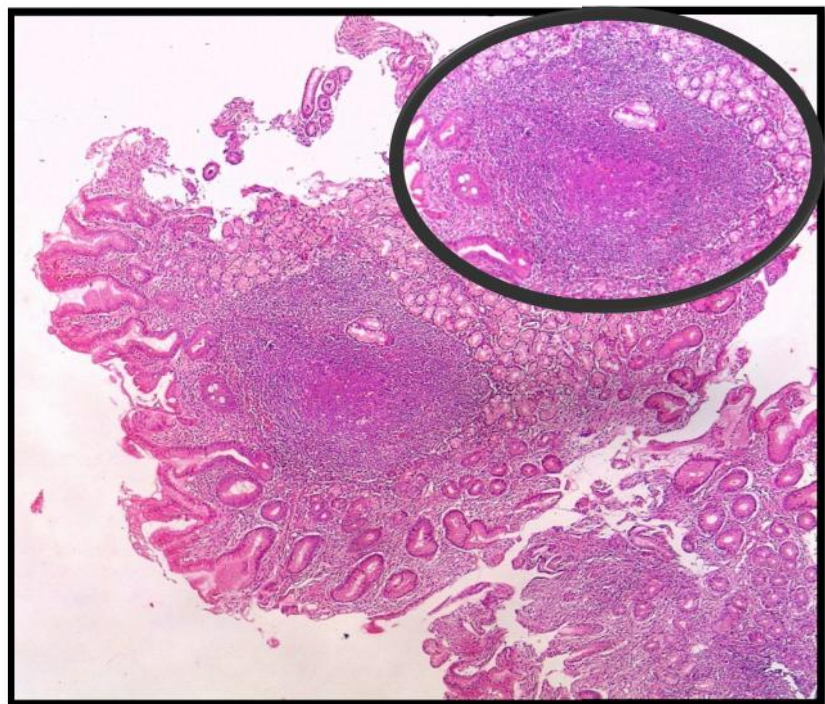


Figure 5. H&E Lymphoid follicles. Inset- lymphoid follicles with germinal centre.(higher magnification)

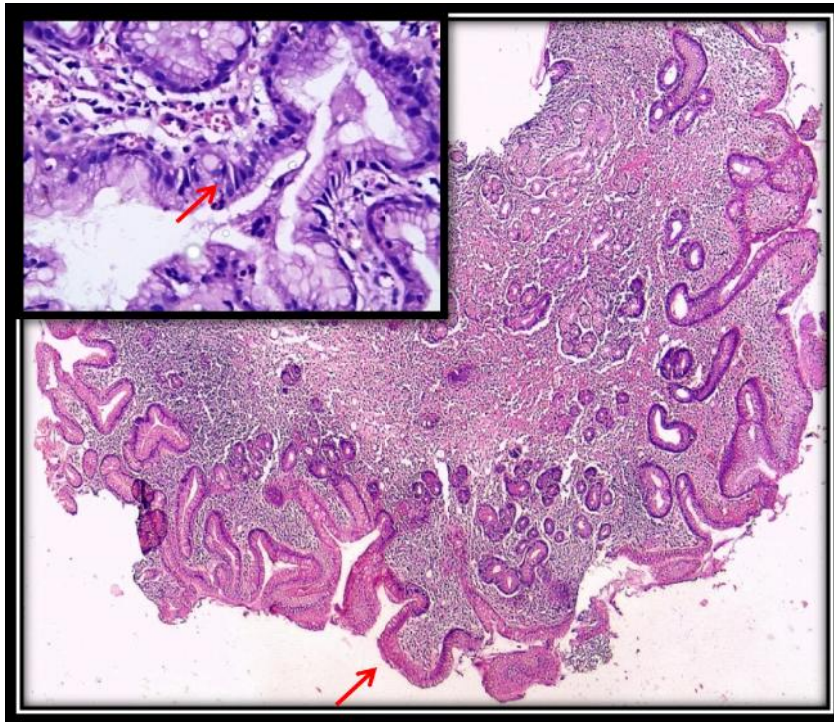


Figure 6a H&E 10x villiform transformation (arrow) . Inset shows intestinal metaplasia (arrow)

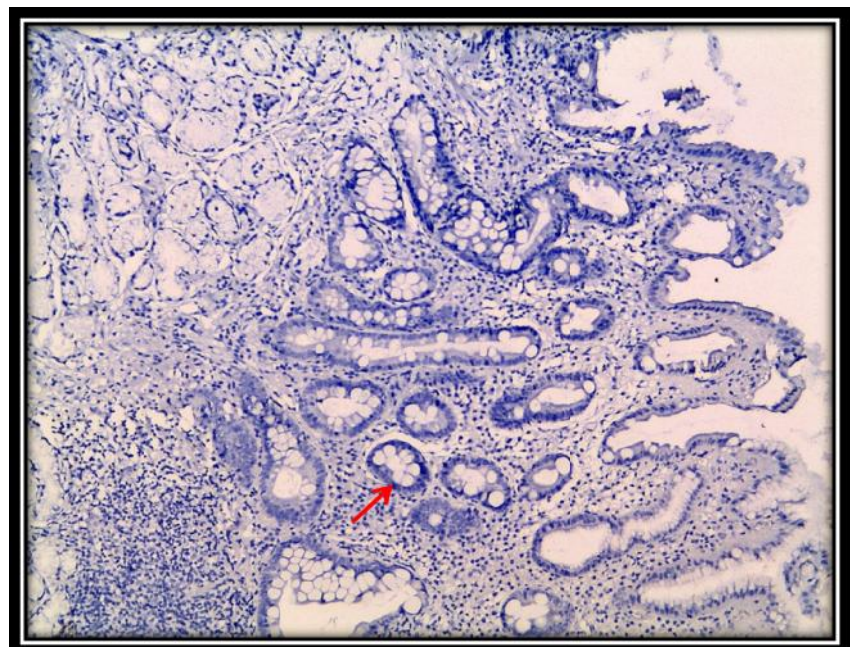


Figure 6b villiform transformation. Intestinal metaplasia (arrow)

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MASTER CHART

S.No	Number id	Age/Sex	Site of Biopsy	H & E	Mod. Giemsa	H.pylori Ab	Other findings
1.	S-4359	16/M	ANTRUM	H.pylori like organism+	+++ in lining epithelium	++++	Mild lymphoid follicle . Intestinal metaplasia
2.	S-4594	52/M	ANTRUM	H.pylori like organism+	++ on surface	++++	Lymphoid hyperplasia
3.	S-3012	56/F	ANTRUM	H.pylori like organism+	++	+++	Focal villiform transformation
4.	S-3476	18/M	ANTRUM	H.pylori like organism+	+ occasional H.pylori like organism	++	-
5.	S-3497	19/M	ANTRUM	H.pylori like organism+	++	++++	Focal villiform transformation / intestinal metaplasia
6.	S-4251	46/M	ANTRUM	H.pylori like organism+	+	+++	Lymphoid aggregates
7.	S-4798	48/F	ANTRUM	H.pylori like organism+	++	++	Intestinal metaplasia
8.	S-3555	48/F	ANTRUM	H.pylori like organism+	++	+++	Lymphoid follicles
9.	S-4675	41/M	BODY	H.pylori like organism+	+	+++	Lymphoid follicles
10.	S-5119	32/F	ANTRUM	H.pylori like organism+	++	++++	Lymphoid aggregates, intestinal metaplasia
11.	S-5222	38/M	ANTRUM	H.pylori like organism+	+++ on surface epithelium	++++	Intestinal metaplasia
12.	S-4571	24/F	ANTRUM	H.pylori like organism+	+ over mucus	+++	Lymphoid aggregates
13.	S-5145	28/M	BODY	H.pylori like organism+	+ occasional organism	+++	Intestinal metaplasia
14.	S-3335	34/M	ANTRUM	H.pylori like organism+	Vague positivity	++	Intestinal metaplasia
15.	S-4214	33/M	ANTRUM	H.pylori like organism+	+++	++	Lymphoid follicles
16.	S-4366	39/F	ANTRUM	H.pylori like organism+	++	++++	Lymphoid follicles
17.	S-5107	41/M	ANTRUM	H.pylori like organism+	+/-	++	Focal villiform transformation
18.	S-4872	43/F	ANTRUM	H.pylori like organism+	+/-	+	Lymphoid aggregates
19.	S-5090	43/M	ANTRUM	H.pylori like organism+	++ over surface	+++	Intestinal metaplasia
20.	S-4813	49/M	ANTRUM	H.pylori like organism+	+++ in gastric pits	+++	Lymphoid follicles

S.No	Number id	Age/Sex	Site of Biopsy	H & E	Mod. Giemsa	H.pylori Ab	Other findings
21.	S-4410	50/M	ANTRUM	H.pylori like organism +	+ over surface mucus	+++	Lymphoid follicles, Intestinal metaplasia
22.	S-3321	20/F	BODY	H.pylori like organism +	++ seen in gastric pits	+++	Lymphoid aggregates with moderate activity
23.	S-2942	24/M	ANTRUM	H.pylori like organism+	+++ over lining epithelium	++++	Focal villiform transformation
24.	S-2783	44/M	ANTRUM	H.pylori like organism+	+++ over lining epithelium	++ in pits	Mild activity
25.	S-5005	49/F	ANTRUM	H.pylori like organism+	++	+++	Mild activity, suggestive changes
26.	S-4537	41/M	ANTRUM	H.pylori like organism+	+++ over surface	++ scattered rods in the pits / debris	Intestinal metaplasia
27.	S-4867	43/M	ANTRUM	H.pylori like organism+	+ over gastric pits	++ scattered in surface epithelium	Lymphoid aggregates
28.	S-3538	46/M	BODY	H.pylori like organism+	++ over surface mucus	++	Moderate activity
29.	S-4548	49/M	ANTRUM	H.pylori like organism+	+++ over gastric pits	++	Focal intestinal metaplasia
30.	S-4422	50/M	BODY	H.pylori like organism+	++	++ scattered over surface	Lymphoid follicles in lamina propria
31.	S-4563	58/M	BODY	H.pylori like organism+	+++	+ seen over surface mucus	Intestinal metaplasia
32.	S-5227	60/M	ANTRUM	H.pylori like organism +	++	+++ in surface epithelium	Intestinal metaplasia, lymphoid aggregates
33.	S-4999	55/F	ANTRUM	H.pylori like organism+	++	+ over pits ++ in mucus	Focal Intestinal metaplasia
34.	S-4365	56/M	BODY	H.pylori like organism+	+	++	Focal villiform transformation, lymphoid follicles
35.	S-4895	57/F	ANTRUM	H.pylori like organism+	++	+++ in surface epithelium	Intestinal metaplasia, lymphoid aggregates
36.	S-3418	51/M	ANTRUM	H.pylori like organism+	+++	++++	Lymphoid aggregates
37.	S-3056	49/F	ANTRUM	H.pylori like organism+	++	+++ in pits	Focal villiform transformation
38.	S-2197	44/M	ANTRUM	H.pylori like organism+	+++	++ over surface	Lymphoid aggregates
39.	S-2739	46/F	ANTRUM	H.pylori like organism	++	+++	Lymphoid aggregates
40.	S-3488	44/M	ANTRUM	H.pylori like organism+	+	+++	Moderate activity, lymphoid aggregates

S.No	Number id	Age/Sex	Site of Biopsy	H & E	Mod. Giemsa	H.pylori Ab	Other findings
41.	S-4611	51/M	ANTRUM	H.pylori like organism+	+ over the surface	++ in pits	Foveolar hyperplasia, Mild activity
42.	S-4874	53/M	ANTRUM	H.pylori like organism+	+	++ in pits	Lymphoid aggregates in lamina propria
43.	S-3440	58/F	BODY	H.pylori like organism+	+ on surface epithelium	++ in pits	Lymphoid aggregates
44.	S-3524	59/F	ANTRUM	H.pylori like organism+	++	+++	Lymphoid follicles in lamina propria
45.	S-3067	60/M	ANTRUM	H.pylori like organism+	++ in pits	+	Intestinal metaplasia
46.	S-5062	61/F	ANTRUM	H.pylori like organism+	+++	+++	Intestinal metaplasia, Focal villiform transformation
47.	S-3562	40/M	ANTRUM	H.pylori like organism+	+++	+++ in pits	Lymphoid follicles in lamina propria
48.	S-3581	37/F	ANTRUM	H.pylori like organism+	++ over surface	+++	Focal Intestinal metaplasia
49.	S-5043	36/M	ANTRUM	H.pylori like organism+	++	+++	Lymphoid aggregates in lamina propria
50.	S-4836	35/M	ANTRUM	H.pylori like organism+	++	+++	Lymphoid aggregates
51.	S-4878	20/M	ANTRUM	H.pylori like organism+	+++	++ over debris	Mild activity
52.	S-3132	33/M	ANTRUM	H.pylori like organism+	+++	++	Mild activity
53.	S-3534	31/M	ANTRUM	H.pylori like organism+	++	+++	Lymphoid aggregates
54.	S-1077	41/M	ANTRUM	H.pylori like organism+	+++ over surface	+++	Lymphoid aggregates in lamina propria
55.	S-3555	48/M	ANTRUM	H.pylori like organism+	++	+++	Focal villiform transformation , moderate activity