COMPARATIVE STUDY OF HEMATOXYLIN & EOSIN, SPECIAL STAINS AND IMMUNOHISTOCHEMISTRY FOR DETECTION OF HELICOBACTER PYLORI IN BOTH NEOPLASTIC AND NON-NEOPLASTIC GASTRIC LESIONS

Dissertation submitted in partialfulfilment
of therequirements for the degree of

M.D. (PATHOLOGY)

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INSTITUTE OF PATHOLOGY
MADRAS MEDICAL COLLEGE
CHENNAI – 600 003

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI

APRIL 2015

CERTIFICATE

This is to certify that this dissertation entitled **"COMPARATIVE STUDY OF HEMATOXYLIN&EOSIN ,SPECIAL STAINS AND IMMUNOHISTOCHEMISTRY FOR DETECTION OF HELICOBACTER PYLORI IN BOTH NEOPLASTIC AND NON-NEOPLASTIC GASTRIC LESIONS**" is the bonafide original work of **Dr.L.R.UMASANKARI**, in partial fulfilment of the requirements for M.D., (Branch III) in Pathology examination of the TamilnaduDr.M.G.R Medical University to be held in April 2015.

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DECLARATION

I, **Dr.L.R.UMASANKARI,** solemnly declare that the dissertation titled "**COMPARATIVE STUDY OF HEMATOXYLIN&EOSIN ,SPECIAL STAINS AND IMMUNOHISTOCHEMISTRY FOR DETECTION OF HELICOBACTER PYLORI IN BOTH NEOPLASTIC AND NON-NEOPLASTIC GASTRIC LESIONS**" is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr.S.PAPPATHI,M.D.,** Professor of Pathology, Institute of Child Health , Madras Medical College. The dissertation is submitted to the TamilnaduDr.M.G.R Medical University towards partial fulfilment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr.L.R.UMASANKARI**

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INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

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CERTIFICATE OF APPROVAL

To
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PG in Pathology,
Institute of Pathology,
Madras Medical College, Chennai-3.

Dear Dr. L.R. Umasankari,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Comparative Study of H&E (Hematoxylin & Eosin) Special Stain and IHC (Immunohistochemistry) for detection of H.Pylori in both Neoplastic and Non-neoplastic Gastric Lesions"** No.30032014

The following members of Ethics Committee were present in the meeting held on 11.03.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr. C. Rajendran, M.D.	-- Chairperson
2.	Prof. Kalaiselvi, MD Vice-Principal, MMC, Ch-3	-- Member Secretary
3.	Prof. Nandhini, M.D. Inst. of Pharmacology, MMC, Ch-3.	-- Member
4.	Prof. Bhavani Shankar, M.S. Prof & HOD of General Surgery, MMC, Ch-3.	-- Member
5.	Prof. V. Padmavathi, M.D. I/c Directory of Pathology, MMC, Ch-3.	-- Member
6.	Thiru. S. Govindasamy, BABL	-- Lawyer
7.	Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

13/3/14

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Member Secretary, Ethics Committee

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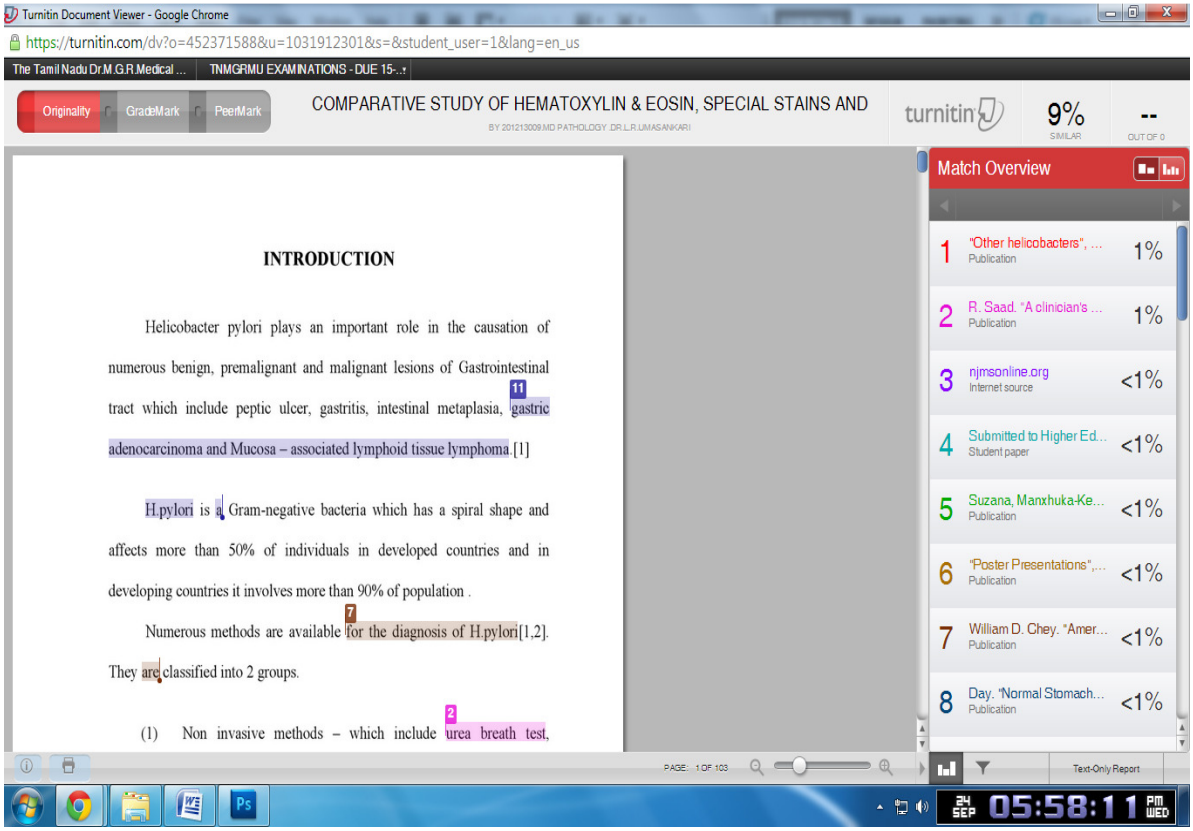
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INTRODUCTION
Helicobacter pylori plays an important role in the causation of numerous benign, premalignant and malignant lesions of Gastrointestinal tract which include peptic ulcer, gastritis, intestinal metaplasia, gastric adenocarcinoma and Mucosa – associated lymphoid tissue lymphoma.[1]
H.pylori is a Gram-negative bacteria which has a spiral shape and affects more than 50% of individuals in developed countries and in developing countries it involves more than 90% of population .
Numerous methods are available for the diagnosis of H.pylori[1,2]. They are classified into 2 groups.
(1) Non invasive methods – which include urea breath test,
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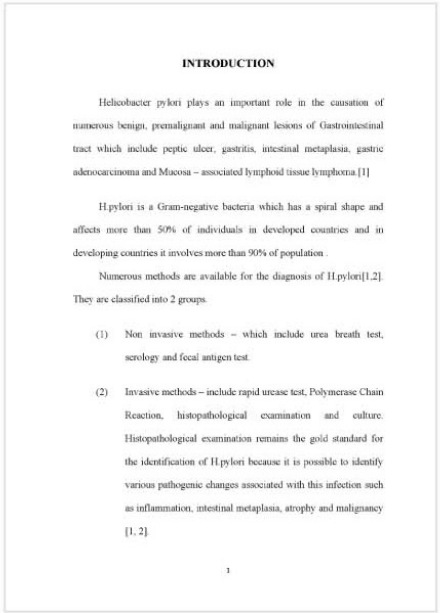

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(2) Invasive methods – include rapid urease test, Polymerase Chain Reaction, histopathological examination and culture. Histopathological examination remains the gold standard for the identification of H.pylori because it is possible to identify various pathogenic changes associated with this infection such as inflammation, intestinal metaplasia, atrophy and malignancy [1, 2].

1

ABBREVIATIONS

HPE	**-Histopathological Examination**
H&E	**-Hematoxylin& Eosin**
IHC	**-ImmunoHistoChemistry**
H.PYLORI	**-Helicobacter Pylori**
WHO	**-World Health Organisation**
PCR	**-Polymerase Chain Reaction**
Cag A	**-Cytotoxin - associated gene**
ELISA	**-Enzyme Linked Immune Sorbent Assay**
HRP	**-Horse Radish Peroxidase**
OMP	**-Outer Membrane Protein**
LPS	**-Lipopolysaccharides**
HPSS	**-Helicobacter Pylori Silver Stain**
IL-8	**-InterLeukin-8**

CONTENTS

COMPARATIVE STUDY OF HEMATOXYLIN & EOSIN, SPECIAL STAINS AND IMMUNOHISTOCHEMISTRY FOR DETECTION OF HELICOBACTER PYLORI IN BOTH NEOPLASTIC AND NON-NEOPLASTIC GASTRIC LESIONS

ABSTRACT

Aims and Objectives : The role of Helicobacter Pylori in the pathogenesis of Gastritis-Peptic ulcer syndrome and its association with the development of upper gastrointestinal tract malignancy warrant efficient method for the identification of the bacteria in biopsy specimens. Four staining methods - Hematoxylin and Eosin, Giemsa, Toluidine Blue and Immunohistochemistry were compared to detect the presence of Helicobacter pylori. The prevalence of H.Pylori in gastric biopsies was also evaluated.

Methods : A total of 70 cases (40 cases of gastritis and 30 cases of gastric adenocarcinoma) were randomly selected for this study and all the four stains were applied.

Results : Helicobacter pylori infection showed an overall prevalence rate of about 67.1%. When compared with Immunohistochemistry, Sensitivity and specificity of Hematoxylin and Eosin was 57.45% and 100% respectively. Giemsa showed sensitivity of about 87.23% and specificity of about 100%. Toluidine blue showed sensitivity of about 78.72% and specificity of about 100%.

Conclusion : Hence in the present study Giemsa was more reliable and cost effective stain when compared with Hematoxylin & Eosin, Toluidine blue and immunohistochemistry. However, Immunohistochemistry carries the highest level of sensitivity in the detection of Helicobacter Pylori especially when the density of organism is low and in clinically suspected cases of Helicobacter Pylori with negative Giemsa staining

Key Words : Helicobacter pylori, Hematoxylin & Eosin, Giemsa, Toluidine blue, Immunohistochemistry.

INTRODUCTION

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In 1994 (WHO) World Health Organisation and the International Agency for Research on Cancer (IARC) classified H.pylori infection as a group I carcinogen in humans [3].

The updated Sydney system graded gastritis based on several parameters which includes inflammation, activity, intestinal metaplasia, atrophy and density of H.pylori [4].

Several histochemical stains are available for detecting the presence of H.pylori in gastric biopsies and resection specimens which include Hematoxylin and Eosin (H&E), toluidine blue, modified Giemsa, Alcian yellow – toluidine blue, Warthin-starry, modified Genta and Immuno histochemistry staining. H&E and Giemsa are more commonly used. Several studies have been conducted about the need for use of special stains and immunohistochemistry in H.pylori detection [5].

The aim of the present study is to compare the efficacy of Hematoxylin and Eosin, Giemsa , toluidine blue and immunohistochemistry for the detection of H.pylori in cases of gastritis and gastric adenocarcinoma.

Aims and Objectives

AIMS AND OBJECTIVES

1) To evaluate the prevalence of H.Pylori in non-neoplastic and neoplastic gastric lesions.

2) Comparing the efficacy of Hematoxylin & Eosin, Special stains (Giemsa, Toluidine blue) & Immunohistochemistry for detection of Helicobacter pylori in non-neoplastic and neoplastic gastric lesions.

Review of Literature

REVIEW OF LITERATURE

EMBRYOLOGY

The stomach is a derivative of primitive foregut which is seen as a fusiform dilatation. During the late 5^{th} week of gestation, the stomach rotates about 90 degree along its longitudinal axis. This results in a position, that right side faces backwards and left side faces the front. During that period of rotation, there is rapid growth of posterior wall than the anterior which results in the formation of greater and lesser curvatures [6].

During the early stages the stomach is located in the midline. Later, there is mobilisation of caudal portion towards up and to right side becoming the antropyloric region. The cephalic portion undergoes rotation to the left and slightly downward forming cardia[6].

ANATOMY

Grossly the stomach consists of the following regions.

- Cardia – seen just distal to the gastroesophageal junction which is narrow and conical shaped.

- Fundus – It is the dome shaped portion of the stomach situated proximally.

- Body or corpus – It forms the portion of stomach situated proximal to incisura angularis.
- Antrum – Occupies the portion of stomach distal to incisura angularis.
- Pylorus – The narrowest portion of the stomach situated most distally.

Lesser curvature forms the superomedial margin and greater curvature forms the inferolateral margin. On the serosal side the junction between the antrum and corpus is noted by a notch called incisura seen in the lesser curvature. The mucosa is thrown into numerous folds termed as rugae.

MICROSCOPY [7]:

The mucosa of the body of stomach has an appearance of 'Moracco leather' under dissecting microscope because of uniform, closely packed regular papillae and at the apex of each has a circular opening of gastric gland.

The mucosa of the antrum has a coarser leaf-like pattern formed by the aggregation of numerous papillae which form each leaf . In scanning electron microscopy the mucosa of the body of stomach has a 'cobblestone' pattern [8].

The (foveolar) surface epithelium :

It is present uniformly throughout the stomach . It has a single layer of cells which are tall columnar with the nuclei basally located and secretes mucus. This epithelium lines the gastric pits and surface papillae.

Each cell shows the presence of microvilli on their luminal aspect which are small and pleomorphic of about 0.05 – 0.15 µm in diameter and 0.2 – 0.6 µm high. There is also a glycocalyceal coat [9].

The Cardiac Zone :

It is seen as a downward extension from the cardio-esophageal junction of about 5 – 30mm [10]. The mucosa of the cardia is called as junctional mucosa because it represents the anatomical boundary between stomach and esophagus. The glands of the cardia are usually branched , tubular and are separated by elongation of muscularis mucosae and by connective tissue [11]. The glands also show cystic dilatation and the deeper part of the mucosa shows the presence of lymphoid follicles. Endocrine cells and acid secreting cells are present few in number.

The body and fundus :

The superficial zone formed by gastric crypts which are lined by surface epithelium constitutes 25% of total mucosal thickness. The deeper zone is composed of straight tubules which are seen perpendicular to the

surface form the rest of 75% thickness. At the bottom of each crypt there is a constriction or neck where the glands open. The body of the stomach shows the presence of four types of cells – the parietal or Oxyntic cells, mucous neck cells, endocrine cells and chief or zymogenic cells [10,12].

Mucous neck cells :

These cells are mainly seen in the upper portion of tubules. They are low columnar and more triangular when compared to the surface epithelial cells which are high columnar in shape. These cells contain neutral mucin .

Some studies consider mucous neck cells as transitional cells, in gastric stem cells differentiation to chief or zymogen cells [13,14]. Mucous neck cells also play a role in the protection of gastric mucosa against gastric acid secretion by their production of numerous luminally active peptides.

Parietal, oxyntic cell / Acid secreting cells[7]

These cells line the upper portion of glands seen in the mucosa of the body of stomach. These cells secrete intrinsic factor , hydrochloric acid and blood group substances . The cells are round, large or pyramidal in shape, having eosinophilic or vacuolated cytoplasm and a central nucleus.

Pepsinogen – Secreting (Chief) Cells :

These cells line the deeper portion of the tubules and are more near the cardiac end of body mucosa . These cells are low columnar or cuboidal

and have neutrophilic cytoplasm. Pepsinogens I and II [15] and proteolytic proenzymes like lipase are produced by these cells.

Endocrine cells :

These cells are widely distributed in all portions of the stomach and are seen as clear round or halo cells between the epithelium and the glandular basement membrane. Enterochromaffin or argentaffin cells contain Serotonin. D cells are seen in the antrum and body which produces somatostatin.

Gastrin is produced by the G cells which are seen mostly in the middle and lower third mucosa of antropyloric region whose density decreases from pylorus to the body of stomach [16].

The antral and pyloric region :

The antral mucosa thickness varies from 200 to 1100 µm of which 40% is contributed by the surface pits. They show branching and are not perpendicular to the surface. The deeper zone is formed by coiled tubules with few show branching. The lining consists of mucin secreting cells which are faintly granular and the nucleus is basally located.

Pyloric mucosa contains occasional parietal cells and their number increases towards the gastroduodenal junction [17].

The Intermediate Zone :

In this zone, half of the mucosal thickness is occupied by the pits and shows the presence of both pyloric and cardiac glands.

Lamina propria :

It is formed by connective tissue network which contains lymphatics, blood vessels, cells of macrophage and immune system and nerve fibrils. Immunoglobulin A (IgA) containing plasma cells are seen more in the antral region [18].

Muscularis Mucosae :

The thickness of muscularis mucosae varies from 30 to 210 µm and does not show inflammatory cells normally.

Submucosa :

This is formed by loose connective tissue, scant adipose tissue and contains blood vessels ,lymphatics and ganglion cells.

Muscle Coats :

The stomach consists of three muscle coats. The inner circular layer covers the entire stomach and lies in continuation with that of esophagus. The outer longitudinal layer extends from esophagus to the duodenum. Oblique fibres which lie internal to the circular layer extend down from cardia and run parallel to lesser curve. At the pylorus the circular muscle

layer undergoes thickening which leads to the formation of proximal and distal loops that unite along the lesser curve [19,20] in a complex or torus.

Serosa :

This lies in continuity with the peritoneum and formed by loose areolar tissue in which lies blood vessels, lymphatics, ,nerve fibres and lined by flattened mesothelial cells.

DISCOVERY OF H.PYLORI [21]

In 1979, a pathologist RobinWarren, identified the presence of curved bacteria in gastric biopsies which were submitted for histopathological examination. These curved bacteria were seen in the surface mucus layer [22].

Barry Marshall and Warren isolated these organisms from biopsy specimens by inoculating them onto selective media and under microaerobic conditions the culture was incubated. After an incubation period of 5 days these colonies were identified [23] and named as campylobacter pyloridis.

After that, several investigations had confirmed the presence of H.pylori in the gastric mucus [24,25].

Marshall and Warren found the association between H.pylori infection and duodenal ulceration [23].

In 1994, it was proved in National Institute of Health Conference that H.pylori was the main etiological factor for peptic ulcer and the affected patients were recommended for treatment to eradicate H.pylori [26].

The association between H.pylori and gastric cancer was proved in 1991 [27,28].

In 1994, H.pylori was declared as a human carcinogen [29] by the International agency for cancer research.

MORPHOLOGY [21]

H.pylori is a microaerophilic, gram negative bacteria which is spiral shaped. In gastric biopsies they usually show blunt rounded ends [30]. H.pylori usually reveals a rod-like shape on culture with solid medium . Coccoid forms usually predominate in solid or liquid culture medium after prolonged incubation period [31]. Coccoid forms are seen as U-shaped structures on electron microscopy and a membranous structure usually connects the ends of both arms. H.pylori organisms are about 2.5 to 5.0 µm in length and width of about 0.5 to 1.0 µm. These organisms show four to six unipolar sheathed flagellae which helps in mobility. Each flagellum is about 2.5 mm thick and approximately 30 µm in length [32].

EPIDEMIOLOGY[33]

Several studies showed that the prevalence of infection with H.pylori is more common in developing countries when compared to that of developed countries.[34,35]

The prevalence rate also varies according to age, sex, race, geographic area, and socioeconomic status . In a study conducted at China, among 98 children it was found that more than 70% of children aged 5-6 years showed infection with H.pylori [36]. Similar infection rates were also seen in adults of that region [37]. This study revealed that most cases of H.pylori infection occur in early childhood life.

The prevalence of H.pylori is usually more in developing countries with a prevalence rate of 70% when compared to developed countries where the prevalence rate is about 40%.

In developed countries, the adult individuals show a low annual rate of seroconversion of about 0.2 – 1.0% .

Good sanitation and better hygiene are usually associated with low incidence of H.pylori infection in developed countries.

In a study conducted at United States, it was evident that H.pylori showed difference in distribution among various races. Whites showed lower seroprevalence of infection with H.pylori when compared to Blacks and Hispanics [38].

Another study at NewZealand [64] also showed the presence of ethnic differences in patients with H.pylori infection. It was more common in pacific islands, least common in Europeans and intermediate in Maori.

The variations in H.pylori prevalence among different races and ethinicity are commonly linked with difference in hygienic practices, socio economic status, or extensive antimicrobial usage during childhood for the treatment of common infections[39].

In a study conducted at United States, it was found that poverty, overcrowding, poor hygiene, residence at rural areas and lack of education were commonly associated with H.pylori infection.

In a study by Graham et al 1991 [40] at Texas it was evident that there was difference in distribution of H.pylori infection among blacks and whites races. Among 246 blacks 70% showed positivity for H.pylori by serology and among 239 whites 34% showed positivity.

In a study conducted at Bangladesh by Ahmad et al 1997 [41] it was found that among 181 outpatients in the age group of 20-44 years 92% showed positivity for H.pylori by serology.

RISK FACTORS [33]

Smoking :

The possibility of association between smoking and H.pylori infection has been assessed by several studies. Some studies showed that H.pylori seropositive individuals were mostly found to be current smokers [42,46] when compared with seronegative persons.

Many recent studies showed that the association of current smoking or tobacco use with H.pylori infection had no significance. [37,43,44,45].

Alcohol

Several epidemiological studies showed that there was no significant association seen between H.pylori infection and consumption of alcohol but many studies showed a nonstatistically significant rate of reduction in risk [43,44,45,46].

Alcohol consumption causes pH reduction resulting in an acidic environment of the stomach which favoures the survival of H.pylori .

Diet

Several studies have found the association between H.pylori and consumption of diet.

Fontham et al [46] and Goodman et al [47,48] found that there was low risk of infection with H.pylori in individuals who consume large amount of fruits and vegetables.

Goodman et al [47] found that high levels of beta-carotene had a protective role against H.pylori.

Jarojz et al [49] in his study showed that in chronic gastritis patients, after 4 weeks of treatment with Vitamin C, H.pylori was eradicated in 30% of individuals.

Begue et al [50] in his study at Peru stated that there was increased risk of H.pylori infection among individuals who consumed food from street vendors due to poor sanitary conditions.

Occupational Exposures

Lin et al [51] in his study found that the prevalence of infection with H.pylori was significantly higher in endoscopists (80%) when compared with dentists (21%).

The increased risk among endoscopists than dentists suggests that in H.pylori transmission, gastric mucus serves as a better medium for transmission than saliva [52].

Waterborne exposures:

Several studies in rural China, Colombia and LimePeru [37,48] revealed the possibility of association seen between water source and risk of infection with H.pylori.

Zhang et al [37] found that H.pylori showed increased seroprevalence (88%) among individuals who consumed water from surface sources when compared with individuals who consumed water from deep wells (73%).

Hygiene :

Several studies showed that poor hygienic practices were associated with increased risk of H.pylori infection mainly during childhood [45,48].

Overcrowding:

Several studies found the significance of association seen between H.pylori infection and crowded environment which increases the rate of transmission among family members [45,48].

Family History:

Brenner et al in his recent study [53] stated that there was increased risk of H.pylori infection among adults who had a parental history of carcinoma stomach than that of subjects without such history.

ROUTES OF TRANSMISSION [54]

The main route of transmission of H.pylori is by contact between person-to-person.

There are 3 possible ways of transmission of H.pylori from one individual's stomach to other individual [21].

(1) Iatrogenic

This is the most common route of transmission that occurs by the usage of endoscopies or tubes which come in contact with one individual's gastric mucosa that can be used for other patient .

Increased rate of infection was also seen in certain occupations like gastroenterologists and endoscopists [21,57].

(2) Fecal-oral

Among 407 children in the age group of 2 months to 12 years, a study was conducted at Peru which concluded that the prevalence rate of H.pylori

infection was higher in children who consumed municipal water when compared with children who consumed water in private wells [56].

Several studies found that in young infected children H.pylori was isolated from the feces [21,57].

Water contaminated with feces may also be a route of transmission.

(3) Oral-Oral

The possibility of spread of H.pylori by oral-oral route has been observed in sharing of same spoon by both the child and mother, intake of premasticated foods which is common among few ethnic groups [21,55] and rarely by aspiration of vomit.

PATHOGENESIS

The virulence of H.pylori depends on the following factors.

- Flagellae – This helps in the mobility of organisms in viscous mucus.
- Adhesins – This helps the bacteria to adhere to surface foveolar cells.
- Urease – This causes metabolism of endogenous urea and liberates ammonia and causes rise in gastric pH.
- Toxins – Play a main role in the development of ulcer and gastric cancer. The toxin mainly involved is Cytotoxin – associated gene (CagA).

Outer membrane protein :

Helicobacter pylori consists of five families of major outer membrane protein. The major family is formed by adhesins. The rest is constituted by iron transporters, porins, flagellum – associated proteins and proteins of unknown function. Lipopolysaccharides (LPS) and phospholipids are found on the outer membrane. H pylori consists of 4-6 sheathed flagellae which helps in their motility [58].

Adhesins :

The pathogenesis of H.pylori infection depends upon the type of strain, host and environmental factors. The presence of flagellae helps the organism to reach the gastric mucoid lining [59]. By means of chemotaxis H.pylori moves away from the acidic pH of the lumen and reach the surface of epithelial cells which have neutral pH [60]. The adherence to the epithelial cell is favoured by adhesins produced by the bacteria.

Urease :

Urease is produced in large amounts by Helicobacter pylori. The endogenous urea is metabolized by this urease which results in the formation of ammonia and carbondioxide. The ammonia reacts with water and forms ammonium and the remaining hydroxyl ions combines with carbondioxide and leads to the production of bicarbonate. This bicarbonate

causes neutralization of gastric acid. Urease enzyme has a major role in the survival of Helicobacter pylori in the acidic environment of stomach.

Helicobacter pylori also produces vacuolating cytotoxin A (VacA), proteases and phospholipases which cause epithelial cell damage [61].

Following attachment of Helicobacter pylori to the gastric epithelium, the cag expressed type IV secretion system "injects" their cell wall peptidoglycan which is an inducing agent of inflammation into the gastric epithelial cells. The cytoplasmic pattern recognition receptor Nod 1 senses these peptidoglycans and promotes inflammation [62] by the expression of cytokines.

Gastric epithelium expresses Interleukin-8 a chemokine which causes potent activation of neutrophils and mediates inflammation [63].

SYDNEY GRADING SYSTEM OF GASTRITIS

Gastritis can be classified into acute and chronic.

Chronic gastritis is further divided into non-atrophic and atrophic gastritis. Non-atrophic chronic gastritis is usually seen in H.pylori infection and atrophic gastritis is of autoimmune etiology. Helicobacter pylori also has a role in the etiology of multifocal atrophic gastritis.

Other forms of gastritis include

- Lymphocytic gastritis
- Radiation gastritis
- Reactive gastritis (chemical)
- Eosinophilic gastritis
- Non-Infectious granulomatous gastritis
- Infectious gastritis

In 1990, at the 9th World congress of Gastroenterology in Sydney, Australia a group of experts devised the Sydney system of grading and classification of gastritis.

In 1994 in Houston, Texas the Sydney system was updated by the experts [65]. Several histopathological variables were graded on a scale of 3. They are graded as mild, moderate and severe

Graded variables include

- Neutrophilic infiltration
- Mononuclear infiltration
- Helicobacter pylori density
- Atrophy
- Intestinal metaplasia and dysplasia

Biopsy Sites :[65,66]

According to Bayerdorffer E-oertil H.Lehn.N. et al, multiple biopsies are usually recommended for accurate detection of H.pylori. Biopsy sites include two corpus and two antral specimens.

Antral biopsies show a very low density of organisms in patients who are under treatment with proton pump inhibitors. In such cases, corpus biopsies are needed for demonstrating the presence of H.pylori infection .

Incisura angularis reveals a greater degree of intestinal metaplasia , atrophy and this is the site which is also more prone for malignant dysplasia. Hence additional biopsies are also taken from this site.

GRADED VARIABLES :

H.Pylori density :

For effective clinical management, it is essential to diagnose the existence of H.pylori in gastric biopsies. The variations in the density of H.pylori may have epidemiological importance and have an association with many diseases .

Polymorphonuclear neutrophil activity :

It measures the rate of acute inflammation. Tissue damage is usually caused by proteases and reactive oxygen species which are derived from neutrophils.

In H.pylori positive individuals neutrophils are seen with in the epithelium, in lamina propria and also in the foveolar lumen forming “pit abscesses”.

The severity of H.pylori infection and the level of mucosal damage usually correlate with the density of neutrophils in the epithelium. Neutrophil activity is a very useful indicator of H.pylori infection.The presence of neutrophils in post-treatment biopsies is highly suspicious of H.pylori infection. Here comes the use of immunostains or special stains for detecting H.pylori.

Chronic inflammation :

Scattered chronic inflammatory cells are normally seen in the gastric mucosa. In H.pylori infection there is increase in the density of chronic inflammatory cells which includes B-lymphocytes, CD4+ and CD8+ T lymphocytes, monocytes, eosinophils, plasma cells and mast cells.

It may take several years for the chronic inflammatory cells to disappear or become normal in gastric mucosa even after complete eradication of H.pylori .

Glandular atrophy :

Atrophy refers to the loss of gastric mucosal glands which leads to mucosal thinning and ultimately causes severe damage to the mucosa. Atrophy results from severe inflammation or follow ulceration or erosion of the mucosa. Another microscopic evidence of atrophy is the presence of intestinal metaplasia which replaces the antral epithelium.

Loss of secretion of acid leads to oxyntic mucosal atrophy followed by intestinal metaplasia and carries a higher level of risk for the development of gastric cancer .

Presence of severe atrophy in antral mucosa and its association with intestinal metaplasia is also more prone for gastric cancer .

Intestinal metaplasia :

This is more common in all forms of chronic gastritis. Intestinal metaplasia is classified into 3 types based on its glycoprotein content and morphology by using mucin histochemistry . Intestinal metaplasia carries increased risk for developing malignancy.

In a study conducted at Slovenia it was found that patients with type III intestinal metaplasia have 2.7 to 5.8 times more risk for gastric cancer development than with subjects having type I & II intestinal metaplasia .

NON-GRADED VARIABLES:

Surface epithelial damage, erosion and mucin depletion

These features are seen in cases of active H.pylori infection and associated with the risk of peptic ulcer which is related to production of cytotoxin. This epithelial damage occurs as a result of inflammation caused by neutrophilic infiltration which measures the "activity".

Lymphoid follicles :

The characteristic feature seen in H.pylori gastritis is the presence of lymphoid follicles with germinal centre formation .

If the follicles are irregular in shape and larger in size and show the presence of extensive mucosal infiltration by dense lymphocytic population one should consider the possibility of Mucosa associated lymphoid tissue (MALT) lymphoma .

Foveolar hyperplasia :

It is identified by the presence of tortuosity and increased length of the foveolae, depletion of mucin in cytoplasm and a relative increase in the size of nuclei. This occurs due to the effect of stimulation by cytokines or inflammatory mediators like transforming growth factor alpha (TFG α) or as a compensatory mechanism to excessive cell exfoliation.

Pseudopyloric metaplasia :

For accurate classification and localisation of atrophic gastritis it is necessary to distinguish between true antral glands and pseudopyloric glands in a biopsy specimen.

Pancreatic (acinar) metaplasia :

It is usually seen in association with chronic gastritis, Intestinal metaplasia and seen in 1 to 2% of gastric specimens .

Endocrine cell hyperplasia :

Several functional changes occur in cases of chronic gastritis which leads to endocrine cell hyperplasia. It is the most prominent feature seen in autoimmune atrophic gastritis.

In autoimmune gastritis patients, smaller percentage of individuals have the chance of progression to carcinoid tumor.

Sydney grading system of gastritis

Table-1

Features	**Grade**		
Chronic Inflammation	Mild	Moderate	Severe
Atrophy	Mild	Moderate	Severe
Activity	< 1/3 of Pits mild	1/3 to 2/3 moderate	> 2/3 Severe
Intestinal Metaplasia	Mild	Moderate	Severe
H.Pylori colonisation	< 1/3 of surface mild	1/3 to 2/3 moderate	> 2/3 Severe

Phenotypes of gastritis :[66,67]

It can be categorized as

Atrophic

Non-atrophic

Non-atrophic gastritis :

Antral predominant non-atrophic gastritis

This is the most common type of presentation of H.pylori gastritis in western world.

Characterised by

1) Absence of atrophy.
2) Antral inflammation ranges from moderate to severe.
3) Corpus may be mildly inflammed or normal.

Mostly these patients are asymptomatic and have estimated lifetime risk of about 20% for developing duodenal ulcer and minimal increased risk for the development of gastric adenocarcinoma [68] when compared with normal population.

Non-atrophic pangastritis :

In case of H.pylori infection, there will be presence of marked inflammation involving the entire stomach with minimal or no difference between corpus and antrum.

H.pylori is highly endemic in areas with poor sanitation and the affected individuals develop pangastritis which serves as a background for the development of atrophy [69].

Atrophic Chronic gastritis :

Gastric mucosal atrophy refers to the loss of appropriate glands [70]. Due to severe inflammation the glands are damaged and have undergone metaplastic change or replaced by connective tissue. Intestinal metaplasia is the most common transformation occurs in the glands but sometimes in oxyntic mucosa, pseudopyloric metaplasia occurs which consists of mucin-secreting antral glands.

Antrum restricted atrophic gastritis :

In this type the atrophic changes are mainly seen in the antrum. These atrophic and metaplastic changes occur as a result of consequence of past or present infection with H.pylori.

Biopsies usually reveal patchy metaplastic atrophy confined mainly to distal portion of mucin secreting mucosa and associated with moderate to severe inflammation. Corpus is usually normal or shows mild inflammation and absence of atrophic changes.

Corpus restricted atrophic gastritis :

In this type, atrophic – metaplastic changes are seen in the oxyntic mucosa without any associated atrophic changes in the antrum or distal stomach. It is mainly of autoimmune etiology and carries increased risk for

gastric cancer . Rarely it is associated with atrophy of antral mucosa arising from concurrent infection with H.pylori.

Multifocal atrophic gastritis :

It was previously called as Environmental chronic atrophic gastritis.

It is more common in individuals living in poor sanitary conditions such as Latin America, Southern Asia, Eastern and southern Europe [71].

Both the antral and corpus mucosal biopsies show the presence of atrophic and metaplastic changes.Oxyntic mucosa reveals severe inflammation. Atrophic gastritis is considered as a major risk factor for the development of intestinal type adenocarcinoma, gastric non-invasive neoplasia and gastric ulcer .

Atrophic pangastritis :

It represents the advanced stage of multifocal atrophic gastritis and carries increased risk for the development of both noninvasive and invasive gastric neoplasia .

Khan MQ et al (1999) conducted a study for identifying H.pylori in cases of gastroduodenitis and nonulcer dyspepsia and found that 74% of

individuals were positive for H.pylori in cases of non-ulcer dyspepsia and 68% in cases of gastroduodenitis [72].

In a study conducted by kalebi A et al (2007) it was found that the major cause of gastritis was H.pylori infection [73] and was common in the antrum with evidence of chronic inflammation in 98% cases and neutrophilic infiltration in 91% of cases.

Yamaoka et al (1997) graded the density of H.pylori in gastritis cases based on the Sydney system [74]. It was graded as absent, scanty, moderate and heavy colonisation.

In his study Warren JR (2000) found that H.pylori plays a major role in the etiology of non-erosive nonspecific gastritis [75].

KOJK et al in his study [76] found the association between cigarette smoking and intake of alcohol with peptic ulcer. Intake of alcohol is related with increased risk of chronic gastritis and these are mainly linked to the inflammatory changes caused by concurrent infection with Helicobacter pylori. Chronic alcoholism also affects normal gastric mucosal barrier and associated with gastric metaplasia.

Adisa et al [77] conducted a retrospective study of 603 antral biopsies. These antral biopsies were stained by different methods which

include Hematoxylin and Eosin method, Grocott's modification of Hexamine silver method and Giemsa method. 572 (94.9%) patients showed the presence of gastritis with highest age incidence seen among 31 – 40 years (24.8%) H.pylori infection was seen in 345 (57.2%) cases and the peak incidence was seen in the age group of 41 – 50 years (26%). Early detection and eradication of H.pylori were effective in the prevention of gastric cancer.

Chow JY et al [78] assessed the effect of cigarette smoking in relation to healing of gastric ulcer. Cigarette smoking causes increased production of nitric oxide, leukoterines and enhances the activity of Xanthene oxidase. It also leads to decreased proliferation of epithelial cells, lowers the blood flow, blood vessels formation and the production of prostaglandins. These are the factors which are essential for formation and healing of ulcer. This proves the harmful effects of cigarette smoking on gastric mucosa.

Rajeshkumar et al [79] conducted a study on 265 patients for the detection of H.pylori by using histopathological examination and urease test. Among 265 cases, 92 patients (34.71%) showed positivity for H.pylori. Out of this 92 patients there were 59 males and 33 females. In H.pylori infected individuals, the minimum age of positivity was 18 years and the maximum age was 74 years. The highest incidence was seen in the age group of 36 – 45 years.

In endoscopy and histopathological examination the features seen were chronic superficial gastritis in 87 patients which was the most common presentation. 8 patients showed features of esophagitis and 11 showed duodenitis. 4 Patients showed the presence of duodenal ulcer and chronic gastric ulcer in 2 patients. Multiple changes were seen in the same patients.

The infection rate was higher (32/92) among individuals living under poor sanitation, high density or overcrowding, low socioeconomic status. Anti-H.pylori treatment was started in all the positive individuals.

Riba et al [80] and colleagues in their study compared the effectiveness of immunohistochemical technique by using polyclonal and monoclonal antibody for detecting Helicobacter pylori. 300 H.pylori positive cases of gastritis were studied by using these antibodies. Monoclonal antibody identified 96.2% of cases and 98.5% cases were detected by polyclonal antibody method. They also compared these 2 methods for better morphology of organism and background staining. Better preservation of morphology of organism and low level of background staining were seen in new H.pylori monoclonal antibody when compared with the polyclonal antibody.

In a study by Kacar et al [81] he included 60 H.Pylori positive and 10 H.pylori negative cases. These cases were tested by using urea breath tests,

histopathological examination, urease test for H.Pylori. Further these tissue sections were submitted for different staining methods like Hematoxylin and Eosin (H&E), modified Giemsa, Toluidine blue and immunohistochemistry. Using double blinding method these samples were assessed by pathologists. Using Kappa statistics the interobserver variations were analysed. It was evident from this study, demonstration of H.pylori on tissue sections was possible regardless of the stain performed. Modified Giemsa stain and Immunohistochemistry are considered as the best methods. The reliability, cost and the applicability of Giemsa stain makes it as the best choice in the detection of H.pylori on gastric biopsies.

Other types of gastritis :[82]

Acute gastritis :

The common etiological factors include ingestion of salicylates, alcohol, bile reflux or intake of anti-inflammatory drugs. Also known as chemical or reactive gastropathy .Microscopy reveals no significant inflammation but foveolar and glandular lumina show some degree of infiltration by neutrophils.[82]

Hemorrhagic gastritis :

It is seen in the setting of chronic gastritis and manifests as a severe life threatening condition. The precipitating factors include alcohol, stress,

anti-inflammatory drugs and cytomegalovirus infection . Microscopy shows the presence of chronic atrophic gastritis.[82]

Lymphocytic gastritis :

It is characterized by the presence of increased intraepithelial lymphocytes seen in the surface and foveolar epithelium. It is commonly associated with H.pylori infection or celiac disease and resembles lymphocytic colitis .

Collagenous gastritis :

Presence of thick band of collagen in the subepithelium is the characteristic feature and eosinophils constitute the major inflammatory infiltrate in the gastric mucosa.

Granulomatous gastritis :

Commonly seen in cases of Tuberculosis , Crohn's disease , mycosis and sarcoidosis [82].

Allergic gastroenteritis :

Associated with degenerative and regenerative changes in the foveolar and surface epithelium .Microscopy shows the presence of eosinophils in the lamina propria.[82]

GASTRIC CARCINOMA

Gastric cancer was the second most common cancer in the world. 60% of cases were usually seen in developing countries [84].

Location :[83]

- The most common location is the antropyloric region.
- Also occurs in the corpus or body of stomach along greater or lesser curvature.

H.pylori infection :[83]

It plays a main etiological role in the development of gastric adenocarcinoma. Several studies emphazise that there is increased risk in patients who had anti-H.pylori antibodies in stored serum samples 10 or more years before the diagnosis of cancer [85,86]. H.pylori causes several phenotypic changes which leads to the development of adenocarcinoma. These changes includes mucosal atrophy , intestinal metaplasia and dysplasia .In cases of gastritis and atrophy, there is elevation of gastric pH which alters the bacterial flora results in colonisation of stomach by anaerobic bacteria which produces active reductases. These active reductases convert food nitrate into nitrite which reacts with amines, amides and urea which leads to the production of carcinogenic N-Nitroso compounds . H.pylori genome is heterogenous and infections with strains

having cag group of genes [87] exhibit strong association with the development of gastric carcinoma . The mechanism commonly involved is epithelial production of interleukin 8 via nuclear factor kappa B pathway.

H-pylori also produces vac A which is a vacuolating cytotoxin which plays a role in gastric carcinogenesis and also causes epithelial cell damage. Inoculation of cag and vac A positive strain in Mongolian gerbils [88] produces intestinal metaplasia and gastric carcinoma which confirms the role of H.pylori in gastric carcinogenesis.

Excessive cell proliferation is usually seen in H.pylori infected individuals. Eradication of H.pylori results in decreased cell proliferation which supports the mitogenic influence of H.pylori on gastric epithelium. Due to potent urease activity exhibited by H.pylori there is release of ammonia which increases cell replication.

Ascorbic acid is an antioxidant which has anticarcinogenic role. It acts by preventing oxidative DNA damage. Intragastric concentrations of ascorbic acid are usually lower in H.pylori infected cases than in non-infected individuals and its level increases to that of non-infected persons after H.pylori treatment .

Dietary factors[83]

Excessive salt intake increases the risk of gastric cancer . Adequate intake of vegetables and fresh fruits are associated with low risk of gastric cancer development .Pickled vegetables, smoked meat or fish are associated with high risk of gastric cancer.

Bile reflux :

The risk for gastric carcinoma is more after 5 – 10 years in case of individuals who underwent Bilroth II operation which causes increase in bile reflux.

Association between Gastric Carcinoma and H.pylori infection :

Yokota et al [89] in his study established the presence of infection with H.pylori in Mongolian gerbils. These animals develop intestinal metaplasia and severe gastritis [89,90].

Watanabe et al [88] in his study found that 30% of Mongolian gerbils infected with H.pylori developed adenocarcinoma. Mostly these tumors were well differentiated adenocarcinoma – Intestinal type.

Sugiyama et al [91] in his study he assessed the carcinogenic effect of H.pylori. The carcinogenic effect of known carcinogens like N-methyl-N-nitrosourea when present in low levels, is enhanced by H.pylori. For H.pylori infected animals, low dose N-methyl-N-Nitrosourea was added in

drinking water for a period of 6 weeks. Adenocarcinoma was developed in 33% of infected animals.

Uemura et al [92] in his study documented that the risk of developing recurrent adenocarcinoma is lowered in individuals in whom H.pylori eradication had been done after mucosal resection of carcinoma done during a screening program.

Hansson et al [93] and parsonnet et al found that duodenal ulcer affords protection from gastric cancer.

Crabtree et al [94] in his study showed the presence of IgG antibody specific to H.pylori in 70% of persons with gastric cancer.

In a prospective study by Uemura and okamoto [95] among 132 cases of early gastric cancer eradication of H.pylori was done in 50% of patients at the time of resection of early gastric cancer. After 2 years it was found that the recurrence of adenocarcinoma was significantly lower than control group in whom H.pylori eradication was not done.

Oda et al [96] in his study showed the role of p53 gene mutation as a causative or associative factor in the etiology of gastric cancer associated with H.pylori infection.

Murakami et al [97] found the presence of p53 gene mutations in 53% of human mucosa infected with H.pylori and in 100% of infected monkey mucosa.

Hoshi T et al (1999) in his study, found that in H.pylori associated chronic gastritis there was increased cell proliferation in response to cell injury. Incomplete intestinal metaplasia and increased proliferative activity result in instability of DNA and leads to the development of gastric adenocarcinoma – intestinal type in the mucosa of individuals infected with H.pylori. So, H.pylori eradication not only has a substantial role in the treatment of gastric ulcers and gastritis but also prevents the development of intestinal type gastric adenocarcinoma [98].

In a study by Kusters JG et al (2006) in developed countries, 60 to 80% of gastric carcinomas occur as a result of long term consequence of infection with H.pylori. In case of H.pylori infection, there is excessive production of reactive oxygen species due to ongoing inflammation which causes DNA damage and initiates the cascade of development of gastric cancer. It was estimated that there was tenfold increased risk of gastric cancer development in H.pylori infected Individuals and H.pylori was declared as class I human carcinogen by the WHO [99].

Histopathology of Gastric adenocarcinoma :[83]

WHO Classification :

Tubular adenocarcinoma :

-Tumor cells are arranged in the pattern of branching tubules. The cells are cuboidal, columnar or flattened due to the presence of intraluminal mucin.

- Cells exhibit varying degree of cytological atypia .

- When prominent lymphoid stroma are seen in these tumors they are termed as carcinoma with lymphoid stroma or medullary carcinoma. Solid carcinoma refers to the poorly differentiated variant.

Papillary adenocarcinoma :

This type shows the presence of papillary processes which are finger like elongated structures with fibrovascular core. These papillae are lined by cuboidal or cylindrical cells. The tumor also shows the presence of acute and chronic inflammatory cells. Tumor cells show varying degree of atypia.

Mucinous adenocarcinoma :

- Extracellular mucin pools should occupy more than 50% of the tumor.

- Tumor cells may be arranged in glandular pattern where the cells lining the glands are mucus secreting columnar. Interstitium also shows the presence of mucin.
- Tumor cells also seen in dyscohesive clusters floating in pools of mucin.

Signet ring cell adenocarcinoma :

Tumor cells containing intracytoplasmic mucin should constitute more than 50% of tumor which may be arranged in small clusters or singly scattered. The morphology of tumor cells is of 5 types.

1) Nuclei seen pushing against the cell membrane giving the appearance of signet ring and the cytoplasm is optically clear, globoid. The mucin is of acidic nature which stains with Alcian blue at pH 2.5.

2) Cells resembling histiocytes with central nuclei are seen in cases of diffuse carcinoma.

3) The cells are small with deep eosinophilic cytoplasm, the cytoplasm shows the presence of granules with neutral mucin.

4) Cells which are small with scant or no mucin.

5) Anaplastic cells with scant or absence of mucin.

The tumor is constituted by varying proportions of these cells.

Lauren Classification :[83]

The Lauren Classification has evaluated the association of gastric cancer with incidence trends, environmental factors and its precursor lesions. It also evaluated the history of carcinoma stomach.

This consists of

- Intestinal type
- Diffuse type.

Tumors which contain both the components in equal proportions are termed as mixed carcinomas.

Indeterminate category includes the tumor that do not fit into either of these two categories.

Intestinal Carcinoma :

Intestinal metaplasia serves as a background for the development of this type of carcinoma. It consists of recognisable glands that show varying degree of differentiation ranges from well to moderate.

Diffuse Carcinoma :

This shows the presence of poorly cohesive tumor cells and absence of glandular formation. Cells are small and round scattered singly or in dyscohesive clusters. Mitotic rate is lower than in intestinal tumors. There is evidence of increased desmoplasia and the inflammatory changes are minimal.

Grading :[83]

Well differentiated :

It is composed of well formed glands which have the appearance of metaplastic intestinal epithelium.

Moderately differentiated :

It forms the intermediate category between well and poorly differentiated forms.

Poorly differentiated :

It is composed of tumor cells that are scattered singly or seen in small groups with mucin secretions. Glandular structures are rarely seen.

Well and moderately differentiated tumors are considered as low grade and poorly differentiated tumors as high grade.

Other Clinical outcomes associated with H.pylori[1]

Duodenal and gastric ulcer :

H.pylori is found to be associated with 90% of duodenal ulcers [101]. In H.pylori negative individuals other causative agents like nonsteroidal anti-inflammatory drug usage and Zollinger-Ellison syndrome are considered .

In tropical countries, most gastric ulcers are associated with infection with H.pylori . In cases of duodenal ulcer, the cytotoxin cagA produced by H.pylori causes more severe inflammation .

Some studies assessed the major causative role of H.pylori in peptic ulcer [102] and all patients of peptic ulcer should be screened and the affected individuals are treated with antimicrobial agents .

Recurrence rate is more common of about 90%, if the organisms persist and complete cure of the ulcer is possible with effective treatment with antibiotics [103,104,105,106].

Lymphoma :

Wotherspoon et al in his study found that H.pylori was positive in 92% of 110 mucosa associated lymphoid tissue (MALT) lymphoma cases when compared with 50% of control groups.

Further studies suggested that the pathogenesis of these tumors was greatly influenced by the continuing antigenic stimulus of H.pylori and effective treatment of this infection causes tumor regression [107].

The German MALT – lymphoma study group also concluded that complete eradication of H.pylori has caused apparent cure in 50% of cases of MALT lymphoma.

Esophageal diseases:

Many studies found that there was inverse association seen between the H.pylori infection with CagA+ strains and Barretts esophagus and adenocarcinoma of esophagus [108,109,110].

The risk of development of gastroesophageal reflux disease was doubled in cases of patients with duodenal ulcer after H.pylori eradication .

The colonisation of H.pylori is usually lower in patients with gastroesophageal reflux disease when compared with the control group [111].

H.pylori colonisation for a long period of time in the stomach decreases the acidity of the stomach which may plays a role in the protection of esophagus.

Asthma and related disorders :

Reibman and colleagues [112] found an inverse relationship seen between infection with H.pylori and the development of eczema, allergic rhinitis, skin allergies and asthma.

Idiopathic thrombocytopenic purpura :

Numerous reports from East Asia during the past 10 years showed that there was significant epidemiological association seen between the diagnosis of idiopathic thrombocytopenic purpura (ITP) [113,114] and H.pylori infection. So, patients of ITP are now evaluated for H.pylori infection and if it is found to be positive, eradication of H.pylori is now considered as one of the treatment modality.

Diagnosis of H.pylori :[1,2]

The diagnostic tests are categorized into 2 types.

1. Endoscopic tests
2. Non endoscopic tests

Endoscopic tests:

These include four diagnostic methods based on biopsy.

- Rapid urease testing
- Histology
- PCR
- Culture

Rapid urease testing:

This test depends upon the urease activity of H.pylori organisms. In this method, gastric biopsies are placed into a medium of agar gel or on a reaction strip which contains urea, pH indicator and a buffer.

In the presence of organism, urea is metabolized by H.pylori's urease activity which results in the formation of bicarbonate and ammonia. This leads to increase in pH which causes colour change in pH sensitive indicator and confirms the existence of active infection due to H.pylori.

The sensitivity of the test can be reduced upto 25% by the use of medications such as proton pump inhibitors, antibiotics and bismuth containing compounds which decrease the urease activity and density of H.pylori.

Several studies found that the negative predictive value and sensitivity of rapid urease test were decreased due to the presence of active bleeding from ulcer at the time of diagnosis [116,117].

Histology:

Several studies found that histopathological examination remains the gold standard method for H.pylori identification [118].

The major advantage of histology when compared to other methods is that it is possible to identify several pathological changes which are diagnostic of H.pylori infection. These changes include atrophy, inflammation, intestinal metaplasia and malignancy .

Certain medications such as proton pump inhibitors, bismuth and antibiotics affect the sensitivity of histology .

As the H.pylori density in stomach is usually low in cases of medications, multiple biopsies are usually taken for exact diagnosis. Multiple biopsies are taken from sites including angularis, greater curvature of antrum and greater curvature of corpus [118].

Culture:

This method is highly specific for H.pylori detection but the sensitivity is low when compared to histology or rapid urease kit .

The major advantage of culture is that in addition to identify the organisms it also finds the sensitivity of organisms to antimicrobials .

Polymerase chain reaction:

This method is highly sensitive and more specific than other diagnostic methods. In a study on chronic gastritis Polymerase Chain

Reaction (PCR) detected H.pylori infection in 20% of cases whose gastric biopsies revealed no organisms by histology .

Polymerase Chain Reaction also plays a role for detecting mutations seen with antimicrobial resistance [119]. It provides a best tool for typing of organisms and testing of virulence of organisms [120].

Non-Endoscopic diagnostic methods :

These include

- Antibody tests
- Urea breath tests
- Fecal antigen test

Antibody tests :

This test detects the presence of IgG antibodies specific to H.pylori in whole blood, serum or urine. These antibodies are usually seen approximately after 21 days of infection and persist for longtime even after eradication [121]. These antibodies can be assessed by both qualitative and quantitative methods. Quantitative methods include Enzyme – linked immunosorbent assay (ELISA) and latex agglutination tests. Qualitative assessment done by using office based kits.

Antibody tests have a limited role in eradication therapy as these antibodies remain positive for longtime even after successful eradication of infection [121].

Urea Breath tests :

This test identifies the organism by means of H.pylori urease activity. After ingestion of urea labeled either with radioactive isotope ^{14}C or non-radioactive isotope ^{13}C in the presence of organisms, there will be production of labeled carbondioxide which is measured in expired breath [2,122].

In pregnant women and children ^{13}C test is mostly preferred [122]. The sensitivity of the test is affected by certain medications like antibiotics, proton pump inhibitors and bismuth containing compounds. It is generally recommended that proton pump inhibitors should be withheld for 7-14 days, antibiotics and bismuth to be withheld for atleast 28 days prior to the urea breath tests [115].

Fecal Antigen Test :

This test identifies the presence of H.pylori antigen in stool by means of enzyme immunoassay using polyclonal anti-H.pylori antibody. Several studies found that fecal antigen test may be useful in confirming eradication during the early period itself where it is seen after 14 days of treatment

[123,124]. Usage of antibiotics, bismuth compounds and proton pump inhibitors affect the sensitivity of the test .

Treatment

Indications :[1]

-In case of peptic ulcer patients who are positive for H.pylori, a significant reduction in the recurrence rate of ulcer was seen after eradication of H.pylori .

- In duodenal ulcer cases antimicrobial therapy is now considered as the major form of treatment . Similar form of therapy is also indicated in cases of H.pylori associated gastric ulcers.

-In cases of gastric MALTomas, eradication of H.pylori causes regression of tumor .

-H.Pylori eradication therapy is also useful in cases of Idiopathic thrombocytopenic purpura .

Some studies [125,126] suggested that the H.pylori infection has a protective role against diarrheal diseases and eradication of H.pylori increases the rate of morbidity and mortality among children in developing countries.

Treatment Regimens [1]

The most common treatment regimens used in H.pylori infections are

Quadruple therapy:

Proton Pump inhibitors twice daily+

Tetracycline 500 mg. thrice daily+

Metronidazole 500 mg. 3 times a day+

Bismuth for a period of 10 days.

Proton pump inhibitors (PPI) triple therapy:

Proton pump inhibitors twice daily+

Clarithromycin 500 mg. two times daily+

Amoxicillin 1 g. two times daily

for a period of 7 to 10 days

Levofloxacin triple therapy:

Proton pump inhibitors two times daily+

Levofloxacin 500 mg. two times daily+

Amoxicillin 1 g. two times daily

for a period of 10 days

Sequential therapy:

Proton pump inhibitors twice daily+

Amoxicillin 1 g. twice daily

for a period of 5 days which is followed by

Proton pump inhibitors twice daily+

Tinidazole 500 mg. twice daily+

Clarithromycin 500 mg. twice daily

for a period of 5 days

Rifabutin triple therapy:

Proton pump inhibitors twice daily+

Rifabutin 150 – 300 mg/day+

Amoxicillin 1 g. twice daily

for a period of 10 days

These agents are potent inhibitors of urease enzyme and also have a direct inhibitory effect on H.pylori .

Gastric hormones such as ghrelin and leptin are affected by eradication of H.pylori which have an effect on satiety and appetite .

Predictors of H.pylori treatment outcome:[2]

The major factors involved in treatment failure are antibiotic resistance and poor patient's compliance.

Dietary factors, consumption of alcohol and smoking also affect the successful eradication of H.pylori .

H.pylori treatment is usually associated with minimal side effects

- Diarrhea and headache are the common side effects seen with Proton pump inhibitors.
- Clarithromycin therapy causes alteration in taste sensation, diarrhea and gastro intestinal symptoms.
- Gastrointestinal upset, nausea, darkened stool, darkening of the tongue are the side effects associated with bismuth therapy.

In a study conducted at United States during the period of 1993 to 1999, H.pylori showed varying rates of antibiotic resistance of about 10% for clarithromycin, 37% for metronidazole, 3.9% for both drugs, 1.4% for amoxicillin [127].

Consequences of longterm Helicobacter infection :[7]

In H.pylori infection the density of bacteria and the severity of inflammation are seen more in the antrum. In case of long standing infection, there is involvement of both antrum and corpus which lead to development of pangastritis.

Some studies suggested that the intensity of inflammation may decrease over a long period of time [128].

Several follow up studies [128,129] showed that in long standing cases, approximately 10% of patients showed spontaneous regression of chronic gastritis. This may be linked to antibiotic therapy taken for other diseases or effective host defence mechanisms.

Other consequences include peptic ulcer, atrophic gastritis, intestinal metaplasia, gastric cancer and lymphoma.

H.Pylori may rarely cause mucosal fold enlargement in the body of stomach. This gives the appearance of hypertrophic gastritis in both radiology and endoscopy. This giant fold gastritis causes several parietal cell changes both histologically and ultrastructurally. These changes include formation of intracytoplasmic vacuoles, canalicular dilatation, shortening and loss of microvilli. Eradication of H.pylori infection causes reversal of these changes to normal .

IMMUNOHISTOCHEMISTRY

Immunohistochemistry (IHC) involves the application of both Immunology and histology. It is mainly used to find the expression of specific antigen and to locate its exact micro anatomical position in the tissue. The cells exhibit antigenic differences which are distinguished by the use of specific antibodies in IHC. The lineage of cell population and the presence of cells that are biologically distinct with in the same lineage are specifically identified by these antigenic differences.

The history of IHC was established in 1940 by COONS who developed an immunofluorescence technique which identifies in frozen sections the presence of specific antigens.

In 1974, the antigens seen in tissues that are routinely processed are demonstrated by Taylor and colleagues.

In 1991, SHI and colleagues established the antigen retrieval technique.

In antigen retrieval method before staining for IHC, the paraffin sections are heated at high temperature which involves simpler technique.

IHC involves the use of antibody which depends upon the specificity and sensitivity of antigen - antibody reaction.

Blocking non-specific background staining :

Background staining occurs because of endogenous enzymes or due to non-specific binding. By means of preincubating sections on optimal working dilution with same species serum, the non-specific binding with polyclonal primary antibody can be reduced.

Peroxidase is an endogenous enzyme which is present in neoplastic and normal tissues. These enzymes are blocked by peroxidase blocking or other methods like Immunogold technique.

There are several methods which are used to block the activity of these endogenous enzymes. Addition of Levamisole in a concentration of 0.1M to enzyme substrate solution blocks the endogenous alkaline phosphatase. Other method is that at room temperature incubation in methanol which contains 0.5% hydrogen peroxide for a period of 10 minutes completely abolishes the endogenous activity of peroxidase.

Detection Systems :

For exact visualisation of the antibodies they are labeled by certain substances which include enzymes which form coloured reactions when combine with suitable substrate (Light Microscopy), fluorescent substances or heavy metals (Electron Microscopy).

IHC methods :

Direct labelling method :

In this method antibody is applied directly to tissue sections and a label is attached by chemical means.

- It is a simple and fast procedure.
- The major disadvantage is separate incubation periods are required by multiple antigens with corresponding antibodies.

Indirect labelling method :

In this method, there is production of secondary antibodies against primary antibody and this secondary antibody labels the enzyme.

- It is a simple and more sensitive method.
- Advantages include higher working dilution of primary antibody, secondary antibodies are produced against primary antibodies of various species, higher versality and easy preparation.

Avidin biotin techniques :

This method involves the use of high affinity binding seen between avidin and biotin. Avidin is chemically conjugated to enzyme and biotin is linked to primary antibody chemically. This involves the binding of avidin to the biotinylated antibody and localising the peroxidase moiety at the antigenic site.

Non-specific background staining produced by the endogenous biotin is the major disadvantage of this method.

Avidin Biotin conjugate procedure :

This method is more sensitive. In this method first there is addition of primary antibody followed by secondary antibody which is biotinylated and then by avidin and biotin horse radish peroxidase conjugate complex that is preformed.

Biotin streptavidin system :

In this system, avidin is replaced by streptavidin and these complexes are more stable.

Immunogold silver staining technique:

In this method, metallic silver is added in multiple layers which enhance the gold particles. It is used for ultrastructural studies.

Polymeric method:

In this technique, via the dextran backbone numerous enzyme molecules bind to a secondary antibody. This method has more advantages.

- Non-specific background staining is minimal.
- Assay steps are reduced in number.
- Sensitivity is high.

In IHC, the tissue processing involves.

- Tissue fixation
- Dehydration
- Paraffin embedding

Fixation:

Better morphological preservation is needed for effective IHC interpretation. The most commonly used fixative is 10% buffered neutral formalin due to the following reasons.

1) Cheap
2) Preservation of morphology is good.
3) Sterilizes the tissues.
4) Preservation of Carbohydrate antigen is better.

The disadvantage is that the antigens are masked by fixation and this is rectified by antigen retrieval techniques.

Antigen Retrieval:

The antigens that are masked during fixation are unmasked by this procedure. There are several methods for antigen retrieval.

1) Microwave antigen retrieval

2) Proteolytic enzyme digestion

3) Pressure cooker antigen retrieval

4) Microwave and trypsin antigen retrieval technique

Materials and Methods

MATERIALS AND METHODS

SOURCES OF DATA:

This study is a combined retrospective and prospective study .The study was carried out in the Institute of Pathology, Madras Medical College, Chennai. A total of 150 cases of gastritis and 90 cases of gastric adenocarcinoma were received during the period of July 2013 to June 2014 and out of this, 40 cases of gastritis and 30 cases of gastric adenocarcinoma were randomly selected for this study.

INCLUSION CRITERIA:

Endoscopic biopsies and resected specimens of gastric lesions

EXCLUSION CRITERIA:

None

METHOD OF DATA COLLECTION:

Relevant clinical details (age, sex) and investigations were collected from the medical records of Institute of Pathology, Madras Medical College, Chennai. Corresponding histopathological slides prepared from formalin fixed paraffin embedded tissue (4 micron thick)of both endoscopic biopsies and resected specimens of gastric lesions were subjected to Hematoxylin &Eosin staining and studied.

Sections from gastritis cases had been categorized using Sydney grading system based on activity, chronic inflammation, metaplasia, atrophy, Helicobacter pylori colonisation and the results were tabulated. Cases of Gastric adenocarcinoma were graded into Well, Moderate and Poorly differentiated.

Special stains (Giemsa, Toluidine blue) and Immunohistochemical study using Helicobacter pylori polyclonal antibody were done in 70 cases (including 40 gastritis cases and 30 cases of gastric adenocarcinoma)

GIEMSA STAINING TECHNIQUE

Giemsa stock solution:

Giemsa stain powder	-	4gm
Methanol	-	250ml
Glycerol	-	250ml

The powder is dissolved in glycerol at 60^{0}C with regular shaking.

To this add methanol.

The mixture is well shaken and then allowed to stand for 7 days.

Filter it 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

TOLUIDINE BLUE METHOD.

Solutions:

Toluidine blue in pH6.8 phosphate buffer.

1% aqueous toluidine blue 1 ml

Sorenson's phosphate buffer pH 6.8 - 50 ml

Steps:

- Deparaffinise and rehydrate through graded alcohol to distilled water.
- Buffered toluidine blue was used for staining for a period of 20 minutes.
- Then wash well with distilled water.
- Dehydrate, clear and mount.

Result:

Organisms – Dark blue

Background – 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 . 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

This study is a combined retrospective and prospective study .The study was carried out in the Institute of Pathology, Madras Medical College, Chennai during the period of July 2013 to June 2014 .40 cases of gastritis and 30 cases of gastric adenocarcinoma were randomly selected for this study to detect the presence of Helicobacter pylori using Hematoxylin and Eosin, Giemsa, Toluidine Blue and Immunohistochemistry.

Age & Sex distribution of 40 patients presented with gastritis

TABLE – 2

Age(years)	Male	Female	Total
0 TO 20	2(5%)	1(2.5%)	3(7.5%)
21 TO 40	5(12.5%)	2(5%)	7(17.5%)
41 TO 60	14(35%)	9(22.5%)	23(57.5%)
> 60	4(10%)	3(7.5%)	7(17.5%)
Total	25(62.5%)	15(37.5%)	40(100%)

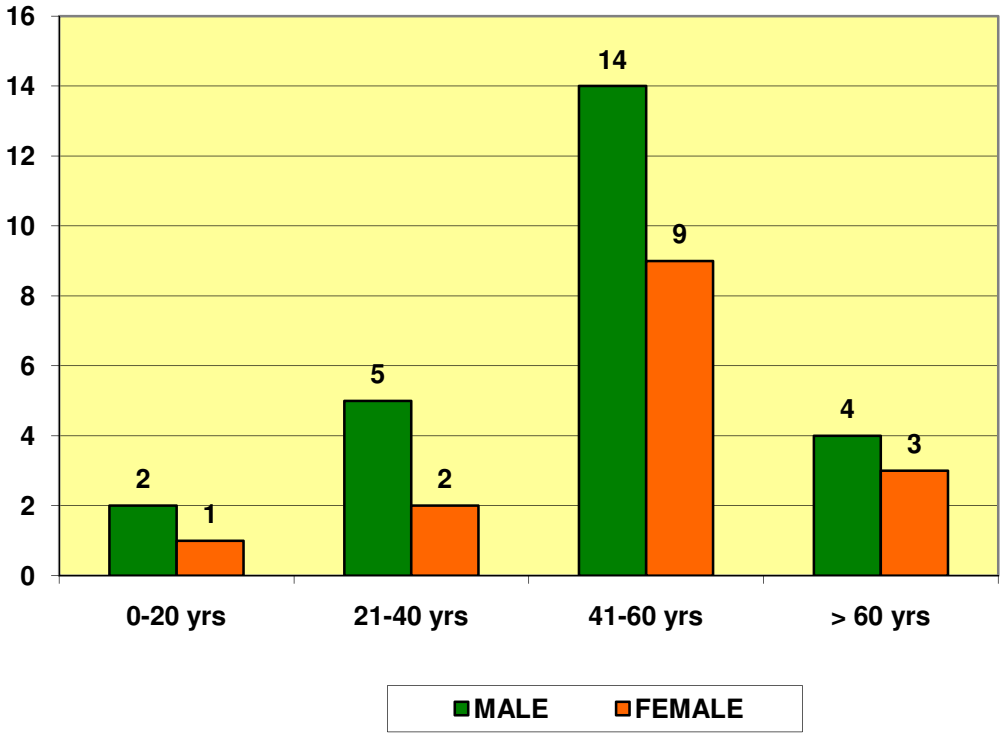
Out of 40 patients of gastritis , highest incidence (57.5%) was seen in the age group of 41-60 years (23 out of 40) in both males and females which is a significant factor.

Incidence of gastritis shows a male predominance in this study (25 out of 40 about 62.5%).

Male:Female sex ratio was about 1.67:1.

Age & Sex distribution of 40 patients presented with gastritis

CHART -1



Age & Sex distribution of 30 patients with gastric adenocarcinoma

TABLE – 3

Age(years)	Male	Female	Total
0 TO 20	0	0	0
21 TO 40	2(6.7%)	2(6.7%)	4(13.3%)
41 TO 60	10(33.3%)	4(13.3%)	14(46.7%)
> 60	11(36.7%)	1(3.3%)	12(40%)
Total	23(76.7%)	7(23.3%)	30(100%)

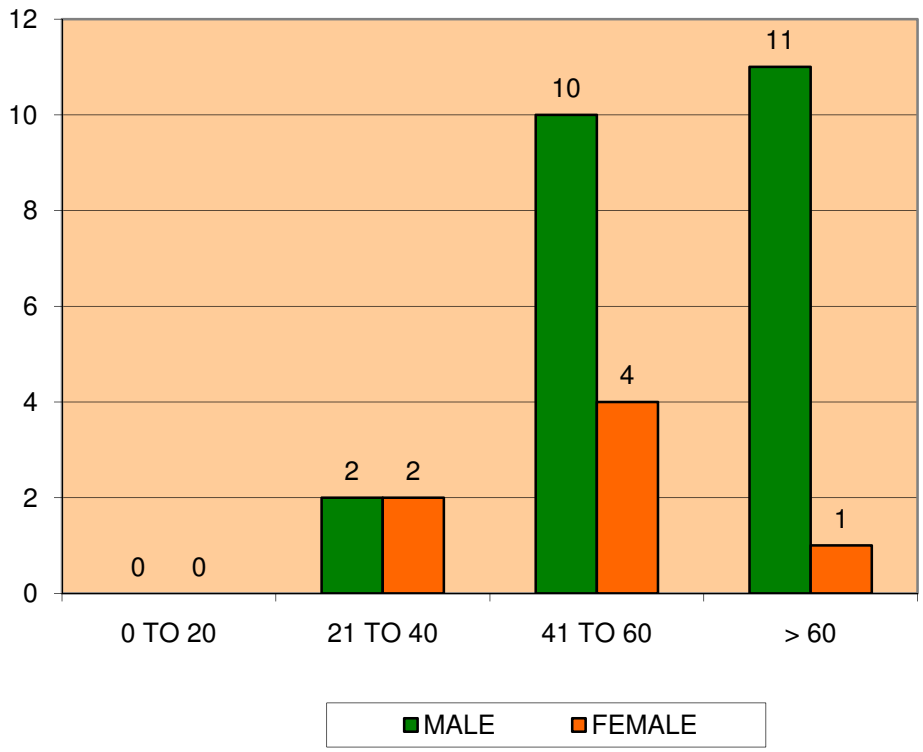
Out of 30 patients with gastric adenocarcinoma highest incidence was seen in the age group of 41-60 years (14 out of 30 about 46.7%) similar to gastritis in this study.

On correlation of age with sex ,males show increased incidence in the age group of more than 60 years(11 out of 23 about 47.8%).

Male:Female sex ratio was about 3.3:1

Age & Sex distribution of 30 patients with gastric adenocarcinoma

CHART -2



Sydney scoring in 40 cases of gastritis presented with symptoms

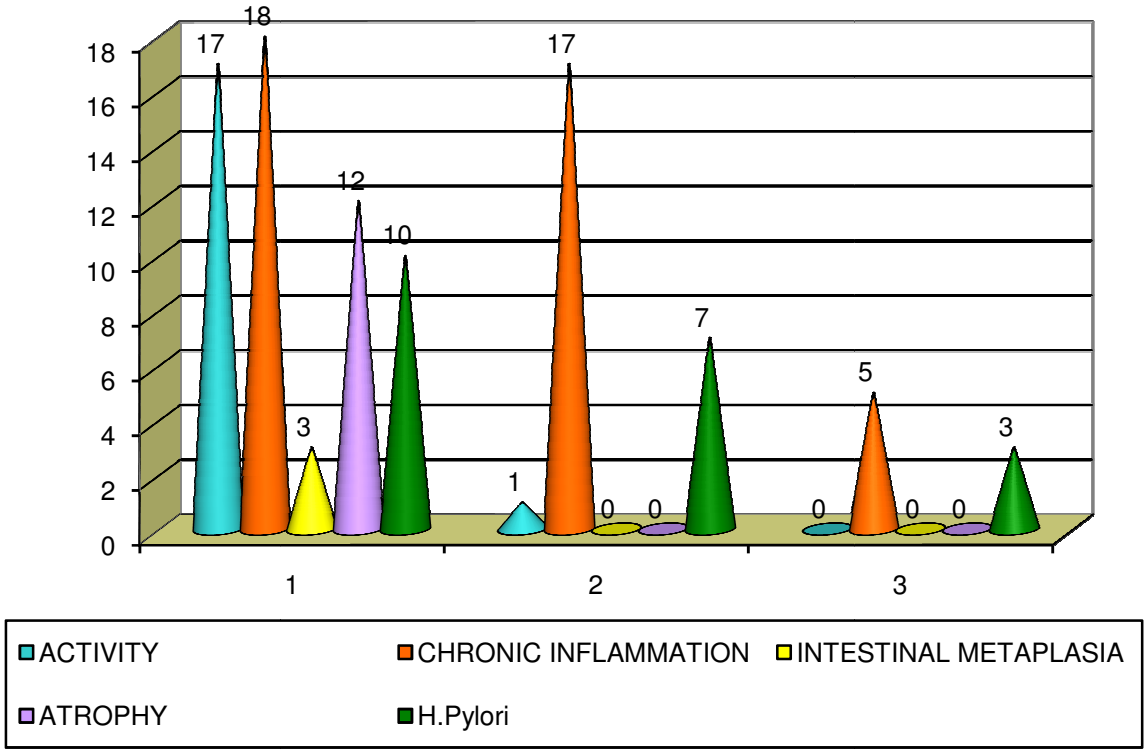
TABLE – 4

Sydney Score	Activity	Chronic Inflammation	Intestinal Metaplasia	Atrophy	Helicobacter Pylori
1	17	18	3	12	10
2	1	17	0	0	7
3	0	5	0	0	3

In this study of 40 cases of gastritis , maximum number of cases in all graded variables like activity, chronic inflammation, atrophy, intestinal metaplasia and the positivity of Helicobacter Pylori on H&E stain were seen in the Sydney score of 1 .

Sydney scoring in 40 cases of gastritis presented with symptoms

CHART-3



Male and Female distribution of positive & negative cases of Helicobacter pylori in Gastritis - H&E, Giemsa, Toluidine Blue & IHC

TABLE – 5

METHOD	H&E		GIEMSA		TOLUIDINE BLUE		IHC	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
MALE(25)	12	13	16	9	15	10	20	5
FEMALE(15)	8	7	10	5	9	6	10	5
TOTAL(40)	20	20	26	14	24	16	30	10

Out of 40 cases of gastritis, positivity for H.Pylori was seen in

20 out of 40 cases (50%) in H& E stain

26 out of 40 (65%) in Giemsa stain

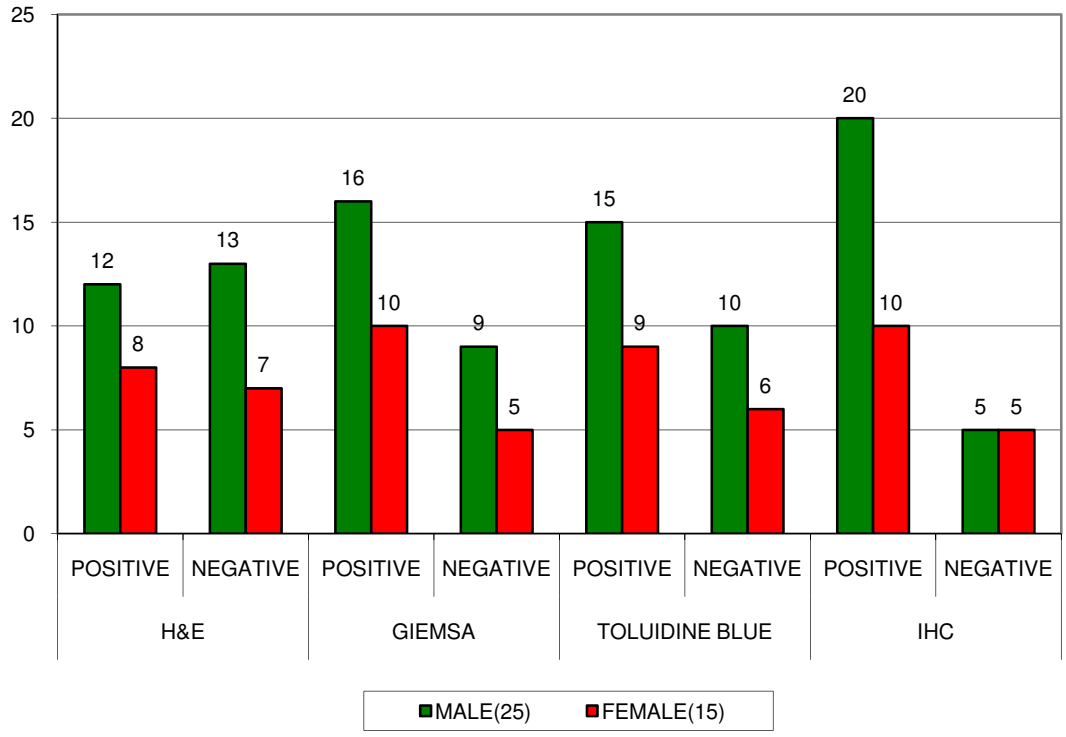
24 out of 40 (60%) in Toluidine blue stain

30 out of 40 cases (75%) in IHC (of which 20 were males and 10 were females).

The values of which show maximum positivity in IHC.

Male and Female distribution of positive & negative cases of Helicobacter pylori in Gastritis - H&E, Giemsa, Toluidine Blue & IHC

CHART – 4



Male and Female distribution of positive & negative cases of Helicobacter pylori in Gastric Adenocarcinoma- H&E, Giemsa, Toluidine Blue & IHC

TABLE – 6

METHOD	H&E		GIEMSA		TOLUIDINE BLUE		IHC	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
MALE(23)	5	18	13	10	11	12	15	8
FEMALE(7)	2	5	2	5	2	5	2	5
TOTAL(30)	7	23	15	15	13	17	17	13

Out of 30 cases of Gastric adenocarcinoma, positivity for H.Pylori was seen in

7 out of 30 (23%) in H&E stain

15 out of 30 (50%) in Giemsa stain

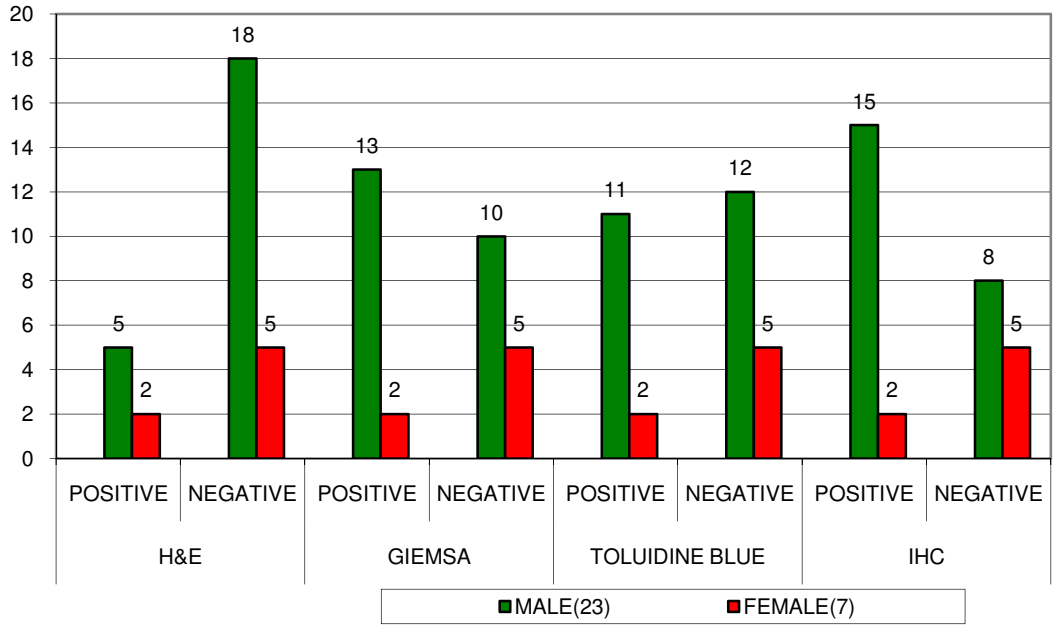
13 out of 30 (43%) in Toluidine blue stain

17 out of 30 cases (56.6%) in IHC (of which 15 were males and 2 were females).

The maximum number of positivity was seen in IHC similar to gastritis.

Male and Female distribution of positive & negative cases of Helicobacter pylori in Gastric Adenocarcinoma- H&E, Giemsa, Toluidine Blue & IHC

CHART– 5



Male & Female ratio of Helicobacter pylori infection in Gastritis Cases

TABLE – 7

SEX	NO. OF PATIENTS	H.PYLORI POSITIVE	H.PYLORI NEGATIVE
MALE	25	20(80%)	5(20%)
FEMALE	15	10(66.6%)	5(33.4%)
TOTAL	40	30(75%)	10(25%)

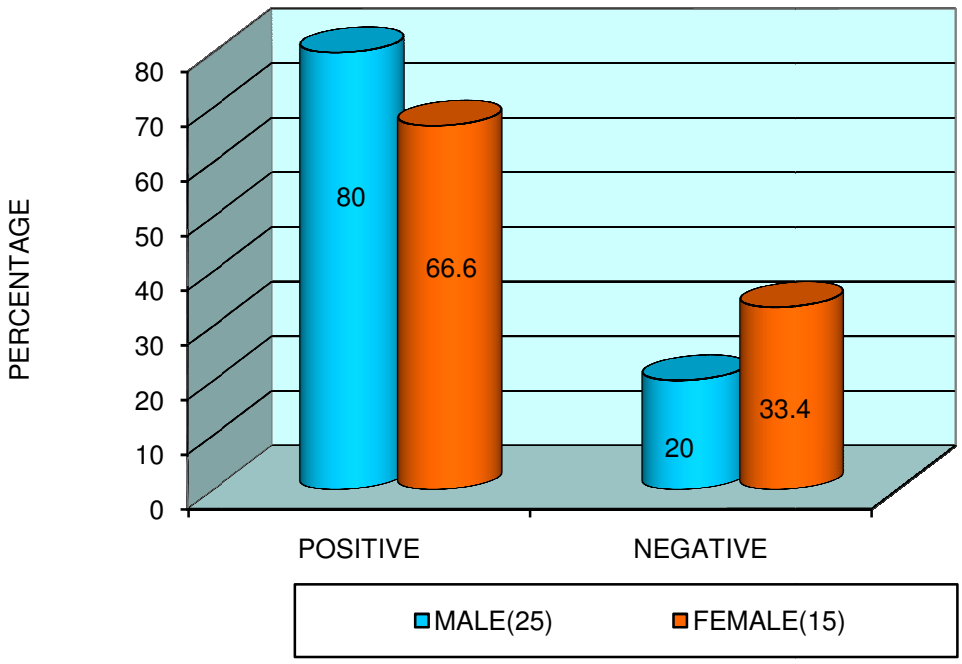
Out of 25 males 20 showed positivity for Helicobacter pylori. The percentage of positivity was 80%.

Out of 15 females 10 showed positivity for Helicobacter pylori. The percentage of positivity was 66.6%.

Male : Female ratio was 1.2:1.

Male & Female ratio of Helicobacter pylori infection in Gastritis Cases

CHART - 6



Male & Female ratio of Helicobacter pylori infection in 30 Gastric Adenocarcinoma cases

TABLE –8

SEX	NO. OF PATIENTS	H.PYLORI POSITIVE	H.PYLORI NEGATIVE
MALE	23	15(65.2%)	8(34.8%)
FEMALE	7	2(28.6%)	5(71.4%)
TOTAL	30	17(56.6%)	13(43.4%)

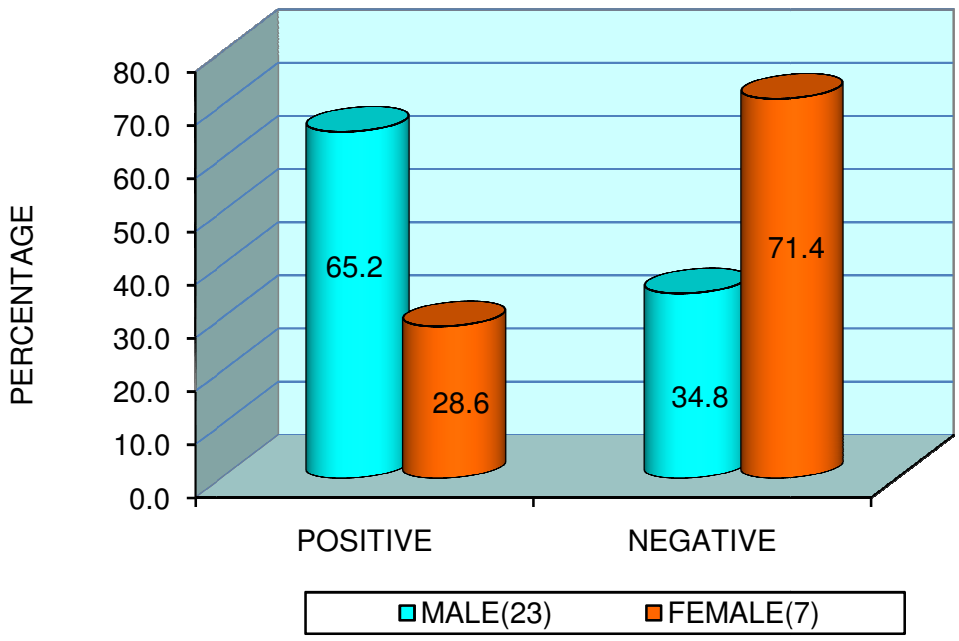
Out of 23 males 15 showed positivity for Helicobacter pylori. The percentage of positivity was 65.2%.

Out of 7 females 2 showed positivity for Helicobacter pylori. The percentage of positivity was 28.6%.

Male: Female ratio was 2.28:1.

Male & Female ratio of Helicobacter pylori infection in 30 Gastric Adenocarcinoma cases

CHART - 7



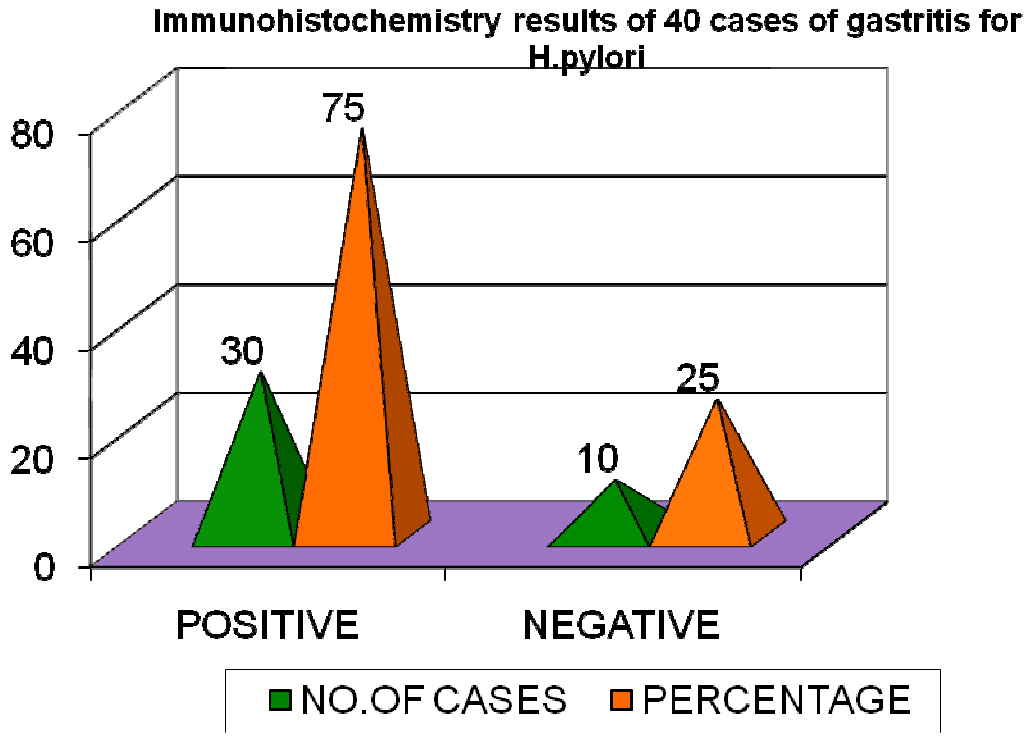
Immunohistochemistry results of 40 cases of gastritis for H.pylori

TABLE –9

	Helicobacter Pylori +ve	Helicobacter Pylori –ve
No. of Cases	30	10
Percentage	75%	25%

Out of 40 cases of gastritis studied for Helicobacter pylori with IHC 30 cases showed positivity and 10 cases were negative. The percentage of positivity was 75%.

CHART - 8



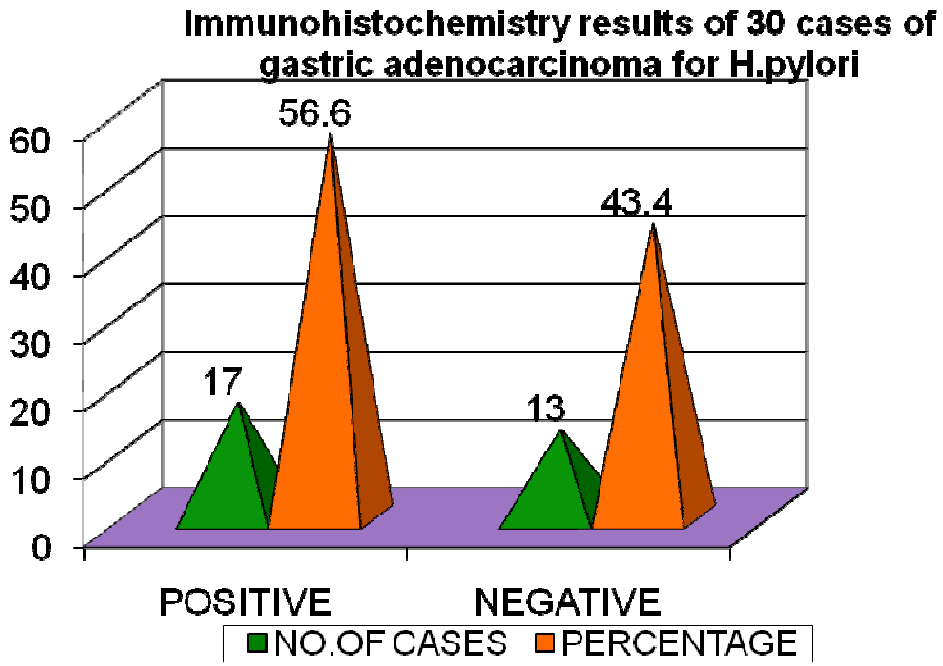
Immunohistochemistry results of 30 cases of gastric adenocarcinoma for H.pylori

TABLE –10

	Helicobacter Pylori +ve	Helicobacter Pylori –ve
No. of Cases	17	13
Percentage	56.6%	43.4%

Out of 30 cases of gastric adenocarcinoma studied for Helicobacter pylori with IHC 17 cases showed positivity and 13 cases were negative. The percentage of positivity was 56.6%.

CHART - 9



Age distribution of Helicobacter pylori infection in 40 cases of Gastritis

TABLE –11

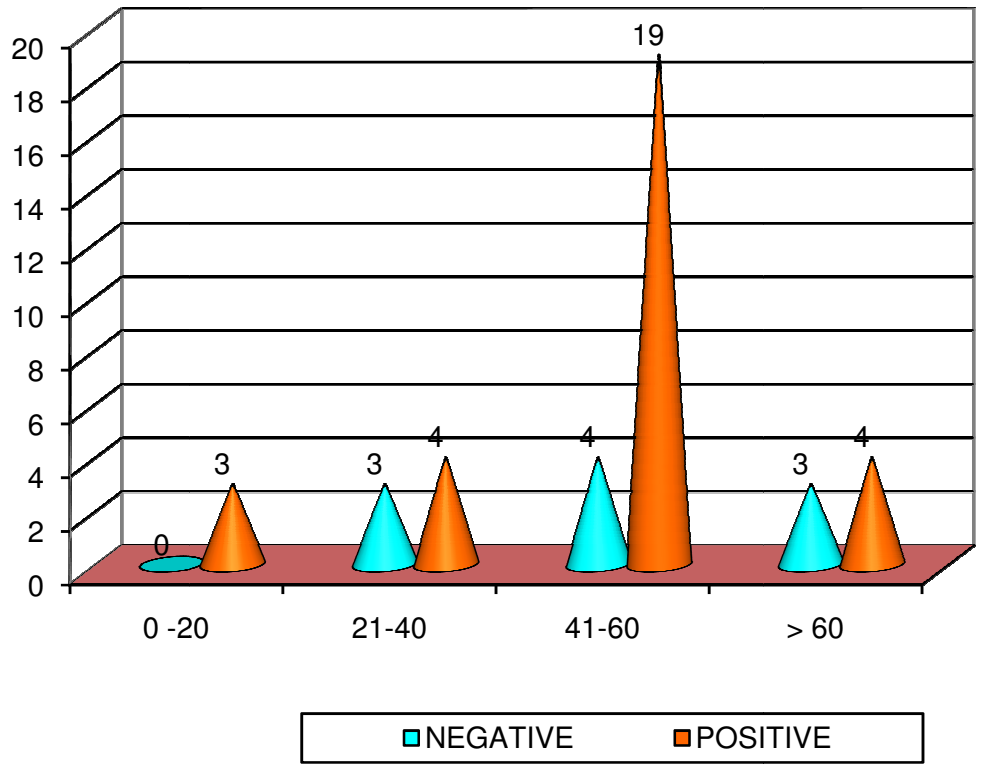
AGE(years)	TOTAL	NEGATIVE	POSITIVE
0 -20	3(7.5%)	0	3(7.5%)
21-40	7(17.5%)	3(7.5%)	4(10%)
41-60	23(57.5%)	4(10%)	19(47.5%)
>60	7(17.5%)	3(7.5%)	4(10%)
TOTAL	40(100%)	10(25%)	30(75%)

Out of 40 cases of gastritis studied for Helicobacter pylori with immunohistochemistry 30 cases showed positivity.

The most common age group affected was seen between 41-60 years.

Age distribution of Helicobacter pylori infection in 40 cases of Gastritis

CHART - 10



Age distribution of Helicobacter pylori infection in 30 cases of Gastric Adenocarcinoma

TABLE –12

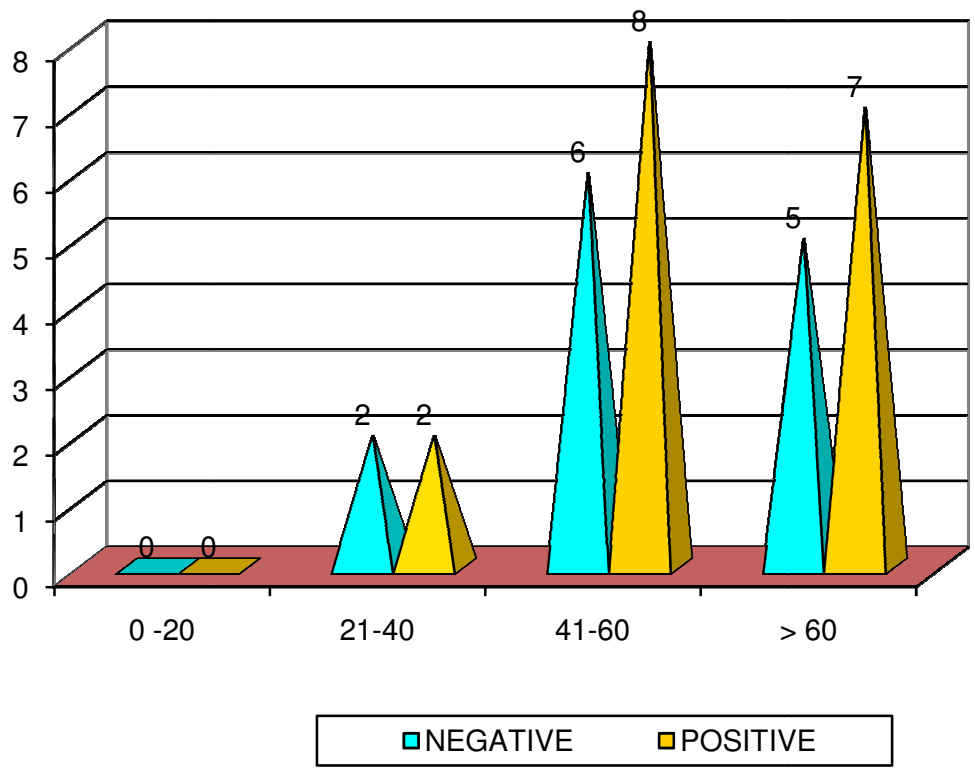
AGE(years)	TOTAL	NEGATIVE	POSITIVE
0 -20	0	0	0
21-40	4(13.3%)	2(6.7%)	2(6.7%)
41-60	14(46.7%)	6(20%)	8(26.6%)
>60	12(40%)	5(16.7%)	7(23.3%)
TOTAL	**30(100%)**	**13(43.4%)**	**17(56.6%)**

Out of 30 cases of gastric adenocarcinoma studied for Helicobacter pylori with immunohistochemistry 17 cases showed positivity.

The most common age group affected was seen between 41-60 years.

Age distribution of Helicobacter pylori infection in 30 cases of Gastric Adenocarcinoma

CHART - 11



Grading of H.Pylori infection in gastric biopsies using Sydney scoring system in various staining methods like H&E, Giemsa, Toluidine Blue and IHC

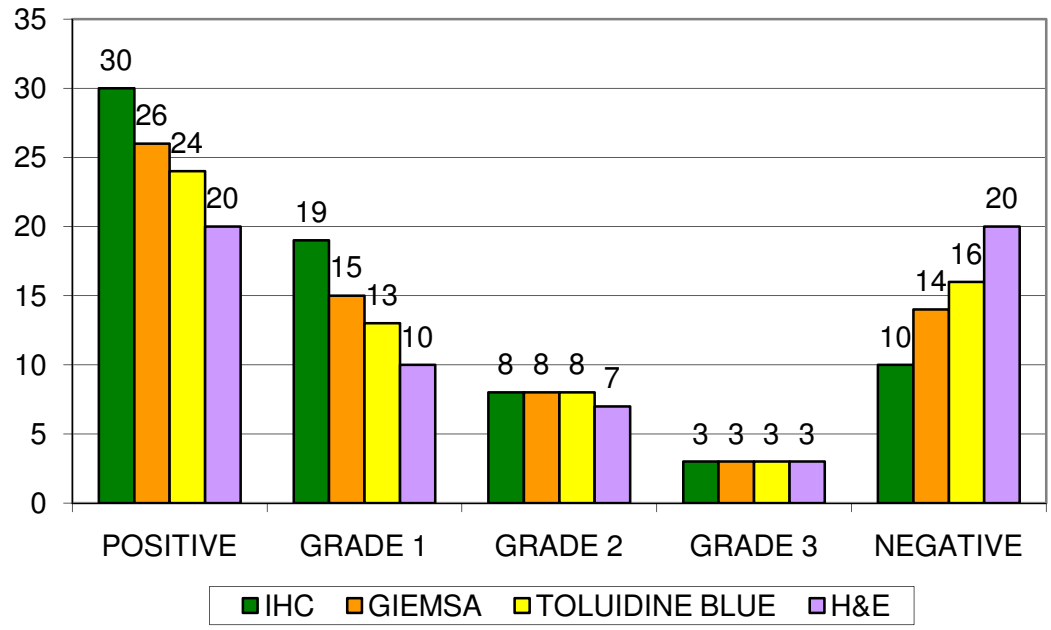
TABLE –13

STAINING METHOD	TOTAL NO. OF CASES	H.PYLORI POSITIVE	GRADE 1	GRADE 2	GRADE 3	H.PYLORI NEGATIVE
IHC	40	30(75%)	19(47.5%)	8(20%)	3(7.5%)	10(25%)
GIEMSA	40	26(65%)	15(37.5%)	8(20%)	3(7.5%)	14(35%)
TOLUIDINE BLUE	40	24(60%)	13(32.5%)	8(20%)	3(7.5%)	16(40%)
H&E	40	20(50%)	10(25%)	7(17.5%)	3(7.5%)	20(50%)

From this table it was shown that detection of Helicobacter pylori using H&E, Giemsa, Toluidine blue and IHC was identical in cases of grade 2 and grade 3 colonisation of H.pylori. Differences in staining pattern were observed in grade 1 when there is low colonisation of H.pylori.

Grading ofH.Pylori infection in Gastric biopsies using Sydney Scoring System in various staining methods like H&E, Giemsa, Toluidine Blue and IHC

CHART –12



Age and Sex distribution among Helicobacter pylori positive Gastritis cases by Immunohistochemistry

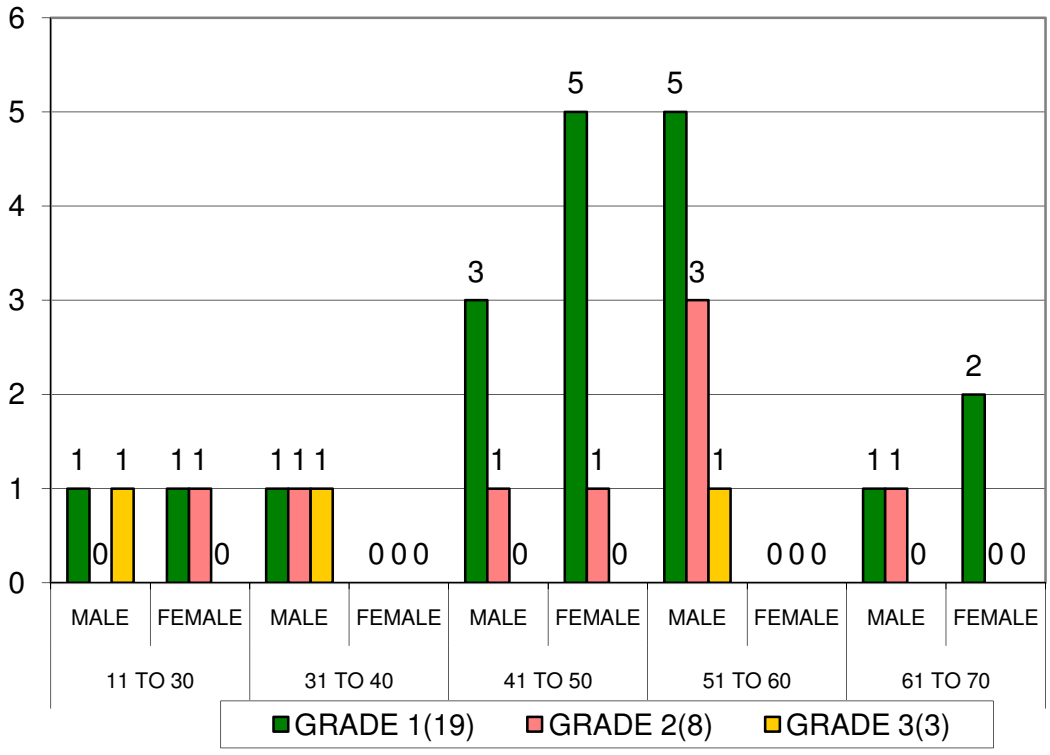
TABLE –14

GRADE AND TOTAL NUMBER OF CASES	11 TO 30		31 TO 40		41 TO 50		51 TO 60		61 TO 70	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
GRADE 1 (19)	1	1	1	0	3	5	5	0	1	2
GRADE 2 (8)	0	1	1	0	1	1	3	0	1	0
GRADE 3 (3)	1	0	1	0	0	0	1	0	0	0
TOTAL (30)	**2**	**2**	**3**	**0**	**4**	**6**	**9**	**0**	**2**	**2**

From this table it was evident that the most common age group infected with Helicobacter pylori was between 41-60 years.

Age and Sex distribution among Helicobacter pylori positive cases by Immunohistochemistry

CHART - 13



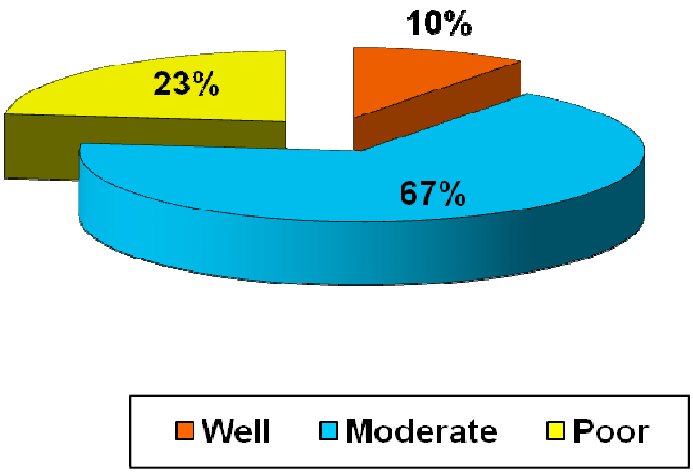
Grading of 30 cases of Gastric Adenocarcinoma

TABLE –15

Grade	Well Differentiated	Moderately Differentiated	Poorly Differentiated
No. of Cases	3(10%)	20(67%)	7(23%)

Out of 30 cases of Gastric adenocarcinoma 3 cases were well differentiated, 20 were moderately differentiated, 7 were poorly differentiated.

CHART - 14



Immunohistochemistry results of 30 cases of Gastric Adenocarcinoma for Helicobacter Pylori in different grades

TABLE –16

GRADE	Total No. of Cases	H.Pylori Positive	H.Pylori Negative
Well Differentiated	3	2(66.6%)	1(33.4%)
Moderately Differentiated	20	11(55%)	9(45%)
Poorly Differentiated	7	4(57%)	3(43%)

From this table it was evident that out of 30 cases ,17 showed positivity for H.pylori.

H.pylori positivity was seen in

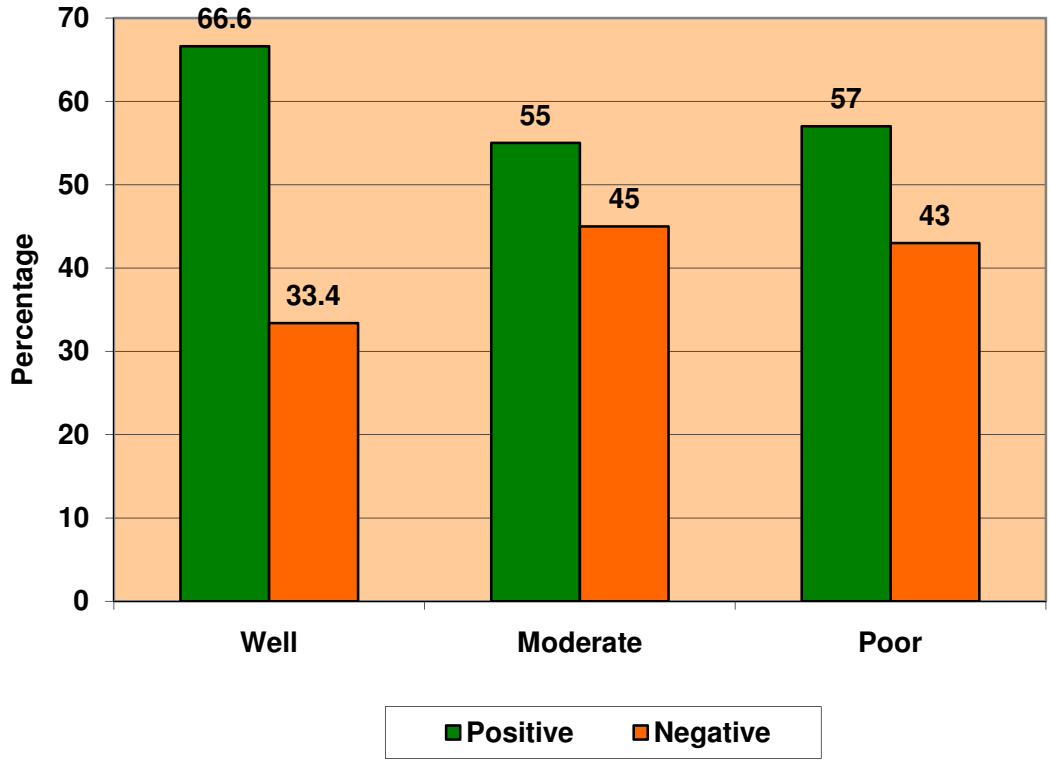
2 out of 3(66.6%) cases of well differentiated grade

11 out of 20 (55%) cases of moderately differentiated grade

4 out of 7 cases (57%) of poorly differentiated grade

Percentage of cases showing H.Pylori Positivity in different grades of Gastric Adenocarcinoma

CHART – 15



COMPARISON OF GIEMSA & IHC IN 40 CASES OF GASTRITIS

TABLE –17

GIEMSA	IHC	
	POSITIVE	NEGATIVE
POSITIVE	26	0
NEGATIVE	4	10

Out of 40 cases , IHC showed positivity for Helicobacter pylori in 30 cases.4 cases which were negative by Giemsa stain was found to be positive in IHC. Both Giemsa and IHC showed negativity in 10 cases.

Sensitivity	-	86.67%
Specificity	-	100%
Positive Predictive value	-	100%
Negative Predictive value	-	71.43%
% of False Positive	-	0.00
% of False Negative	-	13.33

COMPARISON OF TOLUIDINE BLUE & IHC IN 40 CASES OF GASTRITIS

TABLE –18

TOLUIDINE BLUE	IHC	
	POSITIVE	**NEGATIVE**
POSITIVE	24	0
NEGATIVE	6	10

Out of 40 cases, IHC showed positivity for Helicobacter pylori in 30 cases.6 cases which were negative by Toluidine blue stain was found to be positive in IHC. Both Toluidine blue and IHC showed negativity in 10 cases

Sensitivity	-	80%
Specificity	-	100%
Positive Predictive value	-	100%
Negative Predictive value	-	62.50%
% of False Positive	-	0.00
% of False Negative	-	20.00

COMPARISON OF H & E & IHC IN 40 CASES OF GASTRITIS

TABLE –19

H&E	IHC	
	POSITIVE	NEGATIVE
POSITIVE	20	0
NEGATIVE	10	10

Out of 40 cases, IHC showed positivity for Helicobacter pylori in 30 cases.10 cases which were negative by H&E stain was found to be positive in IHC. Both H&E and IHC showed negativity in 10 cases.

Sensitivity	-	66.67%
Specificity	-	100%
Positive Predictive value	-	100%
Negative Predictive value	-	50%
% of False Positive	-	0.00
% of False Negative	-	33.33

COMPARISON OF GIEMSA & IHC IN 30 CASES OF GASTRIC ADENOCARCINOMA

TABLE –20

GIEMSA	IHC	
	POSITIVE	**NEGATIVE**
POSITIVE	15	0
NEGATIVE	2	13

Out of 30 cases IHC showed positivity for Helicobacter pylori in 17 cases.2 cases which were negative by Giemsa stain was found to be positive in IHC. Both Giemsa and IHC showed negativity in 13 cases.

Sensitivity	-	88.23%
Specificity	-	100%
Positive Predictive value	-	100%
Negative Predictive value	-	86.67%
% of False Positive	-	0.00
% of False Negative	-	11.76

COMPARISON OF TOLUIDINE BLUE & IHC IN 30 CASES OF GASTRIC ADENOCARCINOMA

TABLE – 21

TOLUIDINE BLUE	IHC	
	POSITIVE	NEGATIVE
POSITIVE	13	0
NEGATIVE	4	13

Out of 30 cases IHC showed positivity for Helicobacter pylori in 17 cases.4 cases which were negative by Toluidine blue stain was found to be positive in IHC. Both Toluidine blue and IHC showed negativity in 13 cases.

Sensitivity	-	76.47%
Specificity	-	100%
Positive Predictive value	-	100%
Negative Predictive value	-	76.47%
% of False Positive	-	0.00
% of False Negative	-	23.53

COMPARISON OF H & E & IHC IN 30 CASES OF GASTRIC ADENOCARCINOMA

TABLE – 22

H & E	IHC	
	POSITIVE	**NEGATIVE**
POSITIVE	7	0
NEGATIVE	10	13

Out of 30 cases IHC showed positivity for Helicobacter pylori in 17cases.10 cases which were negative by H&E stain was found to be positive in IHC. Both H&E and IHC showed negativity in 13 cases

Sensitivity	-	41.18%
Specificity	-	100%
Positive Predictive value	-	100%
Negative Predictive value	-	56.52%
% of False Positive	-	0.00
% of False Negative	-	58.82

Pictures

Fig. 1 : HPE NO 3423/14 – Adenocarcinoma Stomach

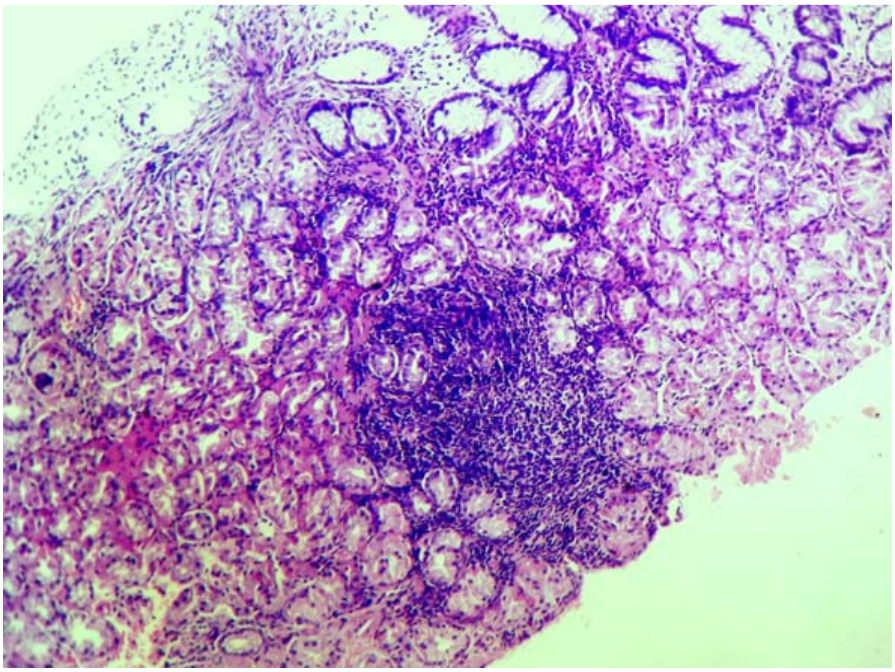


Fig.2 : Lamina propria with lymphoid follicle formation- H&E (100x)

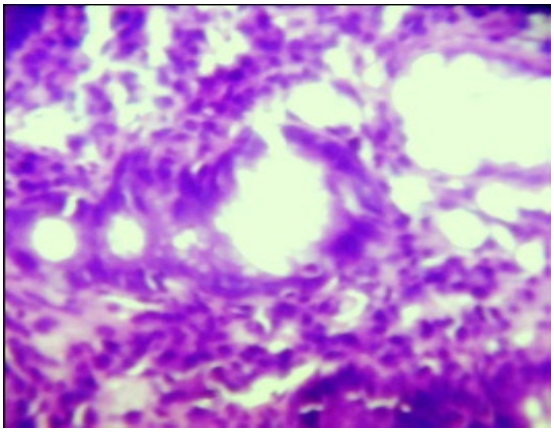


Fig.3 : Presence of Intraepithelial neutrophils- H&E (400x)

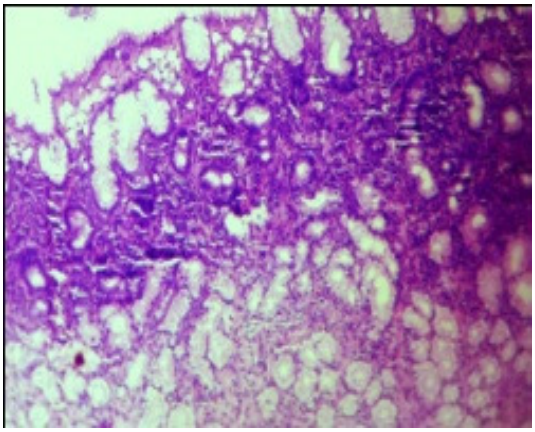


Fig.4 :Laminapropria inflammation- H&E (100x)

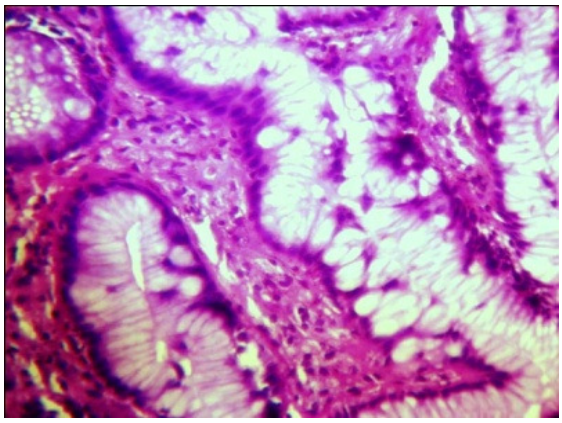


Fig.5: Intestinal metaplasia in antral mucosa- H&E (400x)

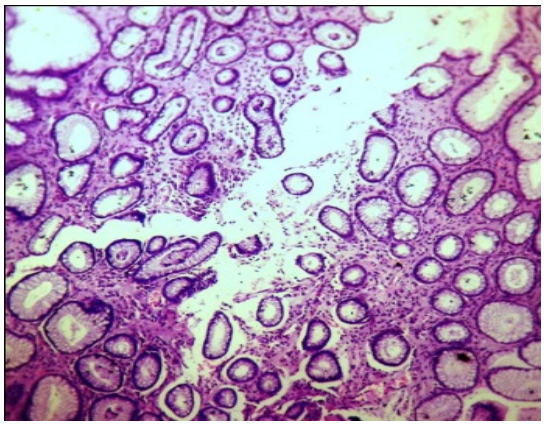


Fig.6 : Atrophy of glands- H&E (100x)

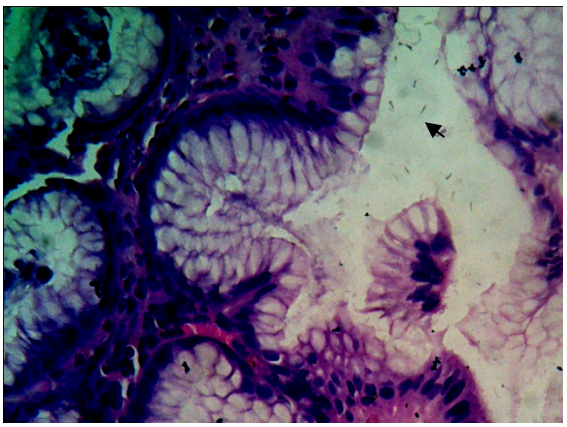


Fig.7 : Helicobacter Pylori colonisation- H&E (400x)

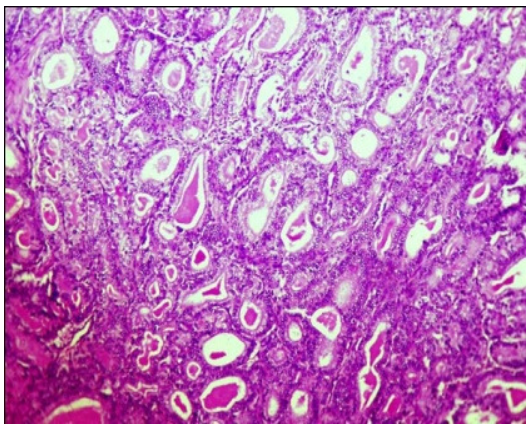


Fig.8 : Infiltrating adenocarcinoma stomach- Well differentiated- H&E (100x)

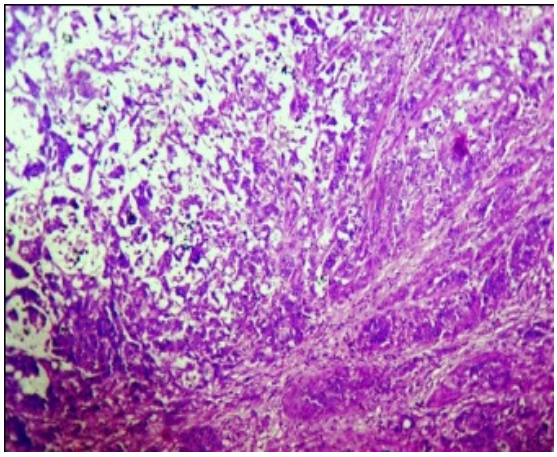


Fig.9 :Infiltrating adenocarcinoma stomach- Moderately differentiated- H&E (100x)

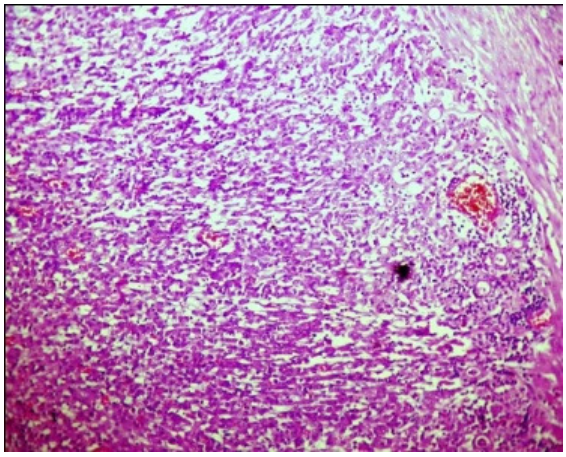


Fig.10 : Infiltrating adenocarcinoma stomach- Poorly differentiated- H&E (100x)

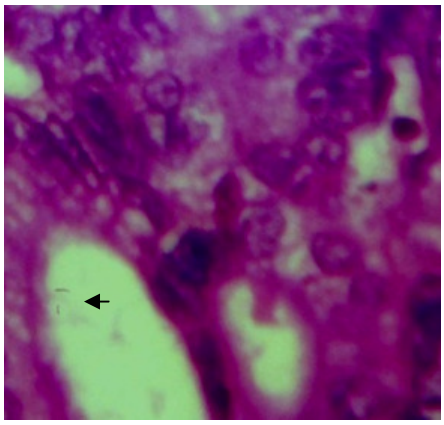


Fig.11 :Helicobacter Pylori colonisation in Gastric adenocarcinoma H&E (400x)

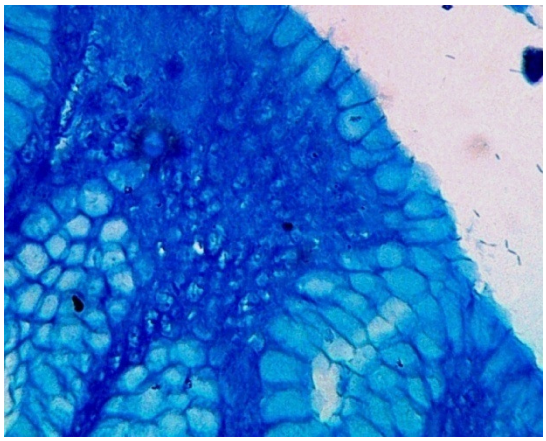


Fig.12 :Helicobacter Pylori grade 1 colonisation- Giemsa (400x)

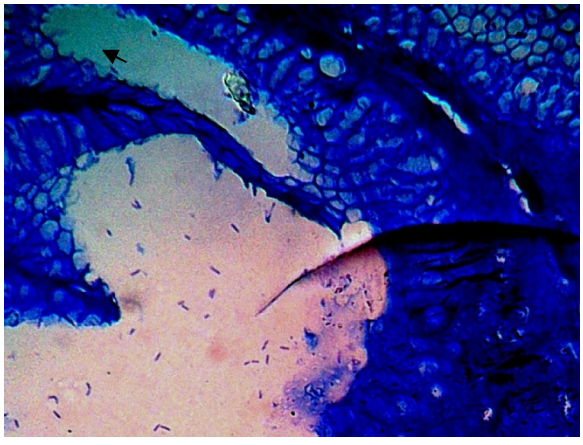


Fig.13 : Helicobacter Pylori grade 2 colonisation- Giemsa (400x)

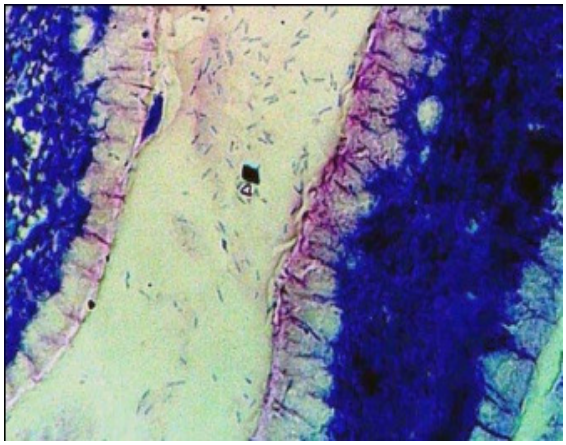


Fig.14 : Helicobacter Pylori grade 3 colonisation- Giemsa (400x)

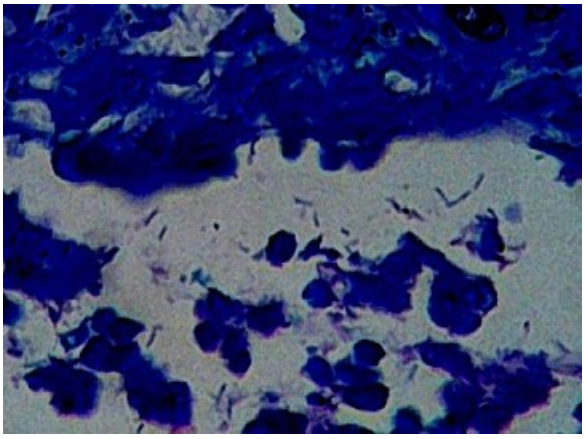


Fig.15 : Helicobacter Pylori colonisation in Gastric adenocarcinoma- Giemsa (400x)

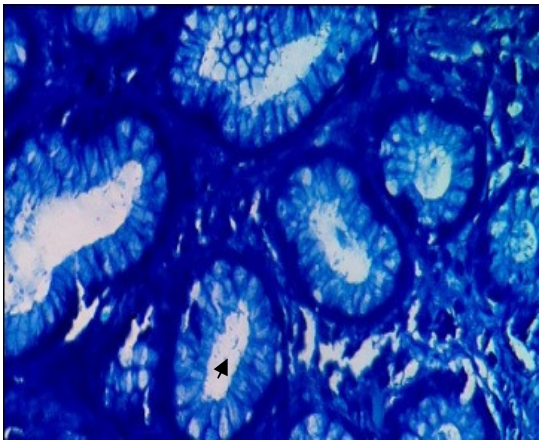


Fig.16: Helicobacter Pylori grade 1 colonisation-: Toluidine blue (400x)

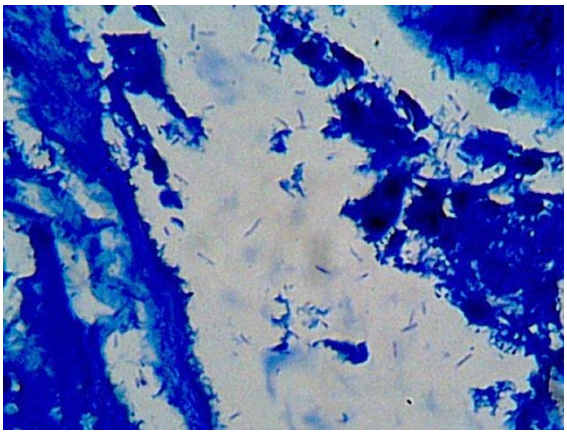


Fig.17: Helicobacter Pylori grade 2 colonisation- Toluidine blue (400x)

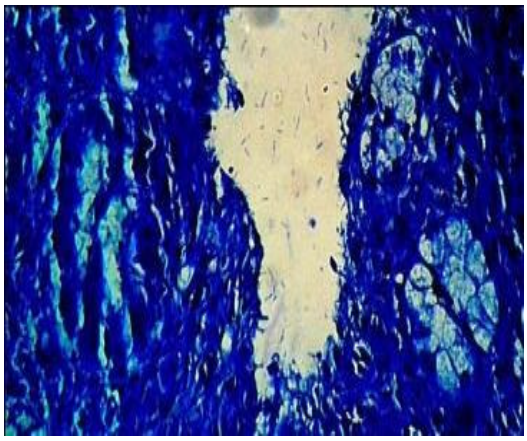


Fig.18: Helicobacter Pylori grade 3 colonisation- Toluidine blue (400x)

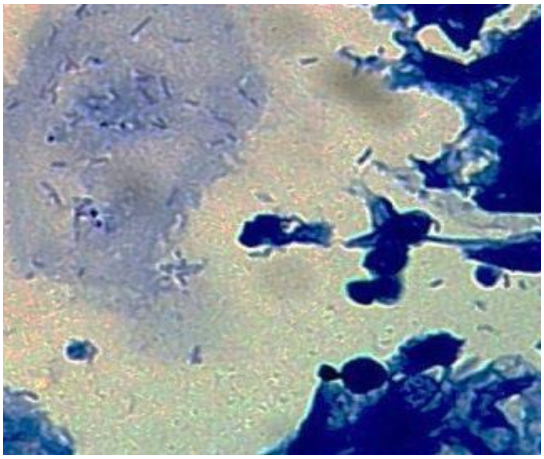


Fig.19 : Helicobacter Pylori colonisation in Gastric adenocarcinoma Toluidine blue (400x)

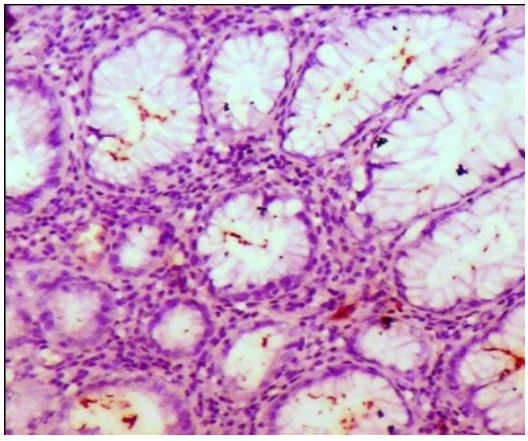


Fig.20 : Helicobacter Pylori grade 1 colonisation- IHC (100x)

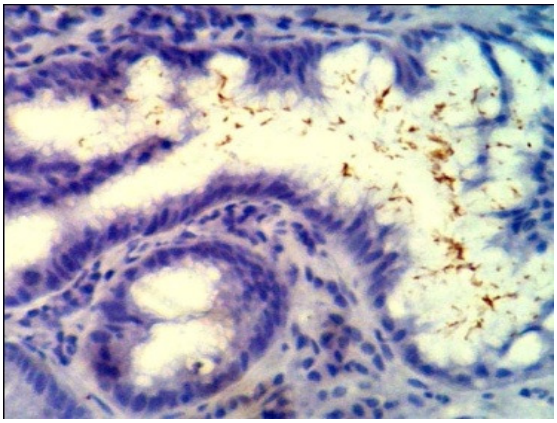


Fig.21 : Helicobacter Pylori grade 2 colonisation- IHC (100x)

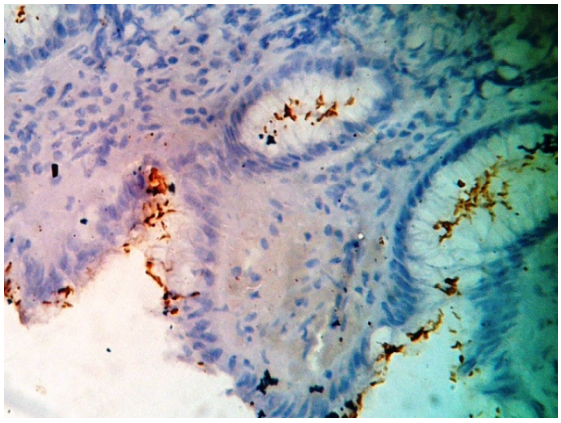


Fig.22 Helicobacter Pylori grade 3 colonisation-: IHC (100x)

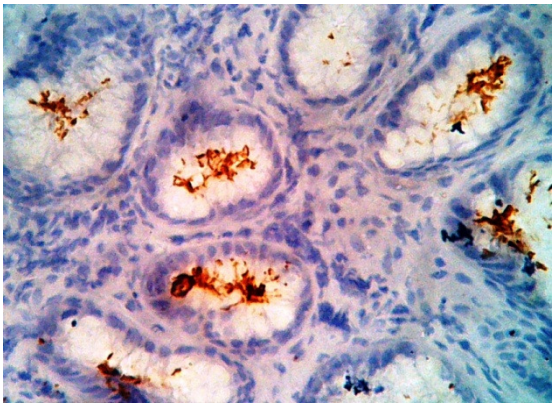


Fig.23 : Helicobacter Pylori colonisation- IHC (100x)

Discussion

DISCUSSION

Helicobacter pylori infection has an important role in the etiology of several diseases of the gastrointestinal tract which include chronic active gastritis, peptic ulcer, Gastric adenocarcinoma and Mucosa - associated lymphoid tissue lymphoma. It affects more than 50% of the world wide human population.

In this study to detect the presence of Helicobacter pylori Giemsa, Toluidine blue and IHC were done for 40 cases of gastritis and 30 cases of gastric adenocarcinoma.

In a study conducted by Adisa et al [77] Helicobacter pylori was positive in 345 out of 603 cases and the prevalence rate was about 57.2%.

In a study conducted by Rajeshkumar et al [79] the Helicobacter pylori was positive in 92 out of 265 cases with a prevalence rate of about 34.71%.

In a study by Orhan D et al 66 out of 78 cases of antral biopsies showed positivity for Helicobacter pylori with a prevalence rate of 84.6% [130]

In a study by Dr,VishwaPriya.M. Godkhindi et al [131] out of 110 cases Helicobacter pylori was positive in 69 cases with an overall prevalence rate of 62.72%.

J.E.TZeng, Y.L. Lin, S.M. Chung et al[132] in their study out of 111 cases, 66 cases showed positivity for Helicobacter pylori with a prevalence rate of 59.5%.

In the present study of 40 cases of gastritis ,30 cases were positive for Helicobacter pylori with a prevalence rate of about 75% and out of 30 cases of gastric adenocarcinoma 17 cases showed positivity for Helicobacter pylori with a prevalence rate of about 56.6%.. The overall prevalence rate was about 67.1%.

Comparison of Helicobacter pylori prevalence with different studies.

Table-23

STUDY	PREVALENCE IN (%)
Adisa et al	57.2%
Rajeshkumar et al	34.71%
Orhan. D et al	84.6%
Vishwapriya M .Godkhindi et al	62.72%
J.E.TZeng, Y.L .Lin, S.M. Chung, et al	59.5%
Present study (70 cases)	67.1%

The prevalence of Helicobacter pylori infection varies worldwide which was more prevalent among developing countries of about 80% seen mostly in middle age group and in industrialised countries the prevalence rate was about 20-50%.

Rajeshkumar et al [79] in his prospective study found that the maximum prevalence of infection with Helicobacter pylori was in the age group of 36-45 years.

Adisa et al [77] in his study assessed that the prevalence of Helicobacter pylori infection was maximum between the age group of 41-50 years.

Tanya Dogar et al [133] in his study found that the mean age of patients infected with Helicobacter pylori was 45.9 years.

Javed et al [134] in his study found that the prevalence of Helicobacter pylori infection was maximum in the age group of 30 -50 years.

In the present study the most common age group affected with Helicobacter pylori was seen between 41-60 years in both groups of gastritis and gastric adenocarcinoma.

Comparison of age groups of persons infected with Helicobacter pylori with other studies.

Table-24

STUDY	Age group
Rajeshkumar et al	36-45 years
Adisa et al	41-50 years
Tanya Dogar et al	Mean Age of 45.9 years
Javed et al	30 – 50 years
Present study (70 cases)	41-60 years

The distribution of infection with Helicobacter pylori varies widely among different races, age groups, different population among various countries and level of socio economic status.

Adisa et al [77] in his study found that among the individuals infected with Helicobacter pylori 46.8% were males and 53.2% were females.

In a study by Tanya Dogar et al [133] it was shown that among the individuals infected with Helicobacter pylori 59% were males and 41% were females.

In a study by Rajeshkumar et al [79] the positivity for Helicobacter pylori was seen among 64.13% of males and 35.87 % of females.

In the present study, among 30 H.Pylori positive cases of gastritis 66.66% were males and 33.34% were females and among 17 H.Pylori positive cases of gastric adenocarcinoma 88.2% were males and 11.8% were females. Overall in the study of total 70 cases 47 showed positivity for Helicobacter pylori. Among these 47 Helicobacter pylori positive cases 74.5% were males and 25.5% were females.

Comparison of male and female distribution of Helicobacter pylori infection with other studies

Table-25

Study	Males (in %)	Females (in %)
Adisa et al	46.8 %	53.2 %
Tanya Dogar et al	59 %	41 %
Rajeshkumar et al	64.13 %	35.87 %
Present study (70 cases)	74.5 %	25.5 %

In a study conducted by Dr.Vishwapriya. M. Godkhindi et al [131] among 35 cases of chronic gastritis 32 cases showed positivity (91.42%) for Helicobacter pylori and among 14 cases of Adenocarcinoma (7.14%) 1 showed positivity for Helicobacter pylori.

In the study by WYatt, JI Semin Diagn pathol [135] Helicobacter pylori positivity was seen in 90% of cases of gastritis ad 50% of cases of Adenocarcinoma.

In the present study among 40 cases of gastritis 30 showed positivity for Helicobacter pylori (75%) and among 30 cases of gastric Adenocarcinoma 17 cases (56.6%) showed Helicobacter pylori positivity.

Comparison of frequency of positivity of Helicobacter pylori among gastritis and gastric adenocarcinoma with other studies.

Table-26

Diseases	Present study	Dr.Vishwapriya.M Godkhindi et al	W.YattJI.Semin Diagan pathol
Gastritis	75%	91.42%	90%
Adenocarcinoma	56.6%	7.14%	50%

Helicobacter pylori has an important role in the pathogenesis of upper gastrointestinal tract malignancy. So, early detection and eradication of this organism is essential for the prevention of gastric cancer.

Due to lack of contrast between the surrounding tissue and the bacteria the H&E stain carries low sensitivity. The specificity is also low due to non specific staining of other bacteria which is seen in the stomach.

Modified Giemsa is a simple, rapid procedure at low cost. It provides reliable results with acceptable levels of sensitivity and specificity.

IHC carries high level of sensitivity and specificity for detection of Helicobacter pylori but it is a time consuming technique and also expensive.

In a study by shukla et al[136] for H.Pylori detection among 102 patients H&E showed sensitivity of about 72.5% and specificity of about 100%.Giemsa showed sensitivity of about 80.4% and specificity of about 100%.

In a study by Pandya et al[137] for H.Pylori among 436 patients it was shown that sensitivity of H&E was 100% and specificity was 87.9%. Giemsa showed sensitivity of about 100% and specificity of about 84.5%.

In a study among 111 patients for H.Pylori by J.E Tzeng et al[132] the sensitivity of H&E was 98.5% and specificity was 100%. Giemsa showed sensitivity of about 98.5% and specificity of about 97.8%.

Raziye Tajalli et al[100] in their study among 54 patients for H.Pylori detection found that the sensitivity of H&E was 41.86% and specificity was 100%. Giemsa showed sensitivity of about 53.49% and specificity of about 95.24%. Toluidine Blue showed sensitivity of about 76.74% and specificity of about 100%.

In this study for detection of H.Pylori, the sensitivity and specificity of various staining methods like H&E, Giemsa, Toluidine Blue and IHC were compared.

In 40 cases of gastritis H&E showed 66.67% sensitivity and specificity of about 100%,Giemsa showed 86.67 % sensitivity and specificity of about 100%. Toluidine blue showed 80% sensitivity and specificity of about 100% .

In 30 cases of gastric adenocarcinoma H&E showed sensitivity of about 41.18% and specificity of about 100%.Giemsa showed sensitivity of about 88.23% and specificity of about 100%. Toluidine blue showed sensitivity of about 76.47 % and specificity of about 100% .

So in the present study of total 70 cases the overall sensitivity of H&E was 57.45% and specificity was about 100% .Giemsa showed 87.23% sensitivity and 100% specificity , Toluidine blue showed 78.72% sensitivity and 100% Specificity .

Comparison of sensitivity and specificity of different stains in the present study cases of Gastritis and Gastric Adenocarcinoma

Table-27

Diseases	Sensitivity (%)			Specificity (%)			PPV(%)			NPV(%)		
	H&E	G	T.B	H&E	G	T.B	H&E	G	T.B	H&E	G	T.B
Gastritis (40)	66.67 %	86.67 %	80 %	100 %	100 %	100 %	100 %	100 %	100 %	50 %	71.43 %	62.5 %
Gastric Adenocarcinoma (30)	41.18 %	88.23 %	76.47 %	100 %	100 %	100 %	100 %	100 %	100 %	56.52 %	86.67 %	76.47 %
Overall results (70 cases)	57.45 %	87.23 %	78.72 %	100 %	100 %	100 %	100 %	100 %	100 %	53.49 %	79.31 %	69.70 %

- H & E – Hematoxylin - Eosin
- G – Giemsa
- T.B – Toluidine Blue
- PPV – Positive predictive value
- NPV – Negative Predictive value

Comparison of sensitivity and specificity of different stains in various studies

Table-28

Study	Sensitivity			Specificity			PPV			NPV		
	H&E	G	T.B	H&E	G	T.B	H&E	G	T.B	H&E	G	T.B
Shukla et al [136]	72.5%	80.4 %	-	100 %	100%	-	100%	100%	-	78.5 %	83.6 %	-
Pandya et al [137]	100%	100%	-	87.9 %	84.5 %	-	65%	59.1 %	-	100%	100%	-
J.E.Tzeng et al [132]	98.5%	98.5 %	-	100 %	97.8 %	-	100%	98.5 %	-	97.8 %	97.8 %	-
Raziye Tajalli et al [100]	41.86 %	53.49 %	76.74 %	100 %	95.24 %	100 %	62.07 %	69.70 %	75%	69.44 %	66.6 %	47.62 %
Present study (70 Cases)	57.45 %	87.23 %	78.72 %	100 %	100%	100 %	100%	100%	100 %	53.49 %	79.31 %	69.70 %

- H & E – Hematoxylin - Eosin
- G – Giemsa
- T.B – Toluidine Blue
- PPV – Positive predictive value
- NPV – Negative Predictive value

Summary

SUMMARY

During the period of July 2013 to June 2014 a total of 150 cases of gastritis and 90 cases of gastric adenocarcinoma were received in the Institute of Pathology, Madras Medical College, Chennai .Out of this 70 cases (40 cases of gastritis and 30 cases of gastric adenocarcinoma) were selected randomly for this study to detect the presence of Helicobacter pylori using Hematoxylin and Eosin, Giemsa, Toluidine Blue and Immunohistochemistry.

- In this study the most common age group infected with Helicobacter pylori was seen between 41-60 years in both gastritis and gastric adenocarcinoma cases.

- In the present study of 40 cases of gastritis, 30 cases were positive for Helicobacter pylori with a prevalence rate of about 75% and out of 30 cases of gastric adenocarcinoma 17 cases showed positivity for Helicobacter pylori with a prevalence rate of about 56.6%.

- In this study of total 70 cases , Helicobacter pylori infection showed an overall prevalence rate of about 67.1%.

- Males showed a maximum percentage of positivity in this study.

- In this study the maximum percentage of H.Pylori positivity was seen in Well differentiated grade of gastric adenocarcinoma.

- From this study it was shown that detection of Helicobacter pylori using Hematoxylin & Eosin ,Giemsa, Toluidine blue and Immunohistochemistry were identical in cases of grade 2 and grade 3 colonisation of H.pylori. Differences in staining pattern were observed in grade 1 when there is low colonisation of H.pylori , where maximum number of cases were detected by immunohistochemistry.

- Hematoxylin and Eosin showed sensitivity of about 57.45% and specificity of about 100% .

- Giemsa showed sensitivity of about 87.23% and specificity of about 100%.

- Toluidine blue showed sensitivity of about 78.72% and specificity of about 100%.

Conclusion

CONCLUSION

In histopathological sections Helicobacter pylori can be identified by various staining methods like Hematoxylin and Eosin (H&E), toluidine blue, modified Giemsa, Alcianyellow – toluidine blue, Warthin-starry, modified Genta and Immunohistochemistry. Toluidine blue and Giemsa methods are inexpensive and reliable. The major disadvantage is little contrast between the tissues and the bacteria.

Immunohistochemistry is the most sensitive technique but it is expensive and time consuming. It is not economical to use immunohistochemistry in all gastric specimens. It is used in certain specific situations like ;

- Low density of organisms
- To identify coccoid forms
- In cases of inactive gastritis

where the other stains carry low rate of detection for Helicobacter pylori.

The cost, reliability and applicability of Giemsa and Toluidine Blue make them as suitable stains for identification of Helicobacter pylori in gastric biopsies.

In this study Giemsa stain carries higher level of sensitivity over toluidine blue and H&E .Giemsa stain is also a less time consuming procedure when compared with IHC.

Hence in the present study Giemsa was more reliable and cost effective stain when compared with Hematoxylin & Eosin, Toluidine blue and immunohistochemistry. However, Immunohistochemistry carries the highest level of sensitivity in the detection of Helicobacter Pylori especially when the density of organism is low and in clinically suspected cases of Helicobacter Pylori with negative Giemsa staining.

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Annexures

ANNEXURE-I

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical diagnosis :

Complaint :

UGI scopy :

Radioimaging :

Specimen : Endoscopic biopsy/others

GROSS DETAILS :

MICROSCOPY : Histological diagnosis

Special stains for Helicobacter Pylori

Giemsa : Positive/ Negative

Toluidine blue : Positive/ Negative

IHC for H.Pylori : Positive/ Negative

FINAL IMPRESSION:

ANNEXURE-II

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical diagnosis :

Complaint :

UGI Scopy :

Radioimaging :

Specimen : Subtotal gastrectomy/Total gastrectomy/others.

GROSS

Specimen size : Greater curvature Lesser curvature

Tumor size :

Appearance :

Resected margins : Proximal Distal

MICROSCOPY : Histological diagnosis

Special stains for Helicobacter Pylori

Giemsa : Positive/ Negative

Toluidine blue : Positive/ Negative

IHC for H.Pylori : Positive/ Negative

FINAL IMPRESSION:

Master Chart

MASTER CHART

S.No:	BIOPSY NO	AGE	SEX	SYDNEY scoring of gastritis-H&E						GIEMSA		TOLUIDINE BLUE		IHC	
				ACTIVITY	CHRONIC INFLAMMATION	INTESTINAL METAPLASIA	ATROPHY	H.Pylori		POS/NEG	GRADE	POS/NEG	GRADE	POS/NEG	GRADE
								POS/NEG	GRADE						
1	89/14	58	M	1	1	0	1	N	0	N	0	N	0	P	1
2	632/14	22	M	1	1	0	1	N	0	N	0	N	0	N	0
3	637/14	56	M	0	1	0	0	N	0	N	0	N	0	P	1
4	833/14	23	F	1	1	0	0	P	1	P	1	P	1	P	1
5	1009/14	46	M	1	2	0	1	P	2	P	2	P	2	P	2
6	1048/14	68	F	0	1	0	0	N	0	P	1	N	0	P	1
7	1052/14	57	M	0	1	0	0	N	0	P	2	P	2	P	2
8	1484/14	18	F	2	2	0	0	P	2	P	2	P	2	P	2
9	2449/14	40	M	0	2	0	0	N	0	P	1	N	0	P	1
10	2492/14	45	F	1	2	0	1	P	2	P	2	P	2	P	2
11	2892/14	60	M	1	2	0	0	P	2	P	2	P	2	P	2
12	3298/14	50	F	1	1	0	0	P	1	P	1	P	1	P	1
13	3449/14	55	M	1	1	0	1	P	1	P	1	P	1	P	1
14	4180/14	50	F	0	1	0	0	P	1	P	1	P	1	P	1
15	4870/14	48	M	0	1	0	0	N	0	P	1	P	1	P	1
16	5381/14	63	M	0	2	0	0	N	0	N	0	N	0	N	0
17	5387/14	65	M	0	1	0	0	N	0	N	0	N	0	N	0
18	5390/14	45	F	0	2	0	0	N	0	P	1	P	1	P	1
19	5391/14	54	M	0	1	0	0	N	0	N	0	N	0	P	1
20	5624/14	57	M	1	3	0	1	P	3	P	3	P	3	P	3
21	5630/14	18	M	1	2	0	0	P	1	P	1	P	1	P	1
22	5886/14	65	F	1	1	0	1	P	1	P	1	P	1	P	1
23	5887/14	23	M	0	1	0	0	N	0	N	0	N	0	N	0
24	5901/14	10	M	1	2	0	1	P	3	P	3	P	3	P	3
25	6099/14	35	F	0	3	0	0	N	0	N	0	N	0	N	0
26	6178/14	65	F	0	2	1	0	N	0	N	0	N	0	N	0
27	846/13	55	M	1	1	0	1	N	0	N	0	N	0	N	0
28	1313/13	55	F	1	2	0	1	N	0	N	0	N	0	N	0
29	1675/13	60	F	0	3	1	0	N	0	N	0	N	0	N	0
30	2836/13	45	M	1	2	0	1	N	0	N	0	N	0	P	1
31	5479/13	33	M	1	2	0	0	P	3	P	3	P	3	P	3
32	8489/13	33	M	0	3	0	0	P	2	P	2	P	2	P	2
33	8595/13	52	M	0	3	0	0	P	2	P	2	P	2	P	2
34	9339/13	43	F	0	2	1	1	N	0	N	0	N	0	N	0
35	9518/13	60	M	1	1	0	0	N	0	P	1	P	1	P	1
36	9862/13	45	F	0	1	0	0	P	1	P	1	P	1	P	1
37	10558/13	65	M	0	2	0	0	P	1	P	1	P	1	P	1
38	10872/13	47	M	0	1	0	0	P	1	P	1	P	1	P	1
39	11108/13	65	M	0	2	0	0	P	2	P	2	P	2	P	2
40	11329/13	43	F	0	2	0	0	P	1	P	1	P	1	P	1

GASTRIC ADENOCARCINOMA CASES								
S.No:	**BIOPSY NO**	**AGE**	**SEX**	**H&E**	**GIEMSA**	**TOLUIDINE BLUE**	**IHC**	**Grade**
41	662/14	38	F	N	N	N	N	M
42	678/14	39	M	N	N	N	N	P
43	862/14	66	M	N	N	N	N	M
44	872/14	58	F	P	P	P	P	P
45	1249/14	62	M	N	N	N	N	P
46	1805/14	60	F	N	N	N	N	M
47	1810/14	40	M	N	P	P	P	M
48	1910/14	65	M	N	N	N	N	M
49	2072/14	60	M	P	P	P	P	M
50	2520/14	40	F	P	P	P	P	P
51	2551/14	60	F	N	N	N	N	M
52	3423/14	65	F	N	N	N	N	W
53	3532/14	66	M	N	N	N	P	W
54	3633/14	65	M	P	P	P	P	P
55	4409/14	60	M	N	N	N	P	M
56	414/13	54	M	P	P	P	P	M
57	1254/13	61	M	N	P	P	P	M
58	1440/13	70	M	N	P	N	P	M
59	1705/13	53	M	N	N	N	N	M
60	2363/13	65	M	P	P	P	P	M
61	3424/13	47	M	N	N	N	N	M
62	3708/13	50	M	N	N	N	N	M
63	4191/13	56	M	P	P	P	P	M
64	7998/13	66	M	N	N	N	N	M
65	8603/13	65	M	N	P	N	P	M
66	9074/13	46	M	N	P	P	P	W
67	9215/13	48	M	N	P	P	P	M
68	9969/13	57	M	N	P	P	P	M
69	10719/13	74	M	N	P	P	P	P
70	11216/13	57	F	N	N	N	N	P

KEY TO MASTER CHART

H&E	-	Hematoxylin and Eosin
IHC	-	Immunohistochemistry
P	-	Positive
N	-	Negative
H.Pylori	-	Helicobacter Pylori

SEX

M	-	Male
F	-	Female

GRADE

W	-	Well differentiated
M	-	Moderately differentiated
P	-	Poorly differentiated