HELICOBACTER PYLORI RELATED GASTRITIS IN ADULTS A CLINICAL,ENDOSCOPIC,HISTOPATHOLOGICAL AND RAPID UREASE TEST STUDY

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CERTIFICATE

This is to certify that the dissertation entitled '**HELICOBACTER PYLORI RELATED GASTRITIS IN ADULTS A CLINICAL, ENDOSCOPIC, HISTOPATHOLOGICAL AND RAPID UREASE TEST STUDY**' is the bonafide original work **Dr. P.RAJESWARI** in Partial fulfillment of the requirements for M.D. Branch – III (PATHOLOGY). Examination of the Tamilnadu Dr.M.G.R. Medical Uninversity to be held in April 2011.

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INTRODUCTION

The understanding of etiopathogenesis of peptic ulcer, expressed as gastritis, gastric ulcer, duodenitis, duodenal ulcer has been revolutionised during last decade with the discovery in 1983, of a new pathogen categorized as Helicobacter Pylori by Warren and Marshall. [67.68] Several reports have subsequently supported the association of H.pylori as a major etiological factor in the development of peptic ulcer disease [50, 78] and recent reports also suggest its association with gastric carcinoma and lymphoma.[82,88] Bacterium has been classified as class I definite gastric carcinogen to human [77,83,97]

A prospective study of adult presenting with upper abdominal pain,[20] dyspepsia, vomiting, haematemesis is undertaken to evaluate the relationship of this symptomcomplex to inflammatory gastroduodenal lesions with special reference to H.pylori infection. The clinical endoscopic findings, rapid urease test and histopathological evaluation of gastric antral specimen with special stains [16] to demonstrate the organism are presented and analysed.

Regarding the histopathology, previous studies on the pathologic changes of gastric mucosa, colonized by H.pylori have markedly focused on inflammatory reaction.

In addition to more common inflammatory cell infiltration it is only recently the histopathologic effect of H.pylori on gastric epithelium at light microscopic level has been stressed and this has been studied systematically,

describing striking changes in surface epthelium and attributing them as specific for H.pylori colonization and correlating them with type of cytotoxin [30,17,18] ,production and risk of peptic ulcer.[50,53] H.pylori infection can be diagnosed by invasive [12,14,66] (requiring endoscopy) and non invasive technique. [22,29,64]

In this study the various methods of identification of H.pylori and histopathological features associated H.pylori in gastric mucosa in patients, presenting with dyspepsia are discussed and described in detail paying particular attention to histopathological effects of H.pylori on epithelial cells and evaluate the gastric mucosa as per updated Sydney system.[23,24,70,88]

In addition, the recent literature regarding epidemiology, clinical features, pathogenesis of infection is also reviewed.

AIM OF STUDY

- The Aim and objective of this study find out the association of H.pylori with gastric lesions in endoscopic biopsy specimen in semi-urban regions.
- To study the specificity and sensitivity of Rapid Urease test.
- To evaluate the usefulness of Giemsa stain in addition to histopathological examination for identification of H.pylori.
- To evaluate the gastric mucosa as per the modified Sydney system.

REVIEW OF LITERATURE

Embryology:

The epithelial lining of the various parts of gastrointestinal tract is that of endodermal origin.

Stomach:

The stomach is first seen as a fusiform dilatation of the foregut just distal to the oesophagus. Its distal border is attached to the posterior abdominal wall by a fold of peritoneum called the dorsal mesogastrium. Its ventral border is attached to the septum transversum by another fold of peritoneum called the ventral mesogastrium.

The stomach undergoes differential growth resulting in a considerable change in the shape and orientation. The original ventral border comes to face upward and to the right and becomes the lesser curvature. The dorsal border now points downwards and to the left and becomes greater curvature. The original left surface becomes its anterior surface and the original right surface becomes the posterior surface.

Anatomy of stomach:

The stomach extends from just left of the midline where it is joined to the oesophagus, to just right of the midline where it is connected to the duodenum. It resembles a large gland.

The concavity of the right, the inner curve is called the lesser curvature, and the convexity of the left, outer curve is the greater curvature. An angle along the lesser curve, the incisura angularis, marks the approximate point at which the stomach narrow prior to its junction with the duodenum. The most distal and narrow portion of the stomach is termed the pylorus.

In the empty state, the stomach is contracted and its mucosa and submucosa are thrown up into distinct folds called rugae. When distended with food, the rugae are "ironed out" and flat. It secretes a complex of digestive enzymes, acid and mucus.

The stomach is divided into 5 anatomical regions (Fig.1)

1. Cardia – Narrow conical portion of the stomach immediately distal to the gastroesophageal junction.
2. Fundus – Domeshaped portion of the proximal stomach that extends superolateral to the gastroesophageal junction.
3. Body or Corpus – Comprises the remainder of the stomach proximal to the incisura angularis.
4. Antrum – The stomach distal to the incisura angularis.
5. Pylorus – The most distal and narrow portion of the stomach.

HISTOLOGY OF STOMACH

The gastric wall consists of mucosa, submucosa, muscularis propria and serosa.

Mucosa

It has two compartments; the superficial foveolar compartment and the deep glandular compartment.

Foveolar compartment

It consists of surface epithelial cells lining the entire mucosal surface as well as gastric pits.

It is relatively uniform throughout the stomach. The surface epithelium shows a regular picket – fence arrangement, with a plentiful diffuse secretion of mucus. All foveolar cells secrete mucus, in contrast to the scattered goblet cells, between absorptive cells, lining the intestine. The cells are tall, with regular basal

nuclei. The epithelial surface appears flat on light microscopy. This epithelium lines the gastric pits (foveolae).

In the body of the stomach, the pits are short tubules, with long, closely packed glands opening into them. Figno7. When seen in three dimensions, the pits in the antrum have a partial cerebriform structure of complex folds. The mucus secreting glands branch from the base of these pits. The superficial parts of the gland form narrow straight tubules, the gland neck, lined by epithelium similar to the outer surface but with smaller cells.

A primary function of the foveolar cells is the secretion of mucus. A dense layer of mucus globules fills the superficial one third to two thirds of the cells. Normal foveolar mucus consists mainly of neutral glycoprotein, which is strongly "Periodic Acid Schiff (PAS)" positive and stain weakly with Alcian blue, whereas intestinal mucins stain strongly with Alcian blue.

A combination of Alcian blue and PAS [ABpH2.5PAS] shows these differences practically and well appreciated. Foveolar mucus stains as a rich magenta red, in contrast the intestinal mucus stains a variety of purple and indigo blue colors as a result of the presence of acid glycoproteins.

One notable exception is the secretion of Brunner's glands in the duodenum, which stains a brighter red than the gastric foveolar mucus. Brunner's glands, however, are deep to the muscularis mucosae and are irregular and glandular outline, in contrast to foveolae.

Glandular Compartment

It exhibits major difference in thickness and in glandular composition in different regions of stomach. It consists of gastric glands, which vary between anatomic regions.

1. Cardiac glands contain mucus secreting cells only.
2. Oxyntic glands (also called gastric / fundic glands) found in the fundus and body and contain parietal cells, chief cells and scattered endocrine cells.
3. Antral or pyloric glands contain mucin secreting cells and endocrine cells.

The normal gastric mucosa shows another unusual feature. It is the only part of the gastrointestinal tract with almost no lymphoid tissue.

HELICOBACTER PYLORI

Before the first isolation and documentation of Helicobacter pylori from the human stomach in 1982, it was assumed that the human stomach was a sterile environment because of the high levels of acid, which would exclude it as an ecologic niche for any organism.

H.pylori was introduced into the scientific community in 1982 by Marshall[67,68] and Warren who described a campylobacter-like bacterium that was seen in large numbers in the gastric mucus of patients with chronic gastritis and duodenal ulcers.

Historical Perspective:

German scientists found spiral-shaped bacteria in the lining of the human stomach in 1875, but they were unable to culture it and the results were eventually forgotten.[40]

Professor Walery Jaworski[41] of the Jagiellonian University in Krakow investigated sediments of gastric washings obtained from humans in 1899. Among some rod-like bacteria, he also found bacteria with a characteristic spiral shape, which he called Vibrio Rugula. He was the first to suggest a possible role of this organism in the pathogenesis of gastric diseases. This work was included in the Handbook of Gastric Diseases, but it had little impact as it was written in Polish.

Several small studies conducted in the early 1900s demonstrated the presence of curved rods in the stomach of many patients with peptic ulcer and stomach cancer.[92]

The interest waned when an American study published in 1954 failed to observe the bacteria in 1180 stomach biopsies.

However the role of bacteria in stomach diseases was rekindled in the 1970s with the visualization of bacteria in the stomach of gastric ulcer patients.

The bacterium had also been observed in 1979 by Australian pathologist Robin Warren, [94] who did further research on it with Australian physician Barry Marshall beginning in 1981. After numerous unsuccessful attempts at culturing the bacteria from the stomach, they finally succeeded in visualizing colonies in 1982 when they unintentionally left their Petri dishes incubating for 5 days over the Easter weekend.

In their original paper, Warren and Marshall contended that most stomach ulcers and gastritis were caused by infection by this bacterium and not by stress or spicy food as had been assumed before.[13]

Although there was some skepticism initially, within several years, numerous research groups verified the association of H.pylori with gastritis.

To demonstrate the role of H.pylori with gastritis, Marshall drank a beaker of H.pylori. He became ill several days later with nausea and vomiting. An endoscopy performed ten days after inoculation revealed signs of gastritis and the presence of H.pylori. These results suggested that H.pylori was the causative agent of gastritis.

Marshall and Warren also showed that antibiotics were effective in the treatment of many cases of gastritis.

In 1987 the Sydney gastroenterologist Thomas Borody[24, 85,86] invented the first triple therapy for the treatment of duodenal ulcers.

In 1994, the National Institute of Health (USA) published an opinion stating that most recurrent duodenal and gastric ulcers were caused by H.pylori and recommended antibiotics in the treatment regimen. Warren and Marshall were awarded the Nobel Prize in Medicine in 2005 for their wonderful work on H.pylori.[94]

EPIDEMIOLOGY

At least half the world's population is infected by H.pylori making it the most widespread infection in the world.[13,48] Actual infection rates vary from nation to nation. The Third World has much higher infection rates than the West (Western Europe, North America, Australia), where the rates are estimated to be around 25%.

Infections are usually acquired in early childhood in all countries. However, the infection rate of children in developing nations is higher than in industrialized nations, probably due to poor sanitary conditions.

In developed nations it is currently uncommon to find infected children. The percentage of infected people increases with age, 50% infected are over the

age of 60 compared with 10% between 18 and 30 years of age. The higher prevalence among the elderly reflects the fact that they were infected from childhood.[7]

Prevalence appears to be higher in African – American and Hispanic population, although this is likely related to socioeconomic rather than racial factors.[13,49,94] The lower rate of infection in the West is largely attributed to higher hygiene standards and widespread use of antibiotics. Despite high rates of infection in certain areas of the world, the overall frequency of H.pylori infection is declining. However, antibiotic resistance is appearing in H.pylori and many metronidazole and clarithromycin resistant strains are seen in most parts of the world.

H.pylori is contagious, although the exact route of transmission is not known. Person-to-person transmission by either the oral-oral or fecal-oral route is most likely.[13,55] Consistent with these transmission routes, the bacteria have been isolated from feces, saliva and dental plaque of some infected people.[49]

Transmission occurs mainly within families in developed nations yet can also be acquired from the community in developing countrie H.pylori may also be transmitted orally by means of fecal matter through the ingestion of waste tainted water. Hence a hygienic environment could help to decrease the risk of H.pylori infection.[13]

MORPHOLOGY

H.pylori is a spiral to curved, rod shaped, gram negative microaerophilic bacterium about 3 microns long with a diameter of about 0.5 micron. It has 4 to 7 polar sheathed flagella, which enable the bacterium to move freely in viscous environments such as gastric mucus.[94]

This bacterium is the human-adapted helicobacter primarily found in the gastric mucosa and areas of gastric metaplasia in the duodenum and occasionally in Meckel's diverticulum and rectum.

BIOCHEMICAL CHARACTERISTICS

H.pylori is urease, catalase and oxidase positive. The urease activity is striking and the amounts produced have allowed accurate diagnosis in patients by direct detection of the enzyme in gastric biopsy specimens and by breath tests using carbon isotopes labeled with urea.

Many roles have been proposed for urease enzyme. It is known to be important for colonization and survival of the bacterium in the gastric environment.[85,90]

The hydrolysis of urea to ammonia by urease could have a buffering effect, protecting the bacterium from acidity.

In-vitro studies have shown that helicobacter pylori cannot survive in acidic condition without the presence of urea, and urea inhibits its growth in alkaline conditions.[94,48,82] Urease also has been proposed as an important virulence factor.

GENETICS

In 1997, the complete genomic sequence of H.pylori strain 26695 was published.[61,68] This bacterium has a single circular chromosome of 1,667,867 base pairs and 1590 predicated coding sequence of which 1091 matched database sequence of genes are known from other organisms.

PATHOPHYSIOLOGY

To colonize the stomach H.pylori must survive in the acidic pH of the lumen and burrow into the mucus to reach its niche, close to the stomach's epithelial cell layer. The bacterium has flagella and moves through the stomach lumen and drills into the mucoid lining of the stomach.[55]

Many bacteria can be found deep in the mucus, which are continuously secreted by mucous cells and removed on the luminal side. To avoid being carried into the lumen, H.pylori senses the pH gradient within the mucus layer by chemotaxis and swims away from the acidic contents of the lumen towards the more neutral pH environment of the epithelial cell surface.[28,57]

H.pylori is also found on the inner surface of the stomach epithelial cells and occasionally inside epithelial cells,[91] It produces adhesions which bind to membrane-associated lipids and carbohydrates and help it adhere to epithelial cells. Adhesin BabA binds to the Lewis b antigen displayed on the surface of stomach epithelial cells.[82]

H.pylori produces large amounts of the enzyme urease, molecules of which are localized inside and outside of the bacterium. Urease breaks down urea (which is normally secreted into the stomach) to carbon dioxide and ammonia (which neutralizes gastric acid). The survival of H.pylori in the acidic stomach is dependent on urease, and it would eventually die without the enzyme. The ammonia that is produced and other products of H.pylori such as protease, vacuolating cytotoxin A(VacA), and certain phospholipases are toxic to the epithelial cells.[42,30,17]

Colonization of the stomach by H.pylori results in chronic gastritis. The severity of the inflammation is likely to underlie H.pylori related diseases.[88,90] Duodenal and stomach ulcers result when the consequences of inflammation allow the acid and pepsin in the stomach lumen to overwhelm the mechanisms that protect the stomach and duodenal mucosa from these caustic substances.

The type of ulcer that develops depends on the location of chronic gastritis, which occurs at the site of H.pylori colonization. The acidity within the stomach lumen affects the colonization pattern of H.pylori and therefore ultimately determines whether a duodenal or gastric ulcer will form.

In people producing large amounts of acid, H.pylori colonizes the antrum of the stomach to avoid the acid – secreting parietal cells located in the corpus (main body) of the stomach. The inflammatory response to the bacteria induces G (gastrin – producing) cells in the antrum to secrete the hormone gastrin, which travels through the bloodstream to the corpus. Gastrin stimulates the parietal cells in the corpus to secrete even more acid into the stomach lumen. Chronically increased gastrin level eventually cause the increase in the number of parietal cells, further increasing the amount of acid secreted. The increased acid load damages the duodenum and ulceration may eventually result.

In contrast, gastric ulcers are often associated with normal or reduced gastric acid production, suggesting that the mechanisms that protect the gastric mucosa are defective. In these patients H.pylori can also colonize the corpus of the stomach, where the acid-secreting parietal cells are located. However, chronic inflammation induced by the bacteria causes further reduction of acid production and eventually, atrophy of the stomach lining, which may lead to gastric ulcer and increases the risk for stomach cancer.[55,70]

About 50-70% of H.pylori strains in Western countries carry the Cag Pathogenicity Island (Cag PAI).[18,53] Western patients infected with strains carrying the Cag (cytotoxin – associated antigen) PAI have a stronger inflammatory response in the stomach and are at a greater risk of developing peptic ulcers or stomach cancer than those infected with strains lacking the island. Following attachment of H.pylori to stomach epithelial cells, the type IV secretion system expressed by the Cag PAI "injects" the inflammatory inducing agent peptidoglycan from their own cell wall into the epithelial cells. The injected peptidoglycan is recognized by the cytoplasmic immune sensor Nodl, which then stimulates expression of cytokines that promote inflammation.

The type IV secretion apparatus also injects the Cag PAI-encoded protein CagA into the stomach's epithelial cells, where it disrupts the cytoskeleton, adherence to adjacent cells, intracellular signalling, and other cellular activities. Once inside the cell the CagA protein is phosphorylated on tyrosine residues by a host cell membrane – associated tyrosine kinase.

Pathogenic strains of H.pylori have been shown to activate the epidermal growth factor receptor (EGFR), a membrane protein with a tyrosine kinase domain. Activation of the EGFR by H.pylori is associated with altered signal transduction and gene expression in host epithelial cells that may contribute to pathogenesis. It has also been suggested that a terminal region of the CagA protein (amino acids 873-1002) can regulate host cell gene transcription independent of protein tyrosine phosphorylation.

Two related mechanisms by which H.pylori could promote cancer are under investigation. One mechanism involves the enhanced production of free radicals near H.pylori and an increased rate of host cell mutation. The other proposed mechanism has been called a "perigenetic pathway" and involves

enhancement of the transformed host cell phenotype by means of alterations in cell proteins such as adhesion proteins. It has been proposed that H.pylori induces inflammation and locally high level of TNF- α and IL-6 (interleukin-6). According to the proposed perigenetic mechanism, inflammation-associated signaling molecules such as TNF- α can alter astric epithelial cell adhesion and lead to the dispersion and migration of mutated epithelial cells without the need for additional mutations in tumor suppressor genes such as genes that code for cell adhesion proteins.

H.pylori colonizes the stomach and induces chronic gastritis. The bacterium persists in the stomach for decades in most people. Most individuals infected by H.pylori will never experience clinical symptoms despite having chronic gastritis. Approximately 10-20% of those colonized by H.pylori will ultimately develop gastric and duodenal ulcers. H.pylori infection is also associated with a 1-2% lifetime risk of stomach cancer and a less than 1% risk of gastric MALT lymphoma.

It is widely believed that in the absence of treatment, H.pylori infection-once established in its gastric niche-persists for life. In the elderly, however, it is likely that infection can disappear as the stomach's mucosa becomes increasingly atrophic and inhospitable to colonization. The proportion of acute infections that persist is not known, but several studies that followed the natural history in populations have reported apparent spontaneous elimination.

While H.pylori has been disappearing from the stomach of humans, the incidence of the related disorders acid reflux disease, Barrett's esophagus, and esophageal cancer have been rising dramatically.

In 1996, Martin J. Blaser advanced the hypothesis that H.pylori has a beneficial effect, by regulating the acidity of the stomach contents, it lowers the

impact of regurgitation of gastric acid into the esophagus. The hypothesis is not universally accepted as several randomized controlled trials failed to demonstrate worsening of acid reflux disease symptoms following eradication of H.pylori.

Nevertheless, Blaser has refined his view to assert that H.pylori is a member of the normal flora of the stomach. He postulates that the changes in gastric physiology caused by the loss of H.pylori account for the recent increase in incidence of several diseases, including Type-2 diabetes, obesity and asthma His group has recently shown that H.pylori colonization is associated with a lower incidence of childhood asthma.

CLINICAL OUTCOMES ASSOCIATED WITH H.PYLORI

Peptic ulcer(Fig. 3,4)

H.pylori associate strongly with duodenal ulcers, than with gastric ulcers. Gastric metaplasia of the duodenal mucosa is common in places near the duodenal ulcers.

Carcinoma

There is a growing body of evidence that H.pylori is a precursor of carcinoma of the body and antrum of the stomach. H.pylori infection causes chronic gastritis and it leads to development and progression of atrophic gastritis and intestinal metaplasia, which are considered precursor lesions of gastric cancer. Some strains of H.pylori may be more carcinogenic than others, especially CagA-positive bacteria.

MALT Lymphoma

Primary Non-Hodgkin Lymphoma (M+NHL) of the stomach is a relatively rare malignant disorder, accounting for about 5% of gastric tumors. The cause of

primary gastric NHL had been unknown because by definition NHLs are malignant clonal diseases of the lymphatic tissue, and the stomach is a site that is normally considered to be devoid of organized lymphoid tissue.[82,88,90]

The association between Mucosa associated lymphoid tissue (MALT) lymphoma and H.pylori was postulated for the first time in 1998 with the recognition that the cause of acquired gastric MALT is chronic infection with H.pylori. Wother-spoon et al were the first to investigate the presence of H.pylori in larger numbers of gastric lymphomas of the MALT type (MALToma). H.pylori was detected in 92% of cases.

They suggested that H.pylori (and Helicobacter heilmannii) might trigger the acquisition of MALT in the gastric mucosa, and this lymphoid tissue is thought to harbor the precursor cells for MALT NHL. These precursor cells changes gradually into malignant lymphoma cells with autonomous and uncontrolled growth by accumulation of genetic alteration, mutations, deletions and amplifications (i.e., trisomy 3 and 7)

GASTRIC PATHOLOGY ASSOCIATED WITH H.pylori

The active changes defined by Whitehead et al are linked closely with H.pylori infection in humans. These changes, together with non-specific chronic gastritis, usually accompany helicobacter infection.

Three features make the diagnosis:

1. The presence of uniform small curved bacilli, closely adherent to the surface of the epithelium
2. A typical infiltration of the epithelium by polymorphonuclear neutrophil.
3. The typical epithelial distortion, which is specific but often absent.

Identification and distribution of organism

The bacteria play an important part in the histopathology of H.pylori gastritis. Small numbers of H.pylori may still be difficult to find. A search with the oil immersion lens usually reveals any organisms. Single Helicobacter-like organisms can be recognized. [Small, pale, curved, well formed bacilli]. Immunoperoxidase antibodies are also available. Polymerase chain reaction provides a sensitive and specific method of identification.

H.pylori show marked variation in number and distribution. They proliferate mostly on the superficial foveolar epithelium. Fewer bacilli grow in the gastric mucosal pits.

Occasional Helicobacter organism grows in the inflamed gland necks but almost never in the glands. They tend to adhere closely to the surface of the epithelial cells, particularly in the antrum, often palisading. Unattached bacilli are commonly seen deep in the mucus.

The H.pylori is of unusual length in occasional biopsy specimens. They may be less than half or almost double the usual length. In most well-fixed specimens, they are small, pale, curved bacilli and remarkably constant in appearance. Single bacillus is easy to identify with oil immersion microscopy.

H.heilmannii is larger and more tightly coiled. They tend to infiltrate into the glands, often in the fundus, and produce remarkably little reaction. In contrast to H.heilmannii, H.pylori is rare in the actual corpus mucosa gastric glands (as distinct from the necks of the glands).

Foreign bacteria are common on gastric biopsy specimens. They show variation in size than H.pylori, which are larger with darker staining than others. They are usually seen above the mucus secretion but not on the epithelial cell surface.

H.pylori grows well on intact antral mucosa and also in the fundus, usually in smaller numbers than in the antrum. When the fundal mucosa is intact, the Helicobacter organisms seen less firmly attached to the epithelial surface. They often float deep in the mucus layer.

H.pylori grows only on gastric-type epithelium, and a dense proliferation of the bacteria stops within one cell of a focus of mature intestinal metaplasia.

Sometimes few bacteria grow on areas of partial or atypical metaplasia, with PAS-positive mucus in the epithelial cells between the goblet cells.

They sometimes grow on the areas of gastric metaplasia in the duodenum, usually patchy and fewer in number than the antrum. Infected areas often show active inflammation, similar to the stomach.

The bacteria rarely grow in the oesophagus but can do so in Barrett's esophagitis with well-formed gastric metaplasia near the gastroesophageal junction.

Grading of morphological variables are carried out in an attempt to improve the agreement among different observers. In the updated Sydney system a visual analogue scale was provided to assist in grading as mild, moderate, marked and a set of guidelines for its application have been designed.

Graded Variables:

1. H.pylori density:

H.pylori is small, curved, slightly basophilic bacillus that is most easily seen in the mucus layer overlying the gastric surface epithelium, with gastric pits, rarely intra cellularly. In a given biopsy the organism may be dense in one area of section and absent or sparse in another.

Evaluation of H.pylori density is performed as mild, moderate or marked in the areas where it normally resides. Using the visual analogue scale provides avoiding the inclusion of entire length of specimen by upgraded Sydney systems.[24 70,82,88,]

Polymorphonuclear Neutrophil infiltration:

Activity implies the presence of Neutrophil polymorphs in a background of chronic inflammation [49,90], Neutrophil activity is almost universal phenomenon in H.pylori gastritis.[72] Neutrophils may be seen in lamina propria within epithelium, (particularly in the region of glandular neck) and within foveolar lumen, where they form pit abscess. Neutrophils are very sensitive indicator. The features described by Whitehead at a as active gastritis almost always are associated with H.pylori infection.

H.pylori and epithelial Neutrophil show an almost absolute correlation. The Neutrophils infiltrate with a typical pattern, not seen otherwise. They usually infiltrate the epithelium of the necks of the glands, adjacent to the base of gastric mucosal pits. As a rule, this infiltration is most obvious in the gastric antrum. The typical infiltration is focal.

Superficial epithelial neutrophil, without involvement of the gland necks are less specific for H.pylori infection. The severity of the changes is of less diagnostic importance. These changes are related to the presence but not the number of H.pylori.

Neutrophil infiltrating the stroma (the mucosal lamina propria) are not clearly related to H.pylori infection. Neutrophil infiltrate into the lumen of some gland necks and may fill the overlying pits. It results in micro abscesses, resembling the crypt abscesses of Ulcerative colitis or Crohn's disease.

Chronic inflammation – The normal gastric mucosa contains only individual scattered chronic inflammatory cells in laminar propria. They are indicators of chronic gastritis. The cellular infiltrate contains effectors of immune response including CD4 , CD8, T-Lymphocytes, B-lymphocytes, plasma cells, monocytes, mast cells, eosinophils**.**

Normal number of gastric mucosal mononuclear cells in laminar propria is viewed as maximum 2-5 lymphocytes, plasma cells, macrophages/ HPF.[91]

>5 lymphocytes per 100 epithelial cells indicate inflammation. Chronic inflammatory cells have been shown to disappear after eradication of H.pylori.

Atrophy:

Intestinal Metaplasia

Specific epithelial changes

Normally epithelial cells look rigid, with a picket-fence arrangement, a flat surface, regular small round basal nuclei, and plentiful superficial mucus secretion. The main specific change in the foveolar epithelium is a disorganization of the structure of the epithelial cells. This may be mild, moderate, or severe and diffuse, patchy, or focal.[90]

The mildest recognizable specific change consists of a definite cobblestone irregularity of the epithelial surface. The surface is no longer flat; the cells bulge out[72].

When the change are severe, the epithelial cells show ameboid features. The cells lose their picket – fence arrangement and the basal nuclear polarization. The epithelium often appears thickened, with irregular nuclei scattered throughout.

Microcrypts

Microcrypts, small spaces or virtual spaces within the foveolar epithelium, are often seen with H.pylori. Adjacent epithelial cells form microcrypts by turning in on each other, producing an apparent intraepithelial mucus-secreting gland. Microcrypts are not specific for Helicobacter, but only infected mucosa shows them easily. Small groups of the bacteria often fill the microcrypts. One of the last places where the bacteria collect is in the microcrypts.

Mucous secretion

Factors that alter cell function tend to reduce secretion. Such factors include epithelial proliferation, inflammatory damage, atrophy and cellular atropia or dysplasia. Reduced mucus secretion by the foveolar cells is observed with H.pylori infection. The gastritis, rather than the Helicobacter may cause this change. They reduced mucous secretion which could be a combination of direct bacterial effect and the associated inflammatory damage.[72]

Reduced mucus secretion with a diffuse cobblestone change gives an appearance resembling a string of beads [PAS stain]. These changes are well seen in the antrum.

Atrophy of gastric mucosa is defined as loss of glandular tissue. Loss of glands may follow erosion or ulceration of mucosa with destruction of glandular layer result from prolonged inflammatory process where individual cells undergo destruction of piecemeal fashion.[70] Helicobacter infiltration and the changes of active gastritis are most obvious in the gastric antrum. The appearance of atrophy in the antrum is deceptive, however. Atrophy is easier to recognize in the corpus, where it is mild, focal or absent.

The glands become separated, with increasing loss of chief and parietal cells. The inflammatory infiltration is mild and superficial. The most severe cases show widespread intestinal metaplasia. In such areas, Helicobacter is sparse or absent, and the stromal inflammation decreases.

Intestinal Metaplasia is a complex process in which superficial and gastric pit lining epithelial changes, both morphologically and histochemically. Metaplastic epithelium can be identified as resemblance of lining epithelial cells of small intestine [intestinal metaplasia]. The basic histologic structure of the gastric mucosa remains intact. Metaplastic changes are mainly seen in the superficial part of the epithelium. When metaplasia extends into the glands, they usually show marked distortion and atrophy, but they do not resemble intestinal crypts, which are much shorter and more regular.

The metaplastic epithelium can show a wide range of appearances. In type I intestinal metaplasia,[72,82] the metaplastic epithelium resemble mature small intestinal epithelium. In type II metaplasia, the epithelium frequently undergoes a partial, incomplete, or atypical metaplastic change. This metaplasia may consist of scattered goblet cells in regular epithelium showing a variable amount of gastric mucus secretion. In type III metaplasia, foveolar-like cells are large with nuclear irregularity, poor mucus secretion and poorly formed goblet cells.

There is marked difference between the superficial epithelium of the stomach and the intestine. Gastric foveolar epithelium secretes neutral glycoproteins diffusely from all cells. The intestine secretes acidic mucins from scattered goblet cells, separated by nonsecretory cells. A standard H&E stain shows the goblet cells and microvilli, but small foci of metaplasia are easy to miss. Specific stains for mucus give a much more sensitive and definite result.

Alcian-PAS provides and excellent method of finding metaplasia quickly and easily in the stomach or duodenum.

The relationship between Helicobacter and metaplasia is complex. H.pylori do not grow on intestinal epithelium, including intestinal metapasia. Because of this fact, larger areas of mature intestinal metaplasia in the stomach show reduced inflammation, with regular epithelium and no active changes.

Other histological changes: - Non-graded variables

Surface epithelial changes: Mucin depletion, loss of nuclear polarity, nuclear enlargement, irregular ragged surface epithelial pits, micro erosion, ulceration. The surface epithelial changes are marked in pit regions than in surface epithelium.

Lymphoid Follicles: Lymphoid aggregates with active germinal centers are characteristics of H.pylori especially in children.

Foveolar hyperplasia: Increased length and tortuosity of the foveolae combined with expansion of the proliferative compartment as an increase in nuclear size relative to mucin depleted cytoplasm - as a result of cytokine stimulation in chronic gastritis (chemical gastritis).

Pseudo pyloric metaplasia: --When the site of gastric biopsy in uncertain glands can be differentiated from true antral glands in which endocrine cells associated with metaplastic glands do not include “G” cells. whereas, they routinely accompany antral glands. Furthermore, pseudopyloric glands contain both Pepsinogen I and II, whereas the true antral glands show only Pepsinogen II. Discriminating between two glands are of great value correctly localizing and classifying atrophic gastritis.

Endocrine cell hyperphasia: In auto immune gastritis, hypochlorhydria or achlor hydria leads to G-cell hyperplasia to which the exposed ECL cells in the oxyntic glands undergo hyperplasia.

Stromal changes

H.pylori causes nonspecific inflammation of the mucosal stroma (lamina Propria). This inflammation varies considerably and usually is most obvious in the antrum. The most striking change is an infiltration of lymphoid cells, which are almost absent in the normal stomach. Often a moderate diffuse infiltration of lymphocytes extends through the full thickness of the mucosa to the mucosa to the muscularis mucosae. Other less common patterns of lymphocytic infiltration include fine diffuse, superficial, patchy or focal and dense diffuse. Small follicle-like concentration of lymphocytes are frequent fully developed lymphoid follicles, with germinal centers, are uncommon.

Congestion and edema are common. Patchy fibrosis accompanies more severe damage, often related to varying degree of glandular distortion and atrophy. Other cells often present in small numbers include eosinophils and mast cells.

Electron microscopy

The normal gastric epithelium consists of a sheet of well-formed cylindrical cells. The superficial surface is flat, with numerous microvilli. The microvilli are not as numerous or well formed as those on the intestinal cells, but they still are plentiful and fairly regular. Fibrils attach into the microvilli, extend through the cytoplasm, past the globules of mucus and the nucleus, and into the

base of the cell. These fibrils appear to provide a skeleton that maintains the cell shape and internal structure, with a flat surface.

With H.pylori infection, the bacteria attach to the epithelial cells, with patches resembling cell junction. They often attach to the microvilli. The microvilli become distorted, thickened, and reduced in number. As the microvilli disappear, the fibrils also disappear. The cells lose their skeleton and become somewhat ameboid. The intercellular junctions and basal junction may weaken, but they remain intact and hold the cell in position. The cell surface bulges out, however, giving the cobblestone appearance often seen with H.pylori infection.

Changes after treatment

Patients with mild pathology reverted to normal within two weeks of treatment. The neutrophil infiltration and the active changes in the epithelium vanished with the bacteria. The foveolar epithelium soon returned to normal, with normal mucus secretion and regular picket-fence appearance. The lymphoid infiltration improved slowly. It was usually mild after twelve months and normal, almost absent, after seven years.

DIAGNOSIS

H.pylori infection can be diagnosed by invasive (i.e., requiring endoscopy) [12,14,16,85] and noninvasive techniques (i.e., techniques that do not require endoscopy).[22,29,64] Each of the available diagnostic techniques has advantages and disadvantages.

Non-invasive techniques

1. Serologic testing
2. Urea breath tests
3. Stool tests

Invasive techniques

1. Urease tests
2. Biopsy
3. Culture
4. Polymerase chain reaction
5. Immunohistochemistry

Serologic testing

This is the commonest method of non-invasive diagnosis for H.pylori. Generally the prevalence of raised IgG in the population tends to be higher in developing countries than in developed countries. Soon after the discovery of H.pylori,[22,85] Jones et al described a complement fixation test that had an accuracy of 80% to 90%. Alternative methods, such as hemagglutination, were available soon after this, followed by more sophisticated enzyme-linked immunosorbent assay (ELISA) methods, such as those first described by Good-win et al.

The various serodiagnostic techniques used in detecting H.pylori are Bacterial agglutination, Complement fixation test, Haem-agglutination, ELISA, Western blotting, Co-agglutination, immuno-fluorescence, Radio immunoassay and Latex agglutination.[80]

Sensitivity and specificity of ELISA depend largely on the nature of the antigenic materials bound to the solid support. Although more expensive, the gold standard for multiple antigens can be obtained in an individual patient.

In cases in which serologic response has been studied, it appears to be similar to any other bacterial infection (i.e., after approximately 14 days, IgM is present and by 21 days, IgG is detectable). IgM declines over the next 3 months so that patients with chronic H.pylori infection usually have no IgM but always have IgG. IgA is variable. The antibody titres preserve their levels even after the eradication of the bacteria by antibacterial therapy. A 50% fall in titer of IgG between 6 and 9 months after treatment is predicted as cure.

Urea breath tests

This test is based on organism's urease activity, which liberates carbon dioxide (Co_2) from urea and produces ammonia to buffer its acidic environment. Ingestion of labeled urea results in the production of labeled CO_2, which then can be detected in the breath. In contrast to antibody based testing, the urea breath test identifies patients with active H.pylori infection[85].

Two forms of labeled urea are available: one contains the stable, nonradioactive isotopes ^{13}C, and the other contains the radioactive isotope ^{14}C. Because of broad exposure of ingested ^{14}C-urea to the gastric mucosa, sampling error theoretically is less of a problem with the urea breath test than with the biopsy based diagnostic methods for H.pylori.

Stool tests

Culture of H.pylori has been obtained from stool samples, but viable organisms are present only in a small percentage of cases. An enzymatic

immunoassay that detects the presence of H.pylori antigen in stool specimen is also available. The test uses polyclonal anti-H.pylori capture antibody absorbed to microwells. The HpSA(Helicobacter pylori Stool Antigen) test has received approval from the US Food and Drug Administration for two indications: Diagnosis of H.pylori infection in symptomatic adults and monitoring response, post therapy in adults [60.]

Urease test

With the observation that H.pylori was a strong urease producer, several groups began to work on the use of urease as a marker of H.pylori in the human stomach. This work ultimately resulted in the rapid urease test and the urea breath test, both are now widely used in the diagnosis of this common infection.[60]

The enzyme was active at physiologic temperatures, with an optimum of 45^{o}C. The enzyme was found to be rapidly denatured in acid, so that it was inactive at any pH less than 4.5. Active urease is located only beneath the mucus layer where the pH is neutral and the H.pylori organism resides. Urease tests can be based on biopsy or can be performed on samples of gastric mucus scraped and retrieved from the stomach at endoscopy.

False positive results may occur when non-H.pylori helicobacter organisms infect the gastric mucosa. Helicobacter heilmannii is also urease positive. Urease reactions are less intense with non-H.pylori helicobacer organisms and are more likely to be positive in the corpus rather than in the antral mucosa.

Patients taking omeprazole often have achlorhydria. Subsequent superficial colonization of the gastric mucus layer with urease producing organisms (e.g., Proteus mirabilis or Klebsiella) can give a false-positive urease

test after 24 hrs of inoculation but generally are negative when the test is read 1 hour after biopsy insertion.

The presence of achlorhydria causes false-negative urease test results because the luminal pH of 7.0 can lead to an extremely high pH adjacent to the organism such that H.pylori is destroyed by the action of its own urease.

Biopsy

The bacteria are an important part of the histopathology of H.pylori gastritis. Bacterial stains for histology must contrast the organisms against the complex background of tissue section. The position of H.pylori on the surface of mucus-secreting cells, makes histological staining a little easier. The method must stain the organism and not the mucus.

Commonly used special stains include Warthin-Starry, Giemsa, Diff-Quik, Genta and El-Zimaity's triple stain. The Warthin-Starry method stains the bacteria black and shows them well. A simplified version of the Giemsa stain works well. The stain should be heated for a short time. One should adjust the time to stain the Helicobacter and not the mucus, using a positive control slide. This method destroys the color balance expected with Giemsa but not required for bacteria. The bacteria stain blue with a white or pale blue background.

The contrast between the bacteria and background tissue is greatest with the Genta stain. In contrast, gastric morphology is better with El-Zimaity's triple stain. The Diff-Quik, an inexpensive histologic, stain, has excellent sensitivity and specificity.[16]

For the detection of scant number of organisms, immunohistochemistry [22,51] proved to be highly specific and sensitive and superior to conventional histochemical methods.

IHC for detection of H.pylori in gastric biopsies has also been shown to improve the rate of identification of the organisms after treatment when histologic examination and cultures were negative.

Histologic evaluation has traditionally been the gold-standard method for diagnosing H.pylori infection.[80,90] The disadvantage of this technique is the need for endoscopy to obtain tissue. Limitations also arise at times because of an inadequate number of biopsy specimens obtained or failure to obtain specimens from different areas of the stomach. In some cases, different staining techniques may be necessary, which can involve longer processing times and higher costs. However, histologic sampling does allow for definitive diagnosis of infection, as well as of the degree of inflammation or metaplasia and the presence / absence of MALT lymphoma or other gastric cancers in high-risk patients. [70,82]

Apart from graded variables describes in the updated revised Sydney system, special attention has been thrown on non-graded variables like surface epithelial changes, mucin depletion, erosions, lymphoid follicles, cells drop out, foveolar hyperplasia, pseudopyloric metaplasia and endocrine hyperplasia.[82]

The recent article states that emerging therapies, including development of a vaccine that may enable the future eradication of the organism as a significant human pathogen. [3]

Polymerase chain reaction

The application of polymerase chain reaction (PCR) with respect to H.pylori is useful for molecular epidemiologic aspects as well as for detection

purposes. PCR can be used to distinguish between strains of H.pylori and in typing and determining reinfections. Current detection methods by PCR [12,14,66,85] are aimed at detecting H.pylori in clinical samples collected by less invasive means, such as gastric juice, saliva, dental plaque, and faeces.

TREATMENT

Cure of H.pylori infection is not easy and requires combinations of antibiotics often with additional non-antibiotic adjunctive agents.

Recommended regimens to treat H.pylori infection

Bismuth triple therapy

Bismuth two tablets four times daily

Metronidazole 250mg four times daily

Tetracyclin 500mg four times daily

Proton pump inhibitor (PPI) triple therapy

PPI twice daily

Amoxicillin 1000mg twice daily (or)

Clarithromycin 500mg twice daily

Quadruple therapy

PPI twice daily

Bismuth two tablets three or four times daily.

Metronodazole 500mg three or four times daily.

Tetracyclin 500mg three or four times daily.

The most effective regimens to cure H.pylori infection are combinations of two antibiotics and adjunctive agents taken for 14 days.[3,85,88]

The most effective and best tolerated combination seems to be twice-a-day combination of 1000mg of amoxicillin and 500mg of clarithromycin [PPI + AC] or 500mg of metronidazole and either 250 or 500mg of clarithromycin [PPI + MC].

Quadruple therapy

The triple therapy is often sufficient unless the organism being treated is resistant to clarithromycin or metronidazole. One regimen that provides effective eradication of H.pylori in either instance is high dose quadruple therapy.

Definition of cure

It is defined, as absence of the organism by tests performed no sooner than four weeks after cessation of antimicrobial therapy.

MATERIALS AND METHODS

In the present study, endoscopic biopsies were taken from 50 patients, who attended gastro enterology department with complaints of nausea, vomiting, dyspepsia, flatulence and fullness were screened with detailed clinical history regarding socio-economics status, housing conditions, water supply etc.

After thorough clinical evaluation, patients suspected to have gastric lesions were subjected to endoscopic biopsy procedure.

Methodology

Endoscopy

- Upper gastro – intestinal endoscopy was performed with flexible fiber optic endoscope manufactured by Pentax model number 29P.
- Informed consent was obtained from patients. Relevant history and clinical details were recorded.
- After overnight fasting, endoscopy was done on the following morning, endoscopic changes were noted in esophagus, stomach, duodenum were recorded.
- Three gastric biopsy specimens were taken from antrum and corpus and one was immediately used for Rapid Urease test (Annexure I) and the other was immediately fixed in 10% buffered neutral formalin for histopathological evaluation.

Histopathologic study of biopsy Specimens

The biopsy specimens that were fixed in 10% buffered neutral formalin were processed in automatic tissue processor for paraffin embedding, then 3-5 μ sections were cut.

The sections were stained with Haematoxylin & Eosin (Annexure II) for evaluation of histopathological features and special stains like Giemsa and Alcian Blue / PAS stain (Annexure III) used to detect H.pylori organisms.

Gastritis was defined and classified according to established histological criteria with revised updated Sydney system.

The density of H.pylori, chronic inflammation, neutrophil polymorphic activity, glandular atrophy and intestinal metaplasia were recorded in all cases of gastritis and graded as mild, moderate and marked scale according to the guidelines provided by the updated revised Sydney system, using the visual analogue scale. The most prevalent appearance on each slide was matched with the graded panel that resembles it most closely. Lesion being active was signified by presence of neutrophils within glandular and surface epithelial layer. Glandular atrophy was identified, when gastric glands were correspondingly decreased in amount and widely separated. An increase in lymphocytes and plasma cells in lamina propria categorizes the gastritis as chronic. Infiltration involving upto 1/3 of gastric pits and surface are designated as mild between ½ to 2/3 as moderate and more than this as severe gastritis.

Apart from graded variables described in the updated revised Sydney system, special attention has been thrown on non-graded variables like surface epithelial changes, mucin depletion, erosions, lymphoid follicles, cells drop out, foveolar hyperplasia, pseudopyloric metaplasia and endocrine hyperplasia.

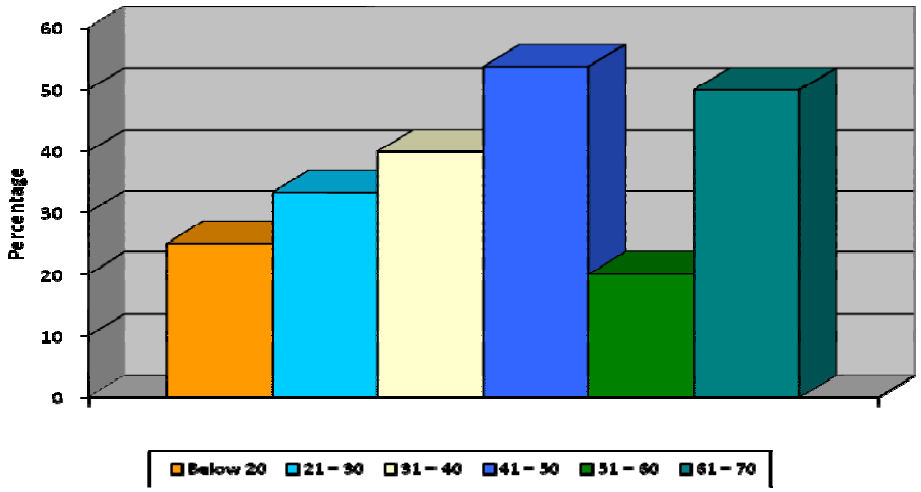
In addition, the recent literature regarding epidemiology, clinical features pathogenesis and pathology of H.pylori infection were also reviewed.

OBSERVATIONS AND RESULTS

This study covered 50 patients clinically suspected to have gastritis and undergone upper-gastro intestinal endoscopy. In the 50 cases, 35 were males with age ranging from 20 years to 70 years (mean age 45 years) 15 were females with age ranging from 20 years to 60 years (mean age 40 years).

When the patients were divided into 6 groups according to their age (< 20, 21 – 30, 31 – 40, 41 – 50, 51 – 60, 61 – 70) there was significant increase in the Helicobacter pylori (H.pylori) positivity in the age group of 41 – 50 years (53.8%) followed by 31 – 40 years (50.0%). ***Table - 1***

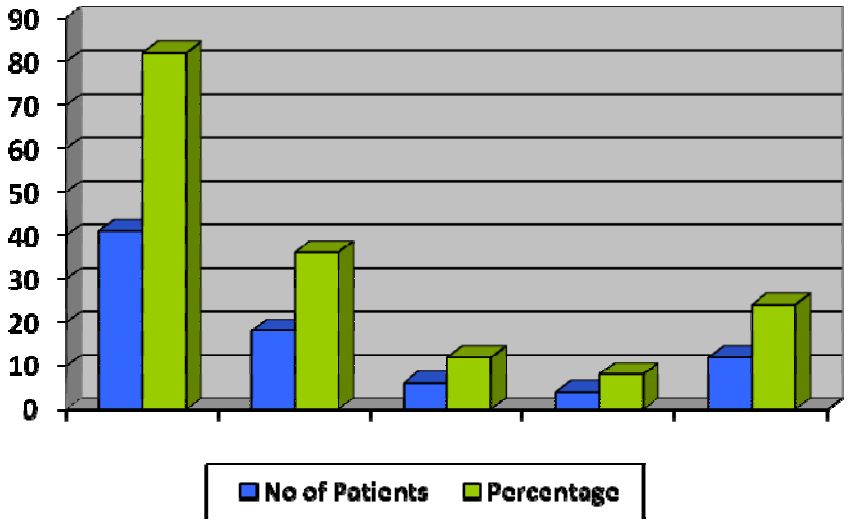
S.No	Age (in Years)	Total no. of cases	H.pylori Positive Cases	Percentage
1	Below 20	4	1	25.0%
2	21 – 30	6	2	33.3 %
3	31 – 40	20	10	50.0 %
4	41 – 50	13	7	53.8 %
5	51 – 60	5	1	20.0 %
6	61 – 70	2	1	50.0 %
	Total	**50**	**22**	**44.0** %



The clinical presentations of the patients are summarized in the following table.

Table-2

S.No	Clinical Presentation	No. of Patients	Percentage
1.	Upper abdominal pain, Bloating sensation, Belching (dyspepsia) more than 3 months	41	82 %
2.	Abdominal discomfort with Vomiting, Nausea	18	36 %
3.	Epigastric Pain + Malena + Heart burn	6	12 %
4.	Epigastric Pain + Haematomesis	4	8 %
5.	Post cibal abdominal distension + Loss of appetite + Iron deficiency anemia	12	24 %



Abdominal pain with dyspepsia more than 3 months is the commonest clinical presentation followed by abdominal discomfort with vomiting and nausea. Abdominal discomfort with anemia was noticed in some cases.

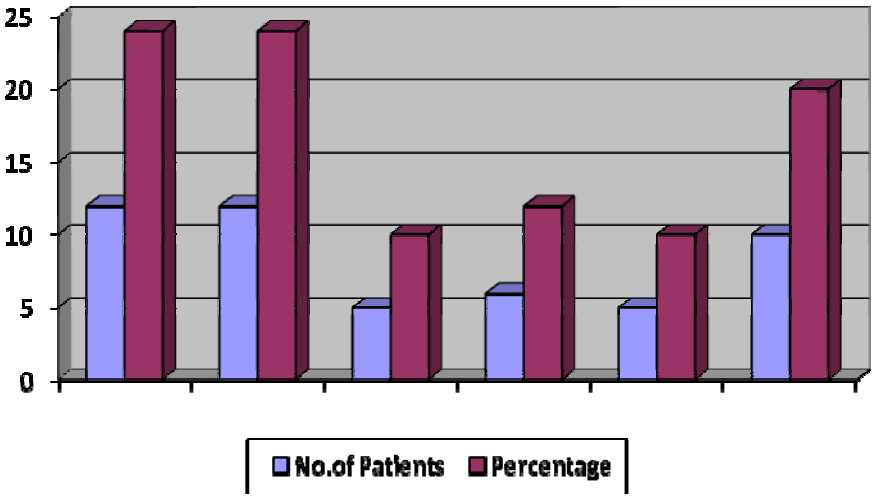
Majority of the patients belonged to lower socio-economic status who used un-hygienic water and lived in over crowded surroundings.

Endoscopic Examination

Detailed endoscopic findings in all the 50 cases are listed in Table-3.

Table-3

S.No	Endoscopic diagnosis	No. of Patients	Percentage
1	Gastric ulcer < 2cm with erosion and edema	12	24 %
2	Antral gastritis with duodenal ulcer	12	24 %
3	Nodularity of gastric mucosa	5	10 %
4	Patchy erythematous gastric mucosa	6	12 %
5	Duodenal erosion with edema	5	10 %
6	Unremarkable mucosa	10	20 %



Upper gastro-intestinal endoscopy revealed **12** cases showed gastric ulcer ranging from 0.5 cm to 2 cm with erosion and edema; **12** cases antral gastritis with

duodenal ulcer; **5** cases showed nodularity of gastric mucosa; **6** cases with patchy erythematous gastric mucosa; **5** cases were with duodenal erosion and edema with ulceration ranging from 0.25 cm x 1 cm to 1.5 cm to 3 cm and **10** patients did not show any endoscopically detected lesion. Fig.3, 4

Endoscopic findings and corresponding histopathologic diagnosis of **50** endoscopic biopsies are listed in the following table (Table-4).

Table-4

S.No	Endoscopic Feature	Histopathological Feature
1.	Gastric Ulcer with erosion – 12	Chronic Active antral gastritis –10 with surface epithelilal changes Normal mucosa – 2
2.	Antral gastritis with duodenal ulcer – 12	Chronic mild gastritis – 8 Atrophic gastritis - 2 Chronic active gastritis – 2
3.	Nodularity of gastri mucosa – 5	Chronic active antral gastritis – 3 Chronic gastritis with Intestinal Metaplasia - 2
4.	Patchy erythematous changes –6	Chronic mild gastritis – 1 Chronic active antral gastritis – 5
5.	Gastric Ulcer Duodenitis – 5	Chronic mild gastritis – 2 Chronic gastritis with Intestinal Metaplasia - 3
6.	Unremarkable mucosa - 10	Normal mucosa – 8 Chronic mild gastritis – 1 Chronic active antral gastritis – 1

In 10 cases, where endoscopy was normal, there was histological evidence of chronic active gastritis in one case and mild gastritis in one case and eight cases shows normal gastric mucosa. These shows an apparent lack of correlation between endoscopic and Histopathalogical diagnosis of gastritis in dyspeptic patients.

RAPID UREASE TEST (RUT):

RUT for detection of H.pylori from endoscopic specimen.

The biopsy specimen was subjected to Urease testing in 50 cases, of which there were 24 positive cases. Among the 24 Urease positive cases, 22 cases were detected Histopathologically for H.pylori.

In the 26 Urease negative gastritis biopsy, Giemsa staining also did not detect H.pylori.

RUT is a simple, cheap test, performed at endoscopy room itself using Helicheck test device. It contains urea solution with indicator that detects alkalinity resulting from formation of ammonia in most infected patients (70%) and gives positive result within 2 Hours. In cases of positive result, it shows a change in colour from yellow/orange to pink/ red. Whereas, in cases of negative result, the colour remains as yellow colour itself. Urease test detects upto 0.3 unit of urease present in sample Fig.5

Limitations of this test

1. The test is pH sensitive and therefore, any contamination in the reaction wells will change the reaction.
2. Biopsy specimen collected in preservatives with acidic or basic pH such as formalin etc should not be used for Heli-Check RUT test device.

Histopathological diagnosis of H.pylori

Though the H.pylori organisms were visible in the H&E stain, demonstration by Giemsa stain is considered as the gold standard for H.pylori detection. It facilitates the identification of H.pylori by darkening the organism.Fig. 11

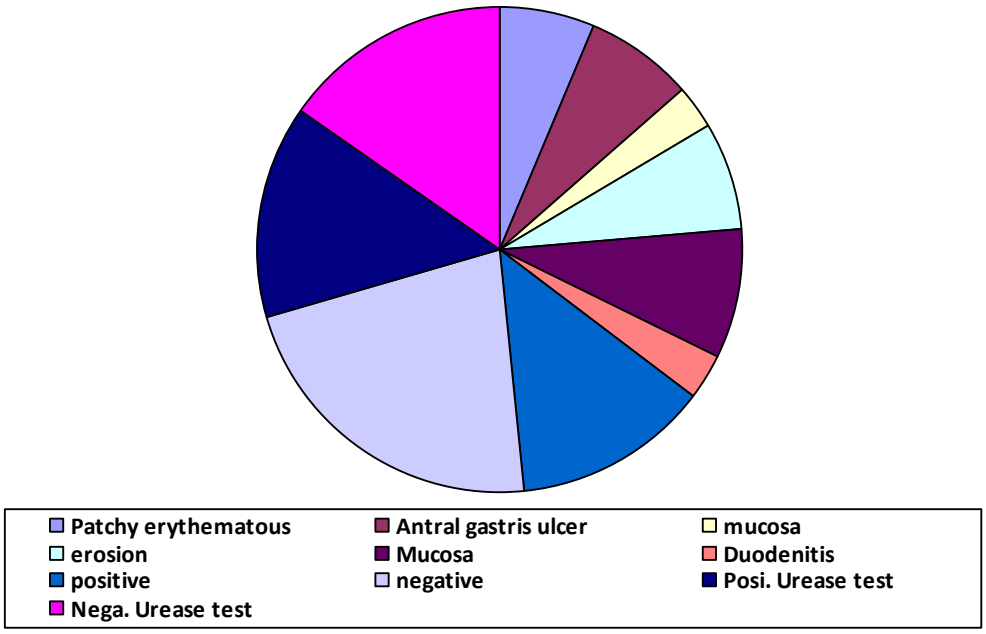
Using Giemsa stain, the spiral shaped bacteria of H.pylori is attached to brush borders of gastric foveolar cells and inside the gastric pits (Luminal side of gastric mucosa). The distribution was mostly patchy and single. Lying close to surface epithelium and more densely distributed within lumen of gastric pits. It also extends less into the deeper portion of the mucosa. The organisms are absent in the areas of intestinal metaplasia.

In this present study, H.pylori was demonstrated by using Giemsa stain in 22 out of 50 biopsies.

Table 5

S.No	Procedure	Diagnosis	No. of patients
1.	Endoscopy (N = 50)	1. Patchy erythematous changes	6
		2. Antral gastritis with D.ulcer	12
		3. Nodularity of gastric mucosa	5
		4. Gastric ulcer with erosion	12
		5. Unremarkable mucosa	10
		6. Duodenitis	5
2.	Giemsa staining (N = 50)	H.pylori Positive	22
		H.pylori Negative	28
3.	Urease testing (N = 50)	Positive Urease test	24
		Negative Urease test	26

Endoscopy Giemsa staining Rapid Urease test



Association between Gastritis & presence of H.pylori

Most of the biopsy specimen, which were positive for H.pylori showed histological evidence of Gastritis

Twenty-four cases showed chronic active antral gastritis and activity implying the presence of high number of Neutrophilic polymorphs in the lamina propria and within epithelium.

Relationship between H.pylori density and severity of Gastritis:

There was no correlation between the degree of inflammation, noted in the histolopathologic study and density of H.pylori organisms.

Histopathology of gastric antral biopsies:

The presence or absence of H.pylori with varying degree of chronic inflammation, Neutrophilic polymorphic activity, glandular atrophy, intestinal metaplasia and gastric surface epithelial changes were recorded in **50** cases. When present, each of these variables was graded on a mild, moderate or severe scale as indicated by the updated Sydney system.

The following table (Table 6) shows details of histopathological findings of all the **50** gastric biopsies. Out of the **50** cases, only **22** cases show gastritis with H.pylori positive.

Histopathologically **10** cases (20%) showed evidence of normal gastric mucosa of which **2** cases (20%) were H.pylori positive. The remaining **40** cases (80%) showed evidence of H.pylori infection with chronic antral gastritis. Out of **40** cases **24** cases (66.67%) showed evidence of chronic active gastritis, of which **16** cases were H.pylori positive. Among the remaining **16** cases, **9** cases (18%) showed chronic non-active gastritis of which **4** cases (44.3%) were H.pylori positive; **5** cases (10%) were detected with Intestinal metaplasia and **2** cases (4%) were detected with atrophy.

Histopathological findings of endoscopic gastric biopsy

Table 6

S.No	Histopathology	No. of cases	Percentage	H.pylori Status	H.pylori %
1.	Normal gastric mucosa	10	20 %	2 positive 11 negative	20 %
2.	H.pylori associated chronic active antral gastritis (CAAG)	24	48%	16 positive 8 negative	66.6%
3.	H.pylori associated chronic non active gastritis (mild)	9	18%	4 positive 17 negative	44.3%
4.	Chronic gastritis with atrophy	2	4%	All Negative	0%
5.	Chronic gastritis with intestinal metaplasia	5	10%	All Negative	0%
6.	H.pylori Negative chronic non active gastritis	28	76%	All Negative	0%

Histopathology of H.pylori associated gastritis:

Chronic mild gastritis

Inflammatory infiltrate is limited to the foveolar region and unaccompanied by glandular atrophy. Epithelial abnormalities like reduced amount of cytoplasmic Mucin and cells with enlargement of nuclei and slightly increased mitosis in foveolar epithelium.

In the early phases chronic mild gastritis inflammatory infiltrate of lymphocytes and plasma cells is typically present within the lamina propria, usually limited to the upper third of the gastric mucosa(Superficial location).Fig. 17

Chronic active gastritis:

- Mucosa shows dense infiltrate of chronic inflammatory cells in which plasma cells are predominant in lamina propria, and also involve full thickness of mucosa. It may surround and separate the glands without causing atrophy. Fig. 18 19
- Lymphoid follicules with germinal centers in deeper portion of mucosa. Fig. 20 21
- Surface and foveolar epithelium infiltrated by neutrophils, which is predominant, termed as “Pit abscess” in which H.pylori organisms are readily identified. Fig. 22
- Other surface epithelial changes like degeneration, regeneration with variable erosions, Neutrophil reaction in lamina propria and epithelial layer. Occasionally epithelial changes like irregular ragged surface; epithelial pits; individual cell dropouts; micro erosions can also occur. Mucosal changes like erosion and ulcerations are seen. Fig. 13 14 19

In the present study, **5** cases of Intestinal Metaplasia and **2** cases of Chronic gastritis with atrophy were identified in the Histopathological examination.

Chronic atrophic gastritis: When inflammation is more extensive and accompanied by glandular atrophy the condition is termed as chronic atrophic gastritis. It is classified as mild, moderate and severe atrophy as per graded variable in updated Sydney system. Fig. 12 21

Glandular atrophy is characterized by increased distance between individual glands and condensation of retilculine fibres in lamina propria. Mucosa shows more obviously thinned and flattened, with extension of infiltrate in the lamina propria to deeper layer. There is often atrophy of glands (loss of gastric glands) fibrosis and variety of cytology changes in the surface epithelial cells.

Intestinal Metaplasia (IM): Is defined as a progressive replacement of gastric mucosa by intestinal epithelium. Either small or large bowl type including goblet cells absorptive (brush border cells), paneth cells, variety of endocrine cells, ciliated cells are also present. IM is classified into complete Type I, incomplete Type II and Type III. This is based on morphological features and mucin content of goblet & columnar cells. Fig.9 10 20

IM - Type 1 Complete – Type I metaplasia gastric mucosa is changed to pattern nearly identical to that of small bowl epithelium, reveals numerous columnar absorptive cells, few goblet mucous cells which secrete mildly acidic mucins such as sialomucins. This subtype has lower cancer risk Fig 20.

IM Type incomplete - Type II IM absorptive cells are absent and consist a mixture of gastric foveolar and colonic type goblet mucous cells. Both mucous cells contain highly acidic mucins such as sialomucins and neutral mucin.

IM Type III - IM characterized by columnar cells that secrete sulfomucin – differentiated from sialomucin by staining with high iron diamine.

IM carries an approximately 10 fold increased risk for development of gastric cancer.

DISCUSSION

Considering the percentage of morbidity and mortality caused by various gastric lesions and high prevalence of H.pylori infection, extent of their association is gaining importance.

The most impressive advance has come from the flexible fibroscope with which it is possible to examine esophagus, stomach and duodenum, and at the same time biopsies for Histopathological Examination can be obtained.

Infection with H.pylori is worldwide chronic infection with the highest incidence in developing countries.[91] It is associated with duodenal ulcer, chronic active gastritis, gastric cancer and gastric lymphoma, which can be easily identified by endoscopic gastric biopsy.

Chronic gastritis is defined as the presence of chronic mucosal inflammatory changes eventually leading to mucosal atrophy [28] & epithelial metaplasia. By far the most important etiological association is chronic infection by the bacillius H.pylori.[92] The organism is a world wide Pathogen that has the highest infection rates in developing countries.[42,77]

Sex distribution

Age and sex related possibility of H.pylori was studied. In the present study, out of 35 male cases 16 cases (45%) are positive for H.pylori and out of 15 female 6 cases (40%) are positive for H.pylori. The male to female ratio is 2:1 which is in contrast to the study literature and study conducted by Abdur Rauf Khan [2]

Age distribution:

The higher prevalence of H.pylori is in the age group of 41-50 years, which had highest percentage (53.8%) and followed by the age group 31-40 years (50.0%) This is in consonance with Richard,Freferick J[81] who states that prevalence of H.pylori increased with advanced age. Anderson states that the prevalence of H.pylori in adults approximates 100% in many developing tropical countries.

The prevalence of H.Pylori in the present study is 44%. It does not coincide with study of Abdul Rahman E, Fakhro[1] et al.,, In their study the prevalence rates are 79.4%.

This high percentage may be due to low socio economic status of the patient and lack of education about hygiene in most of those people. This accordance with James Fox at la[49] Dube C :N F Tanih at al[13] stated that H.pylori spreads from person to person and via a route that depends on hygiene proved by several studies.

In the present study, 45 cases out of 50 shows dyspepsia, abdominal pain, iron deficiency anemia.[41,78] These are the most common symptoms encounter in other studies also.

Perusal of literature shows epigastric pain, which is the most common symptom (**92%)** followed by vomiting (**51**%) and hematemesis (**17**%) in H.pylori associated chronic gastritis.

In the present study epigastric pain was present in most cases (**92**%) followed by vomiting (**36**%) and haematemesis (**8**%).

As per study done by Graham [36]Gill Desai etal [34] who revealed that geographic and social patterns play a role in transmission of H.pylori. According to Anderson, East Asian countries where wide spread sanitation has been introduced, prevalence of H.pylori has shown downward trend.

Transmission from patient to patient after endoscopy has also been described.

As per study the high percentage of H.pylori positive individuals having gastric lesions were found to have history of intake of spicy and non-vegetarian food. H.Pylori infection highly frequent in dyspeptic patients, and it is cardinal risk factor for chronic gastritis.[7]

Endoscopic features:

The advent of the fiber optic gastroscope with biopsy facilities has provided the means of obtaining biopsy specimens under direct vision from any part of stomach.[73]

In this present study, there were 12 cases gastric ulcer with erosion, out of which 8 cases (66%) were positive for H.Pylori and 12 cases of duodenal ulcer in which 9 cases were positive (70%) and small proportion of cases showing patchy erythematous changes, nodularity of gastric mucosa[46] and of unremarkable mucosa were also found in endoscopy examination.

Normal looking gastric mucosa is commonest single endoscopic finding, accounting for 20% cases. Though the results of endoscopic examination may show normal mucosa, [28]histopathological examination may show positive for H.pylori. In these cases, risk of re-infection is always there[94]

The positivity rate for duodenal ulcer is 70% and gastric ulcer is 66% in our study. It is comparable to study by Tytget [98] (1988) who found that 15 patients of duodenal ulcer and 9 out of 11 (81.8%) patients with gastric ulcers have the organism. And in 2002 Sengupta et al [86] studied antral biopsy specimens from 25 patients with symptoms and diagnosis of duodenal ulcer, amongst whom the positivity rate is 84%. In a study by Zhang C, Yamada N et al [96] the prevalence of H.pylori in gastric ulcer is 80.8%. Duodenal ulcer is usually associated with H.pylori infection. Treatment of duodenal ulcer must, therefore include acid reduction and H.pylori eradication all the time.

The most convincing data implicating H.pylori as a cause of cancer are furnished in the case-control studies from Hawaii, California, Great Britain, Taiwa[55] . In the first three studies (mean follow – up years 13, 14, 6 years respectively), serologic evidence of H.pylori infection associated with increased risk of developing gastric cancer, is 2.8 to 6 fold. The fourth nested case control study also identified an elevated risk of cancer (odds ratio = 1.6) but the finding was not statistically significant. This last study was hampered, however by a small number of cases, and short follow-up period. Overall the association between H.pylori and cancer appeared to be restricted to tumors distal to gastric cardia.

One line of research currently favours H.pylori infection as a causal factor in both MALT and non-MALT gastric lymphomas.[55]

When the density of H.pylori is low, application of endoscopic brush cytology helps in rapid detection. [4 21]

Rapid Urease Test (RUT):

At present, there are at least seven diagnostic assays for H.pylori, including bacterial culture, a RUT[66] urea breath test, histopathology, Polymerase chain Reaction [52,] immuno histochemistry using H.pylori antiserum, serology and stool antigen test, which is limited when patients are taking acid-suppressing agents (proton-pump inhibitors)

In the present study, H.pylori is positive in 24 cases (48%) by RUT. It more or less correlates with a study done by U. Arora et al [6] who studied 75 gastric biopsy specimen of same patients complaining of dyspepsia. H.pylori is positive in 52 cases (72%) by RUT.

Gill et all [33,34] have shown that antibodies to H.pylori in serum are present in 80% of Indian subjects with upper G. I. Symptoms.

Some of these methods are based on the high urease activity of H.pylori but because they detect all enzyme activity of urease regardless of its origin, there is the possibility that urease derived from other bacterial species, such as Proteus mirabilis or Klebsiella Pneumonia will confound the result.

Out of 50 cases, 22 were positive on Modified Giemsa stain and 24 positive for rapid urease test. 22 cases were positive for both rapid urease test and Modified Giemsa stain while 2 cases were negative by rapid urease test. Hence sensitivity and specificity of modified Giemsa stain in detecting H.Pylori in gastric lesions used by us in present study shows sensitivity of 94.2% specificity of 96.0% this study conducted by Madan et al [18] who found the percentage of H.pylori positivity with modified Giemsa technique was 100%, as per Grey et al [21] study modified Giemsa stain is simple, reliable, widely used stain. [16]

False negative test (Modified Giemsa Negative and urease positive) in our study could be a result of difficulty in identification of H.pylori. Marshall et al [21] attributed false positive urease test reaction to contamination of biopsy specimen with alkaline bile or growth of other urease-producing bacteria. They proposed addition of pH buffer, which enabled to control these factors. They found no false positive results even in patients with duodenogastric bile reflux and ingestion of milk on evening prior to endoscopy may have enhanced urease contents of mucosa. False positive tests could be attributed to low bacterial load.

The typical histopathologic appearance of H.pylori gastritis is moderate to marked inflammation, as per Tabei M.D et al[90] which is immediately recognizable at high power. Sometimes the acute inflammation may be relatively scant in the corpus compared to antrum & cardia.

The intense acute inflammation will be found in cases, in which the H.pylori are most abundant. Most of these cases have numerous organisms at the luminal surfaces, which can be quickly identified on the H&E stained slide, The haematoxylin and eosin has a high degree of accuracy (98) similar to Giemsa (96) and Genta (97) stain.[16,80]

Owen has pointed out that the bacteria can be easily seen on well differentiated haematoxylin, and eosin sections.

Histopathological study (Histopathology):

The Recent Implication of H.pylori in the pathogenesis of gastritis-peptic ulcer syndrome, and its relevance in development of upper gastro intestinal malignancy warrant efficient methods for the detection and demonstration of organism in biopsy specimens [51,79,80]

A two-way interaction might exist between H.pylori and gastric acid that determines pattern of gastritis and hence clinical outcome. [48].

The prevalence of H.pylori in the present study is 44%. It is similar to a study by Abdul Rahman, E Rakhro et al [1] Histopathologically, 24 cases (48%) out of 50 patients, showed evidence of chronic active gastritis and from these 16 cases (67%) are H.pylori positive. Mild gastritis is evidenced in 9 cases, of which 4 cases (19%) are H.Pylori positive. 10 cases (20%) are normal gastric mucosa, of which 2 cases (40%) are H.pylori positive, 5 cases (10%) of intestinal metaplasia and 2 cases (4%) of atrophy are [44,70] detected.

Abdul Rahman E Fakhro et al [1]studied 102 gastric biopsies in dyspeptic patients. In their study, 66 cases (64.7%) out of 102 patients showed evidence of chronic active gastritis and from these 65 cases (98.5%) are H.pylori positive, Mild gastritis is evidenced in 15 cases (14.7%) of which 9 cases (60%) are H.pylori positive. Our present study results are comparable to this study.

It is also in consonance with another study done by Bayer Dorffer et al [11] who found 82.97% cases of chronic active gastritis were positive with H.pylori.

We conclude that histopathological examination (HPE) is adequate for the initial assessment of gastric biopsies. This is because it is well-tested, cheap, easy staining method, requiring a relative short period of time to perform with highly reproducible results. It has an added advantage of enabling simultaneous assessment of morphological change accompanying H-Pylori infection. [51]

In present histopathological study, out of total 50 cases studied, histopathological diagnosis of gastritis was reported in 45 cases (80% of cases). This is more or less comparable to study done by Wyatt [30] where 90% of patients with gastritis showed antral mucosa colonized by H.pylori.

As found out in our study Dixon et al [24,] stated that neutrophil activity is almost universal phenomenon in H.pylori and that neutrophils are very sensitive indicator of the presence and absence of H.pylori and they disappear within days of cure of infection.

If Neutrophils are seen in post treatment biopsy but organisms are not apparent, a careful search for H.pylori using one of the special stain should be carried out.

In present study no cases with intestinal metaplasia was found to be positive with H.Pylori. This consonance with study done by steer and Noritaka yabulki[80] stated that areas of intestinal metaplasia as rule do not contain H.pylori. Areas of Intestinal metaplasia indicate the longstanding gastritis and also risk of developing gastric carcinoma [80]at an early age if left untreated.

Mucosa associated lymphoid tissue is physiologically absent in normal gastric mucosa, but can develop in response to H.pylori infection and it is considered to be hallmark of H.pylori infection as a result of chronic antigenic stimulation by the organisms. In present study only one case shows gastritis with lymphoid aggregates. Acquired mucosa associated lymphoid tissue is considered as a precursor of gastric low-grade maltoma. Lymphoid reaction to H.pylori infection varies from simple aggregates, follicles with germinal centers to primary gastric lymphoma [1,55,88.] This study analyzes various lymphoid reactions to

H.pylori infection among 50 patients. The study of Stolte et al[88] stated that if sufficient biopsy specimens are examined, follicular gastritis is found in 100% Cases. H.pylori colonized gastric mucosa shows surface epithelial changes in addition to inflammation,[80] which include loss of apical mucin, epithelial cell dropout, formation of epithelial pits, recognized as irregularities on surface epithelium. Changes are mainly seen in pit regions of glands. They are cells which shows pleomorphism with hyperchromatic nuclei, rarely cellular exfoliation and syncytial regenerative changes.

The Sydney system for the classification of gastritis emphasized the importance of combining topographical,[11] morphological and etiological information in to a scheme that would help to generate reproducible and clinically useful diagnoses (Revised Sydney system) [24,82,88] gives guidelines for optimal biopsy sampling of the stomach, use of visual analogue scales for grading the histopathologic features and formation of comprehensive standardized diagnosis.

Good evidence exists in the literature that H.pylori can cause chronic active gastritis. Most compelling and direct evidence are studies by Dr. Marshall et. al and subsequently by Moris and Nicholson. In these studies Moris and Marshall infected themselves with H.pylori and this led to the development of clinical and microscopic gastritis in these subjects and Koch's postulates of the etiology of the disease seemed to be fulfilled.

As further evidence of pathogenicity, secretory IgA directed against H.pylori has been isolated, (3,6-22) and phagocytosis of the organism has been shown by intra gastric neutrophil. Others have successfully eradicated the organism with antibiotics, with resultant improvement of histologic gastritis.

In the present study, it is found that, more or less the antral biopsies colonized by H.pylori, showed evidence of gastritis[91], It confirms the previously reported high prevalence of H.pylori infection in association with antral gastritis further supporting the contention that H.pylori is the etiologic agent of this lesion in most cases.

Helicobacter Pylori is now accepted cause of gastritis and peptic ulcer disease in adult.[27,29,50,97,98]

The following table shows comparative analysis of various studies in children, reporting relationship between H.pylori infection and histological evidence of gastritis percentage in adults. This shows 50% of cases of gastritis show H.pylori positivity in contrast to the pediatric cases. Probably environmental factors, socio economic status, alcohol and smoking modified the development of gastritis with typical symptoms in adults.

S.No	Name of the author and year	H.pylori – infection (No. of cases)	Histo pathological gastritis (No. of cases)	% of H.pylori – infection associated with gastric inflammation
1.	Musgrove et al[75] 1988	54	61	88%
2.	Hartley Cohen et al [40]1989	22	22	100%
3.	Grace W Elta et al[35] 1989	16	16	100%
4.	Siobhan M Gormally et al[87]1995	19	19	100%
5.	C.K. yeung et al 1990	64	64	100%
6.	Present study 2010	22	45	50%

According to Dixon degree of chronic inflammatory cell infiltration is correlated to the extent and density of H.pylori colonization. But we couldn't find

significant correlation between these two factors and the differences can be explained [90]as follows:-

1. Difference immunological as well as histological responses in various age groups, could be due to genetic, social cultural economical, psychological factors.[90]
2. Some patients may have chronic inflammation (gastritis due to other causes and H.pylori, infection) simultaneously.
3. Partially treated patient may show lower degrees of inflammation.

CONCLUSION

Helicobacter pylori is now widely recognised as the most common cause of primary or unexplained gastritis in adults as well as children.

The present prospective study of **50** patients presenting with the upper abdominal pain, dyspepsia were undertaken to evaluate the relationship of this symptom complex to inflammatory gastroduodenal lesions with special reference to H.pylori infection. The clinical and endoscopic findings, Rapid Urease test and histopathological examination of gastric antral biopsy material were all evaluated and the following conclusions are presented.

(1) A positive significant association between abdominal pain and H.pylori gastritis. It was identified in **44**% cases and recurrent epigastric pain was the commonest presenting symptom.

(2) As mentioned in the literature, H.pylori infection can occur in childhood and common in adult of low socio-economic status in poor hygienic living conditions, consuming unhygienic food and drinking water.

(3) In the present study, as in the studies of many others, it was found that H.pylori colonization of gastric mucosa was always associated with chronic gastritis and further supporting the correlation that H.pylori organism is the etiologic agent of this lesion in most cases. H.pylori organisms were also seen in **2** cases with normal mucosa.

(4) In addition, **12** cases were diagnosed by endoscopy to have duodenal ulcer, also had H.pylori antral gastritis. According to literature, duodenal ulcer disease in adults is strongly associated with the presence of H.pylori on antral mucosa.

(5) There was no correlation seen between severity and degree of inflammation and the density of H.pylori organisms.

(6) Common pattern of inflammation consists of mononuclear cell and neutrophils. Polymorpho Neutrophilic activity is almost a universal phenomenon in H.pylori gastritis.

(7) Significant number of antral biopsies in H.pylori gastritis showed focal lymphonodular hyperplasia, which is considered to be a hallmark in this diagnosis and also said to be unique to paediatric and adult H.pylori infection.

(8) The surface epithelial changes of antral mucosa viz loss of apical mucin, cell drop out, formation of epithelial pits which are considered specific for H.pylori colonization were seen in all cases of H.pylori gastritis. The presence of epithelial changes may be used to screen more carefully for H.pylori in a given biopsy if organism is not apparent.

(9) There was a poor correlation between endoscopic findings and presence of histological gastritis, showing that a diagnosis of gastritis cannot be readily excluded in the absence of gastric biopsy.

(10) There was excellent correlation between Urease test and H.pylori gastritis.

(11) Recent article states that there is an emerging therapy in development of vaccine that may enable the future eradication of the organism.

(12) A “test and treat” strategy is recommended for most patients with undifferentiated dyspepsia.With this approach, patients undergo a noninvasive test for H.pylori infection and if positive, are treated with eradication therapy. This strategy reduces the need for antisecretory medications as well as the number of endoscopies. Recently one day drug regimen is used instead of two weeks course of medication in H.pylori eradication

(13) As per updated Sydney system published (in October 1996) semi quantitative scoring system of gastritis remains a useful tool for clinical research. The methods proposed is both feasible and practical when satisfactory set of gastric biopsy is available. Staging and grading of gastritis could represent the concluding message of the histopathological report and also provide useful information about cancer risk.

ANNEXURE I

RAPID UREASE TEST (RUT)

1. Rapid urease test for detection of H.pylori from endoscopic gastric specimen.
2. This is a cheap simple test using Helicheck test device.
3. It contains urea solution with indicator that detects alkalinity resulting from the formation of Ammonia.
4. The positive result shows yellow to pink within 2 hours, maximum 18 to 24 hours
5. Urease test detect upto 0.3 unit of Urease present in sample.

Limitations of this test

1. The test is pH sensitive and therefore, any contamination in the reaction wells will change the reaction.
2. Biopsy specimen collected in preservatives with acidic or basic pH such as formalin etc should not be used for Heli-Check RUT test device.

ANNEXURE II

H & E STAIN

1. Sections to water
2. Stain with Ehrlich’s hematoxylin solution for 30 minutes
3. Wash briefly in water and differentiate in 1% acid alcohol
4. Wash well in water and blue for 10 to 30 seconds
5. Wash in water and stain with 1% eosin solution for 30 seconds to 1 minute
6. Wash quickly in water, differentiate and dehydrate in alcohol. Clear and mount.

ANNEXURE III

GIEMSA STAIN

1. Bring sections down to water through graded alcohols
2. Rinse in pH 6.8 buffered distilled water
3. Stain in working Giemsa stain for 20 to 30 minutes
4. Rinse in distilled water
5. Rinse in 0.5 % aqueous acetic acid until section is pink
6. Wash in tap water
7. Blot until almost dry
8. Dehydrate rapidly

RESULT

Organisms – dark blue

Back ground – pink or pale blue

ALCIAN BLUE / PAS STAIN

PURPOSE

To differentiate between neutral and acid mucosubstances

PRINCIPLE

Acidic mucosubstances are stained by Alcian blue technique and neutral mucosubstances are stained by PAS reaction

PROCEDURE

1. Deparaffinize the sections and bring to water
2. Stain the sections in Alcian blue for 5 minutes
3. Wash the sections in distilled water
4. Place the sections in 0.5% Periodic acid for 10 minutes
5. Wash the sections in running tap water for 5 minutes and rinse in distilled water
6. Place the sections in Schiff reagent for 15 minutes
7. Wash the sections in distilled water and running tap water for 10 minutes
8. Stain the sections with Harris hematoxylin containing acetic acid for 5 minutes, differentiate as appropriate blue.
9. Wash the sections in distilled water 5 to 10 minutes.
10. Rinse in absolute alcohol
11. Clear in Xylene and mount as desired

RESULT

Acid mucin - blue

Neutral mucin - magenta

ANNEXURE III

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S.No	Biopsy Number	Age/Sex	Endoscopic feature	Histopathological features	GIEMSA staining for Hp	Rapid Urease test
1	2723/09	45 Female	Patchy erythematous changes of gastric mucosa	Chronic active gastritis with H.pylori	Positive	Positive
2	2718/09	48 Male	Antral Gastritis with Duodenal ulcer	Chronic active Antral Gastritis	Positive	Positive
3	1619/10	36 Male	unremarkable Mucosa	Chronic atropic Gastritis	Negative	Negative
4	1641/10	22 Male	Gastric ulcer	Chronic Active Gastritis	Positive	Positive
5	1617/10	45 Male	Gastric ulcer	Chronic Active Gastritis	Positive	Positive
6	1618/10	45 Female	unremarkable Mucosa	Normal Gastric mucosa	Negative	Negative
7	1827/10	28 Male	Antral Gastritis Duodenal ulcer	Chronic Active Gastritis	Positive	Positive
8	271/10	40 Female	unremarkable Mucosa	Normal Gastric Mucosa	Negative	Negative
9	1529/10	36 Male	Duodenitis	Chronic Gastritis with intestinal metaplasia	Negative	Negative
10	2085/10	36 Male	Antral Gastritis with Duodenal ulcer	Chronic Active Gastritis	Positive	Positive

S.No	Biopsy Number	Age/Sex	Endoscopic feature	Histopathological features	GIEMSA staining for Hp	Rapid Urease test
11	97/09	45 Female	Patchy erythematous changes of Normal mucosa	Normal Gastric mucosa	Positive	Positive
12	46/09	70 Male	Duodenal ulcer	Chronic mild Gastritis	Negative	Negative
13	1810/10	42 Female	Gastric ulcer	Chronic Active Gastritis	Positive	Positive
14	1621/10	45 Female	Gastric ulcer	Chronic Active Gastritis with IM	Negative	Negative
15	1800/10	38 Male	Duodenal ulcer	Chronic Active Gastritis	Positive	Positive
16	1956/10	60 Male	unremarkable mucosa	Normal Gastric Mucosa	Positive	Positive
17	2181/10	41 Male	Gastritis ulcer	Chronic Active Gastritis	Positive	Positive
18	2496/10	28 Female	Duodenitis	Chronic Gastritis with intestinal metaplasia	Negative	Negative
19	1641/10	20 Male	Gastric ulcer	Chronic Active Gastritis	Positive	Positive
20	348/09	46 Male	Nodularity of Gastric mucosa	Chronic mild gastritis	Negative	Negative
21	1960/10	55 Male	Duodenal Ulcer	Chronic active Gastritis	Positive	Positive

S.No	Biopsy Number	Age/Sex	Endoscopic feature	Histopathological features	GIEMSA staining for Hp	Rapid Urease test
22.	653/10	55/Male	Unremarkable mucosa	Chronic active gastritis	Negative	Negative
23.	1959/10	45/Female	Gastric ulcer	Chronic mild gastritis	Positive	Positive
24.	1799/10	37/Male	Patchy erthematous Changes of gastric mucosa	Chronic active gastritis	Negative	Negative
25.	3309/10	40/Male	Patchy erthematous Changes of gastric mucosa	Chronic mild gastritis	Positive	Positive
26	3623/10	40/Male	Antral gastritis with Duodenal ulcer	Chronic mild gastritis	Negative	Negative
27.	2420/08	38/Male	Patchy erthematous Changes of gastric mucosa	Chronic active gastritis	Negative	Negative
28.	2145/10	22/Female	Gastric ulcer	Chronic active gastritis	Negative	Negative
29.	2179/10	40/Female	Duodenal ulcer	Chronic active gastritis	Positive	Positive
30.	1595/10	20/Male	Nodularity of mucosa	Normal Gastric mucosa	Negative	Negative

S.No	Biopsy Number	Age/Sex	Endoscopic feature	Histopathological features	GIEMSA staining for Hp	Rapid Urease test
31.	763/09	54/Male	Antral gastritis with Duodenal ulcer	Chronic mild gastritis	Positive	Positive
32.	2489/09	32/Male	Unremarkable mucosa	Chronic active gastritis	Positive	Positive
33.	1530/10	36/Female	Duodenitis	Normal gastritis mucosa	Negative	Negative
34.	260/10	28/Female	Gastric ulcer	Chronic active gastritis	Negative	Negative
35.	657/09	58/Male	Unremarkable mucosa	Normal gastric mucosa	Negative	Negative
36.	925/09	35/Female	Nodularity of mucosa	Chronic mild gastritis	Negative	Negative
37.	1544/10	33/Male	Nodularity of mucosa	Chronic active gastritis	Negative	Negative
38.	3182/10	48/Male	Antral gastritis with Duodenal ulcer	Chronic atrophic gastritis	Negative	Negative
39.	2573/08	32/Female	Unremarkable mucosa	Chronic active gastritis	Negative	Negative
40.	150/09	42/Male	Gastric ulcer	Chronic Gastritis with intestinal metaplasia	Negative	Negative
41.	1423/09	38/Male	Duodenal ulcer	Chronic active gastritis	Negative	Negative

S.No	Biopsy Number	Age/Sex	Endoscopic feature	Histopathological features	GIEMSA staining for Hp	Rapid Urease test
42.	2144/10	20/Male	Unremarkable mucosa	Chronic mild gastritis	Negative	Negative
43	2143/10	55/Female	Patchy erthematous Changes of gastric mucosa	Chronic mild gastritis	Negative	Positive
44.	1528/10	37/Male	Unremarkable mucosa	Normal Gastric mucosa	Negative	Negative
45.	2926/09	68/Male	Gastric ulcer	Chronic active gastritis	Positive	Positive
46.	767/10	35/Male	Nodularity of mucosa	Chronic mild gastritis	Negative	Positive
47.	1828/10	50/Male	Patchy erthematous Changes of gastric mucosa	Normal Gastric mucosa	Negative	Negative
48.	2086/10	35/Male	Duodenitis	Chronic active gastritis	Positive	Positive
49.	1957/10	18/Male	Duodenitis	Chronic active gastritis	Positive	Positive
50.	3646/10	32/Male	Gastric ulcer	Chronic gastritis with intestinal metaplasia	Negative	Negative