EVALUATION OF UPPER GASTROINTESTINAL BIOPSY LESIONS WITH SPECIAL REFERENCE TO HELICOBACTER PYLORI INFECTION

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CERTIFICATE

This is to certify that the dissertation entitled **"Evaluation of upper gastrointestinal biopsy lesions with special reference to Helicobacter pylori infection"** submitted by **Dr. K.M. Selvanayaki** to the Faculty of Pathology, The Tamilnadu Dr. M.G.R. Medical university, Chennai in partial fulfillment of the requirement for the award of M.D. Degree in Pathology is a bonafide work carried out by her during the period of August 2007 - July 2009 under my direct supervision and guidance.

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INTRODUCTION

Upper gastrointestinal tract includes the oesophagus, stomach and duodenum. Patients with diseases of upper gastrointestinal system generally present with symptoms of dysphagia, abdominal pain, vomiting and hematemesis.

Upper gastrointestinal (GI) endoscopy is a diagnostic procedure that visualizes the upper part of the gastrointestinal tract upto the duodenum. It is an established mode of investigation and treatment of wide range of upper gastrointestinal conditions. It has replaced upper GI radiography as the primary method of diagnosing upper GI illness.

Helicobacter pylori (H.pylori) is a spiral shaped organism associated with gastrointestinal disease in humans. It causes a chronic gastric infection that usually is life long and many epidemiologic studies have shown that this is probably one of the most common bacterial infection throughout the world, involving 40% to 50% of the population in developed countries and 80% to 90% of the population in developing regions.[88] Over 80% of individuals infected with this bacterium are asymptomatic.

It causes a chronic low-level inflammation of the stomach. The clinical outcomes associated with H.pylori infection include duodenal ulcer, gastric ulcer, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. The bacterium has been classified as a class I definite gastric carcinogen to human.[42]

H.pylori infection can be diagnosed by invasive (i.e., requiring endoscopy) and non-invasive techniques (i.e., techniques that do not require endoscopy with biopsy sampling).[54]

Eradication of H.pylori improved markedly the inflammatory cell infiltration characteristic of H.pylori related gastritis, inhibited recurrence of peptic ulcer[68] and also led to regression of MALT lymphoma.[59]

In this study the various methods of identification of H.pylori and the histopahological features associated with H.pylori in the gastric mucosa in patients presenting with dyspepsia are discussed.

AIM OF THE STUDY

To study the incidence of various lesions in the upper gastrointestinal biopsy materials received.

To estimate the magnitude of Helicobacter pylori infection among dyspeptic patients.

To study the efficacy of invasive and non-invasive methods to detect Helicobacter pylori infection.

To find out the etiological association between Helicobacter pylori and peptic ulcer.

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 anatomic regions (**Fig. 1)**

1. Cardia - Narrow conical portion of the stomach immediately distal to the gastroesophageal junction.
2. Fundus - Dome-shaped 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 a system of pits (foveolae).

In the body of the stomach, the pits are short tubules, with long, closely packed glands opening into them (Fig 7). When seen in three dimensions, the pits in the antrum have a partial cerebriform structure of complex folds. They extend about a third the way through the antral mucosa. Mucus-secreting glands branch from the base of these pits (Fig 8). The superficial parts of the glands form narrow straight tubules, the gland necks, 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 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. Intestinal mucins stain strongly with alcian blue.

A combination of alcian blue and PAS [alcian-PAS] shows these differences practically well. Foveolar mucus stains a rich magenta red. In contrast, intestinal mucus stains a variety of purple and indigo blue colors as a result of the presence of acidic 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 in form, 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. Marshall[51] and Warren[90] 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.[9]

Professor Walery Jaworski[45] 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 ulcers and stomach cancer.[26]

The interest waned when an American study published in 1954 failed to observe the bacteria in 1180 stomach biopsies.[62]

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.[78]

The bacterium had also been observed in 1979 by Australian pathologist Robin Warren, 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.[53]

Although there was some skepticism initially, within several years, numerous research groups verified the association of H.pylori with gastritis and to a lesser extent ulcers.[3]

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[14] 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.[38] Warren and Marshall were awarded the Nobel Prize in Medicine in 2005 for their wonderful work on H.pylori.[83]

EPIDEMIOLOGY

At least half the world's population is infected by H.pylori making it the most widespread infection in the world.[53] 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%.[66]

Infections are usually acquired in early childhood in all countries.[46] 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.[66] The higher prevalence among the elderly reflects the fact that they were infected from childhood.[46]

Prevalence appears to be higher in African-American and Hispanic populations, although this is likely related to socioeconomic rather than racial factors.[76,27] 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.[48] However, antibiotic resistance is appearing in H.pylori and many metronidazole and clarithromycin resistant strains seen in most parts of the world.[55]

H.pylori is contagious, although the exact route of transmission is not known.[56,17] Person-to-person transmission by either the oral-oral or fecal-oral route is most likely.[16] Consistent with these transmission routes, the bacteria have been isolated from feces, saliva and dental plaque of some infected people.[16]

Transmission occurs mainly within families in developed nations yet can also be acquired from the community in developing countries.[22] 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.[16]

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.[37]

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.[20,58]

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 labelled 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.[25]

The hydrolysis of urea to ammonia by urease could have a buffering effect, protecting the bacterium from acidity.[49]

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.[19,57,70] **Urease also has been proposed as an important virulence factor.**

GENETICS

In 1997, the complete genomic sequence of Helicobacter pylori strain 26695 was published.[84] 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.[60]

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.[71]

H.pylori is also found on the inner surface of the stomach epithelial cells and occasionally inside epithelial cells.[65] It produces adhesins 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.[41]

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.[77]

Colonization of the stomach by H.pylori results in chronic gastritis. The severity of the inflammation is likely to underlie H.pylori related diseases.[74] 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.[24] 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.[46] 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.[10] Gastrin stimulates the parietal cells in the corpus to secrete even more acid into the stomach lumen. Chronically increased gastrin levels eventually cause the increase in the number of parietal cells, further escalating the amount of acid secreted.[72] 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.[72] 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.[80]

About 50-70% of H. pylori strains in Western countries carry the Cag pathogenicity island (Cag PAI).[63] 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.[46] 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 Nod1, which then stimulates expression of cytokines that promote inflammation.[88]

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 signaling, and other cellular activities.[4] 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 c-terminal region of the CagA protein (amino acids 873-1002) can regulate host cell gene transcription independent of protein tyrosine phosphorylation.[5,15]

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"[86] 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 levels of TNF-α (tumor necrosis factor- α) and/or IL-6 (interleukin-6). According to the proposed perigenetic mechanism, inflammation-associated signaling molecules such as TNF-α can alter gastric 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.[81]

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.[46] 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.[46]

It is widely believed that in the absence of treatment, H.pylori infection-once established in its gastric niche-persists for life.[60] 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.[33,32]

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.[9]

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.[9,10] 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.[36,21]

Nevertheless, Blaser has refined his view to assert that H.pylori is a member of the normal flora of the stomach.[86] 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.[13,11] His group has recently shown that H.pylori colonization is associated with a lower incidence of childhood asthma.[18]

CLINICAL OUTCOMES ASSOCIATED WITH H.PYLORI

Peptic ulcer (Fig. 3,4)

H.pylori associate strongly with duodenal ulcers, than with gastric ulcers.[53] 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.[42,29,47] 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.[12]

MALT Lymphoma

Primary Non-Hodgkin Lymphoma (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.[23,43,64]

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.[79,93] Wother-spoon et al[92] 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 [69]

The active changes defined by Whitehead et al[91] 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 leukocytes (neutrophils)
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 grow 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 grow 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 grow 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.

Neutrophil infiltration (Fig. 11)

The features described by Whitehead et al[91] as active gastritis almost always are associated with H.pylori infection.

H.pylori and epithelial neutrophils 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 neutrophils, 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.

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

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.

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.

When the changes 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 a microcrypts by turning in on each other, producing an apparent intra-epithelial 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.

Mucus secretion

Factors that alter cell function tend to reduce secretion. Such factors include epithelial proliferation, inflammatory damage, atrophy and cellular atypia 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. The reduced mucus secretion could be a combination of direct bacterial effect and the associated inflammatory damage.

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.

Nonspecific changes:

1. Epithelial changes

(i). Atrophy (Fig. 12)

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.

(ii). Metaplasia (Fig. 13,14)

Metaplasia indicates a change in the gastric epithelium to resemble the lining of the 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, the metaplastic epithelium resemble mature small intestinal epithelium. In type II metaplasia, the epithelium frequently undergoes a partial, incomplete, or atypical metaplastic changes. This metaplasia may consists 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 an 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 metaplasia. Because of this fact, larger areas of mature intestinal metaplasia in the stomach show reduced inflammation, with regular epithelium and no active changes.

2. 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 (Fig.9). 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 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 (Fig.10). 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 cylindric 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.[30]

DIAGNOSIS

H.pylori infection can be diagnosed by invasive (i.e., requiring endoscopy) and noninvasive techniques (i.e., techniques that do not require endoscopy). 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

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, Jones et al[44] described a complement fixation test that had an accuracy of 80% to 90%. Alternative methods, such as hemagglutination, were available soon after this[50], followed by more sophisticated enzyme-linked immunosorbent assay (ELISA) methods, such as those first described by Good-win et al.[34]

The various serodiagnostic techniques used in detecting H.pylori are Bacterial agglutination[44], Complement fixation test, Haem-agglutination, ELISA, Western blotting, Co-agglutination, Immuno-fluorescence, Radio-immunoassay and Latex agglutination.

Sensitivity and specificity of ELISA depend largely on the nature of the antigenic materials bound to the solid support.[39,82] Although more expensive, the gold standard for a serologic test is an immunoblot, in which a visual representation of 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 21days, 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.

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.

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 for 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.

The enzyme was active at physiologic temperatures, with an optimum of 45^0C. 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 helicobacter organisms and are more likely to be positive in the corpus rather than in the antral mucosa.

Patients taking omeprazole often have achlorhydria. With 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 a 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.

For the detection of scant number of organisms, immunohistochemistry proved to be highly specific and sensitive and superior to conventional histochemical methods.[6,85]

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.

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.[8] Current detection methods by PCR are aimed at detecting H.pylori in clinical samples collected by less invasive means, such as gastric juice, saliva, dental plaque, and faeces.

TREATMENT[75]

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

Clarithromycin 500mg twice daily (or)

Metronidazole 500mg twice daily

Quadruple therapy

PPI twice daily

Bismuth two tablets three or four times daily.

Metronidazole 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.

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.

MATERIAL AND METHODS

The present study was carried out in the Department of Pathology, Madurai Medical College, Madurai for a period of two years from August 2007 to July 2009.

During this two year study period, 105 gastric biopsy materials and 105 serum samples were collected for Helicobacter pylori study in patients presenting with dyspepsia.

Patients were clinically diagnosed as suffering from dyspepsia based on the following symptoms: nausea, vomiting, anorexia, heartburn, flatulence, regurgitation, early satiety, fullness and bloating in addition to pain or discomfort.[90] The working proforma is appended in annexure I.

We received a total of 414 biopsy materials from different levels of the upper gastrointestinal tract. The biopsy materials were obtained by using flexible fibreoptic upper gastrointestinal endoscope. Specimens were collected from the Department of Medical Gastroenterology, Government Rajaji Hospital, Madurai.

Study design - Prospective study.

Inclusion criteria:-

Patients of both sex above 12 years of age who were found to have peptic ulcer, gastritis, duodenitis and normal on endoscopy on evaluation of dyspepsia were taken up for the study.

Exclusion criteria:-

1. History of antibiotic ingestion in the previous 4 to 6 weeks.
2. History of ingestion of antacids or H_2 blockers or proton pump inhibitors and Non-steroidal anti-inflammatory drugs over the past 4 to 6 weeks.

Methodology

Informed consent was obtained from all patients included in this study. The relevant history and clinical details were recorded using a structured proforma.

After overnight fasting, oesophagogastroduodenoscopy was done on the following morning. Three gastric biopsy materials, one from corpus and two from antrum of stomach were obtained. One antral specimen was used for urease test in the endoscopic room itself. Remaining biopsy materials were used for histopahological examination. 2ml blood was collected by venipuncture for IgG ELISA serology investigation.

Detection of Helicobacter pylori

Rapid Urease Test

One gastric antral biopsy specimen was taken and placed immediately in 5ml of freshly prepared solution of 10% urea containing 1% phenol red as pH indicator. Change of colour from yellow to pink was observed in the next 24 hrs.

Histopathological examination

Two gastric biopsy materials from corpus and antrum of the stomach were taken for histopahological examination and fixed in 10% neutral buffered formalin. The tissues were processed, paraffin blocked, 5 microns thin sections were cut and stained with Hematoxylin and Eosin (H&E) and Giemsa stains. H&E staining was used for the histological diagnosis of activity of H.pylori infection, mucosal inflammation, glandular atrophy and intestinal metaplasia. Giemsa staining was used for the histological diagnosis of H.pylori infection.

Being active was signified by the presence of neutrophils within the glandular and surface epithelial layer. Glandular atrophy was identified when the gastric glands were correspondingly decreased in amount and/or widely separated.

An increase in lymphocytes and plasma cells in lamina propria categorizes the gastritis as chronic. As an arbitrary guideline, infiltration involving upto 1/3 of the gastric pits and surface are designated mild; between 1/3 and 2/3 moderate and more than this as severe [67]. Lymphoid aggregates were defined as accumulation of lymphocytes without germinal center formation.[61,40]

Serologic testing

Collected blood by venipuncture was allowed to clot and the serum was separated by centrifugation at a speed of 3000 rpm for 5 min at room temperature. The serum samples were stored at -20^{0}C. Using DEMEDITEC H.pylori IgG antibody ELISA test kit, detection and quantitative determination of specific IgG antibodies against H.pylori in serum was done.

The information collected was recorded in a master chart. The results were compared. ‘P’ value analysis was used for statistical calculation to arrive at the conclusion.

Sensitivity, specificity and accuracy were calculated using the following formulae and taking histopahological findings as the gold standard.

Screening test results	**Diseased**	**Not Diseased**
Positive	True Positive (TP)	False Positive (FP)
Negative	False Negative (FN)	True Negative (TN)

Sensitivity = TP / (TP + FN) x 100

Specificity = TN / (FP + TN) x 100

Accuracy = TP + TN / (TP + FP + FN + TN) x 100

OBSERVATION AND RESULTS

Biopsy materials were obtained from different levels of the upper gastrointestinal tract in 414 patients including 144 biopsies from the oesophagus, 42 from oesophagogastric junction, 222 from stomach and 6 from small intestine.

In the oesophagus, 136 biopsies showed malignancy, 4 biopsies showed oesophagitis and 4 biopsies were inadequate for histopahological examination. In the stomach, 1 inflammatory polyp, 95 malignancies, 1 foreign body granuloma, 20 inadequate specimens, 15 normal gastric mucosa and 90 specimens with varying grades of gastritis were noted.

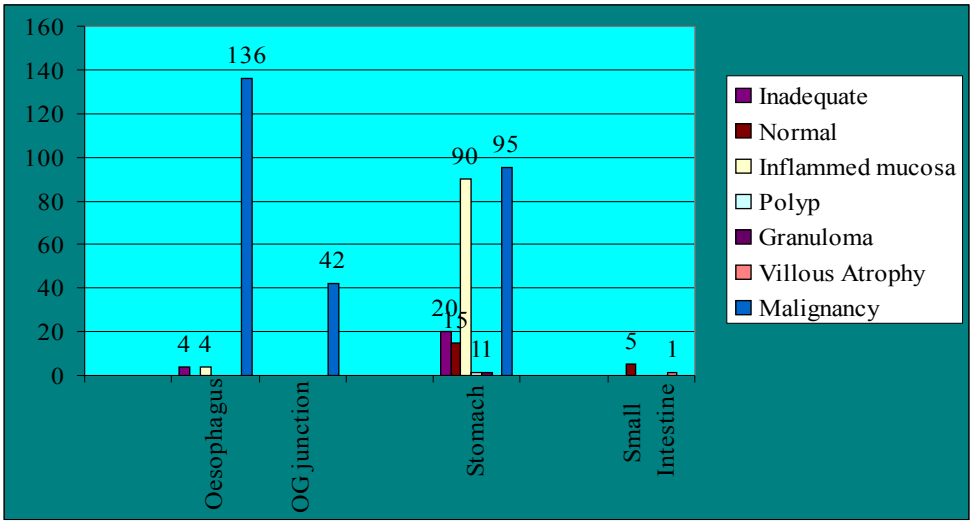
273 biopsy materials showed malignant lesions that were located in the oesophagus (in 136 patients), stomach (in 95 patients) and oesophagogastric junction (in 42 patients). Squamous cell carcinoma was noted in 136 biopsies obtained from oesophagus and 17 biopsies from oesophagogastric junction. Adenocarcinoma comprises 95 biopsies of stomach and 25 biopsies of oesophagogastric junction. In the small intestine 1 villous atrophy and 5 normal mucosa were noted [Table 1 and Chart 1].

Among the patients with oesophageal malignancy, dysphagia was the most common presenting symptom. Dyspepsia and weight loss were the most common symptoms of gastric malignancy.

Table 1: Incidence of various lesions of upper gastrointestinal biopsy materials

Location	Inadequate material	Normal mucosa	Inflammed mucosa	Polyp	Granuloma	Villous Atrophy	Malignancy
Oesophagus	4	-	4	-	-	-	136
OG junction	-	-	-	-	-	-	42
Stomach	20	15	90	1	1	-	95
Small Intestine	-	5	-	-	-	1	-

Chart 1: Incidence of various lesions of upper gastrointestinal biopsy materials



In the present study, a total of 105 Gastric biopsy materials were studied for the detection of H.pylori and the various histopahological features observed in dyspeptic patients.

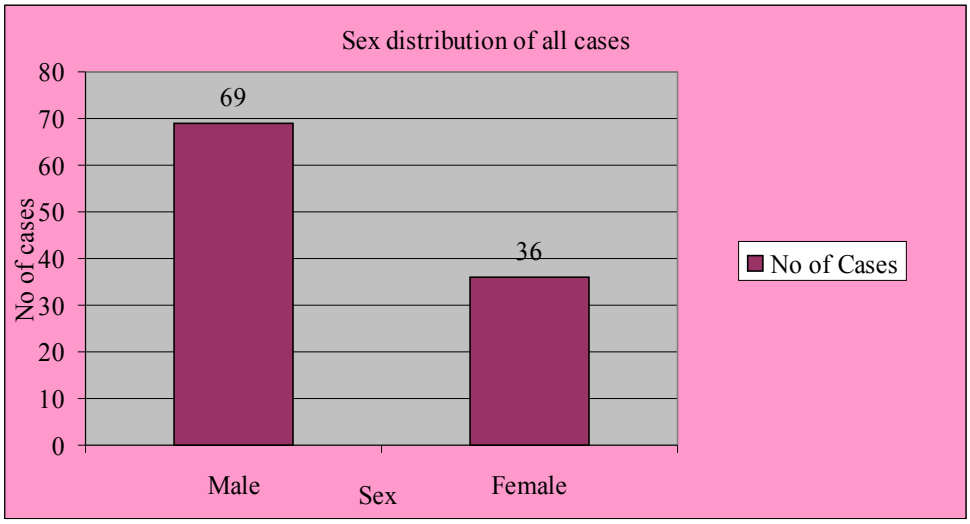
As per the proforma, the clinical data was collected from the patients who had undergone the upper gastrointestinal endoscopic procedure. After studying the biopsy and serum samples following observation were documented.

Sex distribution of all cases

Out of the total 105 cases, 69 (65.7%) cases were males and 36 (34.3%) cases were females [Table 2 and Chart 2].

Table 2: Sex distribution of all cases

Chart 2: Sex distribution of all cases



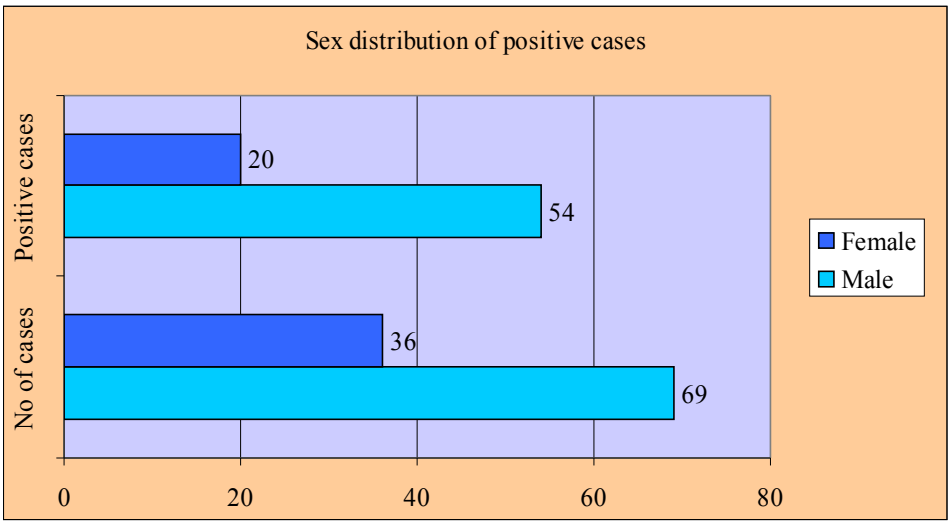
Sex distribution of positive cases

Out of 69 males, 54 (56.3%) males were positive for H.pylori and out of 36 females, 20 (55.6%) females were positive for H.pylori [Table 3 and Chart 3].

Table 3: Sex distribution of positive cases

S.no	Sex	No of cases	Positive cases	Percentage
1.	Male	69	54	56.3
2.	Female	36	20	55.6

Chart 3: Sex distribution of positive cases



Age distribution of all cases

In the present study, the age group of patients ranged from 17 years to 82 years and the maximum numbers of cases were found in the age group of 31- 40 years, comprising a total of 31.4% of the study population.

The least number of cases were in the age groups of 13-20 and above 70 years, each comprising a total of 1.9% of the study population.

Among females, no cases were found in the age groups of 13-20 years and above 70 years.

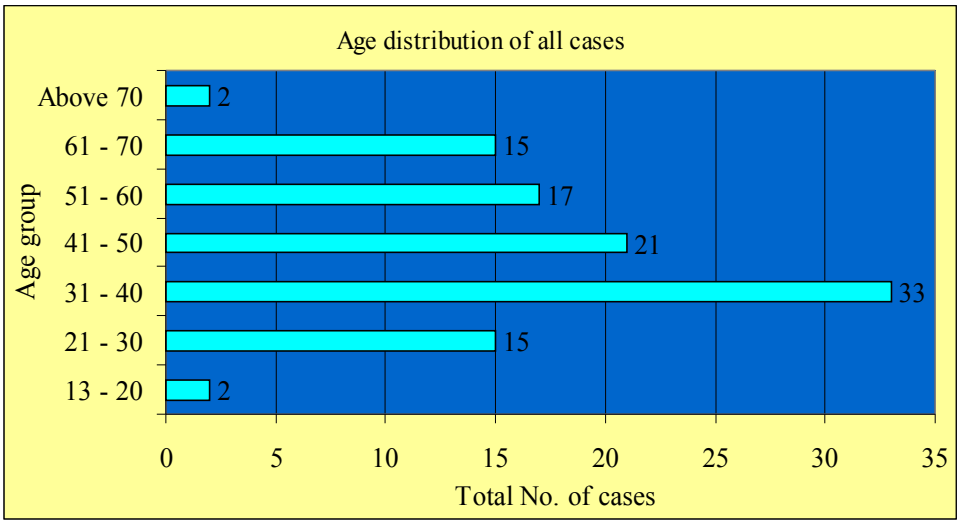
The youngest patient among males was 17 year old and the oldest patient was 82 year old.

The youngest patient among females was 26 year old and the oldest patient was 70 year old [Table 4 and Chart 4].

Table 4: Age distribution of all cases

S.no	Age group (yrs)	No of cases		Total	Percentage
		Male	Female		
1.	13 - 20	2	-	2	1.9
2.	21 - 30	8	7	15	14.3
3.	31 - 40	22	11	33	31.4
4.	41 - 50	13	8	21	20
5.	51 - 60	10	7	17	16.2
6.	61 - 70	12	3	15	14.3
7.	Above 70	2	-	2	1.9
	Total	69	36	105	100

Chart 4: Age distribution of all cases



Age distribution of positive cases

Maximum numbers of positive cases were found in the age group of 51-60 years (88.2%).

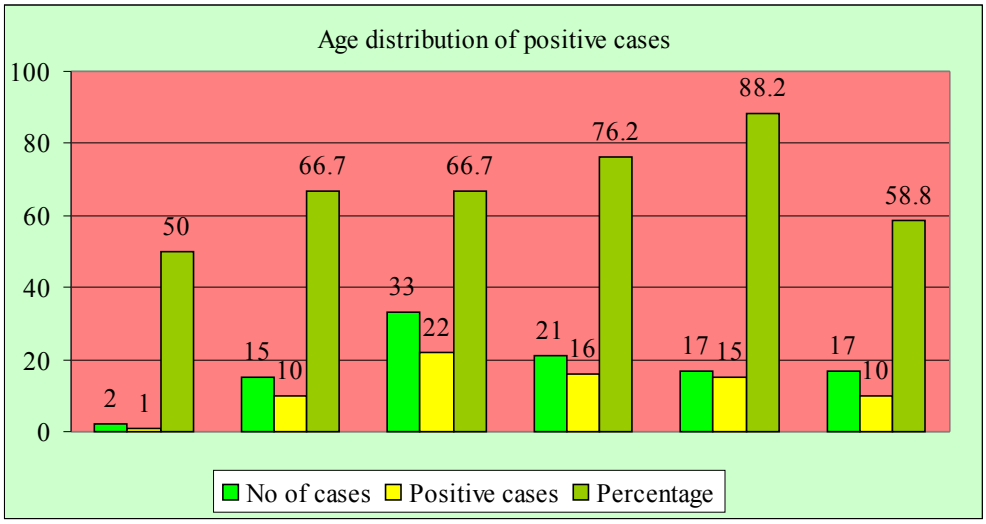
Minimum numbers of positive cases were found in the age group of 13-20 years (50%) [Table 5 and Chart 5].

Table 5: Age distribution of positive cases

Age group	No of cases	Positive cases	Percentage
13 - 20	2	1	50
21- 30	15	10	66.7
31- 40	33	22	66.7
41- 50	21	16	76.2
51- 60	17	15	88.2

>60	17	10	58.8

Chart 5: Age distribution of positive cases



Endoscopic findings

Out of the 105 cases, 54 cases had normal mucosa (Fig.2), 30 cases had duodenal ulcer, 9 cases had gastric ulcer, 8 cases had duodenitis and 4 cases had gastritis.

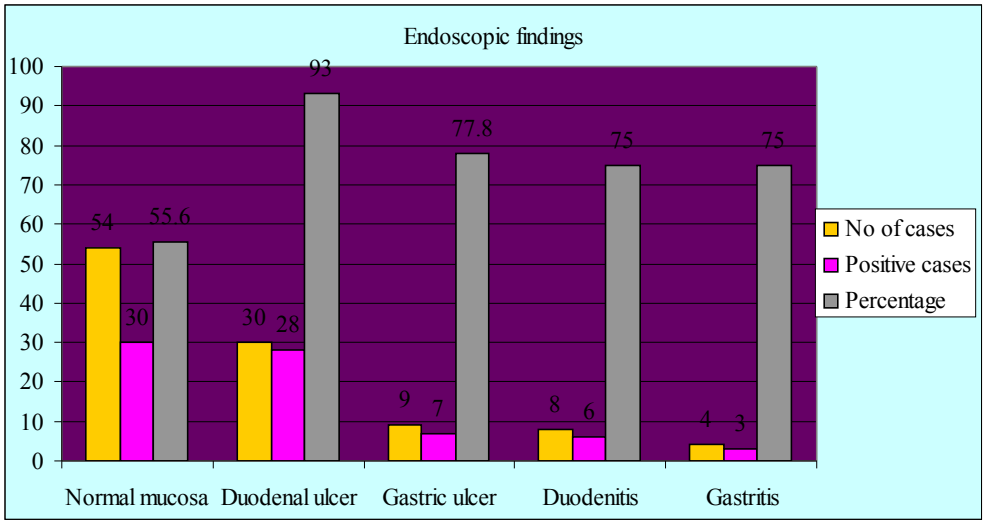
Endoscopic findings of positive cases

Out of 39 peptic ulcer cases (30 duodenal ulcer and 9 gastric ulcer), 35 (89.74%) cases [28 (93%) duodenal and 7 (77.8%) gastric ulcer] showed H.pylori positivity. Among 66 non-ulcer cases (8 duodenitis, 4 gastritis and 54 normal mucosal appearance on endoscopy) 39 cases [6 duodenitis (75%), 3 (75%) gastritis and 30 (55.6) normal mucosal appearance on endoscopy] showed H.pylori positivity [Table 6 and Chart 6].

Table 6: Endoscopic findings

S.no	Endoscopic findings	No of cases	Positive cases	Percentage
1.	Normal mucosa	54	30	55.6
2.	Duodenal ulcer	30	28	93
3.	Gastric ulcer	9	7	77.8
4.	Duodenitis	8	6	75
5.	Gastritis	4	3	75

Chart 6: Endoscopic findings



Rapid urease test

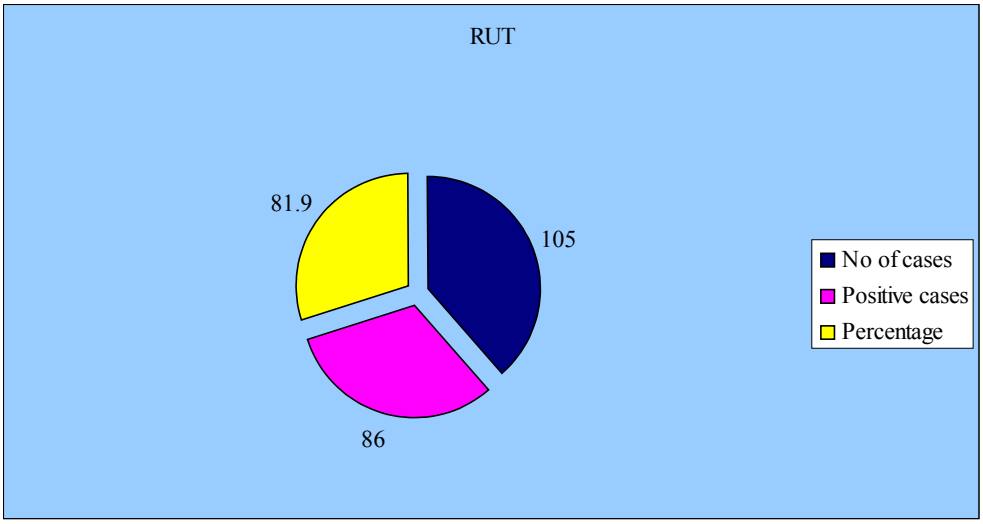
Out of 105 patients, 86 [81.9%] patients were positive for H.pylori by rapid urease test. The pink to red color development represents a positive test (Fig. 5,6) [Table 7 and Chart 7].

Table 7: Rapid urease test (RUT)

S.no	No of cases	Positive cases	Percentage

1.	105	86	81.9

Chart 7: Rapid urease test (RUT)



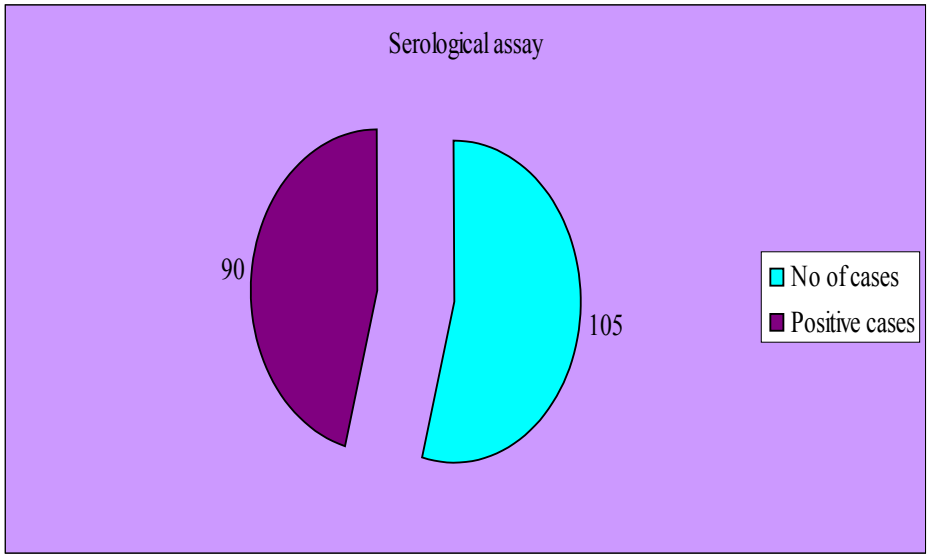
Serological assay

Out of the 105 cases, 90 [85.7%] cases were positive for H.pylori by serological method. The observed cut-off value was 0.516. And the value of the sample higher than that of cut-off value was considered positive indicated by development of yellow color. (Fig.18) [Table 8 and Chart 8].

Table 8: Serological assay

S.no	No of cases	Positive cases	Percentage
1.	105	90	85.7

Chart 8: Serological assay



Histopathology

Using Giemsa stain, the spiral shaped bacteria of H.pylori stained blue, were found attached to the brush border of the gastric foveolar epithelial cells and inside gastric pits (Fig.15,16).

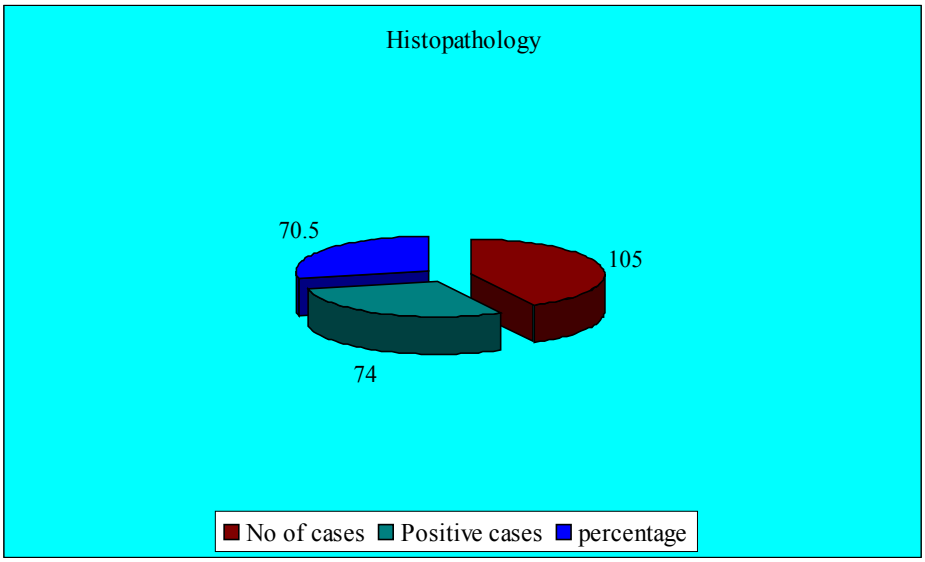
Out of the 105 cases, 74 (70.5%) cases were positive for H.pylori by histopahological examination.

There were 74 positive H.pylori cases in the study group (70.5%) and 31 negative subjects (29.5%). 56.3% of males and 55.6% of females in the study group were positive for H.pylori [Table 9 and Chart 9].

Table 9: Histopathology

S.no	No of cases	Positive cases	Percentage
1.	105	74	70.5

Chart 9: Histopathology



Histopathological features

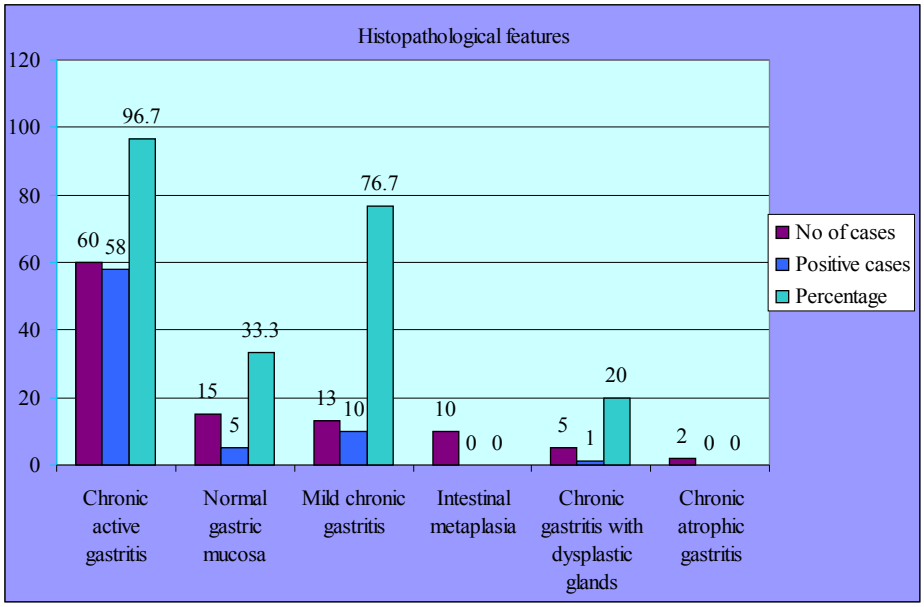
Histopathologically, 60 cases (57.1%) showed evidence of chronic active gastritis, of which 58 cases (96.7%) were H.pylori positive. Mild gastritis was evidenced in 13 cases (12.4%), of which 10 cases (76.7%) were H.pylori positive. 15 cases were normal (14.3%) of which 5 cases (33.3%) were H.pylori positive. Ten cases of intestinal metaplasia (9.5%) and two cases of atrophy (1.9%) were detected, all were H.pylori negative. Five cases of dysplasia (4.8%) (Fig.17) were found, of which one case (20%) was H.pylori positive (Fig.14). Lymphocyte infiltration was more prominent in the antrum [Table 10 and Chart 10].

Table 10: Histopathological features

S.no	Histopathological features	No of cases	Positive cases	Percentage

1.	Chronic active gastritis	60	58	96.7
2.	Normal gastric mucosa	15	5	33.3
3.	Mild chronic gastritis	13	10	76.7
4.	Intestinal metaplasia	10	-	-
5.	Chronic gastritis with dysplastic glands	5	1	20
6.	Chronic atrophic gastritis	2	-	-

Chart 10: Histopathological features



Correlation of Rapid urease test, Histopathological and Serological methods

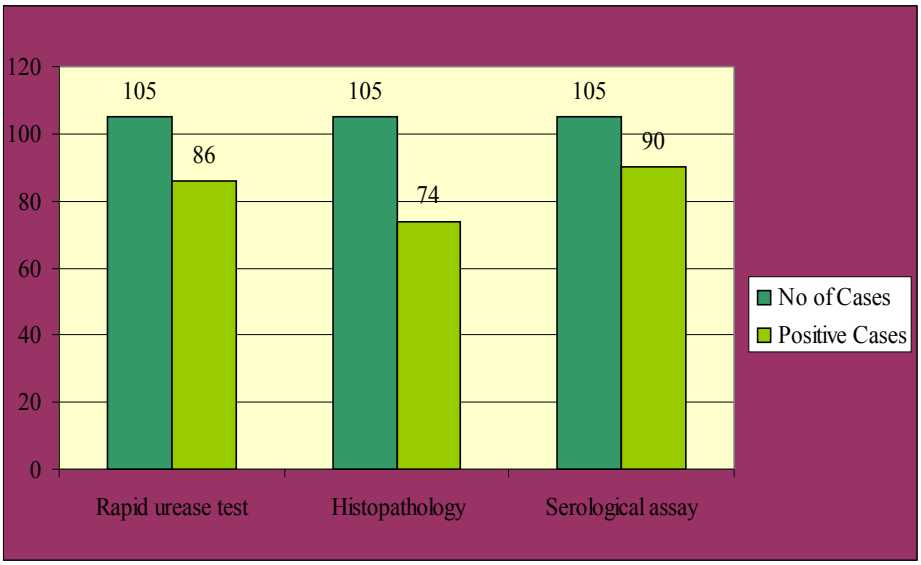
Out of 86 positive cases in rapid urease test, only 69 cases showed H.pylori by Giemsa staining.

Out of 90 positive cases in serological assay, only 73 cases showed H.pylori by Giemsa staining [Table 11 and Chart 11].

Table 11: Correlation of Rapid urease test, Histopathological and Serological methods

S.no	Tests	No of Cases	Positive Cases
1.	Rapid urease test	105	86
2.	Histopathology	105	74
3.	Serological assay	105	90

Chart 11: Correlation of Rapid urease test, Histopathological and Serological methods



DISCUSSION

The most impressive advance has come from the flexible fibroscope with which it is possible to examine the oesophagus, stomach and duodenum and at the same time obtain biopsies for histopahological examination.

Infection with Helicobacter pylori is a world-wide chronic infection with the highest incidence in developing countries. It is associated with duodenal ulcer, chronic active gastritis, gastric cancer and gastric lymphoma. It can be easily identified on biopsy specimens taken at endoscopy.

Chronic gastritis is defined as the presence of chronic mucosal inflammatory changes leading eventually to mucosal atrophy and epithelial metaplasia. By far the most important aetiological association is chronic infection by the bacillus Helicobacter pylori. The organism is a worldwide pathogen that has the highest infection rates in developing countries.

Sex distribution

In the present study, out of 69 males, 54 (56.3%) males and out of 36 females, 20 (55.6%) females are positive for H.pylori. The male to female ratio is 1:1 which is comparable to a study by Abdur Rauf Khan[1]. He studied a total of 528 biopsies. There are 313 males, among whom 217 males (69%) are positive for H.pylori and 215 females, among whom 136 females (63%) positive for H.pylori. The male to female ratio is 1:1.

Age distribution

The higher prevalence of H.pylori is in the age group of 51-60 years (88.2%).

Endoscopic features

Normal looking gastric mucosa is the commonest single endoscopic finding, accounting for 51.4% of all cases.

The positivity rate for duodenal ulcer is 93% and gastric ulcer is 77.8% in our study. It is comparable to a study by Tytget[87] [1988] who found that all 15 (100%) patients of duodenal ulcer and 9 out of 11 (81.8%) patients with gastric ulcer found to have the organism. And in 2002, Sengupta et al[73] 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[94], 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.

Rapid urease test (RUT) and Serological assay

In the present study, H.pylori is positive in 86 (81.9%) cases by RUT and in 90 (85.7%) cases by serological assay. It correlates with a study done by U Arora et al[2]. They studied 75 gastric biopsy specimens and 75 serum samples of same patients complaining of dyspepsia. H.pylori is positive in 52 cases (72%) by RUT and in 57 cases (76%) by serological testing. Gill et al[31] have shown that antibodies to H.pylori in serum are present in about 80% of Indian subjects with upper gastrointestinal symptoms. In our study also the positivity rate is 85.7% which is very well correlated with the previous mentioned study.

Some of those methods are based on the high urease activity of H.pylori,[52,35] 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 pnuemoniae, will confound the result.

Serological identification of anti-H.pylori antibodies is a non-invasive method. However, the antibody titres preserve their levels even after the eradication of the bacteria by antibacterial therapy.

Histopathology

The prevalence of H.pylori in the present study is 70.5%. It is similar to a study by Abdul Rahman E Fakhro et al[28] and a study by Basic H, Katic V et al[7]. In their study the prevalence rates are 79.4% and 71.8% respectively.

Histopathologically, 60 cases out of 105 patients (57.1%) showed evidence of chronic active gastritis, of which 58 cases (96.7%) are H.pylori positive. Mild gastritis is evidenced in 13 cases (12.4%), of which 10 cases (76.7%) are H.pylori positive. 15 cases are normal (14.3%) of which 4 cases (26.7%) are H.pylori positive. Ten cases of intestinal metaplasia (9.5%) and two cases of atrophy (1.9%) are detected, all are H.pylori negative. Five cases of dysplasia (4.8%) are found, of which two cases (40%) are H. pylori positive.

Abdul Rahman E Fakhro et al[28] studied 102 gastric biopsies in dyspeptic patients. In their study, 66 cases out of 102 patients (64.7%) showed evidence of chronic active gastritis, of which 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. 16 cases are normal, of which 3 cases (18.8%) are H.pylori positive. Our study results are comparable to this study.

SUMMARY

A total of 414 upper gastrointestinal biopsy materials are received.

It includes 273 malignant lesions, 20 normal and 94 inflammed mucosa, 24 inadequate materials, 1 inflammatory polyp, 1 foreign body granuloma and 1 villous atrophy.

In the present study, 105 gastric biopsy materials and 105 serum samples are studied for the detection of H.pylori in patients presenting with dyspepsia.

Out of the 105 cases, 86 [81.9%] cases are positive for H.pylori by rapid urease test and 74 (70.5%) cases by histopahological examination.

The maximum numbers of positive cases (90 cases) are diagnosed by serological assay.

Men and women are equally affected by H.pylori infection.

Histopathological examination is the gold standard test for H.pylori detection against which the sensitivity, specificity and accuracy of serologic testing are 98.65%, 45.16% and 82.86% respectively.

The sensitivity, specificity and accuracy of rapid urease test are 93.24%, 45.16% and 79.05% respectively.

In cases of intestinal metaplasia and atrophic gastritis, there are no demonstrable H.pylori organisms.

Among the 105 cases, 39 cases had peptic ulcer (30 duodenal ulcer and 9 gastric ulcer)

The H.pylori positivity rate for peptic ulcer is 89.74% (duodenal ulcer is 93% and gastric ulcer is 77.8%).

CONCLUSION

To conclude H.pylori infection affects both genders equally. The sensitivity, specificity and accuracy of serologic testing are 98.65%, 45.16% and 82.86% respectively. Serological identification of anti-H.pylori antibodies is a non-invasive method. However, the antibody titres preserve their levels even after the eradication of the bacteria by antibacterial therapy. The sensitivity, specificity and accuracy of rapid urease test are 93.24%, 45.16% and 79.05% respectively. Methods based on the high urease activity of H. pylori 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 pnuemoniae, will confound the result. Presence of H.pylori is strongly associated with the occurrence of peptic ulcer ($P < 0.001$).

ANNEXURE I

PROFORMA

S.No:

NAME: AGE: SEX:

ADDRESS: UNIT: WARD:

OP / IP No:

COMPLAINTS:

1. Upper abdominal pain
2. Abdominal discomfort
3. Bloating sensation
4. Early satiety
5. Post prandial fullness
6. Heartburn

ALARM SYMPTOMS:

1. Dysphagia
2. Upper GI bleed (hematemesis / melena)
3. Persistent vomiting
4. Sensation of an abdominal mass
5. Jaundice
6. Loss of weight
7. Loss of appetite

PAST HISTORY:

Systemic illness

Drug intake: (Antibiotics / PPI / Metronidazole)

PERSONAL HISTORY:

Alcohol intake

Smoking

Betel chewing

EXAMINATION:

Nutritional status

Anemia

Jaundice

Abdomen: Epigastric tenderness

Abdominal mass

CLINICAL DIAGNOSIS:

INVESTIGATIONS:

1. Blood urea
2. Serum creatinine
3. Hemoglobin
4. Erythrocyte sedimentation rate (ESR)
5. Ultrasonography of abdomen
6. Upper GI endoscopy
7. Rapid urease test
8. Biopsy findings
9. Stains for Helicobacter pylori

FINAL DIAGNOSIS:

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 1minute.
6. Wash quickly in water, differentiate and dehydrate in alcohol. Clear and mount.

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, overnight
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

Results:

Organism - dark blue

Back ground - pink to pale blue

SEROLOGIC TESTING

Principle of the test

The DEMEDITEC H.pylori IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Helicobacter antigen is bound on the surface of the microtiter strips. Diluted serum is pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Helicobacter antigen takes place. After an hour of incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After washing, the substrate solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

Assay steps

1. Bring all reagents and samples to room temperature.
2. Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
3. Dilute the serum samples with sample diluent in a ratio of 1:101 (e.g. 5uL serum + 500 uL sample diluent).
4. Pipet 100 uL each of the diluted (1:101) samples, standards and controls into the wells. Leave one well empty for the substrate blank.
5. Cover plate with the enclosed foil and incubate at 37^0C for 60 minutes.
6. Empty the wells of the plate (dump or aspirate) and add 300uL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
7. Pipet 100 uL of conjugate into the wells. Leave one well empty for the substrate blank.
8. Cover plate with the enclosed foil and incubate at 37^0C for 30 minutes.

9. Empty the wells of the plate (dump or aspirate) and add 300uL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
10. Pipet 100uL of the substrate into the wells. This time also the substrate blank is pipetted.
11. Incubate without covering at room temperature for 20 minutes in the dark.
12. To terminate the substrate reaction, pipet 100uL of stop solution into the wells. Pipet also the substrate blank.
13. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm).
14. The mean values for the measured absorptions are calculated after subtraction of the substrate blank value.
15. If the value of the sample is higher than that of cut-off value, there is a positive result.

ANNXUREE III

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ANNEXURE IV

MASTER CHART

M - Male F - Female