Comparison among different diagnostic Procedures for *Helicobacter pylori* infection.
SUMMARY

*Helicobacter pylori* is strongly associated with chronic gastritis, peptic ulcer, adenocarcinoma and mucosa–associated lymphoid tissue lymphoma (MALT) of the stomach. The prevalence of infection is high in developing countries demanding reliable diagnostic method. The present study was carried out in the department of microbiology, Mymensingh medical college for the period from July 2006 to June 2007 to find out a suitable method for diagnosis of *Helicobacter pylori* infection. This cross sectional study included 45 patients having upper gastrointestinal symptoms those underwent for endoscopy and subsequently diagnosed as patients with gastritis, peptic ulcer (PU) and non-ulcer dyspepsia (NUD) as symptomatic group. Another age and sex matched 45 individuals without upper gastrointestinal symptoms were included as asymptomatic group. At the time of endoscopy, 2 biopsy specimens were collected from each of the symptomatic patients for rapid urease test (RUT) and direct microscopy. Serum samples from both groups were tested for anti-*H. pylori* Ig G and Ig M by ELISA. Out of 45 patients, rapid urease test (RUT) and direct microscopy were found positive in 38 (84.44%) and 22 (48.89%) patients respectively. Sensitivity and specificity of anti-*H. pylori* Ig G was calculated at different cut-off level. The corresponding values at 5 arbU/ml, 10 arbU/ml, 20arbU/ml and 30arbU/ml were found as 95.55% and 11.11%, 80% and 28.89%, 51.11% and 62.22%, 40% and 75.55% respectively. In case of ELISA for anti-*H. pylori* IgM, sensitivity was found as 73.33 % and specificity 93.33 % respectively. Detection of anti-*H. pylori* IgM was significantly higher in symptomatic group while compared with asymptomatic population (p < 0.001). ELISA for anti-*H.
*pylori* IgM became highly sensitive and specific. It was suggested to use for the diagnosis of *H. pylori* infection among dyspeptic patients. As ELISA for anti-*H. pylori* IgG could not distinguish present and past infection, so we recommended further study including larger asymptomatic population to establish ELISA for anti-*H. pylori* IgG as screening test.
ACKNOWLEDGEMENTS

First of all thanks to Almighty Allah, the beneficent and merciful, for giving me the opportunity and providing me the ample energy and patience to complete the entire thesis work.

I am very grateful and deeply indebted to my honourable teacher and guide Professor Dr Md. Akram Hossain, Head of the Department of Microbiology, Mymensingh Medical College. I have the great privilege and honour to express my whole hearted indebtedness to him for his inspiring encouragement, continuous guidance, active cooperation, constant supervision, valuable suggestions, Constructive criticism and help in carrying out this work successfully.

I am thankful to the honourable members of the Ethical review committee for giving kind approval and I am also obliged to Professor Dr Md. Monwar Hossain, Principal, Mymensingh Medical College, for his kind permission to conduct the present study in this institution.

I would like to express my deepest regards and gratitude to my respected teacher Dr. A.K.M. Musa, Associate Professor, Department of Microbiology, Mymensingh medical College for valuable advice and cordial cooperation.

I express my greatest regards and heartfelt gratitude to my respected teacher Dr. A.K.M. Shamsuzzaman, Assistant Professor, Department of Microbiology, Mymensingh Medical College for his constant guidance, constructive criticism in correcting the manuscript and
active cooperation. My cordial respect to Dr. Md. Chand Mahmud, Assistant Professor, Department of Microbiology, Mymensingh Medical College for valuable suggestions.

I am also grateful to Dr. Md. Shawkat Ali Associate Professor, Department of Gastroenterology and Dr. Chitta Ranjan Debnath, Resident Physician, Department of Medicine, Mymensingh Medical College & Hospital for their active assistance in endoscopic sample collection. I express my grateful thanks to Professor Dr. Kalpana Deb, Head of the Department of Biochemistry, Mymensingh Medical College, for her cordial cooperation and valuable suggestions.

I like to express gratitude to all other lecturers, M.Phil students, laboratory technologists of Microbiology Department, Mymensingh Medical College. I must thank to Librarian and associated staffs of library of Mymensingh Medical College. I wish to express my sincere thanks to Dr. Sayeda Anjuman Nasreen, Lecturer, Department of Community Medicine for her assistance in data analysis.

I am indebted to my parents and my elder sister Major (Dr.) Rownak Azad Anne whose affectionate support inspired me for higher studies. I would like to thank my husband Dr. Mohammad Shoeb Khan for his devoted cooperation and sensible sharing of my pains and pleasures. I would like to express love to my son Shadab whom I could give very little time and attention during this work.

Lastly I am indebted to those patients and control subjects from whom I have collected samples and pray to Almighty for their recovery.

Mymensingh, January 2008. Dr. Asma Azad Sumona
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<tr>
<td>AU</td>
<td>Arbitrary unit</td>
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<tr>
<td>Ca</td>
<td>Carcinoma</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>PU</td>
<td>Peptic ulcer</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>H. pylori</td>
<td>Helicobacter pylori</td>
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<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>H2O2</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>IFN γ</td>
<td>Interferon γ</td>
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<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
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<td>Immunoglobulin G</td>
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<tr>
<td>IL-2</td>
<td>Interleukin-2</td>
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<td>IL-8</td>
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<tr>
<td>IL-12</td>
<td>Interleukin-12</td>
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<td>KDa</td>
<td>Kilodalton</td>
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<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>MALT</td>
<td>--- Mucosa associated lymphoid tissue</td>
</tr>
<tr>
<td>NF-κB</td>
<td>--- Nuclear factor Kappa B</td>
</tr>
<tr>
<td>NSAID</td>
<td>--- Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>NUD</td>
<td>--- Non-ulcer dyspepsia</td>
</tr>
<tr>
<td>OD</td>
<td>--- Optical density</td>
</tr>
<tr>
<td>PCR</td>
<td>--- Polymerase chain reaction</td>
</tr>
<tr>
<td>p</td>
<td>--- Probability</td>
</tr>
<tr>
<td>PPI</td>
<td>--- Proton pump inhibitor</td>
</tr>
<tr>
<td>RNA</td>
<td>--- Ribonucleic acid</td>
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<td>RUT</td>
<td>--- Rapid urease test</td>
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<tr>
<td>SD</td>
<td>--- Standard deviation</td>
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<tr>
<td>Th1</td>
<td>--- T Helper cell 1</td>
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<tr>
<td>Th2</td>
<td>--- T Helper cell 2</td>
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<tr>
<td>TMB</td>
<td>--- Tetramethylbenzidine</td>
</tr>
<tr>
<td>TNFα</td>
<td>--- Tumour necrosis factor alpha</td>
</tr>
<tr>
<td>UBT</td>
<td>--- Urea breath test</td>
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<tr>
<td>WHO</td>
<td>--- World Health Organization</td>
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<td>--------------------------------</td>
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<tr>
<td>&lt;</td>
<td>--- Less than</td>
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<tr>
<td>&gt;</td>
<td>--- More than</td>
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<tr>
<td>µl</td>
<td>--- Microlitre</td>
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<tr>
<td>+ve</td>
<td>--- Positive</td>
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<td>-Ve</td>
<td>--- Negative</td>
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<td>%</td>
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INTRODUCTION

*Helicobacter pylori* is a Gram negative, spiral flagellated bacterium that infects approximately 50 percent or more of the world population (Goodman and Cockburn, 2001). It is one of the most common chronic bacterial infections in human (Brown, 2000). The prevalence of *H. pylori* is 30% in the United States and other developed countries as opposed to >80% in most developing countries (Atherton and Blaser, 2005). Prevalence of infection increases with age and correlates positively with a low socioeconomic status during childhood (Malaty and Graham, 1994). The infection is usually acquired in early childhood and persists throughout life unless treated (Klein *et al*, 1994). The seroprevalence rate in lower socioeconomic class in India was found 79% (Graham *et al*, 1991). In Bangladesh 85% of *H. pylori* infection rate has been found by Urea Breath Test (UBT) among children under five years (Sarker *et al*, 1995) and one pilot study showed seroprevalence of 92% in asymptomatic adults (Ahmed *et al*, 1997).

There is a strong association between the presence of this organism and gastric inflammation (Marshall and Warren, 1984). Host and bacterial factors with interaction of environment contribute pathogenicity. *H. pylori* cytotoxin-associated geneA (cagA), vacuolating toxinA (vacA) and adherence factors to gastric epithelium have been linked to enhanced pathogenicity of the bacterium. Host genetic polymorphism of cytokines, related legends, receptors and enzymes influence *H. pylori* infection (Das and Paul, 2007).
Mode of transmission is most likely from person to person, either by the oral-oral route (through vomitus or possibly saliva) or perhaps the fecal-oral route. The person-to-person transmission is supported by the higher incidence of infection among institutionalized children and adults and the clustering of H. pylori infection within families. Detection of H. Pylori from faeces and dental plaque by culture and PCR suggesting a faecal-oral or oral-oral transmission (Go, 2002; Krajden et al, 1988). Waterborne transmission due to fecal contamination may be an important source of infection especially in parts of the world in which untreated water is common (Goodman et al, 1996). Iatrogenic spread through contaminated endoscopes has been documented but can be prevented by using proper disinfection procedures (Fantry et al, 1995). It has been suggested that infection may be transmitted gastro-orally by gastroesophageal reflux or regurgitation of stomach contents (stone, 1999).

In most individuals H. pylori infection is asymptomatic. However, about 10-15% of infected individuals will sometime experience peptic ulcer disease. It causes >90% of duodenal ulcer and up to 80% gastric ulcers Presently, its role has been established in chronic antral gastritis, duodenal ulcer, chronic gastric ulcer, dyspepsia, gastric cancer and gastric lymphoma (Nobel Foundation, 2005). World Health Organization has added H. pylori as a group I carcinogen (International Agency for Research on Cancer WHO, 1994).

H. pylori infection is the major cause of chronic gastritis, a condition that initiates the pathogenic sequence of events leading to atrophic gastritis, metaplasia, dysplasia and subsequently, cancer (Correa, 1988). Gastric cancer is a major public health issue and the
global burden of gastric cancer is increasing, particularly in developing countries. Gastric cancer remains second (after lung cancer) among causes of cancer death worldwide (Pisani et al, 1999). Pooled analyses of prospective seroepidemiological studies have shown that people with *H pylori* infection are at increased risk of developing non-cardiac gastric cancer (Helicobacter and Cancer Collaborative Group, 2001). Although *H. pylori* is associated with the development of adenocarcinoma of the antrum and body of the stomach, it is also clearly linked with gastric mucosa–associated lymphoid tissue (MALT) lymphomas. It has been reported that regression of low-grade gastric MALT lymphoma can be achieved in 70% to 90% of patients with eradication of *H. pylori* infection (Morgner et al, 2000).

The NIH (National Institute of Health) Consensus Development Panel on *Helicobacter Pylori* in peptic ulcer disease recommends that the diagnosis should be established before therapy is started. There are several procedures both invasive and non-invasive are available for detection of *H. pylori* infection.

Invasive procedures based upon endoscopic biopsy based procedures such as rapid urease test (RUT), culture, polymerase chain reaction (PCR), microscopical examination and histopathology. These procedures are expensive and tedious. Moreover, patchy distribution of the organism may lead to false negative results as they can assay infection only at the biopsy sites. So the sensitivity of invasive procedures is sometimes reduced due to sampling error (Morris et al, 1989).
Since invasive procedures are expensive and tedious, non-invasive procedures to assess *H. pylori* infection have become more popular (Zagari *et al.*, 1999). The more widely available non-invasive procedures for diagnosing *H. pylori* infection are the $^{13}$C or $^{14}$C urea breath test (UBT) and serological assays. A recently developed stool antigen assay has been shown to be a reliable diagnostic tool and is becoming increasingly available and employed. Other non-invasive procedures of more limited use are assessment of saliva or urine analysis for anti-*H. pylori* antibody (Vaira *et al.*, 2000).

*H. pylori* is difficult to cultivate on culture media, so the role of culture in diagnosis of the infection is limited mostly to research and epidemiologic purpose. The sensitivity and specificity of culture claimed to be 60 - 90% and 100% respectively. The success of the technique depends on local technique and access to facilities. Although costly, time-consuming and labor intensive, culture does have a role in antibiotic susceptibility studies and studies of growth factors and metabolism (Perez-Perez *et al.*, 2000). In addition to culturing *Helicobacter* from the gastric biopsy the organism has also been isolated from faeces and rarely dental plaque. This is a topic that requires further study because of the ease of collecting these specimens and their potential use in diagnosis (Vaira *et al.*, 2000).

Microscopical examination of a stained smear made of crushed tissue is convenient and quick. If carried out skillfully, Warthin-Starry silver stain as originally used by Warren gives the best results. Others recommended staining methods include Gram stain, modified Giemsa, acridine orange (fluorescence), cresyl violet and Brown-Hopps method. Immunohistochemical techniques have been developed that use panti *H. pylori*
antibody reacting against somatic antigens of the whole bacteria and have been found to correlate well with the presence of the bacteria. Modified Giemsa staining has been favoured by many researchers because of its ease of performance. The sensitivity of modified Giemsa stain is 85%. However, the specificity of modified Giemsa stain depends on the morphological appearance. The specificity of immunostaining permits detection of low numbers or even single organism (Babic et al, 2005).

Histologic evaluation has traditionally been the gold-standard method for diagnosing *H. pylori* infection. It does allow for definitive diagnosis of infection, as well as of the degree of inflammation or metaplasia. Histological detection of the organism has generally been considered to have lower sensitivity at 80% - 95% with 100% specificity. Most infection can be detected with haematoxylin & eosin (H&E) stain of gastric tissue, but special stains like Giemsa can be used if H&E results are not conclusive. The need for a number of biopsy specimens to be examined by experienced pathologists renders histology expensive and it requires an invasive procedure (El-Zimaity et al, 1999). Limitations also arise at times since *H pylori* is not evenly distributed throughout the stomach, biopsies can miss the site of infection.

Measuring urease production from biopsy specimen can be accomplished by rapid urease test (RUT). This test has the advantages of being inexpensive, fast and provides more rapid results than either histology or culture. The test has shown 90 - 95% sensitivity and 100% specificity. However, recent ingestion of medications that decrease the density and/or urease activity of *H pylori* such as bismuth containing compounds, antibiotics, and
proton pump inhibitors can reduce the sensitivity of the rapid urease test by as much as 25% (Midolo and Marshall, 2000). Thus false negative urease tests may occur mainly due to low numbers of organisms in the biopsy which typically follow treatment (Tokunaga et al., 1998).

Molecular methods like polymerase chain reaction (PCR) have the potential to accurately determine both the presence of infection and the genotype of bacteria and have marked sensitivity and specificity (Gramley et al., 1999). These techniques have been used successfully to detect *H. pylori* DNA in gastric tissues by amplifying genes such as the adhesin genes (Evans et al., 1995), the urease gene (Clayton et al., 1992) and the 16S rRNA gene (Ho et al., 1991). The 16S rRNA gene of *H. pylori* is a highly specific target for amplification and has been used to help reclassify the organism. The 16S rRNA is one of the specific targets to confirm *H. pylori* infection and positive amplification of *H. pylori* specific DNA may be considered as a direct evidence of the presence of the pathogen (Chong et al., 1996). Trials investigating PCR amplification of saliva, feces, and dental plaque to detect the presence of *H. pylori* are ongoing (Bravos and Gilman, 2000).

Urease activity can be detected noninvasively by way of a breath test. In this test, urea that is radioactively labeled with carbon (either $^{14}$C labeled urea or $^{13}$C -labeled urea) is ingested. Bacterial urease splits off labeled carbon dioxide, which can be detected in the breath. Accuracy is not a problem for either of these breath tests since both elicit 95% sensitivity and 100% specificity. The breath test technique reflects current infection and also useful for treatment follow-up purposes (Atherton & Spiller, 1994). Disadvantage of this technique is that it may involve a small amount of exposure to radiation. Although
\(^{13}\)C is a stable isotope and does not emit radiation but its detection requires a mass spectrometer, which may not be readily available.

Serologic tests measure circulating IgG, IgM and IgA antibodies to *H. pylori* and have excellent sensitivity and specificity of above 95%. These are cheap and simple compared to invasive techniques (De Cross & Puera, 1992). The presence or absence of IgM antibodies to *H. pylori* may reflect whether or not an acute infection exists (Alem et al, 2002). Determination of IgG level can act as an important screening procedure (Arora et al, 2003). Serology is not useful for follow-up because antibody titers may not decline for 6 to 12 months or sometimes even years after treatment (Kosunen et al, 1992). The commercially available assays are of different types such as microtitre-plate assays/ enzyme linked immunosorbent assay (ELISA) for uses in a laboratory or near-patient testing kits are based on either latex agglutination or immunochromatography (ICT). All the evidence to date suggests that ICT/Latex kits have very much lower sensitivity and specificity compared with a laboratory-based ELISA test (Stone et al, 1997). Western blotting kits have also been developed and are used to detect the presence of specific virulence markers. The exact role of serology in the management of *Helicobacter pylori* infection has still to be defined although there is evidence that using this as a screening procedure can reduce endoscopy cost and workload (Vaira et al, 1998). Serologic tests are the only tests which are not likely to give false negative results in patients who have taken antibiotics, bismuth compounds or omeprazole in the recent past (NIH Consensus Conference, 1994).
Stool antigen assay is a relatively new diagnostic procedure that uses an enzyme linked immunosorbent assay (ELISA)/ICT to detect the presence of *H. pylori* antigen in the stool with the use of polyclonal anti-*H pylori* antibody. As this test detects bacterial antigen in ongoing infection, it can be used as a reliable means of diagnosing active infection and also useful for treatment follow-up purposes. These tests have sensitivity and specificity comparable to those of other noninvasive tests (Monteiro et al, 2001).

All the current diagnostic procedures have its own advantages, disadvantages and limitations. It has become increasingly clear that no single procedure is optimal (Mégraud et al, 1997) and only a combination of different procedures provides an adequate diagnostic yield (Maastricht Consensus report, 1997).

This study was carried out to analyze the diagnostic value of rapid urease test, microscopical examination of smear and antibody (IgG, IgM) assay against *H. pylori* in serum by enzyme-linked immunosorbent assay (ELISA). The findings of this study will help to choose the appropriate procedures to diagnose the *H. pylori* infection.

**OBJECTIVES**

**General objective**

**To find out a suitable diagnostic procedures for diagnosis of *Helicobacter pylori* infection.**

**Specific objectives**
a) To diagnose *H. pylori* from endoscopic biopsy specimen by rapid urease test.

b) To diagnose *H. pylori* by direct microscopy.

c) To diagnose *H. pylori* infection by anti-*H. pylori* IgG and anti-*H. pylori* IgM antibody detection by ELISA.

d) To compare sensitivity and specificity of mentioned diagnostic procedures.

## REVIEW OF LITERATURE

### 2.1. HISTORICAL BACKGROUND

The presence of spiral-shaped microorganisms in the human stomach was described over 100 years ago. In 1875, German scientists found helical shaped bacteria in the lining of the human stomach. The bacteria could not be grown in culture and the results were eventually forgotten (Blaser, 2005).

In 1893, the Italian researcher Giulio Bizzozero described helical shaped bacteria living in the acidic environment of the stomach of dogs (Wikipedia, 2007). Polish clinical researcher, Professor W. Jaworski, investigated sediments of gastric washings obtained from humans in 1899. Among some rod-like bacteria, he also found bacteria with a characteristic helical 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 did not have much impact as it was written in Polish (Konturek, 2003).
The bacterium was rediscovered in 1979 by Dr. J. Robin Warren, a pathologist at the Royal Perth Hospital in Western Australia, who did further research on it with Clinician / gastroenterologist Dr. Barry J. Marshall beginning in 1981. They isolated the organisms from mucosal specimens from human stomachs and were the first to successfully culture them. 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 (Marshall & Warren, 1984). The medical community was slow to recognize the role of this bacterium in stomach ulcers and gastritis, believing that no microorganism could survive for long in the acidic environment of the stomach. The community began to come around after further studies were done, including one in which Marshall drank a Petri dish of \textit{H. pylori}, developed gastritis, and the bacteria were recovered from his stomach lining, thereby satisfying three out of the four Koch's postulates. The fourth was satisfied after a second endoscopy ten days after inoculation revealed signs of gastritis and the presence of "\textit{H. pylori}". Marshall was then able to treat himself using a fourteen day dual therapy with bismuth salts and metronidazole. Marshall and Warren went on to show that antibiotics are effective in the treatment of many cases of gastritis. In 1994, the National Institutes of Health (USA) published an opinion stating that most recurrent gastric ulcers were caused by \textit{H. pylori} and recommended that antibiotics be included in the treatment regimen. Evidence has been accumulating to suggest that duodenal ulcers are also associated with \textit{H. pylori} infection (Pietroiusti \textit{et al}, 2005). Warren and Marshall were awarded the Nobel Prize in Medicine for their work on \textit{H. pylori} in 2005.
The discovery of *H. pylori* is a consequence of two technical advances. Firstly, the development of fibre-optic endoscopy in 1970s as rigid endoscopes did not reach or take biopsies from the gastric antrum that is the area most commonly infected by *H. pylori*. Secondly, the progress in bacterial culture is important. In the 1970s, the culture of microaerophilic bacteria became routine after the recognition of the fact that *Campylobacter jejuni*, a microaerophilic bacteria, is an important human pathogen, and gas-packs which allow the generation of the proper atmosphere in jars were made commercially available.

When *H. pylori* was first isolated, it was described as ‘*Campylobacter*-like organism’ (CLO) or ‘gastric *campylobacter*-like organism’ (GCLO). It was subsequently named *Campylobacter pyloridis* (Marshall et al, 1984) which was then changed to *C. pylori* after a correction to the Latin grammar (Marshall and Goodwin, 1987). The name *pylori* comes from the Greek word *pylorus*, which means gatekeeper and refers to the pyloric valve. However, after DNA sequencing and other data showed that the bacterium did not belong in the *Campylobacter* genus and it was placed in the new genus, *Helicobacter* and renamed *Helicobacter pylori* (Goodwin et al, 1989).

2.2. EPIDEMIOLOGY

2.2.1. PREVALENCE OF INFECTION

Approximately 50% of the world population is infected with *H. pylori* making it most widespread infection in the world. The risk factors described for acquiring infection
include residence in a developing country, poor socio-economic conditions, overcrowding, ethnic and genetic predisposition (Gold et al, 2000).

Prevalence of infection is higher in developing countries than that of developed with a prevalence rates ranging from 30% to more than 80%. The highest rates of prevalence are in Eastern Europe, Asia, and many developing countries and developing populations in developed countries (e.g. Native Americans, Immigrants). Overall H. pylori prevalence in the USA is estimated at 30–40%, but it remains much higher (i.e., > 50%) in ethnic groups such as African-Americans and Hispanics (Go, 2002). The seroprevalence rate in lower socioeconomic class in India was found 79% (Graham et al, 1991). In Bangladesh 85% of H.pylori infection rate has been found by Urea breath test (UBT) among children under five years (Sarker et al, 1995) and one pilot study showed seroprevalence of 92% in asymptomatic adults (Ahmed et al, 1997).

The infection is almost always acquired in childhood usually before age 10 years (Giacomo et al, 2002) and if untreated, infection is lifelong. In the developing world, up to 70% of children are infected with H pylori by age 15 years and rates of 80% or more are found in adults. Inadequate sanitation practices, low social class and crowded or high-density living conditions seem to be related to a higher prevalence of infection. By contrast, in developed countries infection in children is rare and adult rates are lower about 50% at 50–60 years of age. In the United States, infection is primarily in the older generations about 50% for those over the age of 60 compared with 20% under 40 years. The age association is due mostly to a birth-cohort effects whereby current 60-year-olds
were more commonly colonized as children than current 30-year-olds (Banatvala et al., 1993). Spontaneous acquisition or loss of the bacterium in adulthood is uncommon.

While the prevalence of *H. pylori* infection in developing countries remains relatively constant, it is rapidly declining in the industrialized world (Genta, 2002). The latter is thought to be caused by the reduced chances of childhood infection due to improved hygiene and sanitation and the Spontaneous elimination by widespread use of antimicrobials for treatment of other common infections (Malaty et al., 1999).

### 2.2.2. TRANSMISSION

Exact mode of transmission is yet unknown. Person to person passage of this infection either by the oral-oral route (through vomitus or possibly saliva) or perhaps the faecal-oral route are the most likely modes of transmission. Detection of *H. pylori* from faeces and dental plaque by culture and PCR suggesting a faecal-oral or oral-oral transmission (Go, 2002; Krajden et al., 1988).

The person-to-person transmission is supported by the higher incidence of infection among institutionalized children and adults and the clustering of *H. pylori* infection within families. It seems likely that in industrialized countries direct transmission from person to person by vomitus, saliva or faeces predominates; additional transmission routes, such as water, may be important in developing countries (Parsonnet et al., 1999). Some studies have also indicated a role for a gastro-oral route of transmission by gastroesophageal reflux or regurgitation of stomach contents (Stone, 1999). Iatrogenic
spread through contaminated endoscopes has been documented but can be prevented by using proper disinfection procedures (Fantry et al, 1995).

2.2.3. RESERVOIRS FOR H. pylori

Human is the only known reservoir of the Helicobacter pylori (Logan and Walker, 2001). There is currently no evidence for zoonotic transmission, although H. pylori is found in some nonhuman primates and occasionally in other animals (Handt et al, 1994). Houseflies have been studied as vectors of H. pylori infection. In the laboratory setting they can harbour live H. pylori organisms in their intestines (Grubel et al, 1997) but there is no evidence showing culture of H. pylori from flies exposed to human faeces or from flies in the natural environment. No direct transmission to humans has been demonstrated.

2.3. MICROBIOLOGY OF H.PYLORE

2.3.1 Taxonomy

<table>
<thead>
<tr>
<th>Scientific classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom: Bacteria</td>
</tr>
<tr>
<td>Phylum: Proteobacteria</td>
</tr>
<tr>
<td>Class: Epsilon Proteobacteria</td>
</tr>
<tr>
<td>Order: Campylobacterales</td>
</tr>
<tr>
<td>Family: Helicobacteraceae</td>
</tr>
<tr>
<td>Genus: Helicobacter</td>
</tr>
</tbody>
</table>
Species:  *H. pylori*

**Binomial name**

*Helicobacter pylori*


The genus *Helicobacter* belongs to the subdivision of the *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*. This family also includes the genera *Wolinella, Flexispira, Sulfurimonas, Thiomicrospira*, and *Thiovulum*. The genus *Helicobacter* consists of over 20 recognized species with many species awaiting formal recognition (Fox, 2002). *Helicobacter* spp. Isolated from humans include *H. pylori, H. cinaedi, H. fennelliae, H. heilmannii* (formerly known as *Gastrospirillum hominis*), *H. westmeadii, H. canis, H. Canadensis sp nov., H. pullorum*, and *H. rappini* (formerly known as “*Flexispira rappini*”). Others colonize the intestines of a wide range of animals. Members of the genus *Helicobacter* are all microaerophilic organisms and in most cases are catalase and oxidase positive and many but not all species are urease positive (Marshall and Warren, 1984).

*Helicobacter pylori* was originally classified in the genus *Campylobacter* as the organism resembled in several respects, including curved morphology, growth on rich media under microaerophilic conditions, failure to ferment glucose, sensitivity to metronidazole, and a G + C content of 34%. It was therefore first referred to as *Campylobacter pylori*. But it differs from true *Campylobacter*. Early electron micrographs showed multiple sheathed flagella at one pole of the bacterium, in contrast to the single bipolar unsheathed flagellum typical of *Campylobacter* spp. Major protein bands and fatty acids were also
markedly different. Like *C.jejuni* and *C.coli*, *H.pylori* has 19-carbon cyclopropane acid (19:0cyc) as one of major fatty acids. But it does not possess the methyl-substituted menaquinones present in campylobacter species (Goodwin *et al*, 1986). Subsequent analysis of 16S rRNA sequence also showed that the distance from those of *Campylobacter* species was sufficient to exclude it from the *Campylobacter* genus. It is now phylogenetically closer to *Wolinella* than to *Campylobacter* and should be placed in a new genus *Helicobacter* (Goodwin *et al*,1989) and now named *Helicobacter pylori*.

2.3.2. Morphology

*H. pylori* is a gram-negative bacterium measuring 2 to 4 µm in length and 0.5 to 1 µm in width. Although usually spiral-shaped, the bacterium can appear as a rod while coccoid shapes appear after prolonged in vitro culture or antibiotic treatment (Kusters *et al*, 1997). These coccoids cannot be cultured in vitro and are thought to represent dead cells although it has been suggested that coccoid forms may represent a viable, nonculturable state (Enroth *et al*, 1999).The organism has 2 to 6 unipolar, sheathed flagella of approximately 3 µm in length, which often carries a distinctive bulb at the end. The flagella confer motility and allow rapid movement in viscous solutions such as the mucus layer overlying the gastric epithelial cells (O’Toole *et al*, 2002).

2.3.3. Cell envelope, outer membrane and LPS

The overall composition of the cell envelope of *H. pylori* is similar to that of other gram-negative bacteria. It consists of an inner cytoplasmic membrane, periplasmic space with peptidoglycan and an outer membrane. The outer membrane consists of phospholipids and LPS. The outer membrane phospholipid moiety contains cholesterol glucosides
(Bukholm et al, 1997) which is very rare in bacteria. The lipidA moiety of *H. pylori* LPS has low biological activity compared to lipid A from other bacteria (Muotiala et al, 1992). The periplasmic space is filled with electron-dense granular bodies (Bode et al, 1993). These bodies are known to be aggregates of polyphosphate and may serve as a reserve energy source. A peptidoglycan layer was only occasionally visible. A thick, very fine filamentous or reticular fringe corresponding to the bacterial glycocalyx was seen surrounding the *H. pylori* cells. At the adhesion loci of *H. pylori* to gastric epithelial cells, *H. pylori* connects to the epithelial cells by very fine, thickly arranged filaments or more closely with a contact zone. The epithelial cells show indentations or pedestals. The well-developed, thick bacterial glycocalyx appears to strongly interact with external cellular components and may play an important role in the adhesion of *H. pylori* to epithelial cells.

### 2.3.4 Cultural characteristics

Successful growth of *H pylori* depends on a combination of factors --- such as rapid processing of specimen, use of fresh media and special incubation environment. The organism is best cultured in a microaerophilic and humid atmosphere (usually 5% O₂, 10%-15% CO₂, 80%-85% N₂, and 70%-100% humidity). High humidity is essential for growth, so plates should not be dried off after pouring. *H.pylori* requires supplemented media. Supplements are blood, haemin, serum, starch or charcoal. For the routine isolation and culture of *H. pylori*, non selective media such as chocolate agar, Columbia or brucella agar supplemented with either (lysed) horse or sheep blood have been useful. Selective media such as Skirrow’s media consists of vancomycin, trimethoprim,
polymyxin B, and amphotericin B also support the growth. Liquid media usually consist of either of brucella, Mueller-Hinton or brain heart infusion broth supplemented with 2 to 10% calf serum also suitable. Plates should be incubated in a humidified, microaerophilic atmosphere at 37°C for 3-7 days. *H. pylori* forms small (∼1-mm), translucent, smooth colonies (Han *et al.*, 1995). All strains grow at 37°C, some grow poorly at 30°C and 42°C but none grows at 25°C (Owen, 1995). They are slightly hemolytic on blood agar. Motility is best demonstrated in broth culture.

### 2.3.5. Biochemical Characteristics

*H. pylori* does not react in most conventional biochemical test. However it is oxidase and catalase positive. All *H. pylori* strains appear to produces urease, alkaline phosphatase, DNAase, leucine aminopeptidase and gamma-glutamyl-aminopeptidase. The extremely powerful urease (100 times more active than that of Proteus vulgaris) is a highly characteristic feature of the organism. A recent crystal structure study demonstrated that the *H. pylori* urease enzyme has a unique supramolecular structure that may be important for survival in acidic conditions (Ha *et al.*, 2001). It is biochemically different from the other common urease producing bacteria and much more active (Mobley *et al.*, 1988).

Urease is a nickel-containing enzyme that consists of 12 UreA and 12 UreB subunits. The UreA and UreB subunits have molecular masses of 27 kDa and 62 kDa (Dunn *et al.*, 1990) respectively and the subunits are encoded by an operon containing the ureA and ureB genes (Labigne *et al.*, 1991). In the presence of urea it enables *H. pylori* to tolerate pH values down to 2.6 because the production of ammonia raises the pH in the immediate vicinity of the bacterial cell. The importance of this action for colonization of the
stomach is obvious and the urease of *H.pylori* to be an important virulence factor for the development of gastritis. The enzyme is strongly immunogenic and is probably the best antigen for the serodiagnosis of *H.pylori* infection (Dent *et al*, 1988).

### 2.3.6. Habitat

#### 2.3.6.1 Human sources

The mucous layer of the gastric epithelium of the human stomach particularly the antrum, is the principal known habitat of *H.pylori*. Almost all isolations are from gastric biopsy specimens but *H.pylori* has occasionally been cultured from saliva and dental plaque (Krajden *et al*, 1989). Thomas *et al* (1992) successfully isolated *H.pylori* from stools of Gambian children. However other investigators concluded that there was no substantial shedding of *H.pylori* in faeces (Van Zwet *et al*, 1994).

#### 2.3.6.2 Other sources

*H.pylori* has been isolated occasionally from different animal species, including rhesus monkey, pig, baboon and domestic cats (Curry *et al*, 1987).

### 2.3.7. Virulence Factors

#### 2.3.7.1 Flagella

*H. pylori* possesses multiple flagella at one end which make it motile (Blaser, 1992). Sheathed flagella combined with spiral morphology allow it to burrow and live deep beneath the mucus layer closely adherent to the epithelial surface (Lee *et al*, 1995).

#### 2.3.7.2 Urease

*H.pylori* produces large amount of urease enzyme. This enzyme metabolizes urea (which is normally secreted into the stomach) to carbon dioxide and ammonia which neutralizes gastric acid and essential for gastric colonization (Lee *et al*, 1993). The ammonia that is
produced is toxic to the epithelial cells. It has been suggested that ammonia or ammonium containing substances abundantly produced as a result of \textit{H. pylori} urease activity may act as a cancer promoters enhancing rates of cell division (Tsujii \textit{et al}, 1992).

\textbf{2.3.7.3 Phospholipases & proteases}

Breaks down the glycoprotein lipid complex of the mucus gel, thus reducing the efficacy of this first line defence.

\textbf{2.3.7.4 Adhesins and outer membrane proteins}

The \textit{H. pylori} genome encodes a large array of outer membrane proteins. The 78-kDa BabA protein probably represents the best-characterized \textit{H. pylori} adhesion protein; it is encoded by the \textit{babA} gene. BabA mediates binding to fucosylated Lewis B blood-group antigen on gastric epithelial cells (Ilver \textit{et al}, 1998). There are two distinct \textit{babA} alleles, \textit{babA1} and \textit{babA2}. The product of \textit{babA1} is identical to \textit{babA2} but cannot interact with Lewis. \textit{H. pylori babA2+}strains are associated with an increased risk of peptic ulcers and distal gastric adenocarcinoma, whereas \textit{babA2} strains are more often associated with uncomplicated forms of gastritis (Hocker and Hohenberger, 2003). Several other members of the outer membrane protein family also mediate adhesion to epithelial cells.

\textbf{2.3.7.5 Cytotoxin-associated geneA (cagA) and its related pathogenicity island (cag PAI)}

A specific region of the bacterial genome, the pathogenicity island, encodes the highly immunogenic protein CagA (Covacci \textit{et al}, 1993). This gene is present in approximately
50 to 70% of *H. pylori* strains (Ching *et al*, 1996). Some of the genes in the cagPAI region encode a type IV bacterial secretion apparatus which can translocate immunoreactive 120-145 kDa protein cagA into host target cells. Phosphorylation of cagA may activate host signaling pathways and subsequently influence host cellular functions, including proliferation, apoptosis, cytokine release, and cell motility (Suerbaum *et al*, 2002). Individuals infected with cagA+ strains are more likely to have gastroduodenal ulceration than those who are cagA−.

2.3.7.6 **vacuolating cytotoxinA (vacA)**

Most of the *H. pylori* strains produce vacA, which induces epithelial cell vacuolation and cell death (Israel *et al*, 2001). Several forms of VacA exist. VacA expression is determined by variations in the signal sequence (s1a, s1b, s1c, s2) and mid-region (m1, m2) of the vacA gene and pathology is most strongly associated with s1a/m1 strains. Infection with s1a/m1 strains is associated with intense inflammation and duodenal ulceration (Chan *et al*, 2002). Tripositive strains which have cagA+, vac A1, babA2+ in single *H. pylori* species, further increase the risk of developing gastroduodenal ulcers and distal gastric cancer (Prinz *et al*, 2001).

2.3.7.7 **LPS**

Although lipopolysaccharides (LPS) of gram negative bacteria often plays an important role in the infection. *H. pylori* LPS has low immunogenic activity compared to other organisms.
2.4. PATHOPHYSIOLOGY OF \textit{H. pylori} INFECTION

\textit{H. pylori} colonization induces chronic superficial gastritis, which includes both mononuclear and polymorphonuclear cell infiltration of the mucosa. The pattern of gastric inflammation is associated with disease risk: antral-predominant gastritis is closely linked with duodenal ulceration whereas pangastritis is linked with gastric ulcer and adenocarcinoma. Infection in infancy is thought to lead pangastritis (low acid secretion status), whereas acquisition in latter childhood may lead to a predominantly antral gastritis (high acid load) only (Poddar and Thapa, 2000).

Clinical outcome of \textit{H. pylori} infection (gastritis, PUD, gastric MALT lymphoma, gastric carcinoma) is determined by a complex interplay between bacterial and host factors.

2.4.1 Bacterial factors

Among microbial virulence factors identified so far, the \textit{H. pylori} cytotoxin-associated geneA (cagA), its related pathogenicity island (cag PAI), vacuolating toxinA (vacA) and factors involved in adherence of \textit{H. pylori} to gastric epithelial cells, have been linked to enhanced pathogenicity of the bacterium. These virulence factors, in conjunction with additional bacterial constituents can cause mucosal damage. Urease which allows the bacteria to reside in acidic stomach generates NH$_3$ which can damage epithelial cells (Mobley \textit{et al}, 2001). The bacteria produce surface factors that are chemotactic for neutrophil and monocytes which in turn contribute to epithelial cell injury. \textit{H pylori} makes Phospholipases & proteases that breaks down the glycoprotein lipid complex of the mucus gel, thus reducing the efficacy of this first line defence. Although
lipopolysaccharides (LPS) of gram negative bacteria often plays an important role in the infection. *H.pylori* LPS has low immunogenic activity compared to other organisms.

### 2.4.2 Host factors

Host genetic factors might affect *H. pylori* colonization and development of diseases. Genetic polymorphism of the cytokines and other related legends, receptors and enzymes might influence *H. pylori* infection. The associations with the polymorphism of Interleukin 1 α (IL-1α), IL-1β, IL-IRN, IL-8, IL-10, and tumor necrosis factor α (TNF-α) and TNF-β have been reported. Polymorphism of other related genes, CD14, chemokine receptor 2 (CXC-R2), nuclear factor κB (NF-κB) and Toll like receptor4 (TLR4) are potential influencing factors of *H. pylori* infection (Hamajima *et al*, 2003). TNF-α and Interlukin-1β are pro-inflammatory cytokine as well as a powerful inhibitor of gastric acid secretion (Beales *et al*, 1998). Host genetic factors that affecting these cytokines may determine why some individuals infected with *H. pylori* develop gastric ulcers or cancers while others do not.

The pathogenesis of duodenal ulceration is becoming clearer. *H.pylori* causes antral gastritis with depletion of somatostatin (from D cells). Since somatostatin inhibits gastrin (from G cells) release, gastrin level are higher. The subsequent hypergastrinaemia stimulates acid production by parietal cells. Duodenal acid load leads to protective gastric metaplasia in the duodenum, the duodenum become colonized by *H.pylori* leading to duodenitis and eventually duodenal ulcer (Savarion *et al*, 1997). Patients with duodenal ulcer have impaired bicarbonate secretion in the proximal duodenum in response to
acidification of the duodenum. Unlike other pathophysiological defect, this impairment seems to be especially specific to duodenal ulcer patients and eradication of *H. pylori* will return duodenal bicarbonate secretion to normal (Hogan *et al*, 1996).

The pathogenesis of gastricl ulceration is less well understood. *H. pylori*-associated gastric ulcers usually occur at the junction of antrum and corpus type mucosa. *H.pylori* causes a pangastritis leading to gastric atrophy and hypochlorhydria. This allows bacteria to proliferate within the stomach, these may produce mutagenic nitrates from dietary nitrates predisposing to the development of gastric cancer.

In summary, the final effect of *H.pylori* on the gastrointestinal tract is variable and determined by microbial and host factors. The type and distribution of gastritis correlate with the ultimate gastric and duodenal pathology observed. Specifically the presence of antral-predominant gastritis is associated with DU formation. Gatritis involving primarily the corpus predisposes to the development of gastric atrophy and ultimately gastric carcinoma.

2.4.3. EFFECTS ON GASTRIC PHYSIOLOGY

In addition to producing local injury of gastric mucosa, *H. pylori alter* normal gastric secretion. Interestingly the location and severity of the infection seem closely associated with the ultimate clinical outcome, most likely because of effects on gastric physiology. Many studies have shown that patients with a duodenal ulcer who are infected with *H. pylori* have an increased serum level of gastrin, which in turn leads to increased acid
output (Peterson et al, 1993). These patients tend to have a milder phenotypic expression of their gastritis, with inflammation mostly in the antrum or distal part of the stomach (Schultze et al, 1998). In contrast patients with gastric adenocarcinoma, a known complication of *H. pylori* infection, tend to have pangastritis, with involvement of the acid-secreting body of the stomach as well as the antrum. This condition leads to atrophy of parietal cells (which are responsible for producing acid) and gastrin-producing cells of the antrum (which stimulate acid secretion) and eventually produces achlorhydria.

### 2.5. IMMUNE RESPONSE

The immune response to *H. pylori* includes both the production of antibody (local and systemic) and a cell mediated response. *Helicobacter pylori* infection, once acquired, persists indefinitely. Although there is a definite and immediate immune response to *H. pylori*, but it is ineffective in clearing the bacterium. *H. pylori* survives in the host and causes chronic inflammation by altering signaling pathways, down regulating inflammation, and dysregulating host immune responses (Ceponis et al, 2003).

#### 2.5.1 Humoral Immune Response:

It was initially assumed that a protective immune response against *H. pylori* would predominantly be mediated by antibodies. Subsequent experiments have indicated that the relevance of the humoral system for protective immunity is only marginal. Antibodies can effectively prevent infection and reduce colonization in animal models (Marnila et al, 2003).
*H. pylori* infection results in an induction of a Th1-polarized response that does not result, however, in clearance of the infection (Lindholm *et al*, 1998). This is striking, as it is the cellular rather than the humoral immunity that has been reported to play the principal role in sterilizing immunity. It is now generally accepted that the development of *H. pylori*-induced gastritis depends predominantly on Th1 cells and Th1 cytokines (Smythies *et al*, 2000). Although a Th2-polarized response protects against this specific pathology, this does not necessarily imply that Th2 cells are responsible for protection after immunization. In fact, Th1-polarized rather than Th2-polarized, T cells recruit mononuclear cells to the site of infection, resulting in elimination of the bacteria (Akhiani *et al*, 2002).

The presence of increased numbers of B-lymphocytes and plasma cells in the gastric mucosa is evidence of an active humoral immune response in chronic *H. pylori* infection. Sampling of gastric secretions from *H. pylori*-infected individuals also reveals an active mucosal antibody response, primarily of the IgA isotype. Secretory IgA anti-*H. pylori* antibodies are found also in saliva.

Most patients infected with *H. pylori* produce an easily identified systemic humoral immune response, composed primarily of IgG. Serum IgA may be detected in fewer than half (range, 39%-82%) of infected patients, and serum IgM is found rarely. These findings are consistent with a chronic infection usually acquired in early childhood. In naturally acquired infection, an initial serum IgM response is observed and seroconversion from IgM to IgG is demonstrated between 22 and 33 days after infection.
Circulating anti-\textit{H. pylori} IgG persist at a constant level for years during infection (Tummala \textit{et al}, 2004).

\subsection*{2.5.2 Immune modulation}

\textit{H. pylori} infection always results in a strong immune response of the host against the infecting strain, but this response seldom results in clearance of the infection. It can even be argued that much of the pathology associated with \textit{H. pylori} infection results from the activities of the host's immune system rather than from direct bacterial activity (Kusters \textit{et al}, 2006). The intensity of the host immune responses can culminate in one of several ways

1. The most common result is chronic superficial gastritis, which is an inflammation of the stomach lining due to the infiltration of lymphocytes, plasma cells, eosinophils, and monocytes into the mucosal lining of the stomach, which causes injury to the gastric glands.

2. The immune response can actually benefit \textit{H. pylori} by releasing nutrients locally for the organism.

3. The host could be harmed by the immune response due to the direct damage of epithelial cells, which affects their function and vitality. The host, in order to avoid this type of cell damage, will often down-regulate its immune response, making it even more difficult to completely eliminate \textit{H. pylori} from the affected area.
4. The immune response can also cause inflammation of the duodenum, leading to duodenal ulcers.

5. Atrophic gastritis, which is a nonspecific inflammation of the entire lining of the stomach, may be the result of the infiltration of lymphocytes into the stomach.

6 MALT-type and other lymphomas, which are tumors of the mucosal and lymphoid tissues, can also result from \textit{H. pylori} infection.

The effects of infection by \textit{Helicobacter pylori} represent a delicate equilibrium between the host's inability to remove the organism and its ability to contain the damage caused by the pathogen. It is the integrity of this equilibrium that allows \textit{H. pylori} to persist in most cases for a lifetime in their hosts. \textit{H. pylori} is thought to downregulate inflammation and control the host's immune response through a wide range of virulence factors that are involved in both provoking and maintaining a proinflammatory immune response.

\textbf{2.5.3 Cell-mediated Immune Responses in \textit{H. pylori} Gastritis}

\textit{H. pylori} infection stimulates the gastric epithelium to secrete a multitude of cytokines that initiate and direct the inflammatory reaction within the gastric mucosa. Chemokines such as IL-8, leukotrienes, and complement activation products represent potent chemo attractants for neutrophils and lymphocytes. Since it is now known that Lewis antigens are involved in adhesion and colonization, the increased adherence may lead to an increased bacterial burden and leads to the activation of the nuclear factor-\(\kappa\)B (NF-\(\kappa\)B) and host signal transduction pathways. IL-8 production is enhanced and results in the
characteristic neutrophil infiltration and inflammation of *H. pylori* gastritis. *H. pylori* induces also IL-12 which in turn elicits interferon- gamma (INF\(\gamma\)) production.

*H. pylori* infection results in a Th1-predominant host immune response in the gastric mucosa, characterized by the induction of interferon IFN\(\gamma\) and IFN\(\gamma\)-regulated genes. Differentiation of naive T cells into activated Th1 cells requires the presence of IL-12, which is predominantly produced by mononuclear cells. The presence of *H. pylori* in the gastric mucosa is associated with strong IL-12 production (Karttunen *et al*, 1997) and the presence of large numbers of Th1 cells (Meyer *et al*, 2000). A Th1-predominant immune response is associated with elevated levels of the proinflammatory cytokines IL-12, IL-18, and TNF\(\alpha\). The severity of gastritis associated with *H. pylori* infection was correlated with mucosal expression of the TNF\(\alpha\) subunit CD68 and IFN (Lehmann *et al*, 2002). The Th1 response is a normal adaptive immune response, however, when this response is too strong it will produce a severe chronic inflammation. Th1 response has also been associated with progression to atrophic gastritis and gastric cancer (Houghton *et al*, 2002).

2.5.4 Regulatory T cells

IL-10-producing T cells seem to be crucial in the control of *H. pylori*-induced inflammation and enable the bacterium to persist in the gastric mucosa. For example, *H. pylori* is unable to persist in IL-10-deficient mice suggesting that it is an important regulator of the mucosal immune response. (Chen *et al*, 2001; Ismail *et al*, 2003). CD25\(^+\) regulatory T cells probably play a key role in this, as mice lacking CD25\(^+\) cells developed more severe gastritis while having reduced bacterial loads in the gastric mucosa (Raghavan *et al*, 2003). Similarly, removal of CD25\(^+\) cells from *H. pylori*-positive
volunteers resulted in increased in vitro proliferation and gamma interferon production, indicating that *H. pylori* infection results in downregulation of the immune response through interaction with regulatory T cells (Stromberg *et al.*, 2003).

### 2.6. CLINICAL ASSOCIATIONS

#### 2.6.1 *Helicobacter pylori*–Associated Conditions

**Association is accepted**
- Gastric adenocarcinoma
- Gastric mucosa–associated lymphoid tissue lymphoma (MALT)
- Gastritis
- Peptic ulcer disease

**Association is controversial**
- Cardiovascular disease
- Gastroesophageal reflux disease (GERD)
- Iron deficiency anemia
- Nonulcer dyspepsia

#### 2.6.2 Gastritis and Gastric Cancer

Colonization of the gastric mucosa by *H. pylori* first results in the induction of an inflammatory response. The initial acute gastritis is followed by active chronic gastritis, which lasts for life if the infection is not treated (*Kuipers et al.*, 1995). Most infected persons remain asymptomatic. Some infected persons may even clear the infection, with seroreversion rates in the range of 5% to 10%. It is not known if this seroreversion is spontaneous or results from elimination of the organism by antibiotic agents used to treat other conditions (*Everhart et al.*, 2000).
The typical course of disease in infected patients begins with chronic superficial gastritis, eventually progressing to atrophic gastritis. This progression appears to be a key event in the cellular cascade that results in the development of gastric carcinoma. Existing data indicate a 90-fold increase in rates of gastric carcinoma in patients with severe, multifocal atrophic gastritis, compared with normal controls (Sipponen et al., 1985). The mechanism of tumorigenesis appears to involve DNA damage induced by different cytokines and free radicals released in the setting of chronic inflammation in susceptible persons (Scheiman and Cutler, 1999). Although *H. pylori* is associated with the development of adenocarcinoma of the antrum and body of the stomach, it is also linked with gastric mucosa–associated lymphoid tissue (MALT) lymphomas (Parsonnet and Hansen, 1994). *H. pylori* stimulate lymphocytic infiltration of the mucosal stroma. This infiltration may act as a focus for cellular alteration and proliferation, ultimately resulting in neoplastic
transformation to lymphoma (Zucca et al, 1998).

Fig. Model representing the role of *H. pylori* and other factors in gastric carcinogenesis, based on the cascade proposed by Corea, 1988

It appears that *H. pylori* also produce proteins that stimulate growth of lymphocytes in the early stages of neoplasia. It has been reported that regression of low-grade gastric MALT lymphoma can be achieved 70% to 90% with eradication of *H. pylori* infection (Morgner et al, 2000).

2.6.3 Peptic Ulcer Disease

The relationship between *H. pylori* infection and peptic ulcer disease has been studied exhaustively and it is now accepted that the organism is the major cause but not the only
cause of peptic ulcer disease worldwide. Eradicating the infection can alter the natural course of peptic ulcer disease by dramatically reducing its recurrence rate in treated patients compared with untreated patients (Vander et al, 1997). This reduction occurs in patients with duodenal and gastric ulcers who have no history of nonsteroidal anti-inflammatory drug use.

The mechanism by which *H. pylori* induces peptic ulcer disease is incompletely understood but most likely involves a combination of genetic predisposition of the host, virulence factors of the organism (e.g., VacA and CagA proteins), mechanical damage to the mucosa, and alterations of gastric and duodenal secretions (Cohen, 2000).

2.6.4 Non-ulcer Dyspepsia

Nonulcer dyspepsia comprises a variety of symptoms, including dysmotility-like, ulcer-like and reflux-like symptoms. Many possible causes have been suggested for nonulcer dyspepsia, including lifestyle factors, stress, altered visceral sensation, increased serotonin sensitivity, alterations in gastric acid secretion and gastric emptying, and *H. pylori* infection. A recent review also highlighted the role played by psychosocial impairment (e.g. depression, somatization, anxiety) in patients with nonulcer dyspepsia (Olden and Drossman, 2000). In a study, linking *H. pylori* infection to nonulcer dyspepsia, patients with the latter condition were twice as likely to be positive for the organism (Armstrong, 1996). However, despite such epidemiologic evidence, treatment studies have failed to consistently show that eradication of *H. pylori* results in improvement of nonulcer dyspepsia symptoms (Talley et al, 1999). Consequently,
eradication of the organism cannot be considered the standard of care in all patients with nonulcer dyspepsia, because *H. pylori* infection is only a single part of the multifactorial etiology of the disease.

### 2.6.5 Gastroesophageal Reflux Disease (GERD)

Much attention has been focused on the possible relationship between infection with *H. pylori* and gastroesophageal reflux disease (GERD) in its various manifestations (e.g. esophagitis, Barrett’s esophagus). The incidence of esophagitis may, in fact, increase after eradication of the organism (Labenz *et al.*, 1997.) Treatment of *H. pylori* infection can lead to exacerbation of GERD in many patients. Conversely, other studies using endoscopic findings, pH probe measurements, and histology to determine the presence of *H. pylori* did not find any association between GERD (in any of its manifestations) and infection with *H. pylori* (Oberg *et al.*, 1999; Gisbert *et al.*, 2001). However, more definitive studies are necessary to define the relationship.

### 2.6.6 Other Disease Associations

Some Investigators have further postulated a relationship between *H. pylori* infection and cardiovascular disease (Ameriso *et al.*, 2001) and Iron deficiency anemia (Annibale *et al.*, 1999). These associations however, require much more study before a causal relationships is established.

### 2.7. Diagnosis of *H. pylori* Infection
The NIH (National Institutes of Health) Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease recommends that, rather than instituting empirical therapy for this infection, the diagnosis should be established before therapy is started. Symptoms are not adequate to establish a diagnosis of either peptic ulcer disease or *H pylori* infection. When gastric or duodenal ulcer is found in a patient without a history of nonsteroidal anti-inflammatory drug use, an effort should be made to document *H pylori* infection (Anthony *et al*, 1996).

There are two general ways in which a diagnosis of infection by *Helicobacter pylori* can be made: by using either an invasive or non-invasive procedure. The invasive procedures involve an endoscopy and biopsy. Histologic evaluation, culture, polymerase chain reaction (PCR), and rapid urease tests are typically performed on tissue obtained at endoscopy. The non-invasive procedures that can be used to diagnose the infection are serology, detection of labelled metabolic products of urea hydrolysis in the breath (*13C* or *14C* urea breath test), detection of *H. pylori* antigen in a stool specimen and trials investigating PCR amplification of saliva, feces, and dental plaque to detect the presence of *H. pylori* are ongoing (Bravos and Gilman, 2000).

All of the available diagnostic techniques have shown an acceptable reliability, at least when used in a dedicated setting. However, it has become increasingly clear that no single test is optimal (Mégraud *et al*, 1997) and only a combination of different techniques provides an adequate diagnostic yield (Maastricht Consensus report, 1997). The choice of the test to be used is not straightforward and may vary according to the clinical condition and local expertise.
2.7.1 INVASIVE PROCEDURES

The detection of *H. pylori* infection in biopsy tissue samples has been based on histology, culture or rapid urease test which are widely available and employed worldwide. Other recently developed diagnostic methods—for example, molecular analysis employing PCR—are still restricted to a research setting. There has been a suggestion that there are some factors capable of affecting the performance of all biopsy-based tests, such as site, number and dimension of biopsies or a previous recent eradication attempt, which can significantly decrease the diagnostic accuracy. Therefore, once the appropriate test has been chosen for the clinical setting, care must be taken to optimize its diagnostic accuracy.

2.7.1.1 Histology

Histological examination of tissue biopsy samples (usually four, taken from different parts of the stomach lining) permits detection of the bacterium together with evaluation of tissue damage. It has traditionally been the gold-standard method for diagnosing *H. pylori* infection. The organism can be identified on a routine hematoxylin-eosin stain. The histological stain originally used to detect *H. pylori* was the Warthin-Starry silver stain, but nowadays a more commonly used stain is the Giemsa stain which compared with culture showed a sensitivity of about 90% (Laine *et al.*, 1997). Other stains that have recently been proposed include the Genta's stain, which has the advantage of obtaining both an excellent visualization of *H. pylori* and the histological appearance of the tissue (El-Zimaity *et al.*, 1999) and the alcian yellow-toluidine blue stain which has comparable sensitivity to the Giemsa stain, is cheap, technically easy and shows high contrast...
(Vartanian et al, 1998) The specificity of histology can be increased by immunochemical staining using monoclonal antibodies although these stains are not used routinely (Negrini et al, 1989).

The disadvantage of this technique is the need for endoscopy to obtain tissue. Limitations also arise at times since *H pylori* is not evenly distributed throughout the stomach, biopsies can miss the site of infection (Hardin and Wright, 2002).

Histological detection of the organism generally been considered to have lower sensitivity at 80 - 95% with 100% specificity. The need for a number of biopsy specimens to be examined by experienced pathologists renders histology expensive.

2.7.1.2 Culture

*H. pylori* soon loses viability when exposed to the environment and biopsies should be cultured quickly to maximize the recovery of bacteria. The organism is recoverable from biopsy material stored in Stuart's transport medium for 24 h at 4°C but above 15°C viability is poor even after 6 hours (Soltesz et al, 1992). Since the organism is fastidious and difficult to grow in the laboratory, the role of culture in diagnosis of the infection is limited mostly to research and epidemiologic considerations. Although costly, time consuming and labor intensive, culture does have a role in antibiotic susceptibility studies and studies of growth factors and metabolism.

Several selective and differential media have been proposed (Holton, 1997) and the media are usually, but not exclusively, based upon either Columbia or Brain Heart Infusion agar base containing either blood or blood products or an additive such as starch,
charcoal, cyclodextrin or bovine serum albumin. Selectivity is provided by the addition of different combinations of antibiotics.

In addition to culturing Helicobacter from the gastric biopsy the organism has also been isolated from faeces and rarely dental plaque. This is a topic that requires further study because of the ease of collecting these specimens and their potential use in diagnosis.

Culture is 100% specific in diagnosing H. pylori infection and can be regarded as being no more than 60 - 90% sensitive. Unfortunately, it is not so for sensitivity which can vary widely between centres due to local expertise (Lerang et al, 1998).

2.7.1.3 Sensitivity testing of H. pylori

The culture of H. pylori for sensitivity testing is clinically relevant for two main reasons. Firstly, as a guide to treatment for an individual patient and secondly, to assess trends in antibiotic resistance, which is important when choosing empirical therapy. Although H. pylori is apparently sensitive to a wide range of antibiotics there is a lack of correlation between *in vitro* sensitivity and therapeutic outcome because of ‘pharmacological’ resistance, i.e. inactivation of the antibiotic by acid in the stomach or failure to achieve concentrations of the antibiotic at the site of infection. Similarly, treatment failure can occur because the strain is resistant to the antibiotic. The failure to eradicate H. pylori is associated with a high relapse rate for peptic ulcer disease, whereas successful eradication ensures a low relapse rate of disease. It is therefore clinically important to know the local antibiotic susceptibility and their trends, to be able to choose the most appropriate regimen. Clearly in an area where the prevalence of antibiotic resistance is
high it is more important to determine the antibiotic sensitivity of the patient's isolate compared with an area where the prevalence of antibiotic resistance is low. The antibiotics that are commonly used to treat *H. pylori* are tetracycline, amoxycillin, metronidazole and clarithromycin. Significant resistance and subsequent treatment failure has been reported for the latter two antibiotics (Vaira *et al*, 2000).

### 2.7.1.4 Rapid urease test (RUT)

Rapid urease testing takes advantage of the fact that *H. pylori* is a urease-producing organism. Gastric biopsies are obtained and placed into urea-containing medium or on a reaction strip containing urea, buffer, and a pH-sensitive indicator. If urease is present, urea is metabolized to ammonia and bicarbonate leading to an increase in pH of the medium and a subsequent color change in the pH-dependent indicator phenol red. Results are available in 1 to 24 hours.

Commercially available rapid urease test kits include the HUT-test, CLOtest, HpFast and PyloriTek. Their overall pretreatment sensitivity is 93% to 97% and their specificities greater than 95% (Midolo and Marshall, 2000). Though the different tests are comparable in their overall performance, they have some practical differences. For example, PyloriTek yields a positive result more quickly than two of the agar gel-based tests, CLOtest and HpFast (Laine *et al*, 1996).

More recent modifications of the rapid urease test using a chemiluminescent pH indicator demonstrate a more rapid result and a 50-fold higher sensitivity compared with the routine tests (Roda *et al*, 1998). The rapid urease test can also be performed per-
endoscopically with the use of a pH sensitive biosensor with a sensitivity and specificity of 92% and 95%, respectively and this test takes one minute to perform (Sato et al, 1999).

Medications that decrease the density and/or urease activity of *H pylori*, such as bismuth containing compounds, antibiotics, and proton pump inhibitors, can reduce the sensitivity of the rapid urease test by as much as 25%. Thus false negative urease tests may occur and are mainly due to low numbers of organisms in the biopsy which typically follow treatment (Tokunaga et al, 1998). Despite these potential limitations, the ease of use, relatively low cost and rapid reaction time of the rapid urease test make it a practical and cost-effective means of diagnosing *H pylori* infection if endoscopy is necessary.

### 2.7.1.5 Molecular based techniques

*Helicobacter* can be detected by *in situ* hybridization using biotinylated probes, with a reported sensitivity and specificity of about 95% and 100% respectively. However, the technique is difficult and it is not routinely used (Park and Kim, 1999).

*Helicobacter* is more usually detected using the polymerase chain reaction (PCR) which has been used to detect *H. pylori* in gastric biopsies, gastric juice, dental plaque and faeces (Li et al, 1996). Depending on the primer used, the method has a reported sensitivity and specificity. PCR has been used diagnostically (Gzyl et al, 1999) and as a method of typing (Ge and Taylor, 1998) either from cultures or directly from a specimen. It is more technically complex to perform than the rapid urease test, histology or culture and may offer only a marginal advantage in terms of sensitivity if used on gastric
biopsies. However, it is perhaps more useful where there are likely to be small numbers of organisms.

Several other DNA enzyme immunoassays have been developed to detect *H. pylori*; however, their clinical role is not yet well defined and their use is still restricted to the research laboratory.

It can also be used to identify different strains of bacteria for pathogenic and epidemiologic studies. Additional information that may be clinically relevant can be obtained by determining the strain of *Helicobacter* that is colonizing the patient. *Helicobacter* can broadly be divided into Type I and Type II strains. Type I strains phenotypically expresses both the *cagA* phenotype and produces the *vacA* vacuolating cytotoxin. The Type II strains, although genotypically *vacA* positive, lack the *cagA* genotype and are phenotypically *cagA and vacA* negative. Type I strains have been shown to be more virulent, i.e. more likely to be associated with gastro-duodenal ulceration and gastric cancer than the Type II strains in some studies (Gunn *et al*, 1998) but not in others (Graham *et al*, 1996). However, now a days the clinical role of knowing the cagA/vacA status of the patients is still unclear.

Point mutations have been linked to phenotypic antibiotic resistance in *H. pylori* and a further possible use for nucleic amplification methods is in the detection of drug resistant bacteria. Clarithromycin is used as part of an effective short course eradication regimen and the prevalence of resistance ranges from 3 to 10% depending upon the country of origin. Resistance is caused by post-transcriptional adenylation and point mutations (A-G transitions) in a conserved loop of 23SrRNA. As the specific mutations linked to
resistance are known, the mutant sequences can be detected by standard PCR methods using specific primers of the ‘mutant’ sequences.

2.7.2 NONINVASIVE PROCEDURES

Endoscopy is expensive, unpleasant to patients and carries a small but definite risk of complications; non-endoscopic tests to assess \textit{H. pylori} infection have therefore been welcomed.

In addition to their defined role in epidemiological studies, it appears that non-invasive procedures are probably the best option to monitor eradication in most clinical settings with the possible exclusion of patients with gastric or complicated ulcers who may require control endoscopy.

Several studies have investigated the possibility of pre-endoscopic non-invasive screening to reduce the endoscopic workload without affecting the safety of patients. However, no generally accepted recommendations are available and these screenings should be restricted to a research setting (Vaira \textit{et al}, 1997).

The more widely available non-invasive Procedures for diagnosing \textit{H. pylori} infection are the urea breath test and serological assays. A recently developed stool assay has been shown to be a reliable diagnostic tool and is becoming increasingly available and employed. Other non-invasive procedures of more limited use are assessment of saliva or urine analysis for anti-\textit{H. pylori} antibody.

2.7.2.1 Urea Breath Test (UBT)
A urea breath test, like the rapid urease test, identifies the active *H. pylori* infection through the organism’s urease activity. In this test, a patient with suspected infection ingests either $^{14}$C labeled urea (radioisotope with a long half-life) or $^{13}$C-labeled urea (stable non-radioactive isotope). Bacterial urease splits off labeled carbon dioxide, which can be detected in the breath.

The $^{14}$C labeled urea breath test consists of the oral administration of $^{14}$C urea, followed by sampling of the exhaled breath at timed intervals. The breath samples are then analyzed in a liquid scintillation counter. Liquid scintillation counter can provide results in less than 20 minutes or the specimen can be sent to Reference laboratories which will provide an analysis to the physician within 24-36 hours. A disadvantage of this technique is that it may involve a small amount of exposure to radiation.

The $^{13}$C-urea breath test is identical to the $^{14}$C-urea breath test except that $^{13}$C is a non-radioactive isotope of $^{12}$C and its detection requires a mass spectrometer rather than a scintillation counter. Because of the expense of a mass-spectrometer its use is restricted to research centres although some companies offer to analyse expired breath samples if sent to them by post. The test has the advantage of being nonradioactive and can safely be used for children and pregnant women. As $^{13}$C is a naturally occurring isotope it is measured as a ratio of $^{12}$C which is the most abundant isotope and it is necessary to compare the value obtained 30 minutes after ingestion of the urea with the baseline of prior ingestion.
Overall, the performance characteristics of both tests are similar with sensitivity greater than 95% and specificity 100% (Gomes et al., 2002). The urea breath test also provides an accurate means of post-treatment follow up to determine effect of treatment.

As the urea breath tests rely on robust urease activity by *H. pylori*, their sensitivities may decreased by medications that reduce organism density or urease activity, including bismuth containing compounds, antibiotics and proton pump inhibitors. It is currently recommended that that bismuth and antibiotics be withheld for at least 1 month and proton pump inhibitors for 7 to 14 days prior to the urea breath test (Laine et al., 1998).

### 2.7.2.2 Serology

Patients infected with *H. pylori* have antibodies to the organism. These antibodies can be detected in serum or whole-blood samples by use of a biochemical assay and many different ones are available. Tests for the detection of antibodies to *H. pylori* have excellent sensitivity and specificity of above 95% and are cheap and simple compared with invasive techniques (DeCross & Puera, 1992).

Serologic tests offer a fast relatively easy results and are the only tests which are not likely to give false negative results in patients who have taken antibiotics, bismuth compounds or omeprazole in the recent past (NIH Consensus Conference, 1994).

A number of different commercially available assays exist for antibody detection. Enzyme linked immunosorbent assay (ELISA) based kits are the most numerous of the commercially available tests. Originally the kits used crude antigen preparations but many of the newer kits use a more purified antigen preparation giving increased
specificity but a lower sensitivity. Near patient test kits are based on either latex agglutination or immunochromatography. Generally, they have low sensitivities compared with laboratory tests. Commercial western blotting kits have also been developed and are used to detect the presence of specific virulence markers. The exact role of serology in the management of *Helicobacter* infection has still to be defined, although there is evidence that, used as a screening procedure, it can reduce endoscopy cost and workload (Vaira *et al*, 1998).

2.7.2.3 Commercially available sero-diagnostic kits

2.7.2.3.1 Laboratory ELISA kits

Circulating IgG, IgM and IgA antibodies to *H. pylori* can be detected by ELISA to determine both acute and chronic infection. Several kits are commercially available. Commercially available ELISA tests show that all the kits have comparable sensitivities 90–100% but have more variable and lower specificities 76–96% (Feldman *et al*, 1995.)

The main differences between these kits are the use of antigens. Several different antigen preparations have been tested. Initially crude or semicrude antigens containing whole cells, sonicates and acid-glycine extracts were most often used. However nonspecific cross reactions are frequently seen in these assays. Intention was to reduce non-specific cross reactions, and more purified antigens are used.

Using more purified antigen prepared by fast protein liquid chromatography (FPLC) or monoclonal antibody capture generally showed lower sensitivities and increased specificity. However it is possible that a judicious combination of purified antigens may
maximize both sensitivity and specificity, and many second generation serological tests use combinations of purified antigens. The best results have been obtained when combinations of purified antigens are used.

*H. pylori* infection develops a short-lived IgM response preceding development of an IgG antibody response (Gold et al., 1997). Titres of IgG fall relatively slowly and approximately 6 months after *H pylori* eradication, there will be reduction of 50% in the antibody titre (Kosunen et al., 1992). Because of this slow reduction after successful eradication, serology testing is not generally useful in confirming the success of eradication within a few weeks of treatment. However, ELISA test determining IgG, a good test for screening.

### 2.7.2.3.2 Near-patient testing

Near-patient tests are technically simple to perform. Most of the tests are one step tests using whole blood but others require serum separation that diminishes their usefulness as near-patient kits. All the evidence to date suggests that they have very much lower sensitivity and specificity compared with a laboratory-based ELISA test. Overall, greater accuracy can be obtained with laboratory-based tests compared with near-patient tests which at present seem not accurate enough to be routinely recommended (Stone et al., 1997).

### 2.7.2.3.3 Salivary antibody assay

Tests for antibodies to *H pylori* in saliva are equally effective as those for antibody in serum. Some results have demonstrated sensitivities and specificities lower than a serum assay (Christie et al., 1996). However, using a special applicator (OraSure salivary device,
Epitope Inc) to collect salivary antibodies, a sensitivity and specificity of 100% and 61% has been obtained when compared with a UBT (Malaty et al, 1998). In saliva, IgG antibodies must be measured as measuring IgA antibodies does not distinguish positive from negative cases (NIH Consensus Conference, 1994).

2.7.2.3.4 Sero-assays for pathogenicity markers

In certain populations where there is a strong correlation between colonization by cagA and serious gastro-duodenal disease, determining the infecting strain may give additional valuable clinical information. Several serological tests exist for determining the cagA status of a patient either as an Elisa test or a Western blot. A comparison of two anti-cagA elisa strains (HeloriCTX -Eurospital It and Radim 2) showed sensitivity and specificity of 100%, 76% and 90%, 94%, respectively (Basso et al, 1999). Anti-CagA and -VacA antibody response can be assessed also by a Recombinant Immunoblot Assay Strip (RIBA) which in recent studies showed good sensitivity and specificity (Fusconi et al, 1999). However the clinical value of knowing the cagA/vacA status is not yet clear.

2.7.2.4 Stool Antigen test

Stool antigen testing is a relatively new methodology that uses an enzyme linked immunoassay (ELISA )/ ICT to detect the presence of *H. pylori* antigen in the stool with the use of polyclonal anti-*H pylori* antibody. As this test detects bacterial antigen in ongoing infection, it can be used as a reliable means of diagnosing active infection and also useful for treatment follow-up purposes.
These tests have sensitivity and specificity comparable to those of other noninvasive tests (Monteiro et al, 2001). The first to be commercially available was the Premier Platinum HpSA EIA (Meridian Diagnostics, USA) which uses an affinity purified polyclonal antibody attached to microwell plates to which is added a faecal suspension followed by a peroxidase conjugated antibody and substrate. This kit has been assessed in several studies giving a reported sensitivity and specificity of 94% and 91%, respectively (Vaira et al, 1999).

Although the fecal antigen test shows great promise, issues slowing its widespread use include the unpleasantness of handling and storing stool, limited availability and variable state-to-state reimbursement (Valle et al, 2003).

**2.8. MANAGEMENT**

**2.8.1 General Treatment Principles**

Determining the optimum treatment of *H.pylori* infection is difficult, because the organism lives in an environment not easily accessible to many medications and emerging bacterial resistance presents an added challenge. Moreover, many of the recommended regimens leading to problems with patient’s compliance and coping with unpleasant adverse effects. Despite these obstacles, current regimens can obtain cure rates in excess of 85% (Fennerty et al, 1999) in most patient populations. The most clear-cut indications for treatment are *H.pylori* related acute or chronic duodenal ulcer and gastric ulcer and low-grade B cell lymphoma. Many guidelines now recommend *H.pylori* treatment in uninvestigated simple dyspepsia following noninvasive diagnosis. Others also recommend treatment in functional dyspepsia in which patients
may get 5 to 10% benefit from such treatment. People with a strong family of gastric cancer should be treated for *H. pylori* in the hope that this therapy will reduce their risk.

### 2.8. 2 Antibiotic Agents

Although *H. pylori* is sensitive to a wide range of antibiotics in vitro, they all fail as monotherapy in vivo. The lack of efficacy of monotherapy is related to the niche of *H. pylori*, residing at lower pH in a viscous mucus layer. The combination of two or more antimicrobial agents increases rates of cure and reduces the risk of selecting for resistant *H. pylori*. The chief antimicrobial agents used in these regimens are amoxicillin, clarithromycin, metronidazole, tetracycline and bismuth. Primary resistance to amoxicillin and tetracycline remains uncommon, but the frequency of clarithromycin resistance is now around 10 percent in most European countries and the United States and even higher in Japan. Metronidazole resistance ranges between 20 percent and 30 percent and is more frequent in developing countries because of the frequent use of nitroimidazoles to treat other diseases (Meyer *et al*, 2002). Resistance of *H. pylori* to macrolides is caused by point mutations in the 23S ribosomal RNA genes. Resistance to metronidazole is caused primarily by mutations in nitroreductase genes (rdxA and frxA) that interfere with the intracellular activation of nitroimidazoles (Méraud *et al*, 2001).

### 2.8. 3 Adjunctive Agents

The most popular agents currently used in combination with antibiotic agents are the proton pump inhibitors, with omeprazole being the most widely studied drug. Omeprazole acts not only by directly inhibiting bacterial microsomal enzymes but also by
raising intragastric pH, thus facilitating the action of antibiotic agents, reducing gastric secretions, and increasing antibiotic concentrations in the stomach. Other adjunctive agents include histamine receptor antagonists and ranitidine bismuth citrate, which has antisecretory properties in addition to the antibacterial action of bismuth (ie. interruption of the bacterial cell wall).

2.9. Current Regimens

2.9.1 First-Line Therapies

Most clinicians treat *H. pylori* infection with a triple-drug or even quadruple-drug approach. The 1998 guidelines from the American College of Gastroenterology judged the following 3 regimens to be optimal:

2.9.1.1 Proton-Pump-Inhibitor–Based Triple Therapies

Administration of a proton pump inhibitor simultaneously with two antibiotics (PPI-clarithromycin-amoxicillin or PPI-clarithromycin- metronidazole) for 14 days treatment is the recommended first Line Therapies. Large, randomized trials confirmed the effectiveness of treatment twice daily for seven days with 20 mg of omeprazole, given either with 1 g of amoxicillin and 500 mg of clarithromycin, or with 400 mg of metronidazole and 250 mg of clarithromycin (Lind *et al*, 1996). Several comparative trials have demonstrated the equivalence of 30 mg of lansoprazole twice daily, 40 mg of pantoprazole twice daily, 20 mg of rabeprazole daily, and 20 mg of esomeprazole twice daily with omeprazole in these triple therapies (Misiewicz *et al*, 1997).
In several studies combinations of a PPI, clarithromycin, and metronidazole; a PPI, clarithromycin and amoxicillin were judged to be similar with rates of cure of 78.9 to 82.8 percent (Laheij et al, 1999). Increasing the dose of clarithromycin to 1.5 g per day improved rates of cure, but increasing the doses of the other antibiotics did not. In another pooled analysis, no effect of larger doses of proton-pump inhibitors was observed among the triple therapies (Unge, 1998). The duration of therapy remains controversial. In Europe, 7-day treatment is recommended (Bazzoli, 2001), whereas in the United States, 14-day courses have been found to be better than shorter courses and are approved by the FDA. In a recent meta-analysis, 14-day treatment achieved rates of cure 7 to 9 percentage points better than 7-day treatment (Calvet et al, 2000). Primary resistance to clarithromycin and metronidazole decreases rates of cure by 50 percent and 37 percent respectively (Dore et al, 2000). The indication for therapy, bacterial factors, patient compliance, and geographic differences can further affect rates of cure (Lee et al, 1999).

### 2.9.1.2 Ranitidine Bismuth Citrate–Based Therapies

Ranitidine bismuth citrate in dual therapy with clarithromycin for two weeks has been approved by the FDA (Peterson et al, 1996). It has been suggested that ranitidine bismuth citrate, clarithromycin and either metronidazole, amoxicillin, or tetracycline for 2 weeks; performs as well as corresponding proton-pump-inhibitor–based therapies (Gisbert et al, 2000). Ranitidine bismuth citrate–based regimens may be less influenced by antibiotic resistance than their proton-pump-inhibitor–based counterparts (Sung et al, 1999). No ranitidine bismuth citrate–based triple therapy has been approved by the Food and Drug Administration (FDA).
2.9.1.3 Bismuth-Based Triple Therapies

Bismuth in association with metronidazole and tetracycline compares well with therapies based on proton-pump inhibitors or ranitidine bismuth citrate, even if the duration of treatment is reduced to seven days. This inexpensive regimen remains an important option. Efficacy is negatively affected by metronidazole resistance (Houben et al, 1999). Furazolidone, a nitrofuran derivative, has also been proposed for use in bismuth-based triple therapies. Triple therapy for two weeks, consisting of 100 mg of furazolidone four times daily, amoxicillin, and bismuth, was successful in 86 percent of cases. However, furazolidone, particularly when combined with bismuth for two weeks, is associated with substantial side effects. Standard bismuth-based therapy and its furazolidone-containing alternatives were recommended at the 1999 Latin American Consensus Conference (Coelho et al, 2000).

2.9.2 Second-Line Therapies

Eradication is more difficult when a first treatment attempt has failed, usually because of either poor patient compliance or the development of antibiotic resistance. Therefore, a 10-to-14-day treatment course is advocated for second-line therapies. However, the optimal strategy for retreatment after the failure of eradication has not yet been established.

Because the failure of therapy is often associated with secondary antibiotic resistance, retreatment should ideally be guided by data on susceptibility. However, such information is often unavailable, so Bismuth based quadruple therapy (PPI-bismuth-metronidazole-
tetracycline) have been suggested as optimal second-line therapy. It leads to satisfactory eradication rates about 76 percent (Hojo et al, 2001). This second-line therapy was recommended at major consensus conferences (Lam and Tally, 1998) although it may prove disappointing, given the failure of regimens containing metronidazole.

Another approach to retreatment without susceptibility testing is to prescribe a second course of proton-pump-inhibitor–based triple therapy, avoiding antimicrobial agents against which prior therapy may have induced resistance and avoiding less effective combinations, such as amoxicillin and tetracycline. If a clarithromycin-based regimen is used first, a metronidazole-based regimen should be used afterward, or vice versa (Hojo et al, 2001).

Alternative approaches to second-line, proton-pump-inhibitor–based therapies using a different combination of available antibiotic agents or increasing the duration of treatment have been reported recently. Rifabutin, given in association with amoxicillin and pantoprazole for 10 days, achieved an 86 percent rate of cure, even in patients with resistant strains (Perri et al, 2001).

Owing to the variety of clinical situations and antibiotics available in different countries, no specific recommendation is given for third line therapy except to perform susceptibility testing. Several studies have shown that higher eradication rates are obtained when antibiotics are chosen based on susceptibility testing rather than chosen empirically (Toracchio et al, 2000; Romano et al, 2003). This may be a cost effective approach (Breuer and Graham, 1999). The high impact of clarithromycin resistance led to the proposal to perform culture and antimicrobial susceptibility testing when the
resistance rate reaches 15–20%. Culture and sensitivity may help in decision making after the failure of a second line therapy.

2.11. EMERGING THERAPIES

2.11.1 Antibiotics and Other Agents

As emerging drug resistance continues, new therapeutic regimens incorporating existing antibiotic agents and newly developed compounds are essential to eradicate *H. pylori* infection. Two other classes of antibiotics have emerged in the treatment of *H pylori* infection: a fluoroquinolone, levofloxacin and a rifamycin, rifabutin. These antibiotics have been evaluated for the most part in first choice treatments with PPI and amoxicillin rather than rescue treatments, with a good success rate. However, rifabutin is an antibiotic which can select resistance among Mycobacteria, so it must be used cautiously. *H pylori* resistance to rifabutin may occur but is rare.

2.11.2 Vaccines

Vaccination against *H. pylori* has been performed in many animal models and in a few human trials. Although several studies showed the benefit of prophylactic as well as therapeutic vaccination in animals, bacterial eradication has not been achieved in humans.

Several issues remain in regard to a safe and effective vaccine against *H. pylori* infection. Firstly, a safe mucosal adjuvant or vector to stimulate an immune response must be identified. Secondly, the optimal route of administration needs to be defined; studies in
mice show promise with nasal and rectal routes, which would avoid the possible post immunization gastritis likely with an oral route (Sutton, 2000). In addition different regimens need to be developed to ensure complete sterilization of the gastric mucosa; the latter step has not generally been attempted in murine models. Clinical trials are underway to answer these and other questions, with the goal of producing an inexpensive, safe and effective vaccine seeming to be within reach.

Colonization of the stomach with *H. pylori* results in the induction of a strong but unprotective inflammatory response, mainly polarized toward Th1 cells (Mohammadi *et al*, 1996). It is currently believed that effective vaccination depends on the induction of a humoral and Th2 cell immune response. Mucosal immunization with a variety of antigens in combination with mucosal adjuvants such as cholera toxin (AB5 toxin, CT), the heat-labile toxin of *Escherichia coli*, or Freund adjuvants which induce a Th2 response, prevents or cures an infection by *Helicobacter* spp., while Th1 response-inducing adjuvants enhance inflammation rather than eliminating it. The first indications that these mucosal immunizations against *Helicobacter* induce a Th2 response were seen in studies showing specific salivary secretory immunoglobulin A (IgA) and serum IgG1 antibodies after oral immunization of mice (Ferrero *et al*, 1995; Lee *et al*, 1995). Subsequent studies indicated that mucosal immunization with urease resulted in a Th2 CD4+ T-cell response that effectively eliminated an ongoing *Helicobacter felis* infection in BALB/c mice (Saldinger *et al*, 1998). Apparently, if a vaccine against *H. pylori* drives the immune response toward a Th2 response it can both prevent and eradicate *H. pylori* infections. Other indications for therapeutic use of *H. pylori* vaccines come from experiments with chronically infected animals, in which either a significant proportion could be cured of
gastric *Helicobacter* infections or the effectiveness of antibiotic regimens was shown to be greatly enhanced (Ghiara et al., 1997). Thus, even in the absence of complete eradication, therapeutic vaccination may already be beneficial, as it reduces the numbers of bacteria exposed to antibiotics and thus decreases the possibility of inducing antibiotic-resistant *H. pylori* organisms. Although one should be aware of the limitations of animal models with regard to therapeutics, preliminary human vaccine trials have already been performed and further results are awaited (Keller et al., 2001)

### 2.11.3. Probiotics

Another approach would be to use probiotics. Probiotics prevent infection with pathogenic bacteria both through activation of the host's immune system and through direct competition of the probiotic bacteria with the pathogen. There is good evidence that *H. pylori* is killed by lactobacilli both in vitro and to a limited extent in vivo (Sakamoto et al., 2001). It is unlikely that such an approach will lead to complete eradication of *H. pylori* bacteria in the stomach but complete eradication is not needed to prevent disease either reduction of the amount of bacteria or altering of the immune response might result in a significant decrease in inflammation, thus reducing the induction of peptic ulcers and cancer.
MATERIALS AND METHODS

3. 1.  Place and Period

The study was carried out in the Department of Microbiology, Mymensingh Medical College for a period from July 2006 to June 2007. This study was approved by protocol approval committee of the Department of Microbiology and Ethical review Committee of Mymensingh Medical College.

3. 2.  Type

The study was designed as Cross sectional type.

3. 3.  Population

Two groups of population were included in the study. A total of 45 symptomatic patients and 45 asymptomatic population of either sex from 18 to 65 years of age were included in the study. The Specimens were collected from private chamber of Gastroenterologists of Mymensingh Medical College Hospital (MMCH). All patients were informed of the objective of the study and subsequently provided written informed consent.

3. 4. symptomatic group / endoscopic group

A total of 45 patients having upper gastrointestinal symptoms were included in the study as symptomatic group who fulfilled the inclusion criteria.
3.4.1. **Inclusion Criteria for symptomatic group / endoscopic group**

Patients with a variety of symptoms relating to upper gastrointestinal tract, advised by the physicians for endoscopic examination and subsequently diagnosed as patients with gastritis, peptic ulcer (PU) and non-ulcer dyspepsia (NUD).

3.4.2. **Exclusion Criteria for symptomatic group / endoscopic group**

Patients with gastric carcinoma and Patients taking *H. pylori* eradication treatment and consumption of antibiotics, proton pump inhibitors, bismuth containing compounds or sucralfate during the 4 weeks preceding the endoscopy were not included in the study.

3.5. **Asymptomatic group / Non-endoscopic group**

To study a representative sample of healthy population for active infection and serologic evidence of exposure to *H. pylori*, 45 individuals of either sex from 18-65 years were included in this group. All of them were be free from upper gastrointestinal symptoms and did not undergo endoscopic examination.

3.6. **Data collection and recording**

All relevant history, clinical findings and laboratory records of every subject was systematically recorded in a pre designed data sheet (Appendix-I) for subsequent analysis by computer programme SPSS version 12.0.
3. 7. Specimens

Gastric Biopsy specimen for rapid urease test (RUT) and smear were collected from symptomatic / endoscopic group. Venous blood for serological tests was collected from both groups.

3. 7.1 Procedure of gastric biopsy specimen collection

Examination of upper GIT of the patients was done by Endoscopy. Upper GI endoscopy is a visual examination of the upper intestinal tract using a lighted, flexible fiber optic or video endoscope. Patients selected for the study were examined with video endoscope (Pantax EPK 150C, Japan). The newer video endoscopes have a tiny, optically sensitive computer chip at the end. Electronic signals are then transmitted up the scope to the computer, which then displays the image on a large video screen. Presence of lesions in the gastro duodenal mucosa was noted. The ulcer was diagnosed by a circumscribed break in the mucosa with apparent depth and covered by exudates. An open channel in these scopes allows other instruments to be passed through in order to take tissue samples. Biopsy tissue was taken from the gastric antrum within 5 cm of pylorus (Sengupta, 2002). Two biopsy specimens were collected, one for rapid urease test (RUT) and another for smear preparation. The specimen for RUT was placed on top of urea agar media contained in the tube and the other was directly smeared on cleaned glass slides. As endoscopic biopsy is an invasive procedure therefore, biopsy was not taken from asymptomatic / Non-endoscopic group.

3. 7.2 Procedure of collection of blood and separation of serum
About 3 to 5ml of venous blood was collected from each patients and asymptomatic subjects using sterile disposable syringe and placed into a clean dry test tube for separation of serum. Tubes containing blood were kept at room temperature for 1 hour to allow clotting of blood. Then it was centrifuged at 1500 rpm for 15 minutes and serum was separated and kept in a sterile eppendorf tube at –20°c until analyzed (Chessbrough, 2000).

3.8. Rapid Urease Test

Rapid urease test is a rapid test for diagnosis of *Helicobacter pylori*. The basis of the test is the ability of *H.pylori* to secrete the urease enzyme, which catalyzes the conversion of urea to ammonia and bicarbonate. A biopsy of mucosa was taken from the antrum of the stomach and placed into Christensen’s urea agar media (Appendix-II) and incubated at 37°c. Results indicated by the colour change of the medium were often available within 20 minutes to 4 hours and always within 24 hours (Versalovic, 2003).

- Pink colour…………………………Positive urease test
- No pink colour………………………Negative urease test

3.9. Microscopic examination of the stained smear

Biopsy specimens (antral mucosa) were crushed between two slides. Slides were fixed in 95% ethyl alcohol and were stained by modified Giemsa stain (Appendix-V) and Gram stain (Appendix-III) and examined under microscope for S-shaped organisms.

3.9.1 Modified Giemsa Staining technique (Gray et al 1986)

3.9.1.1 Composition
Giemsa powder…………………………. 2gram
Distilled water………………………….. 100ml

Giemsa powder is dissolved in distilled water. It is kept in room temperature and filtered before use.

3.9.1. 2 Staining procedure

Slides were fixed in 95% ethyl alcohol and then incubated in 2% Giemsa solution in distilled water for 30 minutes at room temperature. After rinsing in tap water the slides were quickly dehydrated and examined under microscope (Gray et al 1986). *H. pylori* stained blue using modified Giemsa stain.

3. 10. Serological tests

3.10.1. ELISA test for Detection of anti-*H.pylori* IgG

Serum anti-*H.pylori* IgG was detected by ELISA *H.pylori* IgG Kit. The Kit was manufactured by Orgenics, Inc. France. Lot no: 060809W. Test procedure was as per kit manual.

3.10.1.1 Principle of the test

ELISA *H.pylori* IgG test determines IgG antibodies to *Helicobacter pylori*. Microplates are coated with *H.pylori* immunodominant antigens derived from tissue culture of a virulent strain. In the 1st incubation, the solid phase is treated with diluted samples and anti-HP IgG are captured (if present) by the antigens which are detected by the addition of anti human IgG antibody labeled with Horseradish peroxidase (HRP). The enzyme
captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-HP IgG antibodies present in the sample. IgG in the sample may therefore be quantitated by means of a standard curve calibrated in arbitrary units per milliliter (arbU/ml) as no international standard is available.

3.10.1.2. Test procedure

1) Samples were diluted 1:101 into a properly defined dilution tube (example: 1000 µl sample diluent + 10 µl sample).

2) Required number of Microwells were placed in the microwell holder.

3) Hundred microlitre (100 µl) of Calibrators (CAL1, CAL2, CAL3, CAL4, CAL 5, CAL6) were dispensed into A1, B1, C1, D1, E1 and F1 accordingly. Then 100 µl of diluted samples were dispensed in each properly identified well.

4) The microplate was Incubated for 60 min at +37°C (1st incubation)

5) The microplate was washed with an automatic washer by delivering and aspirating 300 µl/well of diluted washing solution.

6) Hundred microlitre (100 µl) of Enzyme Conjugate (anti-human IgG conjugated with horse radish peroxidase) was added into each well. Then this red coloured component had been dispensed in all the wells were checked.
7) The microplate was incubated for 60 min at +37°C (2nd incubation).

8) The microplate was washed with an automatic washer by delivering and aspirating 300 μl/well of diluted washing solution as in step 5.

9) Hundred microlitre (100 μl) of Chromogen/Substrate mixture (0.03% TMB and 0.02% H2O2) was added into each well. Then the microplate was incubated at room temperature (18-24°C) for 20 minutes.

10) Hundred microlitre (100 μl) of sulphuric acid was added into all the wells to stop the enzymatic reaction. Addition of acid turned the positive calibrators and the positive samples from blue to yellow.

11) The colour intensity of the solution in each well was measured at 450 nm filter.

3.10.1.3. Interpretation of results

Samples with a concentration lower than 5 arbU/ml were considered negative for anti-\textit{H.pylori} IgG antibody. Samples with a concentration at or above 5 arbU/ml were considered positive for anti-\textit{H.pylori} IgG antibody.

3.10.1.4 ELISA test for detection of anti-\textit{H.pylori} IgM
Serum anti-\textit{H.pylori} IgM was detected by ELISA \textit{H.pylori} IgM Kit. The Kit was manufactured by Orgenics, Inc. France. Lot no: 070847K. Test procedure was as per kit manual.

\textbf{3.10.1.5. Principle of the test}

ELISA \textit{H.pylori} IgM test determines IgM antibodies to \textit{Helicobacter pylori}. Microplates are coated with \textit{H.pylori} immunodominant antigens derived from tissue culture of a virulent strain. In the 1st incubation, the solid phase was treated with diluted samples and anti-HP IgM are captured (if present) by the antigens which are detected by the addition of anti human IgM antibody labeled with Horseradish peroxidase (HRP). The enzyme captured on the solid phase, acting on the substrate / chromogen mixture, generates an optical signal that is proportional to the amount of anti-HP IgM antibodies present in the sample. The presence of IgM in the sample may therefore be determined by means of a cut-off value able to discriminate between negative and positive samples. Neutralization of IgG anti-HP, carried out directly in the well, is performed in the assay in order to block interferences due to this class of antibodies in the determination of IgM.

\textbf{3.10.1.6 Test procedure}

1) Samples were diluted 1:101 into a properly defined dilution tube (example: 1000 µl sample diluent + 10 µl sample).

2) Required number of Microwells were placed in the microwell holder Leaving A1 well empty for the operation of blanking.
3) Fifty microlitres (50 µl) of Neutralizing Reagent was dispensed in all the wells except the wells used for blanking and the Controls.

4) Hundred microlitres (100 µl) of Negative Control and 100µl of positive Control was dispensed in each properly identified well.

5) The microplate was incubated for 60 min at +37°C (1st incubation).

6) The microplate was washed with an automatic washer by delivering and aspirating 300 µl /well of diluted washing solution.

7) Hundred microlitre (100 µl) of Enzyme Conjugate (anti-human IgM conjugated with horse radish peroxidase) was added into each well carefully except the A1 well and covered with the sealer. Then this red coloured component had been dispensed in all the wells were checked except the blank well.

8) The microplate was incubated for 60 min at +37°C (2nd Incubation).

9) The microplate was washed with an automatic washer by delivering and aspirating 300 µl/well of diluted washing solution as in step 5.

10) Hundred microlitre (100 µl) of Chromogen/Substrate mixture (0.03% TMB and 0.02% H2O2) was added into each well including the blank well. Then the microplate was incubated at room temperature (18-24°C) for 20 minutes.
11) Hundred microlitre (100 µl) of Sulphuric Acid was added into all the wells to stop the enzymatic reaction. Addition of acid turned the control serum and the positive samples from yellow to blue.

12) The colour intensity of the solution in each well was measured at 450nm filter.

3.10.1.7 Interpretation of results

Results were calculated from the mean OD450 nm value of the Negative Control (NC) by means of a cut-off value determined with the following formula:

\[
\text{Cut-Off} = \text{NC} + 0.250
\]

RESULTS

The present study was conducted on a total of 90 subjects. Among them, 45 were asymptomatic and 45 were symptomatic subjects of either sex from 18 to 65 years of age.

Table no. 1 Figure 1 shows the age and sex distribution of the symptomatic group. Among them, 34 were male and 11 were female. Majority of the subjects (15) were in the age group between 31-40 years. Minimum numbers of subjects (09) were in the age group of >51 years. Male to female ratio was 3.09: 1.

Table no. 2 shows the age and sex distribution of the asymptomatic group. Among them, 29 were male and 16 were female. Majority of the subjects (21) were in the age group of
<30 years. Minimum numbers of subjects (03) were in the age group of >51 years. Male to female ratio was 1.81: 1.

Table no. 3 shows the socioeconomic condition of the subjects. In symptomatic group, people from lower class were 36 (80%) and rest 9 (20%) were from middle class, none were from upper class. In asymptomatic group, people from lower class were 21 (46.67%) and from middle class were 20 (44.44%), upper class people were 4 (8.89%).

**Table - 1    Age and sex distribution of the symptomatic group**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male (Yrs)</th>
<th>Female (Yrs)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>08 (72.73)</td>
<td>03 (27.27)</td>
<td>11(24.44)</td>
</tr>
<tr>
<td>31-40</td>
<td>12 (80.00)</td>
<td>03 (20.00)</td>
<td>15(33.33)</td>
</tr>
<tr>
<td>41-50</td>
<td>09 (90.00)</td>
<td>01 (10.00)</td>
<td>10(22.22)</td>
</tr>
<tr>
<td>&gt;51</td>
<td>05 (55.56)</td>
<td>04 (44.44)</td>
<td>09 (20.00)</td>
</tr>
<tr>
<td>Total</td>
<td>34 (75.56)</td>
<td>11(24.44)</td>
<td>45 (100.0)</td>
</tr>
</tbody>
</table>

Ratio Male : Female = 3.09 : 1

Figures in the parenthesis indicate percentage.

* minimum age- 18 years, maximum age- 65 years, mean age 41.09 years, SD ± 13.41

*Majority of the subjects in the age group of 31-40 years- 33.33 %
*Minimum numbers of subjects in the age group of >51 years- 20%

*Male- 75.56%

*Female- 24.44%

Figure-1 Age and sex distribution of the symptomatic group
## Table 2 Age and sex distribution of the asymptomatic group

<table>
<thead>
<tr>
<th>Age group (Yrs)</th>
<th>asymptomatic group (n= 45)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>&lt;30</td>
<td>14(66.67)</td>
<td>07 (33.33)</td>
<td>21 (46.67)</td>
</tr>
<tr>
<td>31-40</td>
<td>10 (62.50)</td>
<td>06 (37.50)</td>
<td>16(35.55)</td>
</tr>
<tr>
<td>41-50</td>
<td>03 (60.00)</td>
<td>02 (40.00)</td>
<td>05 (11.11)</td>
</tr>
<tr>
<td>&gt;51</td>
<td>02 (66.67)</td>
<td>01 (33.33)</td>
<td>03 (6.67)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>29 (64.44)</td>
<td>16 (35.56)</td>
<td>45 (100.0)</td>
</tr>
<tr>
<td><strong>Ratio</strong></td>
<td>Male : Female = 1.81 : 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures in the parenthesis indicate percentage.

* minimum age- 18 years, maximum age- 65 years, Mean age 32.73 years, SD ± 10.27
* Majority of the subjects in the age group of <30 years - 46.67%
* Minimum numbers of subjects in the age group of >51 years - 6.67%
* Male- 64.44%
*Female - 35.56%

Table – 3 socioeconomic condition of the subjects.

<table>
<thead>
<tr>
<th>Socioeconomic condition*</th>
<th>symptomatic group (n = 45)</th>
<th>asymptomatic group (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower class</td>
<td>36 (80)</td>
<td>21 (46.67)</td>
</tr>
<tr>
<td>Middle class</td>
<td>9 (20)</td>
<td>20 (44.44)</td>
</tr>
<tr>
<td>Upper class</td>
<td>00 (00)</td>
<td>04 (8.89)</td>
</tr>
</tbody>
</table>

Figures in the parenthesis indicate percentage.

* Socioeconomic condition is categorized into three classes according to monthly income of family (Islam, 1992).

Lower class = monthly income < 3000 Tk
Middle class = monthly income 3000-20000 Tk
Upper class = monthly income >20000Tk
Table no. 4 shows habit of the subjects. In symptomatic group, 34 (75.56%) were either smoker and/or tobacco (jarda) habituated. The rest 11 (24.44%) were non smoker or not habituated with tobacco (jarda). In asymptomatic group, 12 (26.67%) were either smoker and/or habituated with tobacco (jarda). The rest 33 (73.33%) were non smoker or not habituated with tobacco (jarda). Thus either smokers and/or tobacco (jarda) chewer were found to have significant \( \chi^2 = 21.52, p < 0.001 \) association of *H. pylori* infection.

Table no. 5 shows sensitivity of rapid urease test (RUT) 84.44%. Specificity could not be carried out as this test was not performed in asymptomatic group due to invasive procedure.

Table no. 6 shows the sensitivity of direct microscopy of gastric biopsy specimen on Gram stained and modified Giemsa stained smears 48.89%. Specificity could not be carried out as this test was not performed in asymptomatic group due to invasive procedure.

Table no 7 shows sensitivity and Specificity of Anti-\(*H. pylori*\) Ig G at different cut-off value. At cut off value 5arbU/ml, sensitivity and specificity was 95.5% and 11.11% respectively; at cut off value 10arbU/ml, sensitivity and specificity was 80% and 28.89%
respectively; at cut off value 20arbU/ml, sensitivity and specificity was of 51.1% and 62.22% respectively and at cut off value 30arbU/ml, sensitivity and specificity was 40% and 75.55%.

Table – 4    Habit of the subjects.

<table>
<thead>
<tr>
<th>Habit</th>
<th>symptomatic group (n = 45)</th>
<th>asymptomatic group (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker and/or Tobacco (jarda) habituated</td>
<td>34 (75.56)</td>
<td>12 (26.67)</td>
</tr>
<tr>
<td>Non smoker/ not habituated with tobacco(jarda)</td>
<td>11 (24.44)</td>
<td>33 (73.33)</td>
</tr>
</tbody>
</table>

Figures in the parenthesis indicate percentage.

(p < 0.001 by Chi- square test)
Table –5  Sensitivity of rapid urease test (RUT)

<table>
<thead>
<tr>
<th>Test result</th>
<th>symptomatic group (n = 45)</th>
<th>sensitivity</th>
<th>specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUT of biopsy specimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>38 (a)</td>
<td>84.44%</td>
<td>*Could not be done.</td>
</tr>
<tr>
<td>RUT of biopsy specimen</td>
<td>7 (c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45 (a + c)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RUT: Rapid urease test
* This test was not performed in asymptomatic group due to invasive procedure.
a= True positive
c= False negative

Sensitivity = a/a+c × 100
### Table 6: Sensitivity of microscopy of biopsy specimen

<table>
<thead>
<tr>
<th>Test result</th>
<th>Symptomatic group (n = 45)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy of biopsy specimen Positive</td>
<td>22 (a)</td>
<td>48.89%</td>
<td>*Could not be done.</td>
</tr>
<tr>
<td>Microscopy of biopsy specimen Negative</td>
<td>23 (c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45 (a + c)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This test was not performed in asymptomatic group due to invasive procedure.

a= True positive

| Sensitivity = a/(a+c) × 100
Table –7  Sensitivity and specificity of ELISA Anti-*H.pylori* Ig G at different cut-off value.

<table>
<thead>
<tr>
<th>ELISA Anti-<em>H.pylori</em> IgG Cut-off value in arbU/ml</th>
<th>symptomatic group (n= 45)</th>
<th>asymptomatic group (n= 45)</th>
<th>sensitivity</th>
<th>specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(+)</td>
<td>(-)</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 arbU/ml</td>
<td>43 (a)</td>
<td>2 (c)</td>
<td>40(b)</td>
<td>05 (d)</td>
<td>95.55%</td>
<td>11.11%</td>
</tr>
<tr>
<td>10 arbU/ml</td>
<td>36 (a)</td>
<td>09 (c)</td>
<td>32(b)</td>
<td>13 (d)</td>
<td>80%</td>
<td>28.89%</td>
</tr>
<tr>
<td>20 arbU/ml</td>
<td>23 (a)</td>
<td>22(c)</td>
<td>17 (b)</td>
<td>28 (d)</td>
<td>51.11%</td>
<td>62.22%</td>
</tr>
<tr>
<td>30 arbU/ml</td>
<td>18 (a)</td>
<td>27 (c)</td>
<td>11(b)</td>
<td>34(d)</td>
<td>40%</td>
<td>75.55%</td>
</tr>
</tbody>
</table>

(p > 0.05  by Chi-square test)

arbU = Arbitrary Unit, (+) = Positive, (-) = Negative

N.B: Sensitivity and specificity was calculated by the following formula

\[
\text{Sensitivity} = \frac{a}{a+c} \times 100 \quad \text{Specificity} = \frac{d}{b+d} \times 100
\]
Positive predictive value (PPV) = $\frac{a}{a+b} \times 100$

Negative predictive value (NPV) = $\frac{d}{c+d} \times 100$

Table no 8 shows sensitivity and specificity of Anti-\textit{H.pylori} Ig M. The sensitivity and specificity was 73.33 % and 93.33 % respectively. Positive predictive value and Negative predictive value was 91.67 % and 77.78 %.

Table 9 shows Comparison of sensitivity and specificity of different Diagnostic procedures for \textit{H. pylori} infection. Sensitivity of rapid urease test (RUT) and direct microscopy of gastric biopsy specimen was of 84.44 % and 48.8% respectively. Sensitivity and specificity of Anti-\textit{H.pylori} Ig G varied at different cut-off value. The sensitivity and specificity of Anti-\textit{H.pylori} Ig M was 73.33 % and 93.33 % respectively.
Table – 8  Sensitivity and specificity of ELISA Anti-

<table>
<thead>
<tr>
<th>Test result</th>
<th>symptomatic group (n= 45)</th>
<th>asymptomatic group (n= 45)</th>
<th>sensitivity</th>
<th>specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA Anti-\textit{H.pylori} IgM +ve</td>
<td>33(a)</td>
<td>3(b)</td>
<td>73.33 %</td>
<td>93.33 %</td>
</tr>
<tr>
<td>ELISA Anti-\textit{H.pylori} IgM -ve</td>
<td>12 (c)</td>
<td>42(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45(a+c)</td>
<td>45 (b+d)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(p < 0.001 by Chi- square test)

ELISA: Enzyme linked immunosorbent assay

Calculation of the result

Cut-off value = mean OD value of Negative Control + 0.25

\[ = 0.271 + 0.25 \]

\[ = 0.521 \]

N.B: Sensitivity and specificity was calculated by the following formula

\[ a = \text{True positive}, \ b = \text{False positive}, \ c = \text{False negative} \text{ and } \ d = \text{True negative} \]

\[ \text{Sensitivity} = \frac{a}{a+c} \times 100 \]

\[ \text{Specificity} = \frac{d}{b+d} \times 100 \]

Positive predictive value = \[ \frac{a}{a+b} \times 100 = 91.67 \% \]
Negative predictive value = \( \frac{d/c}{d+c} \times 100 = 77.78 \% \)

Table – 9  Comparison of sensitivity and specificity of different Diagnostic procedures for \( H.pylori \) infection

<table>
<thead>
<tr>
<th>Diagnostic procedures</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUT</td>
<td>84.44%</td>
<td>*Could not be done</td>
</tr>
<tr>
<td>Microscopy of biopsy</td>
<td>48.89%</td>
<td>*Could not be done</td>
</tr>
<tr>
<td>ELISA Anti-HP IgG at Cut-off</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5arbU/ml</td>
<td>95.5%</td>
<td>11.11%</td>
</tr>
<tr>
<td>10 arbU/ml</td>
<td>80%</td>
<td>28.89%</td>
</tr>
<tr>
<td>20 arbU/ml</td>
<td>51.11%</td>
<td>62%</td>
</tr>
<tr>
<td>30 arbU/ml</td>
<td>40%</td>
<td>75.55%</td>
</tr>
<tr>
<td>ELISA Anti- ( H.pylori ) IgM</td>
<td>73.33%</td>
<td>93.33%</td>
</tr>
</tbody>
</table>

* These tests were not performed in asymptomatic group due to invasive procedures.
**DISCUSSION**

Different diagnostic Procedures for diagnosing *H. pylori* infection are available. Initially, patients with uncomplicated dyspeptic disease should undergo serologic testing for the presence of ant-*H pylori* antibodies in serum or whole blood. If the serum IgG result is negative and the clinical suspicion remains high, a urea breath test or faecal antigen test for direct detection and diagnostic confirmation would be recommended. Serum IgM and IgA testing is also considered. Biopsy specimens may be assayed for rapid urease test or direct microscopical examination with several stains for demonstration of the organism.
In the present study, in symptomatic group, majority of the patients were in the age group between 31-40 years and below 30 years in asymptomatic group (Table 1 and Table 2). We found 33.33% cases in the age group 31-40 years. Gill et al (1993) from India reported most of the clinically suspected cases (58%) in the age group between 30-39 years having very close correlation with the present study. Another study by Kate V et al (2001) from India, reported 74% cases in the age group 16-30 years. The prevalence of *H. pylori* increases markedly with age with the maximum colonization occurring in young adults (Kate V et al, 2001). In the developing countries, up to 70% of children are infected with *H pylori* by age 15 years and rates of 80% or more are found among middle-aged adults as compared with 20 to 50 percent in industrialized countries (Suerbaum and Michetti, 2002). In this study, male patients were more (76%) than female patients (24%). Similar findings of male dominance (91%) over female (9%) patients were reported in another study by Morshed et al (1997) from Bangladesh although *H. pylori* infection is independent of gender (Graham et al, 1991). The increase number of male patients over female in this study might be due to frequent going out of male persons. Male are the active and main earning member of the most of the family still now, so they are more privileged to visit physician chamber for treatment.

In this study, majority of the patients (80%) were from lower class and rest 20% from middle class and none were from upper class (Table 3). The prevalence of the infection is correlated with low socioeconomic status during childhood, high density of living and low household income (Malaty, 2007). Poor hygiene and crowded conditions may facilitate transmission of infection among family members and is consistent with
intrafamilial and institutional clustering of \textit{H. pylori} infection. In a study by Mahalanabis \textit{et al} (1996) from Bangladesh reported high \textit{H. pylori} infection rate in children, in a poor Bangladeshi community and explained have an association with contaminated environment, crowding, lack of proper sanitation and lack of sufficiently clean water.

In the present study, 76\% of symptomatic subjects were either smoker and/or habituated with chewing tobacco (jarda) whereas in asymptomatic group 27\% were smoker and/or habituated with chewing tobacco (jarda) (Table 4). Smokers and/or tobacco (jarda) chewer were found to have a higher risk of \textit{H. pylori} infection ($p < 0.001$) than nonsmokers or not habituated with tobacco (jarda). This finding is in agreement with Martin (1989) from USA who reported there was a relationship between \textit{H. pylori} infection and smoking but not with Chodos (1988) from USA who reported no significant relationship. Moreover, Smoking has a destructive effect on the immunity of gastric mucosa and lining layers and hence increases its susceptibility to infection by \textit{H. pylori} (El-Barrawy \textit{et al}, 1997).

In this study, 38 (84\%) symptomatic subjects became positive when tested by rapid urease test (Table 5) that differed from the study by Morshed \textit{et al} (1997) from Bangladesh where they found RUT positive in 55.6\% cases and another study by Sivaprakash and Rao (1994) from India found positive in 38.7\% cases. This relatively lower rate of positivity might be due to the reason that they observed RUT result for 6 hours whereas we observed RUT result for 24 hours in our study. RUT positive result correlates with the study from Netherlands by Kamiya \textit{et al} (1993). They found sensitivity of RUT 84.6\%. In another study from UAE by Zaitoun (1993) reported the sensitivity of RUT of 94\% that was higher than our result.
In the present study, the sensitivity of direct microscopy of gastric biopsy specimen on Gram stained and modified Giemsa stained smears was found 48.89% (table 6). Another study by Oyedeji et al (2002) from Nigeria reported sensitivity of 61%. Another study by Arora et al (2003) from India reported sensitivity of direct gram stained smear 20% that seemed quite lower than our study. The result differed from the study by Sengupta et al (2002) from India, Van Horn and Dworkin (1990) from New York as they reported sensitivity of 84% and 88% respectively. Presence of low number of organism in biopsy specimen and not much expert in microscopy might be the reason of not getting satisfactory result by direct microscopy in our study.

In the present study, we calculated sensitivity and specificity of Anti-\textit{H.pylori} Ig G at different cut-off level. The corresponding values at 5 arbU/ml, 10 arbU/ml, 20 arbU/ml and 30 arbU/ml were found as 95.55% and 11.11%, 80% and 28.89%, 51.11% and 62.22%, 40% and 75.55% respectively (table 7).

In the present study, when cut-off level was considered as $\geq5$ arbU/mL, according to the manufacturer’s guidelines, the test showed high sensitivity in terms of very low specificity. Similar findings correlate with a study by Morshed et al (1997) from Bangladesh. They reported sensitivity 100% and specificity 13.6% for anti-\textit{H.pylori} IgG by ELISA holding only culture result as gold standard. Kullavanijaya et al (2004) from Thailand had reported sensitivity 96.8% and specificity 73.1% which does not support our study. Considering the cut-off value as10 arbU/ml, the sensitivity and specificity almost correlate with a study by Dhar et al (1998) from Kuwait. They found 88% sensitivity and 17% specificity and concluded that there is a poor correlation between the
presence of *H. pylori* infection and the antibody response which could be explained either because of low sensitivities and specificities of the commercial kits used for the measurement of antibodies to *H. pylori* in the serum or because of poor immunological response in their patients to *H. pylori* antigens. In the present study, we found sensitivity of 51.11% and specificity of 62.22% by ELISA for *Anti-H.pylori* IgG when cut-off value was considered 20 arbU/ml, almost similar finding was found in a study by Babay Hannan et al (2000) from Saudi Arabia. They reported 50% sensitivity and 86% specificity which they explained past infection in comparison to recent infection. At this same cut-off value (20 arbU/ml), another study from India by Arora et al (2003) reported sensitivity of 76% which does not correlate with our study. Considering the cut-off value 30 arbU/ml, we found sensitivity of 40% and specificity of 75.55%. The result was not satisfactory.

It has been suggested that the usefulness and practicality of the tests are related to the background prevalence of *H. pylori* infection in a specific geographic area (Rauws and Tytgat, 1990). The infection rate of *H. pylori* in the general population of Bangladesh has been found to be very high. Ahmed et al (1997) from Bangladesh found *H. pylori* specific IgG antibody by ELISA in 92% of asymptomatic subjects who attended at the health check-up centre of Bangladesh Institute of Diabetes, Endocrine and Metabolic Disorders (BIRDEM). Geographic variations in the performance of commercial serological tests have received much attention recently, particularly in developing nations. Serological assays developed from *H. pylori* strains of the West and validated in Caucasian populations might have problems when applied in Asian patients. Most of these tests suffered from a poor specificity due to frequent false-positive results (Leung
et al, 2001). Bodhidatta et al (1993) showed that the use of an in-house serological assay which was based on an indigenous *H. pylori* strain was superior to a commercial test in a Thai population (sensitivity 98% vs. 85%; specificity 76% vs. 66%). In another study by Leung et al (1999) evaluated three commercial ELISA tests (developed and validated in USA) for *H. pylori* infection in Chinese population with disappointing results (sensitivities: 50–75%; specificities: 68–97%). On the other hand, Groves et al (1997) from USA reported 100% sensitivity of an ELISA test developed from a pool of Chinese *H. pylori* strains when applied in Chinese patients. Apart from the ethnic differences, genetic heterogeneity of *H. pylori* strains from different parts of the world seems to attribute to this discrepancy. Thus, it is likely that antigenic profiles of bacterial strains from different regions vary and conventional serological tests, which are developed and validated for a specific population, may not be applicable universally (Leung et al, 2001). The diagnostic efficacy of the ELISA test is highly dependent on the *H. pylori* antigen used. In this study, we applied ELISA *H. pylori* IgG kit (developed in France) which was based on an immunodominant antigen derived from tissue culture of a virulent strain of *H. pylori*. The cut-off value (≥5 arbU/mL) that recommended by the manufacturer, were originally validated in French population with dyspepsia. At this low antibody titer, the test showed very unfavorable low specificities despite their satisfactory sensitivities in diagnosing *H. pylori* infection in our population. The impact of changing cutoff point’s was also examined in the present study. The wide range in sensitivities and specificities of the commercial ELISA-kits have prompted using local strains for the *H. pylori* antigen and the cutoff level used for serodiagnosis in the general population needs to be adjusted for better diagnostic performance (Hoang et al, 2004).
In the present study, detection of anti-\textit{H. pylori} IgG was not significant (p > 0.05) by chi square test) for diagnosing \textit{H. pylori} infection in symptomatic population. The findings of higher seropositivity in asymptomatic population (compared to seropositivity rates found in symptomatic individuals) might be due to having \textit{H. pylori} infection in asymptomatic population without upper gastrointestinal tract symptoms. Many studies reported that determination of IgG levels can act as an important screening procedure (Arora \textit{et al}, 2003). In our study, a small number of populations (both patients and asymptomatic group) were included. Further large scale studies may be needed to detect serum IgG antibodies to \textit{H. pylori}.

In the present study, we found sensitivity of 73.33% and specificity of 93.33% by ELISA for anti-\textit{H. pylori} IgM (Table 8). Few studies have examined the seroprevalence of IgM antibodies to \textit{H. pylori}. Alem \textit{et al} (2002) from USA found IgM antibodies to \textit{H. pylori} significantly higher in symptomatic patients (10.4%) than in asymptomatic individuals (1.1%) and also reported the percentage of sera positive for IgM alone was higher in symptomatic than in asymptomatic groups (3.8 vs 0.22%). They concluded that ELISA can be used for the detection of specific IgM to \textit{H. pylori} and the presence or absence of IgM antibodies to \textit{H. pylori} may reflect whether or not an acute infection exists (Alem \textit{et al}, 2002). In our study, anti-\textit{H. pylori} IgM was found positive in 6.67% percent (3 out of 45) of the asymptomatic population which almost correlates with a study done by Andersen \textit{et al} (1996). They studied on unselected Danish populations and found increased IgM antibodies 7.6 percent and also suggested that inclusion of IgM antibody
measurements in future serologic *H. pylori* screening may improve diagnostic sensitivity considerably

In the present study, detection of anti-*H. pylori* IgM was highly significant (p < 0.001 by chi square test) for diagnosing *H. pylori* infection in symptomatic population. The presence of IgM antibodies is reported to be correlated with acute infection while IgG antibodies become present at different titers shortly after primary infections and last in blood for many years. In a study by Anderson *et al* (1996) from Denmark, presence of increased IgM antibody level to *H pylori* unaccompanied by an increase in specific IgG antibody level was interpreted as a serologic sign of primary *H pylori* infection. They also reported that concomitantly increased IgM and IgG antibodies were assumed to reflect reactivation of a chronic *H. pylori* infection or reinfection / superinfection with *H. pylori*. (Anderson *et al*, 1996).

**CONCLUSION AND RECOMMENDATIONS**

From the present study it may be concluded that sensitivity and specificity of ELISA for anti-*H. pylori* IgM is satisfactory and it can be used for the diagnosis of *H. pylori* infection among the dyspeptic patients of young age in whom gastric cancer is unlikely. Endoscopy remains the method of choice for elderly dyspeptic patients and for those with possible gastric or duodenal pathology. Detection of anti-*H. pylori* IgG
can not distinguish present and past infection and can be used only for screening purpose. Since the present study included a small sample size, so further study with large sample is recommended for determination of serum anti-\textit{H. pylori} IgG as an important screening procedure. Among invasive procedures, RUT is a reliable method because of its good sensitivity and specificity.

As the infection rate of \textit{H. pylori} in the general population of Bangladesh has been reported to be very high so the following recommendations are suggested:

1. Person with dyspepsia should be investigated by serological test for the presence of anti-\textit{H. pylori} antibodies and treated if present.
2. Cigarette smoking and taking tobacco or jarda should be avoided.
3. Poor hygiene and crowded conditions must be improved.
4. Proper sanitation and use of sufficiently clean water must be ensured to reduce the risk of \textit{H. pylori} infection.

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