HELICOBACTER PYLORI: DIAGNOSIS, SEROPREVALENCE, MODEL THERAPY FOR DUODENAL ULCER PATIENTS IN BANGLADESH

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I hereby humbly declare that this thesis, entitled "*Helicobacter pylori*: diagnosis, seroprevalence and model therapy for duodenal ulcer patients in Bangladesh", is based on work carried out by me and that no part of it has been presented previously for any higher degree.

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This is to certify that Dr. Mian Mashhud Ahmad has completed this thesis as fulfilment of the requirement for the degree of Doctor of Philosophy (PhD) under my guidance. His work is genuine and up to the mark.

This thesis is submitted in fulfilment of the requirements for the degree of Doctorate of Philosophy (PhD) granted by the University of Dhaka. The work has been carried out in the Department of Gastrointestinal, Liver and Pancreatic Diseases, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka Medical College and Hospital (DMCH) and Bangladesh Institute of Research and Rehabilitation in Diabetes Endocrine and Metabolic Disorders (BIRDEM), Dhaka, from 1995 to 1999.

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ABSTRACT

1. ABSTRACT

Bangladesh is one of the developing countries having reported highest point prevalence of duodenal ulcer among the population above the age of 15 years. Therefore, to investigate different aspects of *H. pylori* in Bangladeshi patients, studies were undertaken in successive phases. At first, standardization of different diagnostic methods were done. Evaluation of efficacy of different diagnostic tests have shown that rapid urease test (RUT) was found to be the most sensitive (95.5%) and specific (82.6%) and had very high positive (84%) and negative (95%) predictive values when compared with culture results as the *gold standard*. The results have also shown that more than 95% *H. pylori* seropositivity in both of the duodenal ulcer and non-ulcer dyspepsia patients.

Prevalence of *H. pylori* infection have shown more than 90% *H. pylori* seropositivity among our asymptomatic adults. It is also noticed that by the age of 20, all were infected.

Comparison between dual therapy (omeprazole + amoxicillin [OA], ranitidine + amoxicillin [RA]) and triple therapy (omeprazole + tinidazole + amoxicillin [OTA] and colloidal bismuth subcitrate + amoxicillin + metronidazole [CAM]) regimens were studied in a pilot clinical trial. *H. pylori* eradication rates were: OA - 58.3% and RA - 69.2%, OTA - 84.6% and CAM - 83.5%.

Antibiotic susceptibility studies have shown that 54 to 75 percent of *H. pylori* strains isolated from our patient were metronidazole resistant *in vitro*.

Furazolidone (F), a nitrofuran, has potential cytoprotective effect on gastroduodenal mucosa and known to have antibacterial activity against *H. pylori*. Clinical trial comparing furazolidone based triple therapy regimens (CAF, OAF and RAF) over nitroimidazole based regimens (OAM and OAT) was done in the next phase. Overall, *H. pylori* eradication rates were >90% in furazolidone based group and >85% in nitroimidazole based group. More than 80% *H. pylori* eradication was achieved in patients with pretreatment metronidazole-resistant *H. pylori* strains, treated with nitroimidazole based triple therapies (OAM and OAT). Sixteen patients (17%) were reinfected over 12 months (11 within 6 months, 2 at 9 months and 3 after 1 year) following successful eradication of *H. pylori* infection.

INTRODUCTION

PEPTIC ULCER AND *HELICOBACTER PYLORI*

2. INTRODUCTION

2.1. PEPTIC ULCER DISEASE

Peptic ulcers are defects in the gastroduodenal mucosa extending through the muscularis mucosae that persist as a function of the acid-peptic activity in gastric juice. Since the 1980s, a revolution has overturned understanding of peptic ulcer. Peptic ulcer occurs in two common forms: one is associated with *Helicobacter pylori* and the other is with consumption of nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin (Warren and Marshall, 1983; Soll, 1998).

2.1.1. Ulcer pathogenesis

Normal gastric mucosal defense and repair mechanisms protect the mucosa from acid-peptic injurious factors, such as environment and different noxious agents we ingest. Except hypersecretory states, acid-pepsin only cause ulcers when mucosal defense, repair and healing mechanisms are disrupted by *H. pylori*, NSAIDs or possible other factors, such as Herpes-Simplex virus-1 (HSV-1), cytomegalovirus (CMV) infection, etc.

Critical factors in the pathogenesis of peptic ulcer are *H. pylori* infection and use of NSAIDs (Calam, 1998). Robust (high normal or high) acid secretion is necessary to develop *H. pylori*-associated duodenal ulcer. Critical covariables in

the pathogenesis of duodenal ulcer are decreased duodenal bicarbonate secretion and duodenitis or gastric metaplasia. Smoking as a single environmental factor interfere with mucosal defense, healing or secretory regulation (Soll, 1998).

2.1.2. Pathology of peptic ulcers

Histologically, acute lesions, such as occurring with aspirin injury, are generally multiple and shallow, with minimal surrounding inflammation or fibrosis. In contrast, four histologic zones have been described surrounding chronic peptic ulcers: a superficial layer of fibrin and exudate, with successive underlying zones of fibrinoid necrosis, granulation tissue and fibrosis (Tarnawski *et al.*, 1995). Chronic ulcers are usually single, although 5-20% may be multiple. Healing ulcers are initially covered with a single layer of undifferentiated epithelial cells, followed by variable resolution of the underlying inflammation (Tarnawski *et al.*, 1995; Soll, 1998).

2.1.3. Ulcer location

The localization of ulcers impacts pathophysiologic and clinical features. Four subsets of ulcers require consideration:

1) Duodenal ulcer (DU)
2) Distal gastric ulcer (GU), comprising distal antral or prepyloric ulcers
3) Proximal GU, frequently occur on the lesser curvature, near the angularis
4) Ulcers in the gastric cardia, more inclined to complicate than ulcers in other locations (Johnson, 1965; Csendas *et al.*, 1987; Boyd *et al.*, 1991)

2.2. *HELICOBACTER PYLORI*

2.2.1. *Helicobacter pylori*: an overview

The organism

For many decades, the dictum 'no acid, no ulcer' dominated thinking in the pathogenesis of peptic ulcer. In the eighties, two major developments occurred; the discovery of *Helicobacter pylori* and the characterization of proton pump. Proton pump inhibitor (PPI), revolutionized the therapeutic approach to peptic ulcer disease (PUD) (Farthing, 1998).

Taxonomy of *Helicobacter pylori*

A general prokaryotic phylogenic tree of 253 representative species was derived by maximum likelihood analysis of 16S rRNA sequences which positions *H. pylori* in the delta and epsilon subdivision of the purple bacteria (proteobacteria) (Olsen *et al.*, 1994). The most closely associated genera were *Wolinella* (represented by *W. succinogenes*), *Campylobacter* and *Thiovulum* - a little known sulphate-dependent marine bacterium. This bacterium also comprises RNA superfamily VI described by Vandamme *et al.* (1991) which is an independently constructed classification based on the use of systemic rRNA-DNA hybridization analyses (Owen, 1992). The most important stage in the development of the taxonomy at least in the formal nomenclature of these microorganisms was the proposal by Goodwin and colleagues (1989) to establish

a new genus called *Helicobacter* and that *Campylobacter pylori* was transferred to that genus as *Helicobacter pylori*. The creation of the new genus was argued mainly on the basis of the 16S rRNA (small subunit) sequence data, but several additional taxonomic features, that were unique to *Helicobacter*, further supported the proposal; sheathed flagella (up to seven) were present, rapid urease activity, a distinct SDS-PAGE protein profile with seven major bands, resistance to polymyxin B is a useful feature for identification of helicobacters (Burnens and Nicolet, 1993), the respiratory quinone thermoplasmaquinone-6 (TPQ-6) were not present, unique fatty acid (major fatty acids present were 14:0 and cyc 19:0 but 3-OH-14:) and 16:1 acids were absent) (Owen *et al.*, 1995), an external glycocalyx produced *in vitro* in liquid media and GC content of chromosomal DNA of 35-44 mol% (Owen, 1998).

Microbiological features of *Helicobacter pylori*

The microbiological features include the morphological, cultural, physiological and biochemical characteristics.

Morphological features

The name *Helicobacter* refers to the morphology of the organisms which are helical *in vivo*, though often rod-like *in vitro* (Owen *et al.*, 1995). *H. pylori* is generally described as a Gram-negative, s-shaped or curved rod (0.5-0.9 μm wide and 2-4 μm long) with one to three turns when observed *in vivo*. Spiral forms are also less obvious and cells appear more as singly curved rods. With

light microscopy, cells of *H. pylori* typically have about five to six polar (lophotrichate) flagella filaments at two days of growth. Cells are mostly actively motile although some cultures may appear to be non-motile in hanging drop preparations. Other forms of *H. pylori* seen in culture and occasionally *in vivo* include spherical, V-shaped, U-shaped and straightened forms (Owen *et al.*, 1995).

Cultural characteristics

Colonies of *H. pylori* from primary cultures on supplemented blood agar at 37°C, usually take 3 to 7 days to appear and are circular (1-2 mm), convex and translucent in appearance. There is slight hemolysis in blood agar around colonies, which are generally visible in older cultures. In older cultures, *H. pylori* undergoes a morphological change from bacillary to coccoid forms with an associated loss in culturability (Catrenich and Malin, 1991). Coccoid forms may be viable but more resistant forms of *H. pylori* reflecting a temporary adaptation to a hostile environment (Owen *et al.*, 1995).

Molecular characteristics

Seven species of *Helicobacter* have been reported to be associated with gastric mucosa of a variety of mammalian species and ten species have been associated with the intestinal mucosa (Owen, 1998). The complete genome sequence of *H. pylori* has recently been reported. The organism has a circular genome with more than 1.6 million base pairs.

The most striking features that the genomic analysis has revealed is the extent of molecular mimicry that exists between *H. pylori* and humans and add additional understanding to the mechanisms of acid tolerance, antigenic variation and new ideas on pathogenesis (Jiang *et al.*, 1996; Owen, 1998).

Unraveling of the genome of *H. pylori* has permitted the development of molecular techniques for diagnosis and typing. A variety of techniques are now available including restriction length polymorphism analysis with amplification using polymerase chain reaction (PCR), ribotyping and direct PCR typing. These molecular genetic approaches have enabled investigation of the molecular epidemiology of *H. pylori* infection, particularly the identification of reservoir and modes of transmission (Ge and Taylor, 1998).

Physiological features

H. pylori is a microaerophilic bacterium that grows best in an atmosphere of 5% oxygen with 5-10% CO_2 on blood-containing media such as Oxoid Brain-Heart Infusion Agar (BHI) containing 5% horse blood supplemented with 1% Iso-Vitalex (Owen *et al.*, 1995). The organisms grow optimally at 37°C after 3 to 7 days. All strains grow over a relatively narrow temperature range of 33-40°C, some grow poorly at 30°C and 42°C, but none grow at 25°C (Owen, 1995). *H. pylori* grows over a wide pH range of 5.5-8.5 with good growth between pH 6.9 and 8.0 depending on the culture medium (Goodwin *et al.*, 1989).

Biochemical characteristics

H. pylori is inactive in most of the conventional biochemical tests used to characterize medical bacteria, in particular, those used for other microaerophilic bacteria such as *Campylobacter* (Barrow and Felthem, 1993). However, *H. pylori* produces catalase and oxidase and is notable for its highly active urease and alkaline phosphatase activity. The enzyme urease accounts for over 5% of its total bacterial protein (Dunn *et al.*, 1990) and appears to be essential for gastric colonization.

Antibiotic susceptibility

Although *in vitro* antibiotic susceptibilities are of clinical relevance, but the fact is that laboratory tests of antibiotic activity may not accurately reflect the *in vivo* activity (Owen *et al.*, 1995). Antibiotic susceptibilities of *H. pylori* are well documented for treatment purposes (Morgan *et al.*, 1987). It is widely recognized that some strains of *H. pylori* differ in their susceptibilities to metronidazole, an antimicrobial commonly included in the treatment regimens, particularly triple therapies (Bell *et al.*, 1992). Activity against polymyxin B is of some taxonomic use because most strains of *Helicobacter* including *H. pylori* are resistant (300 IU disk), so it can be used to discriminate them from *Campylobacter* (Burnens and Nicolet, 1993). Resistance to clarithromycin has been found in up to 15% of strains in some parts of the world, jeopardizing its clinical efficiency (Mégraud, 1997). Resistance to metronidazole is even more widespread. Some reports claim metronidazole resistance of virtually all of the

strains in tropical countries. The clinical significance of this is controversial because only strains with a high level of resistance may be of clinical concur (Mégraud, 1997).

Habitat of *Helicobacter pylori*

Human sources

The mucous layer of the gastric epithelium of the human stomach, particularly of the antrum, is the principal known habitat of *H. pylori*, which has been isolated from gastric sites from diverse populations throughout the world (Owen *et al.*, 1995). The organism can be cultured from metaplastic gastric epithelium in the duodenum and from gastric fluid (Cover and Blaser, 1995). *H. pylori* has occasionally been cultured from saliva (Krajden *et al.*, 1989) and from dental plaque (Shames *et al.*, 1989; Majumdar *et al.*, 1990). Thomas *et al.* (1992) successfully isolated an organism from stools of Gambian children and identified as *H. pylori*. However, other investigators concluded that there was no substantial shedding of *H. pylori* in feces (van Zwet *et al.*, 1994). *H. pylori* has also been recovered from the blood culture of a patient with malignant lymphoma of the upper abdomen including the stomach (Ndawula *et al.*, 1994).

Other sources

H. pylori has been isolated occasionally from different animal species, including rhesus monkey, pig, baboon and domestic cats (Curry *et al.*, 1987; Bronsden and Schoenknecht, 1988; Handt *et al.*, 1994). *In vitro* experiments have shown

that *H. pylori* survives for several days in water, saline, foods and seawater if these are kept cool, but becomes nonculturable after one to three days at room temperature (West *et al.*, 1992).

Route of transmission of *Helicobacter pylori*

The route of transmission of *H. pylori* is assuming greater importance as the public health implications of infection become clearer. No predominant route of acquisition of *H. pylori* has been defined. Possibilities include oral-oral, fecal-oral, iatrogenic and vector transmission. Transmission appear to be via fecal-oral route or via the oral-oral route (Mendall and Pajares-Garcia, 1995).

Oral-oral transmission

Studies supporting the concept of oral-oral transmission of *H. pylori* have come from work examining gastric secretions, oral secretions and dental plaque. *H. pylori* has been shown to be present in the gastric juice of up to 58% of patients infected with *H. pylori* (Varoli *et al.*, 1991). Direct contact with such secretions has been implicated in the higher prevalence of *H. pylori* reported in gastroenterologists than in aged-matched controls; 52% compared with 21%, respectively (Mitchell *et al.*, 1989). In a study, the isolate from both the stomach and saliva were found to be indistinguishable by restriction enzyme analysis (Shames *et al.*, 1989). Majumdar *et al.* (1990) reported *H. pylori* from dental plaque of 100% of 40 healthy individuals.

Fecal oral route

The possibility of fecal-oral spread remains of great interest, but there are little data to support the concept. Thomas *et al.* (1992) reported the isolation of *H. pylori* from diarrheal stool of infected children age ranged 3-27 months in Gambia. Children from high-income families whose homes were supplied with municipal water were 12 times more likely to be infected than were those whose water supply came from community well. In Chile, Hopkins *et al.* (1993) found an association with increased infection and eating uncooked vegetables, which they relate to contamination of irrigation water with untreated sewage.

Iatrogenic spread

The outbreaks of achlorhydria reported by Ramsey *et al.* (1989) is an example of experimental *H. pylori* infection. *H. pylori* may be transmitted from one person to another via contact with endoscopes. Restriction enzyme analysis of bacterial DNA demonstrated that some individuals studied in a gastroenterologist practice were infected with identical strains of *H. pylori* (Langenberg *et al.*, 1990). With manual endoscope washing, such transmission occurs in 1 to 3% of endoscopies but does not appear to occur in modern endoscopy units where endoscopes are mechanically sterilized and washed (Langenberg *et al.*, 1990).

Vector transmission

Handt *et al.* (1994) recently found organisms indistinguishable from *H. pylori* in the stomach of group of cats. The housefly has been shown recently to have the

potential for mechanical transmission of *H. pylori*. Grubel *et al.* (1997) developed a hypothesis that flies might be able to pick up *H. pylori* in human waste, particularly from untreated sewage, mechanically carry it and then deposit contaminated fly excreta on food or even directly into the oral mucous membrane of young children, thus perpetuating the cycle of infection. This hypothesis is most acceptable to areas of the world with poor sanitation.

Pathogenesis

It is now clear that *H. pylori* is the major etiologic agent in the pathogenesis of peptic ulcer disease, but specific mechanisms in the progression from mucosal colonization to ulcer formation are still to be understood. It has also become clear that the degree of gastric inflammation and injury may vary with *H. pylori* strain type, and this has been corroborated by both *in vitro* and *in vivo* observations. With the exception of cytotoxin, the particular bacterial mediators that induce these differential responses have not been identified. *H. pylori* is a highly adapted bacterial parasite that over the millennia has developed a number of specific traits that enable it to survive in the hostile environment of the stomach and to colonize its natural niche of human gastric mucous. These traits include a specialized motility which facilitates the bacterium's passage across the viscous mucous (Lee *et al.*, 1995). In addition, *H. pylori* produces a modified urease enzyme which provides temporary protection from gastric acidity by the local generation of ammonia from endogenous urea (Lee *et al.*, 1993). Specific adhesins possibly play a role in gastric colonization (Hessey *et al.*, 1990). Although certain factors appear to predispose the host to infection by *H. pylori*,

clearly the bacterium possesses a number of virulence factors that allow the organism to colonize the gastric mucosa, evade host defense and damage host tissue. Together these factors allow *H. pylori* to persist in the host, establishing a chronic infection. The ability to withstand gastric pH and motility are the mechanisms of *H. pylori* to elude the primary defenses of the human stomach and to establish persistent infection. Acute ingestion of *H. pylori* leads to transient hypochlorhydria occurring secondary to parietal cell dysfunction, which may be in response to gastric inflammation. In untreated hosts, hypochlorhydria resolves within several months and intraluminal pH decreases to within the normal range (Morris *et al.*, 1989). Another pH altering mechanism is the production of copious amounts of urease by *H. pylori*. The ammonia generated by this enzyme is required for the bacteria to survive in the acidic environment of the stomach. To facilitate locomotion within the gastric mucus and to counteract peristalsis, *H. pylori* possesses one to five flagella with terminal bulbs at a single pole (Blaser, 1992). Once the bacterium has traversed the gastric mucus, a small percentage of organisms attach intimately to the surface of gastric epithelial cells. Although no adherence factor has been proven to be required for colonization, several putative adhesins and their ligands on host cells include hemagglutinins (Evans *et al.*, 1988). A lipid-binding adhesin (Linghood *et al.*, 1993) and an Lewis blood group antigen-binding adhesin (Boren *et al.*, 1992). Other factors may include an acid-inhibitory protein which can inactivate the gastric proton pump (Cave and Vargas, 1989), again neutralizing hazardous acid. The production of high levels of heat shock proteins may explain the ability to transiently tolerate the conditions met by the bacterium during the early stages of colonization (Cave and Vargas, 1989).

Host response to *Helicobacter pylori* infection

The immune response to *H. pylori* is of great interest, firstly, because leukocyte products are likely to damage host tissues, and secondly, because of the potential for immunization against *H. pylori*. The ideal host response provides protection to clear an infection without causing excessive inflammation. The inability to clear the infection and subsequent development of gastroduodenal disease probably results from inappropriate regulation by gastric T cells and their effects on immune and epithelial cell function. Several immune and inflammatory reactions probably contribute to damage the gastric and duodenal tissue. First, the infection and associated cytokines induce chemokines, such as IL-8 and growth-regulated gene α that, in turn, lead to neutrophil accumulation and activation. Subsequent transepithelial migration and release of neutrophil mediators can disrupt epithelial cell function. Second, gastric B cells detected during infection with *H. pylori* are often autoreactive. Moreover, many of these antibodies are IgG and capable of activating complement, would be highly destructive to the epithelial and underlying tissue. Gastric T cells become activated by the epithelial cells which lead to the production of cytokines such as IFN-gamma and IL-2 that promote cell-mediated immunity. Through these cytokines, the host response combines with the bacteria to increase cell-mediated immunity and epithelial cell death via apoptosis. The consequences of increased cell death include aberrant repair of the epithelium, which may lead to atrophy and/or metaplasia. This abnormal tissue would lack the full complement of cytoprotective mechanisms and may increase the risk of peptic ulceration (Ernst

et al., 1997). *H. pylori* infection leads to the development of gastric autoimmunity. The bacterium produces a 58 kDa heat shock protein (hsp) which closely resemble human hsp 60, and most people with *H. pylori* infection have antibodies against the bacterial antigen (Macchia *et al.*, 1993).

2.3. *HELICOBACTER PYLORI* AND PEPTIC ULCER DISEASE

H. pylori has a global distribution and infects human gastric mucosa exclusively but there is some evidence for infection in cats (Owen, 1998). Sixty percent of world population have *H. pylori* infection. Prevalence varies widely in different parts of the world, with average rates of 40-50% in western countries rising to more than 90% in the developing world.

The mode of transmission of *H. pylori* is vigorously debated, although current evidence suggests that direct person-to-person contact is the predominant mechanism. Transmission routes vary being largely oral-oral in the industrialized world and fecal-oral in the developing world (Mitchell *et al.*, 1992; Goodman *et al.*, 1996; Lindkvist *et al.*, 1996; O'Donohoe *et al.*, 1996).

Age of acquisition is critical in determining the clinical outcome of infection. The majority of infected individuals do not develop clinically apparent disease, but there is now indisputable evidence that 6-20% of infections result in peptic ulceration and a smaller proportion (less than 1%) are associated with gastric cancer (Farthing, 1998).

Infection in a given individual will result either in the peptic ulcer pathway with associated increased acid output, or the chronic atrophic gastritis-carcinoma pathway with associated hypo- or achlorhydria (Farthing, 1998; McColl *et al.*, 1998). Other conditions linked to *H. pylori* infection are: low-grade MALT gastric lymphomas, regresses in many instances following eradication (Wotherspoon *et al.*, 1993; Wotherspoon, 1998). Association of non-ulcer

dyspepsia (NUD) with *H. pylori* infection remains controversial. In western countries, 30-60% of NUD are *H. pylori* positive. There is no typical symptom pattern and pathophysiological marker which identifies infected from noninfected NUD. Short-term eradication studies have been disappointing, although there is evidence that benefit may accrue a year or more following cure (The European *Helicobacter pylori* Study Group, 1997; Farthing, 1998; Talley *et al.*, 1998).

Host-pathogen interaction

Gastric inflammation is an inevitable consequence of *H. pylori* infection (Crabtree, 1996). Adherence of organism produces a direct injurious effect on the epithelium, complicated by production and release of a vacuolating cytotoxin, *Vac*A. *H. pylori* urease is thought primarily to enhance the organism's acid tolerance by ammonia production and this at the same time, toxic to the epithelium (Atherton, 1998; Farthing, 1998). Direct damage to the epithelium results in release of chemokines for neutrophils, monocytes and lymphocytes. Neutrophil infiltration is an important component of gastric inflammation and epithelial cell damage. Mononuclear cells are secondary source of inflammatory mediators and initiate specific immune responses, particularly T-cell activation.

The severity of gastric mucosal inflammation is *H. pylori* strain dependent. Organisms carrying the *Cag*A gene induce a more profound proinflammatory cytokine response and a more severe gastritis (Bodger and Crabtree, 1998; Farthing, 1998).

Autoimmunity, an important aspect of the *H. pylori*-host response, may play a role in the epithelial cell destruction and mucosal damage. Molecular mimicry of host antigens has been demonstrated in the gastric epithelium of mice and humans, and monoclonal antibodies raised against *H. pylori* recognize a gastric epithelial cell epitope (Negrini *et al.*, 1991, 1996).

PATHOPHYSIOLOGY

2.3.1. *Helicobacter pylori* and gastric secretion

Gastrin

Helicobacter pylori gastritis, which is confined to the antrum and unaccompanied by atrophy, results in hypersecretion of acid. This is the pattern of gastritis seen in subjects with duodenal ulceration (Dixon, 1991). The increased acid secretion in subjects with antral predominant nonatrophic gastritis is mainly due to the *H. pylori* gastritis stimulating increased release of the hormone gastrin which circulates and stimulates the body of the stomach to secrete acid (El-Omar *et al.*, 1993, 1995).

Subjects with *H. pylori* antral gastritis have increased basal, meal stimulated and gastrin-releasing peptide (GRP) stimulated serum gastrin concentrations. The hypergasteremia fully resolves within 2-14 days of commencing *H. pylori* eradicating therapy indicating that it is caused by the infection (Levi *et al.*, 1989; McColl *et al.*, 1989, 1991; Chittajallu *et al.*, 1991; El-Omar *et al.*, 1993, 1995; Graham *et al.*, 1993). The increased circulating gastrin associated with

H. pylori is mainly due to an increase in gastrin-17 (Mulholand *et al.*, 1993). This form of gastrin originates mainly from the antral mucosa and its selective increase is consistent with the infection predominantly affecting this region.

The release of gastrin by antral mucosa is under physiological inhibitory control in order to prevent excessive gastric acid secretion. Gastrin release is suppressed with antral luminal pH falls below 3.0. In addition, there is inhibitory control exerted on gastrin release by cholecystokinin and other enterogastrones released from the small intestine. The inhibition of gastrin release exerted by both gastric acid and cholecystokinin is mediated mainly via the release of somatostatin by D cells within the antral mucosa. These D cells lie in close proximity to the G cells and the somatostatin they release exerts paracrine inhibitory control on both gastrin synthesis and release (McColl *et al.*, 1998).

The mechanism by which *H. pylori* results in depletion of antral somatostatin has still to be elucidated, but there are at least three potential mechanisms. The first proposed by Calam's group is that *H. pylori* raises mucosal surface pH by virtue of its high urease activity and ammonia synthesis (Levi *et al.*, 1989). Elevated antral pH leads to atrophy of antral D cell by blocking the chronic trophic stimulus exerted by gastric acid.

The second mechanism by which *H. pylori* antral gastritis might alter G and D cell function is via the local production of various cytokines (IL-6 and TNFα) (Crabtree *et al.*, 1991; Crowe *et al.*, 1995). The third mechanism by which *H. pylori* might suppress antral somatostatin is related to its recently reported

production of 'N-alpha-methyl histamine', which is a potent H_3-receptor agonist (Courillon-Malet *et al.*, 1995). Such receptors have been demonstrated on human antral D cells and their activation inhibits somatostatin release and consequently increases gastrin release (Schubert *et al.*, 1993, 1994).

Acid secretion

It is now recognized that there are complex two-way interaction between *H. pylori* gastritis and gastric acid secretion. *H. pylori* gastritis, the relative extent to which the gastritis involves the antral and body mucosa, affects acid secretion. Acid secretion again affects *H. pylori* gastritis and the extent of involvement of the body versus antral mucosa (McColl *et al.*, 1998).

This interaction is seen when subjects with infection are treated with proton pump inhibitor (PPI) therapy. The pharmacological suppression of acid secretion transforms the antral predominant gastritis to body predominant gastritis, lead to further low acid secretion. This interaction explains different response to the infection and different disease outcomes. Subjects with premorbid natural high acid secretion will develop an antral-predominant body-sparing gastritis and this form of gastritis stimulates further increase in acid secretion leading to duodenal ulcer disease. Subjects with premorbid low acid secretion will develop a body predominant gastritis and this further reduces the acid secretion leading to hypochlorhydria and risk of gastric cancer. In the majority of subjects with normal premorbid acid secretion, the gastritis will involve both the antral and body mucosa, result in no significant change in acid

secretion and thus no clinical disease (Hui *et al.*, 1991; Kuipers *et al.*, 1995; Logan *et al.*, 1995; McColl *et al.*, 1998). Other factors contribute to complex interaction and influence disease outcome are diet, smoking, antisecretory drugs, bacterial strains and autoimmune responses (Dixon, 1991; Negrini *et al.*, 1996).

2.3.2. *H. pylori*, gastritis and peptic ulcer

H. pylori causes a chronic, active gastritis that involves predominantly the antrum or the entire stomach. *H. pylori* induces diffuse antral gastritis (antritis), has been recognized as ulcer-associated gastritis. Association of antritis holds equally for both DU and GU. The antritis associated with DU is usually mild to moderate in intensity, severe with occasional antral gland disruption, atrophy and intestinal metaplasia in GU (Kuipers *et al.*, 1995).

2.3.3. Oxyntic gastritis

Progression occurs in GU not in DU. Although the predominant inflammatory process in peptic ulcer occurs in the antrum, patients with GU commonly have moderate body gastritis progresses with age, eventually leading in some individuals to fundic gland atrophy and hyposecretion of acid and pepsin (Tatsuta *et al.*, 1986; Fiocca *et al.*, 1992; Kuipers *et al.*, 1995). In contrast, the acid-secreting oxyntic mucosa in DU is usually less inflamed, a pattern consistent with robust acid secretion and inflammation usually progresses slowly. There is increasing evidence that the high level of acid secretion *per se* retard progression of gastritis in DU (Fiocca *et al.*, 1992; Kuipers *et al.*, 1995).

2.3.4. *H. pylori*, duodenitis and gastric metaplasia in DU

Duodenitis and gastric metaplasia (GM) occur in normal persons but are more extensive in those with DU, chronic inflammation (duodenitis) and chronic injury by gastric acid are important factors for GM. *H. pylori* appears to be an important factor associated with both duodenitis and the extent of GM. Support for this association comes from the findings that cure of infection and profound, prolonged inhibition of acid secretion independently reduce the extent of GM (Carrick *et al.*, 1989; Wyatt *et al.*, 1990; Khulusi *et al.*, 1996).

2.3.5. *H. pylori* association with peptic ulcer

Proof of a causal role for *H. pylori* in peptic ulcer rests on two points: (1) a tight association - the majority of ulcer patients are *H. pylori* infected (Rauws *et al.*, 1988), and (2) cure of *H. pylori* infection reduces ulcer recurrence. The prevalence of *H. pylori* for both DU and GU is much greater than for age-adjusted controls. The presence of *H. pylori* gastritis determines the risk for development of peptic ulcer. The yearly incidence of peptic ulcer in *H. pylori*-infected adults is only about 1%.

Conclusions regarding mechanisms by which it induces peptic ulcer are largely restricted to studies observing the consequences of its cure (Soll, 1998). Reasonable hypotheses have been proposed for the virulence of *H. pylori* for inducing gastritis. Several virulence factors for gastritis have been proposed although it is unclear which are most important. For example, production of

cytotoxin, *Cag*A has been found in the large majority of ulcer patients, but it is also frequently found in subjects without peptic ulcer (Blaser, 1996; Peck *et al.*, 1996).

2.4. DIAGNOSIS OF *HELICOBACTER PYLORI*

Diagnosis

Different invasive and noninvasive diagnostic tests are available for diagnosis of *H. pylori* infection in the individual patient. Several large-scale comparative studies have shown that invasive and noninvasive tests performed equally well, with a sensitivity and specificity in the range of 90% (Hirsch *et al.*, 1993; Thijs *et al.*, 1996). Choice of a particular test depends on local availability and expertise, as well as on clinical circumstances.

2.4.1. Invasive tests

Endoscopic or invasive tests are culture, histology and biopsy urease test, best for primary diagnosis of infection, and endoscopy allows assessment of treatment indications (Glupczynski, 1998).

Rapid urease test

For the diagnosis of *H. pylori* in clinical practice among patients who benefit from an endoscopy, the basic test is the urease test. It is an inexpensive and quick test and is also easy to perform. Sensitivity and specificity are linked, and depend on the time laps before the results of the test are read. With a delay of 1 hour or less, the specificity is very good but the sensitivity is not optimal. The test is based on the unusually potent urease activity of *H. pylori*. This enzyme hydrolyzes available urea to release two ammonia molecules and one carbon

dioxide from one molecule of urea. The free ammonia increases the pH in the medium and the pH indicator, phenol red changes color. The great advantage of this test is that it can be performed in the endoscopy room as soon as the biopsy has been taken. The biopsy is placed on an urea agar and a color change (from yellow to pink) is recorded after 30 minutes and after 2 hours. The sensitivity of the test before treatment is in the range of 75-80% (Marshall *et al.*, 1987) and the specificity is excellent. A positive result requires the presence of approximately 10^5 colony forming units (CFU) (Mégraud, 1995). Alternatively, if the test is read after more than 24 hours, not only is the advantage of rapidity lost, but while the sensitivity increases, the specificity decreases. Rapid urease test only answers the question of the presence or absence of the bacteria without giving an indication on the status of the mucous and the susceptibility to antimicrobial agents.

Direct microscopic examination

Direct microscopic examination of a specimen can be useful in the detection of certain bacteria, but has limited sensitivity. It involves looking for spiral-shaped bacteria on a smear that has been Gram stained. The smear is prepared by grinding the biopsy specimen in normal saline and placed on a glass slide keeping the mucous side down. After Gram staining, the spiral and curved bacteria appear Gram-negative and are unusually found in large numbers in different zones of the slide. This is a quick, simple and inexpensive test with sensitivity of about 80% (Montgomery *et al.*, 1987). This method loses sensitivity if few bacteria are present.

Culture

Culture is considered to b the 'gold standard' for detection of *H. pylori*. In theory, culture is the most sensitive technique for isolation of *H. pylori* because even one organism in the specimen will multiply to produce a population of billions and they give a positive result. Van Zwet *et al.* (1993) have reported an excellent level of sensitivity for culture and considered it to be the best method. The specificity of culture is undoubtedly the best of all of the techniques because once colonies are established, it is possible to perform all of the tests required for the identification of the bacterium including all of the molecular tests. Culture is essential for research purpose and it is the culture that led to the rediscovery of *H. pylori*.

Although it is still generally considered as tedious procedure, culture can now-a-days be performed with minimal difficulties in centers with a standard microbiology laboratory.

The risk of sampling error is probably lower than generally supposed. It is advisable to take two biopsy samples for culture. Culture from either the antrum or the corpus has an excellent diagnostic yield in untreated patients, but sampling of both gastric sites is recommended following treatment in order to optimize the detection of *H. pylori* (Mégraud, 1996). Another factor that may influence the success rate of culture involves the transport conditions from endoscopy room to laboratory. Several liquid or semi-solid transport media have been recommended, but it is not clear whether a specific medium composition is

superior for this purpose. However, the recovery rates of *H. pylori* from gastric biopsy were not adversely affected by a delay of culture up to 24 hours when transport media were held at a room temperature. For transportation or storage for longer than 24 hours, a lower temperature might be an important factor for survival.

However, culture of *H. pylori* provide useful information of strains susceptibility to antimicrobial agents which is helpful for effective treatment regimen for *H. pylori* eradication.

Histology

Histological examination of the mucosal biopsy specimen for the presence of *H. pylori* and/or gastritis is generally considered the gold standard for diagnosis of *H. pylori*.

It also provides information on long-term consequences of inflammation such as atrophy, intestinal metaplasia, dysplasia and an eventual malignant process. The sensitivity of histological techniques can be high. The Sydney System proposes that two biopsies should be taken from each site within the stomach (body and antrum) to avoid any sampling error (Price, 1991). The specificity of histological tests like the sensitivity depends on the expertise of the pathologist. It can be difficult to identify *H. pylori* when the few bacteria present are of atypical morphology. Sensitivity and specificity of the histological diagnosis are high, in the range of 95% provided the test is done by an experienced pathologist (Christen *et al.*, 1992). An important advantage of histology is that it allows the

identification of chronic active gastritis. Its presence almost always indicates *H. pylori* infection and the bacteria should be carefully sought. The problems in using histology arise because the results depend on the observer; the same specimens may have different results, which depend on the pathologist's remark. Obtaining multiple biopsy specimens and use of Warthin-Starry stain and Genta stain (combination of hematoxylin and eosin, silver stain and Alcian blue) maximize diagnostic yields. In ordinary practice, the sensitivity level of histology is too low to enable it to be used as the sole method for detection of infection (taylor *et al.*, 1987). In the event of a negative result, another test should be done.

Polymerase chain reaction (PCR)

Polymerase chain reaction assay has been shown to be a valuable method for detection of various microorganisms including *H. pylori*. PCR has been applied successfully to identify the bacterium in feces at a significantly greater frequency that would be expected from direct culture (Mapstone *et al.*, 1993). Specific DNA sequences of *H. pylori* can be amplified by using pairs of specific oligonucleotides containing 10-15 bases (primers). The analysis of the amplification products allows the detection of *H. pylori* DNA with a great sensitivity and specificity (Glupczynski, 1998). Bacteria do not have to be viable, and coccoidal forms can also be detected. The biopsy should be homogenized and/or lysed using a specific buffer in order to make DNA accessible. A reaction mixture contains a thermostable DNA polymerase (*Taq* polymerase), nucleotides, primers, and the sample to be tested for *H. pylori*

DNA. A series of 30 to 40 amplified cycles are performed, each one comprising denaturation of the DNA strands, annealing and elongation. The amplification products are then analyzed. The minimum analysis required is the identification of a DNA band on electrophoresis which has the molecular weight as predicted by the primers used. It is possible to confirm the identity of the amplified product by hybridizing it to a labeled internal probe specific to the amplified product (Mégraud, 1997). Several kinds of primers have been proposed for the identification of *H. pylori*. The most common are derived from the urease gene (Clayton *et al.*, 1992) and the 16S rRNA gene (Ho *et al.*, 1991). The detection of *H. pylori* using PCR is comparable to culture and histology in terms of sensitivity. The use of a second pair of primers from a different gene enhance sensitivity of the method. The advantage of PCR is that it allows molecular typing on the status, either by digesting of the amplified products with restriction endonuclease, or by sequencing (Glupczynski, 1998). Strict endoscope cleaning and laboratory procedures are required to prevent cross-contamination.

Nucleic acid hybridization

Nucleic acid hybridization is a very simple, specific and prone-to-automation colorimetric hybridization assay which can be used to detect amplified *H. pylori* DNA (Lage *et al.*, 1996). The technique is suitable for detecting *H. pylori* infected patients and also for monitoring the eradication of the pathogen after treatment. The method combines a sensitive sandwich DNA hybridization and a colorimetric protocol. The method proved to be more sensitive than gel detection of PCR products.

2.4.2. Noninvasive tests

Urea breath test (UBT) and serology are the global tests, assess the global presence of *H. pylori* in the stomach even when the bacteria are irregularly distributed on the gastric mucosa (Glupczynski, 1998).

Urea breath test (UBT)

Normal human stomach is devoid of urease. Presence of urease activity is a marker of active infection, with UBT, urea labeled with either ^{13}C or ^{14}C is ingested. If urease is present in the stomach as a consequence of *H. pylori* infection, labeled CO_2 will be split off and absorbed into the circulation, where its presence can be determined by analysis of expired breath (Bell *et al.*, 1987; Graham *et al.*, 1987).

^{13}C UBT is a nonradioactive test, where $^{13}CO_2$ is detected by mass spectrometry. ^{14}C UBT has low-dose (1 microci) radioactivity, is less expensive and detected by scintillation counter. This test is avoided in children and women of childbearing potential.

Performance characteristics of both tests are similar and not prone to sampling error. These tests are accurate for assessing post-treatment *H. pylori* status. Antisecretory drugs, bismuth and antibiotic ingestion lead to false negative result. However, UBT is still not available widely (Bell, 1998).

Serology

Chronic *H. pylori* infection elicits a circulating IgG antibody response that can be quantitated by ELISA tests. Tests on serum IgA or IgM antibodies are unreliable. Serologic tests are as sensitive and specific as biopsy-based methods. Serologic testing has been recommended for initial pre-endoscopy or pretreatment screening in dyspeptic patients. But these tests are less useful to confirm cure after antimicrobial therapy. Although it has been reported that a fall in paired titers of 20% or more 6 months after completion of therapy may be sensitive in confirming cure of the infection. This seems difficult to apply routinely because collecting and storing pretreatment specimens may be problematic in clinical practice and also because 6 months is a long time to wait for results (Meijer *et al.*, 1997; Glupczynski, 1998; Howden and Hunt, 1998).

2.4.3. Diagnostic strategy

Slow growth, fastidious nature and special growth requirements have made culture of *H. pylori* difficult. Isolation by microbiological culture and histological detection of the organism in gastric mucosal biopsy specimens are still the gold standard for diagnosis of *H. pylori* infection (Westblom, 1991).

The selection of the appropriate test in a given patient depends on the clinical situation. For patients in whom an endoscopy is clinically indicated to diagnose or treat a peptic ulcer, the test of first choice is rapid urease test (RUT), if RUT is negative, confirm the infection by histology or culture. For patients with

gastric ulcer (GU), extra biopsy specimens should be taken from ulcer edge to rule out malignancy. Culturing for antibiotic susceptibility testing is not currently practical but may become necessary if resistance to metronidazole or clarithromycine increases.

For patients in whom endoscopy is not indicated for other clinical reasons, the procedure should not be performed solely to diagnose *H. pylori* infection. A urea-breath test (UBT) is the best for documenting active infection. Serologic tests are quick, inexpensive, are the initial screening test of choice (Howden and Hunt, 1998; Peerson and Graham, 1998).

2.4.4. Principles of testing for *H. pylori* infection

Recommendation

Diagnostic testing for *H. pylori* infection should only be performed if treatment is intended. Testing for *H. pylori* infection is indicated in patients with active peptic ulcer disease, a past history of documented peptic ulcer, or gastric MALT lymphoma.

Testing of *H. pylori* is not indicated in asymptomatic individuals without a past history of peptic ulcer disease, or in patients on long-term treatment with proton pump inhibitor for gastroesophageal reflux disease (GERD). GERD patients who also have duodenal or gastric ulcers should be tested for *H. pylori* infection and treated appropriately if positive (Howden and Hunt, 1998).

2.5. TREATMENT OF *HELICOBACTER PYLORI* INFECTION

Prior to the discovery of *H. pylori*, the treatment of peptic ulcer was with a range of ulcer healing agents which included H_2-receptor antagonist, proton pump inhibitor, sucralfate, colloidal bismuth subcitrate and antacids. Treatments with these agents for six to eight weeks resulted in healing of 80-90% of peptic ulcers. However, more than 80% of such patients had one or more recurrences within a year of stopping treatment. As a result, many patients needed repeated courses of treatment and a proportion of these required continuous treatment either because of frequent recurrences or because of risk of complications.

The strong relationship of *H. pylori* with peptic ulcer disease is inferential and is largely based upon a plausible hypothesis, the strong relationship with *H. pylori*-induced gastritis and faster healing of PUD with *H. pylori* suppression (Marshal *et al.*, 1988; Graham *et al.*, 1991; Ahmad *et al.*, 1999) and markedly decreased recurrence rates of ulcer disease after *H. pylori* eradication (Lambert *et al.*, 1987; Hopkin *et al.*, 1996). Rates of ulcer recurrence in patients whose initial ulcers healed during conventional antisecretory therapy range from 60 to 100 percent per year, but ulcers recur in less than 15% of patients in whom the *H. pylori* has been eradicated successfully by antibacterial treatment (Coghlan *et al.*, 1987; Lambert *et al.*, 1987; Marshal *et al.*, 1988; Rauws and Tytgat, 1990; Hopkin *et al.*, 1996).

Since the discovery of *H. pylori* in 1983 by Warren and Marshall (1983), many therapeutic regimens have been tried and advocated in different parts of the

world (Tygat *et al.*, 1993; Hatlebakk *et al.*, 1995; Rauws and Vander Hulst, 1995; Rokkas *et al.*, 1995; Yousfi *et al.*, 1995; Bianchi *et al.*, 1996; Kumar *et al.*, 1996; Markham and McTavish, 1996). Classical triple-therapy with bismuth compound results in eradication varying from 30-75% (Harris, 1998). A substantial proportion of patients receiving such therapy have significant side-effects. Classical triple-therapy is significantly less effective against pretreatment metronidazole-resistant strains (MRS) of *H. pylori* (Hentschel *et al.*, 1993; Salman-Roghani *et al.*, 1997).

Dual-therapy refers to the combination of omeprazole or ranitidine bismuth citrate (RBC) and either amoxicillin or clarithromycine. These regimens were reported to overcome problems that had bedevilled classical triple-therapy such as side-effects, MRS of *H. pylori* and patient compliance with more complex regimens. However, recent data revealed 39-46% *H. pylori* eradication by omeprazole + amoxicillin therapy given for 2 weeks and 56% by omeprazole + clarithromycine. On the other hand, RBC with amoxicillin eradicated *H. pylori* in about 65% of cases but with clarithromycine, the figure became about 80% (Harris, 1998).

Observations emerged from multiple treatment trials were:

1) Only regimens that give consistently good results from country to country and from study to study should be used.

2) Successful cure of infection requires three or four agents.

3) Successful regimens include clarithromycine and/or metronidazole.

4) Although a cure rate of 80% was considered acceptable, rates of 90% or higher are now achievable, especially if the organism is susceptible to the antibiotics used.

5) Resistance to metronidazole or clarithromycine may lead to reduced efficacy with either antibiotic.

6) Compliance is important for successful cure of the infection.

Eradication or cure of infection is defined currently as absence of the organism by tests performed no sooner than 4 weeks after cessation of antimicrobial therapy (The European *Helicobacter pylori* Study Group, 1997; The Report of Digestive Health Institute International Update Conference on *Helicobacter pylori*, 1997; Peerson and Graham, 1998).

2.5.1. Indications of anti-*H. pylori* therapy

The National Institute of Health (NIH) Consensus Development Conference in 1994 (NIH, 1994) recommended that all patients with documented duodenal or gastric ulcers who have *H. pylori* infection should receive antimicrobial therapy to cure the infection.

European experts on *H. pylori* (European *H. pylori* Study Group - EHPSG) met in September 1996 (The European *Helicobacter pylori* Study Group, 1997) to formulate recommendation regarding management of *H. pylori* infection. It has become known as the Maastricht Consensus Conference Recommendation.

Strong recommendations on the basis of unequivocal evidences were: PUD active or not, bleeding PUD, MALT lymphoma, gastritis with severe abnormalities and after resection of early gastric cancer. Advisable recommendations on the basis of equivocal evidences were: long-term PPI for GERD, after PUD surgery, NUD, family history of gastric carcinoma, NSAIDs therapy (planned or existing) and patient's wishes (NIH, 1994; The European *Helicobacter pylori* Study Group, 1997; The Report of Digestive Health Institute International Update Conference on *Helicobacter pylori*, 1997).

2.5.2. Therapeutic regimens to treat *H. pylori* infection

The aim of the treatment of *H. pylori* is eradication of the bacterium from the foregut. Treatment of *H. pylori* infection is difficult for two main reasons. First, the bacterium lives below the gastric mucus adherent to the gastric epithelium and access of antimicrobial drugs to this site is restricted, both from the lumen of the stomach and from the gastric blood supply. Second, *H. pylori* may have acquired resistance to the commonly used antimicrobial agents, such as nitroimidazoles and macrolides (clarithromycine) (Harris, 1998; Mégraud, 1998).

A marked difference has been found between the rate of resistance to nitroimidazoles in developed and developing countries. Resistance rates can be as high as 80-90% in developing countries as reported in Africa (Burkina Faso, Zaire) (Mégraud, 1998). In developed countries, the rate of resistance ranged from 10-50% in a European multicenter study in 1991 (Glupczynski *et al.*,

1992). This difference may be linked to the high level of general use of metronidazole in developing countries to treat parasitic infections. Resistance due to mode of reduction of compound. Genetic basis is unknown. Resistance influences treatment success to lesser extent (Glupczynski *et al.*, 1992; Mégraud, 1998).

The frequency of resistance to macrolides varies from country to country and seems to parallel the use of these agents to treat other infections in the past.

Reported *H. pylori* resistance rate is 10-15% in Southern Europe. Genetic basis of clarithromycine resistance is point mutation on 23SrRNA. Resistance dramatically influences treatment success (Szczebara *et al.*, 1997; Mégraud, 1998).

The ideal therapy for *H. pylori* eradication should be simple, safe, free from side-effects, with 100% efficacy and low cost. The ideal treatment regimen has not yet been defined. Eradication percentage is reported using an intent-to-treat - ITT (worst-case) analysis, whereby all patients treated are included in the analysis. ITT provides a more realistic assessment of the *H. pylori* eradication than a per-protocol analysis - PPA (best-case), whereby only those patients taking the majority (or all) of the drugs and returning for follow-up are included. A PPA provides data about the efficacy of particular regimen under ideal circumstances, but the results may not be reproducible outside of clinical trials. Reasonable targets would be $\geq$90% cure rate on PPA and $\geq$80% cure rate on ITT (Mégraud *et al.*, 1997).

Current eradication regimens are discussed under the heading of dual, classic-triple, PPI-based triple and quadruple therapy, depending on the number and dose of antimicrobial agents used concurrently in the treatment.

Dual-therapy, the 2-week combination of omeparazole, ranitidine bismuth citrate and either amoxicillin or clarithromycine, eradicates *H. pylori* in 50-80% of patients (Rauws and Vander Hulst, 1995; Markham and McTavish, 1996). Classical triple-therapy consists of bismuth compound, metronidazole and either amoxicillin or tetracycline, with eradication rates varying from 30-95%. It is associated with side-effects, is highly dependent on patient's compliance and is significantly less effective in the presence of metronidazole-resistant strains of *H. pylori*, where eradication may be 50% (Harris and Misiewicz, 1995; Salman-Roghani *et al.*, 1997).

At present, different triple-therapy regimens consisting of either a proton-pump inhibitor (PPI) or ranitidine bismuth citrate (RBC) with clarithromycine and either amoxicillin or metronidazole/tinidazole, had received approval from the world experts on *H. pylori* for the treatment of patients with *H. pylori*-associated PUD. These recommended regimens have known efficacy of faster ulcer healing and resulted in fewer recurrences (Howden and Hunt, 1998).

The highest eradication rates were achieved with PPI-based triple-regimens consisting of a PPI, clarithromycine and either amoxicillin or metronidazole for 2 weeks (Harris, 1998).

Second-line therapy (if first-line fails): Two approaches are available in the choice:

1) If susceptibility results are available, regimen determined according to sensitivity.

2) If susceptibility results are not available, metronidazole or clarithromycine (whichever was not used initially)-based therapy, such as OMC or OAC regimens to be given.

Alternative triple-therapy regimen with ranitidine 300 mg daily combined with metronidazole 500 mg 3 times daily and amoxicillin 750 mg 3 times daily for 2 weeks was shown to eradicate 90% of *H. pylori* (Harris, 1998; Mégraud, 1998; Peerson and Graham, 1998).

Quadruple-therapy regimens for *H. pylori* eradication consisting of PPI, CBS, tetracycline and metronidazole, are associated with side-effects and compliance problems. Reported cure rates are 85-95%. Quadruple therapy is best reserved for third-line (de Boer *et al.*, 1995; Bolin *et al.*, 1997; Graham *et al.*, 1997; Harris, 1998).

2.5.3. Duration of anti-*H. pylori* therapy

There is good evidence for the efficacy of 14-day triple-regimens including a PPI or RBC (Howden and Hunt, 1998). EHPSG has recommended 7-day therapy (The Report of Digestive Health Institute International Update Conference on *Helicobacter pylori*, 1997) and US-based trials have suggested that eradication

rates are acceptable with a 10-day PPI-based regimen. Studies have failed to show a statistically significant difference in eradication rates between a 7-day and 14-day duration (Laine *et al.*, 1996; Fennerty *et al.*, 1997). Further studies are necessary to resolve the issue.

2.5.4. Follow-up of patients after treatment for *H. pylori* infection

Currently, routine post-treatment testing is only recommended in patients with a history of ulcer complications, gastric MALT lymphoma, or early gastric cancer. Patients with recurrent symptoms after treatment of *H. pylori* infection will also need further evaluation (Wotherspoon *et al.*, 1993; Rollan *et al.*, 1997; Thiede *et al.*, 1997; Howden and Hunt, 1998).

2.6. REINFECTION

Knowledge of reinfection rates is important for understanding both the epidemiology of *H. pylori* infection and patient management. To date, there have been a substantial number of studies in the developed countries that have examined the *H. pylori* reinfection rate in patients treated for PUD. In the developed countries, reinfection after successful eradication of *H. pylori* appears unusual. At the present time, there have been few studies examining the rate of *H. pylori* reinfection in the developing countries (Graham *et al.*, 1992; Mitchell *et al.*, 1992).

In developed countries the reinfection rates are as low as 1-2%, whereas substantially higher recurrence rates (20-30%) are observed in developing countries (Penston, 1994; Vander Hulst *et al.*, 1996; Peerson and Graham, 1998).

More recently, in a meta-analysis of several studies, Hopkins *et al.* (1996) noted overall recurrence rates of 6% duodenal ulcer and 4% gastric ulcer after successful eradication of *H. pylori* compared with more than 60% when infection persisted.

Apart from diminishing recurrence, eradication of *H. pylori* leads to faster healing of PUD than suppression of acid secretion alone (Hopkins *et al.*, 1996; Huang *et al.*, 1996; Calam, 1998).

2.7. VACCINE DEVELOPMENT AGAINST *HELICOBACTER PYLORI* INFECTION

A long-term worldwide solution to *H. pylori*-related disease can be effected only by prevention of acquisition of the infection. Antimicrobial therapy of existing infection is cumbersome, expensive and ineffective in countries where reinfection rates are high.

One possible solution is to develop a preventive vaccine. Animal models are being used to develop vaccines by defining appropriate antigens (e.g. urease, heat-stable proteins, *Vac*A) and to find effective and safe adjuvants. It appears that the infected stomach does not mount an effective secretory IgA response to the infection.

Animal models are being used to develop vaccines. Vaccination causes effective mucosal response, leads to prevention and cure of infection. Animal experimentation applicable to humans are yet to be evaluated (Corthesy-Theulaz *et al.*, 1995; Lee and Buck, 1996; Peerson and Graham, 1998).

2.8. *HELICOBACTER PYLORI* INFECTIONS IN THE DEVELOPING COUNTRIES

It has become increasingly evident that *H. pylori* infections are more prevalent in developing countries, infecting those populations very early in life. The epidemiology of *H. pylori* infection is clearly distinct between the developed and the developing world. In developed countries, *H. pylori* infection is found in about 20-25% of asymptomatic adults and increases with age in the elderly to approximately 50% by the age of 50 years. Poor socioeconomic status and sanitation seem to be the critical risk factors in acquiring the infection. By way of contrast, in the developing world, *H. pylori* infection seems to be nearly universal, beginning in early childhood.

Using a ^{13}C urea-breath test and serologic assays to indicate infection (in Peru, the Gambia and Bangladesh), it was found that children become infected during their few months of life. They then may temporarily clear the infection but again become reinfected and by the age of 3 years are stably and persistently infected. Approximately 90% children in these areas are infected by 3-5 years of age (Albert *et al.*, 1994).

Peptic ulcer disease is common in Bangladesh. Epidemiological study has shown a point prevalence of 12% for duodenal ulcer and 3.5% for gastric ulcer among individuals above the age of 15 years (Hasan *et al.*, 1985). However, prevalence of *H. pylori* infection in Bangladeshi adults is not known. The recognition that *H. pylori* plays a pivotal role in the pathogenesis of peptic ulcer disease makes its diagnosis necessary in these circumstances.

A cure of peptic ulcer disease by eradication of *H. pylori* has been proclaimed. Recommended treatment for peptic ulcer by various authorities in developed countries is now eradication of *H. pylori* (The European *Helicobacter pylori* Study Group, 1997; NIH Concensus Development Panel, 1994; The Report of Digestive Health Institute International Update Conference on *Helicobacter pylori*, 1997). Cure of *H. pylori* infection is not easy and requires combination of one or two antibiotics with one or two nonantibiotic adjunctive agents.

Given the higher prevalence of *H. pylori* infection in most developing countries, one might expect the reinfection rate of *H. pylori* to be high. Published studies from the developing countries have included a limited number of subjects and in some cases have only had short follow-up periods (Mitchell *et al.*, 1992).

Helicobacter pylori Study Group of Bangladesh, a multidisciplinary group of 10 experts, consisting of gastroenterologists, microbiologists, immunologists and histopathologists from Bangabandhu Sheikh Mujib Medical University (BSMMU) and Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) was setup in 1994 to investigate *H. pylori* infection in Bangladeshi population, evaluation of diagnostic methods, to find out the most effective eradication therapy and recurrence of infection after its successful eradication (Morshed *et al.*, 1995).

AIMS OF THE STUDY

3. AIMS OF THE STUDY

a) Evaluation of different methods for diagnosis of *Helicobacter pylori* infection in Bangladeshi patients.

b) To see the prevalence of *H. pylori* infection in asymptomatic population.

c) To compare the efficacy of different anti-*H. pylori* therapy regimens with a view to find out the most effective *H. pylori* eradication regimen(s) for Bangladeshi patients and to see the recurrence of *H. pylori* infection after its eradication.

SUBJECTS AND METHODS

4. SUBJECTS AND METHODS

4.1. STUDY SITE

Different phases of the study were performed in the Department of Gastrointestinal, Liver and Pancreatic Diseases, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka Medical College Hospital (DMCH) and Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka.

Laboratory works of the study were performed in the Department of Immunology and Microbiology of BIRDEM.

4.2. SAMPLE COLLECTION

4.2.1. Endoscopy and collection of biopsies

At endoscopy, the presence of lesions in the gastroduodenal mucosa was noted. Differentiation was made between erosions and true ulcers in the classification of disease. The criteria for inclusion in the ulcer group were a circumscribed break in the mucosa with apparent depth and covered by an exudate. Either complete epithelization of the duodenal mucosa or formation of white scar was used as the criterion for ulcer healing. Patients who had no endoscopically definable findings were classified as non-ulcer dyspepsia (NUD).

The endoscope and biopsy forceps were sterilized before taking biopsy from each patient by soaking them in disinfectant solution (Cidex, 2% glutaraldehyde, USA).

Biopsy specimens were taken for rapid urease test (RUT), culture and histology. The specimen for RUT was placed in a small vial containing urea agar media (Difco, USA) to detect the urease activity of *H. pylori* as described by Kawanishi *et al.* (1995) and another specimen was placed in a vial containing Stuart transport media (Merck, Germany).

Biopsy specimen for histology was placed in 10% buffered formalin tube.

Specimens were transported to the laboratory immediately after collection.

4.2.2. Blood collection for serum separation

Approximately 3-5 ml of venous blood was drawn aseptically by venipuncture from each subject for serological study. Sterile disposable syringe and needle were used to draw blood.

Fresh serum was obtained by subsequent centrifugation of blood at 1500 X *g* for 10 minutes. Sera samples were transferred to 1.5 ml microcentrifuge tube (Eppendorf, Germany) in duplicate and stored at -20°C until the ELISA test was performed.

4.3. TRANSPORTATION OF SAMPLE

4.3.1. Preparation of transport media

Dehydrated Stuart transport medium (Merck, Germany), a transport medium that provides a moist, reduced oxygen environment and suppresses bacterial contamination, was used to keep the viability of the *H. pylori* well, which is susceptible to O_2 and desiccation. The media was prepared as per the manufacturer's instructions (Appendix-II).

4.3.2. Transportation of biopsy tissue

Biopsy tissue was collected from the biopsy forceps with a sterile needle (Microlance 3, Becton Dickinson, Spain) and transferred to transport medium. The specimen was dipped in 2-3 cm of the transport medium to prevent the reduction of viability and immediately brought to the laboratory for processing.

4.4. SAMPLE PROCESSING

About 200 μl of sterile physiological saline (0.95% NaCl) was taken in a small glass tissue grinder (Thomas Scientific, USA). Biopsy tissue was taken out from transport media by a digital micropipette and transferred to sterilized tissue grinder and after grinding, a small portion (20 μl) of the specimen was taken by a digital micropipette and smeared on a clean glass slide and heat-fixed for direct microscopic examination. Rest of the specimen was inoculated into Brucella agar (Difco, USA) supplemented with 10% sheep blood and four antibiotics (Taylor *et al.*, 1987).

4.5. CULTURE OF *HELICOBACTER PYLORI*

4.5.1. Preparation of Brucella agar media

Dehydrated Brucella agar (4.3%) medium was prepared according to the manufacturer's instruction (Appendix-III). The medium was supplemented with 10% sheep blood and antibiotics, namely, vancomycin (10 μg/ml), polymyxin-B (5 μg/ml) and trimethoprim (5 μg/ml) (Skirrow, 1977). Skirrow's antibiotic supplement (Appendix-III) for use in Brucella agar was slightly modified by the addition of amphotericin B (5 μg/ml) (Appendix-III) (Dent and McNulty, 1988).

Sheep blood was added for enhanced recovery of *H. pylori* and antibiotics were added to prevent bacterial and fungal contamination. The prepared media was then aseptically poured into sterile petri dishes. The media were stored at 2-8°C until use.

4.5.2. Inoculation and incubation

The lid of an anaerobic jar (CampyPak Jar, BBL Microbiology System, Maryland) was opened and the steel rack kept inside the jar was brought out. Then the inoculated Brucella agar plates were placed in the rack and set inside the jar. A gas generation Pak, Campylobacter Microaerophilic System (Difco, USA) was used to produce a microaerophilic condition. The Pak was cut along the broken line as indicated and 10 ml of tap water was added into the Pak. Immediately, the envelope was placed in upright position in anaerobic jar and lid was sealed according to manufacturer's instruction. Tablets of gas generation

Pak produced an atmosphere of approximately 5% oxygen and 10% carbon dioxide. The plates were then incubated at 37°C for 3-7 days in microaerophilic condition.

Growth of *H. pylori* was observed after 3 days. If no growth occurred within 3 days, the plates were reincubated again for 2-4 days. The appearance of an opaque, translucent, convex and circular (0.5-1.0 mm diameter) colonies indicated presumptive evidence of *H. pylori*.

It has been observed that carbon dioxide (Co_2) is important for the growth of *H. pylori* and successive culture has been possible in 5% Co_2 incubator (M.G. Morshed, unpublished data). Therefore, simultaneously we have attempted to culture *H. pylori* from biopsy specimens with use of a candle jar at high humidity. Steel anaerobic jar was used in candle jar system to ensure high humidity; facial tissue was soaked in sterile water and then placed in a plastic petri dish of which upper lid was perforated. Inoculated plates and flamed candle were placed in the steel anaerobic jar carefully and tightly closed the lid of the jar immediately for generating low oxygen and high Co_2 atmosphere and transferred the jar into the incubator.

Direct microscopic examination

Smear preparation and fixation

A small portion (20 μl) of the ground tissue was taken and smeared on a fresh, clean and oil-free glass slide covering 15-20 mm area of the slide. The smear

was allowed to air-dry completely and heat-fixed rapidly by passing the slide through the flame of a burner three times and then cooled before staining.

Gram staining

The fixed smear was flooded with crystal violet staining solution and kept for 60 seconds. The slide was rapidly washed with gentle running clear tap water. All the water was tipped off and the smear was flooded with Lugol's iodine solution for 60 seconds. The iodine was washed of similarly as before. The smear was rapidly flooded with rectified spirit (95.6%) for destaining for 10 seconds and washed immediately in the similar way. Finally, the smear was flooded with safranin counter-staining solution for 1 minute (Cheesbrough, 1993). The slide was washed off with clear water and air-dried. The smear was then examined under a bright-field microscope.

Microscopic observation

Gram-stained smear was observed first with the 40X objective and later, a drop of immersion oil was added onto the smear and examined with 100X objective for stained bacteria. The findings of Gram-negative spiral rod-shaped bacteria gave presumptive evidence of *H. pylori*. Observation was scored as negative if no such spiral bacteria were found on the smear.

4.6. RAPID UREASE TEST (RUT)

Dehydrated urea agar (Difco, USA) medium (Appendix-II) was aseptically prepared according to the manufacturer's instruction. High temperature destroys urea, so the prepared urea agar-base solution containing urea was sterilized by membrane filtration using Millipore membrane filter, 0.22 μm pore size (Sigma, USA). The medium (2.5 ml) was then aseptically poured into sterilized 5 ml screw-cap glass vial. The medium was allowed to solidify by keeping the tubes in upright position. They were then used for direct rapid urease test for the detection of *H. pylori* from biopsy material.

For biochemical identification of *H. pylori* grown on Brucella blood agar plates, urea agar media were poured into screw-cap tubes and kept in a slanted position. They were then stored at 4°C until use.

The biopsy specimen was aseptically placed on the urea agar medium inside the vial. The medium containing a urea substrate that is cleaved by urease into carbon dioxide and ammonium, causing a change in pH. The test was recorded as positive if the color changed from yellow to pink within 2 hours at room temperature. Tube that showed no change in color within 2 hours was checked again after 24 hours for the late positive result. Long incubation increase the sensitivity of the method, but can decrease the specificity because contaminating bacteria might grow and can give a false-positive result.

4.7. BIOCHEMICAL TESTS

Colonies of *H. pylori* were identified biochemically by performing urease, oxidase and catalase tests.

4.7.1. Urease test

Urea agar slant was inoculated with pure culture of the test organism by making single streak. The tube was then kept at room temperature for 10 minutes. The change in color of the medium from yellow to pink within this time indicated a positive urease test.

4.7.2. Oxidase test

A small amount of freshly prepared oxidase reagent (Appendix-IV) was soaked in a piece of Whatman filter paper No. 2. A portion of the test organism was picked up from Brucella agar medium by means of a sterile wooden toothpick and streaked onto the filter paper soaked with the reagent. A positive result was recognized by a dark-purple color that developed within 5-10 seconds (Cheesbrough, 1993).

4.7.3. Catalase test

A drop of 3% hydrogen peroxide solution was taken by a digital micropipette into a dry clean slide. Using a sterile loop, a pure colony of the test organism was picked up and immersed in the hydrogen peroxide solution. The production of bubbles indicated a positive result (Cheesbrough, 1993).

4.8. DRUG SENSITIVITY TEST OF *HELICOBACTER PYLORI* ISOLATES

Kirby-Bauer method was used to determine the drug sensitivity patterns of *H. pylori* (Burnett and Nicolet, 1993; Cappuccino and Sherman, 1996). Modified Brucella agar medium (supplemented with 10% sheep blood and antibiotics) was used for this purpose. Antibiotics and their disc potencies were: penicillin G (10 unit), amoxicillin (10 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), tetracycline (30 μg), nitrofurantoin (300 μg), nalidixic acid (30 μg) and metronidazole (5 μg) (Oxoid, England).

The plate was inoculated directly with the test organism by making numerous horizontal and vertical streaks over the entire agar surface to obtain a uniform inoculum.

Commercially available filter paper discs impregnated with known concentrations of antimicrobial were applied to the surface of the inoculated plate at appropriate spatial arrangement by means of sterile forceps. The discs were gently pressed down onto the agar with the forceps to ensure that the discs adhere to the surface of the agar. Plates were then incubated in an inverted position for 3 days at 37°C in microaerophilic condition. The antimicrobial impregnated into the discs diffused into the surrounding medium could kill or inhibit the growth of microbes creating a clear zone of inhibition in the lawn of bacterial growth or be inactivated by the bacterial enzymes. The sensitivity or resistance shown by the *H. pylori* isolates was recorded as defined by the manufacturer.

4.9. HISTOLOGY

Biopsy specimens were fixed in 10% buffered formalin, oriented and embedded in paraffin. Sections of 3-4 micron thick were stained with hematoxylin and eosin for histological evaluation and with modified Giemsa for identification of *H. pylori*.

Chronic gastritis was defined by mucosal infiltration of increased number of lymphocytes and plasma cells with or without shortening of cyst length. Active chronic gastritis was diagnosed if polymorphs were present in the lamina propria and in intraepithelial sites or in both. All sections were examined by the same investigator who was unaware of the endoscopic or clinical findings.

4.10. DETECTION OF ANTI-*H. PYLORI* IgG ANTIBODIES BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

The serum sample test was based on the ELISA technique by using an ELISA test kit (EIAGEN, Clone System, S.P.A, Casalecchio Di Reno, Italy). This assay system was based on qualitative detection of IgG-specific antibodies to *H. pylori*. The test was performed according to manufacturer's instruction. *H. pylori* antigens were immobilized on the wells of micro-well plate. Diluted patient's serum was added to the wells. IgG antibodies specific to *H. pylori*, if present, bind to the antigens on the micro-wells. The intensity of the color corresponds directly to the amount of antibodies present. The cutoff values of more than 15 arbitrary unit (AU) per milliliter (ml) was considered as positive test.

4.11. ^{13}C UREA BREATH TEST (Appendix-V)

Materials

Test meal: Two hundred milliliter of pasteurized Bangladeshi full-cream milk (in bag); stored in cold room. This was used to prevent rapid gastric emptying.

Vacutainer tube

15 ml siliconised tubes

Tube labels

Tube 1	:	Date, name and number of patient, and '0' minute
Tube 2	:	Date, name and number of patient, and '30' minute

Sampling straw (large plastic drinking straw)

Not used to mix urea solution

Methods

After an overnight fast, an oral dose of urea solution consisting of 100 mg ^{13}C-urea (99AP; MassTrace, Woburn, MA, USA) in 20 ml water was given with a test meal of 200 ml pasteurized full-cream milk. Breath samples were collected before and 30 minutes after ingestion of the substrate. The ratio of ^{13}C to ^{12}C in the baseline and 30-minute samples were determined by isotope ratio

mass spectrometer (Breath MAT^{Plus}; Finnigan, Bremen, Germany). $^{13}Co_2/^{12}Co_2$ ratios were expressed as delta $^{13}Co_2$ values relative to the PDB standard. An increase of the delta $^{13}Co_2$ value at 30 minutes over the baseline value of more than 3.5% was considered positive for *H. pylori* (Sarker *et al.*, 1995).

4.12. STUDY DESIGN

The study was carried out in three phases:

Phase-I

Helicobacter pylori in dyspepsia subjects: A pilot endoscopic biopsy study to evaluate the efficacy of culture, histology, rapid urease test and serology for the diagnosis of *H. pylori* infection in Bangladesh (Published in: (a) Lancet 1995; 346:511, enclosed in Appendix-I as Paper-I, (b) Jpn J Med Sci Bio 1997; 50:50-5, enclosed in Appendix-I as Paper-II).

Phase-II

Prevalence of *Helicobacter pylori* infection in asymptomatic Bangladeshi adults: A pilot serological survey (Published in: J Epidemiol 1997; 7:251-4, enclosed in Appendix-I as Paper-III).

Phase-III

Comparative efficacy of different anti-*Helicobacter pylori* therapy regimens in the treatment of *H. pylori*-associated duodenal ulcer patients in Bangladesh. This study was carried out in two phases:

Phase-IIIa

Comparative study of different dual- and triple-therapy regimens (Published in: Bangladesh J Med 1999; 10:4-9, enclosed in Appendix-I as Paper-IV).

Phase-IIIb

A comparison between furazolidone-based triple-therapy and nitroimidazole-based triple-therapy for the treatment of *Helicobacter pylori*-associated duodenal ulcer patients in Bangladesh: A prospective 1-year follow-up study (Abstract published in Indian J Gastroenterol 2000; 19(Suppl 2):A62-3, enclosed in Appendix-I as Paper-V).

Phase-I

The present study was undertaken with the aim of establishing bacteriological culture and histopathological facilities for the isolation and detection of *H. pylori* in gastric biopsy specimens and of comparing the efficacy of various diagnostic methods from a Bangladeshi perspective.

The study population comprised of 25 duodenal ulcer (DU) (mean$\pm$SD 40$\pm$18 years), 16 non-ulcer dyspepsia (NUD) (mean$\pm$SD 35$\pm$15 years) and 1 gastric ulcer (GU) (23 years), and 3 gastric carcinoma (mean$\pm$SD 60$\pm$15 years) patients.

Culture of biopsy specimens from 45 patients were done by using conventional GasPack system. To culture *H. pylori* in candle jar system, equal number of biopsy specimens from 10 patients (6 DU and 4 NUD) were inoculated into Skirrow's modified agar media.

For rapid urease test (RUT), one antral biopsy specimen from each of the 45 consecutive dyspepsia patients was placed on the urea agar medium.

Antral biopsy specimens from 37 patients were examined histologically.

Serum samples were collected from 42 dyspepsia patients and 15 consecutive pregnant mothers and their newborns for *H. pylori* IgG ELISA.

Susceptibility study of 12 clinical *H. pylori* isolates were done by disk-diffusion method.

Phase-II

This serological survey was undertaken to see the seroprevalence of *H. pylori* infection in a cross-section of asymptomatic adults in Bangladesh.

The serum samples were collected from 181 consecutive healthy young male individuals who attended during the period of August to November 1995, for medical check-up at Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) Health Care Centre (BHCC) to work as immigrant worker in Malaysia. Subjects came from different parts of Bangladesh. Information about their age and socioeconomic

status were noted. The serum samples test was based on the ELISA (enzyme-linked immunosorbent assay) technique by using ELISA test kit (EIAgen, Clone System S.P.A., Casalecchio Di Reno, Italy).

Phase-III

Two consecutive clinical trials with different anti-*H. pylori* treatment regimens were undertaken to see their efficacy in *H. pylori*-associated duodenal ulcer patients.

Phase-IIIa

The earlier one was a pilot comparative study of different dual- and triple-therapy regimens. This study was conducted from December, 1995 through December, 1996. Patients with at least one endoscopically documented duodenal ulcer with a diameter of 5 mm or more and a positive *H. pylori* test result (rapid urease test) were included in the study. Patients with gastric ulcer, or who had taken bismuth or antibiotics during the 4 weeks before endoscopy, were not included. Those who were regularly taking nonsteroidal anti-inflammatory drugs or corticosteroids also excluded from this study. Other exclusion criteria were surgical treatment of ulcer-related conditions, pregnancy and penicillin allergy. Eighty-two consecutive patients with endoscopically proven duodenal ulcer from endoscopy units of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) and Dhaka Medical College Hospital (DMCH). The study subjects gave informed

consent to be randomly assigned to receive omeprazole (O) 20 mg bid, colloidal bismuth substrate (C) 120 mg qid, ranitidine (R) 150 mg bid, amoxicillin (A) 500 mg qid, metronidazole (M) 400 mg tid and tinidazole (T) 500 mg bid in the form of two triple-therapy (OTA and CAM) and two dual-therapy (OA and RA) for 2 weeks.

Two antral biopsy specimens were taken for RUT at each endoscopy (0-week and 6-week) visit to confirm the diagnosis and eradication of infection.

Phase-IIIb

The second prospective clinical trial was undertaken to compare the efficacy of furazolidone-based triple-therapy over nitroimidazole-based triple-therapy regimens in the eradication of *H. pylori* infection in duodenal ulcer patients.

A total of 116 patients with endoscopically confirmed *H. pylori*-associated duodenal ulcer disease were prospectively recruited and randomized to receive omeprazole (O) 20 mg bid, ranitidine (R) 150 mg bid, colloidal bismuth subcitrate (C) 120 mg qid, amoxicillin (A) 500 mg qid, furazolidone (F) 100 mg qid, metronidazole (M) 400 mg tid and tinidazole (T) 500 mg bid, in the form of 3 furazolidone-based CAF, OAF, RAF and 2 nitroimidazole-based OAM and OAT tripe therapies for 2 weeks. Similar inclusion and exclusion criteria (Phase-IIIa) were followed for these patients.

Compliance to therapy was evaluated by counting the returned tablets. Any adverse event (sign and symptoms, such as nausea, vomiting, bad taste, diarrhea, headache, etc.) was recorded on the case record form and classified as

mild (awareness of sign or symptoms but easily tolerated), moderate (discomfort sufficient to cause interference with normal activities) or severe (incapacitating, with inability to perform normal activities).

Endoscopy was performed at the entry visit and was repeated at 8 week, 12 week and at or before 1 year when clinical symptoms reappeared.

Patients whose ulcer remained unhealed at 8 week, continued the antisecretory drug therapy for an additional 4-week period and reendoscopy was done at 12 week.

Two biopsy specimens were taken from antrum for RUT and culture study. Simultaneous serial ^{13}C-urea breath test was performed at 4-week interval from 0 week to 1 year to detect eradication of infection and reinfection. *H. pylori* eradication was defined by the negative urea breath test result and the absence of microorganism both at RUT and culture 8 to 12 weeks after therapy had begun. Cutoff interval used to define cure rate was 12 weeks.

4.13. STATISTICAL ANALYSIS

Chi-square test was done to find out any difference in the prevalence of different age groups of population. Difference between the diffeernt treatment groups in the incidence of duodenal ulcer healing, *H. pylori* eradication and simultaneous healing and eradication were also assessed with Chi-square test. Firhser's exact test was performed to compare the number of healed and eradicated cases between dual- and triple-therapy regimens. Influence of age, sex,

socioeconomic status and smoking on *H. pylori* eradication and ulcer haling were evaluated by logistic regression analysis (SPSS 7.5 for Windows). A two-sided P value of 0.05 was the criterion for statistical significance.

RESULTS

5. RESULTS

5.1. PHASE-I: Evaluation of the tests for diagnosis of *Helicobacter pylori* infection in Bangladesh

Culture is considered to be the 'gold standard' for detection of *H. pylori* infection in gastric biopsy specimen. Several authors have reported an excellent level of sensitivity for culture and considered it to be the best method. The present study was undertaken to evaluate the efficacy of culture, histological examination, the rapid urease test (RUT) and serology for the diagnosis of *H. pylori* infection. A total of 45 consecutive patients with various upper gastrointestinal symptoms were included in this study.

Table-I shows the baseline characteristics and UGI symptoms of the study population. Among the 45 dyspepsia subjects, majority were male and belonged to average socioeconomic class. Mean duration of upper gastrointestinal (UGI) symptoms were 6 ± 4 years in DU and GU, 3 ± 3 years in NUD patients.

Among the various UGI symptoms periodicity of pain and nocturnal pain were present in 60% (15/25) and 64% (16/25) of DU and in 25% (4/16) and 19% (3/16) of NUD patients ($P < 0.05$ and $P < 0.01$, respectively).

Table-II shows the detection of *H. pylori* by different methods. Sixteen (64%) DU, 6 (37.5%) NUD and 1 (25%) gastric cancer patients were culture positive in conventional GasPak system. Microbiological culture of biopsy specimens

Table-I. Distribution of age, sex, upper gastrointestinal symptoms and smoking history in different dyspepsia group

Parameters	DU (n=25)	NUD (n=16)	Gastric ulcer (n=1)	Gastric cancer (n=3)	P value
Age (years) (Mean±SD)	39.66 ±18.14	35.44 ±15.21	23	60±15	
Men/Women	23/2	14/2	1/0	3/0	
Upper GI symptoms:					
Duration (years) (mean±SE)	5.83 ±4.84	31.00 ±2.90	5.83 ±4.84	1.00	
Periodicity of pain N(%)	15(60)	4(25)	0	0	P<0.05 (DU vs NUD)
Nocturnal pain N(%)	16(64)	3(19)	1(100)	0	P<0.05 (DU & GU vs NUD)
Hunger pain N(%)	11(44)	5(31)	1(100)	0	
Antacid relief pain N(%)	10(40)	4(25)	0	0	P<0.05 (DU vs NUD)
Heartburn N(%)	10(40)	10(63)	0	2(67)	P<0.05 (DU vs NUD)
History of vomiting N(%)	9(36)	2(12.5)	1(100)	1(33)	
History of upper GI bleeding N(%)	6(24)	0	0	2(67)	P<0.05 (DU vs carcinoma stomach)
History of smoking N(%)	12(48)	4(25)	0	2(67)	P<0.05 (DU & carcinoma stomach vs NUD)

Table-II. Detection of *H. pylori* infection in dyspepsia patients by different diagnostic methods

Endoscopic findings	Number of cases	Culture	RUT	Histological examination	Serology
Duodenal ulcer	25 (55.6%)	16	16	20	25
Non-ulcer dyspepsia	16 (35.6%)	6	6	13	15
Other forms of dyspepsia (gastric ulcer and gastric cancer)	4 (4.9%)	1	3	4	3
Total	45	23	25	37	42
Percentage	100	51.1	55.6	82.2	93.3

from 4 DU, 3 NUD and 2 other dyspepsia group has shown growth of contaminants. In 'candle jar' incubation system, out of 10 patients, 6 showed profuse growth of characteristic colonies of *H. pylori*. The isolated bacteria were identified as *H. pylori* on the basis of colony morphology, Gram reaction, urease, catalase and oxidase tests. RUT of 45 patients showed that 16 DU, 6 NUD and 3 other dyspepsia group of patients were RUT positive. At histology 20 DU, 13 NUD and 4 (all) other dyspepsia group of patients were *H. pylori* positive. Serology showed that the prevalence rates of *H. pylori* IgG antibody were 100% (25/25) in DU, 94% (15/16) in NUD and 75% (3/4) in other dyspepsia group of patients. The overall detection rate of *H. pylori* infection in different dyspepsia groups were 51%, 55.6%, 82.2% and 93.3% in culture, RUT, histology and serology, respectively.

Table-III shows the susceptibility study of 12 clinical *H. pylori* isolates to commonly used antimicrobials. Sensitivity profiles were metronidazole (done in 12) - sensitive 3, resistant 9; cotrimoxazole (done in 12) - sensitive 1, resistant 11; ceftriaxone (done in 10) - sensitive 9, resistant 1; ciprofloxacin (done in 8) sensitive 8; nitrofurantoin (done in 4) - sensitive 4; ampicillin (done in 10) - sensitive 10; penicillin (done in 5) - sensitive 5.

Table-IV shows the gastric histological findings. The histological evidence of chronic active gastritis were seen in 19 DU, 13 NUD and 4 (all) other dyspepsia group of patients. Atrophic gastritis was present in 4 DU, 1 NUD and 2 other dyspepsia group. One DU and 1 gastric cancer patients had intestinal metaplasia in antral histology.

Table-III. Sensitivity profile of *H. pylori* infection

Pattern	Sensitive	Resistance
Metronidazole	3	9
Cotrimoxazole	1	11
Ceftriaxone	9	1
Ciprofloxacin	8	0
Nitrofurantoin	4	0
Ampicillin	10	0
Tetracycline	10	0
Penicillin	5	0

Table-IV. Prevalence of chronic active antral gastritis, antral atrophic gastritis and intestinal metaplasia in different dyspepsia groups

Parameters	DU (n=25)	NUD (n=16)	Gastric ulcer (n=1)	Gastric cancer (n=3)
Chronic active antral gastritis N(%)	19(76)	13(81)	1(100)	3(100)
Atrophic gastritis N(%)	4(16)	1(7)	1(100)	1(33)
Intestinal metaplasia N(%)	1(4)	0	0	1(33)

Table-V shows the anti-*H. pylori* IgG assay of 15 paired serum samples from mothers and babies. Both mother and baby were positive in 10, both negative in 4 and mother positive and baby negative in one paired sample.

Table-VI shows the comparison of test results and the diagnostic efficacy of RUT, culture, histological examination and serological studies. The sensitivity, specificity, and positive and negative predictive values were, respectively, 95.5%, 82.6%, 84% and 95% for RUT, 95.5%, 30.4%, 56.8% and 87.5% for histology and 100%, 13.6%, 54.8% and 100% for serological study.

Table-V. Anti-*H. pylori* antibody IgG of maternal and cord blood (clone system) [Cutoff: >10 AU/ml (pediatrics), >15 AU/ml (adult)]

Total cases = 15 x 2	Serum level (AU/ml)	Comment
M 1	50	P
B 1	34	P
M 2	41	P
B 2	10	P
M 4	15	P
B 4	13	P
M %	12	N
B 5	8	N
M 6	32	P
B 6	23	P
M 7	80	P
B 7	61	P
M 8	101	P
B 8	40	P
M 9	4.2	N
B 9	3.5	N
M 10	7.8	N
B 10	8.3	N
M 12	6.8	N
B 12	8	N
M 13	18	P
B 13	15	P
M 14	29	P
B 14	13	P
M 15	20	P
B 15	11	P
M 16	29	P
B 16	26	P
M 17	11	P
B 17	7	N

P = Positive
N = Negative
M = Mother's blood
B = Cord blood

Table-VI. Comparative efficiency of diagnostic tests for *H. pylori*

Test	Culture	RUT	Histological examination	Serology
Positive	23	25	37	42
Negative	22	20	8	3
True positive	-	21	21	23
True negative	-	19	7	3
False positive	-	4	16	19
False negative	-	1	1	0
Sensitivity	-	95.5%	95.5%	100.0%
Specificity	-	82.6%	30.4%	13.6%
PPV	-	84.0%	56.8%	54.8%
NPV	-	95.0%	87.5%	100.0%

PPV = Positive predictive value
NPV = Negative predictive value

5.2. PHASE-II: Prevalence of *Helicobacter pylori* infection in asymptomatic Bangladeshi adults

Epidemiological reports reveal that *H. pylori* is distributed among all population in the world. *H. pylori* prevalence rate in the developed countries is generally lower than that in the developing countries, reflecting socioeconomic status, the poorer a population, the earlier is the age of infecting *H. pylori*, resulting in the higher prevalence rate (Paper III) (Vandammee*et al.*, 1991; Jiang*et al.*, 1996; Owen, 1998).

Bangladesh is one of the developing country having a highest prevalence rate (80%) of *H. pylori* infection among under-5-year children (Graham*et al.*, 1997). Recent study has shown a very high seropositivity rate (>92%) in both the ulcer and non-ulcer dyspepsia patients in Bangladesh (Table-I, Paper II). The present pilot serological survey was undertaken to see the seroprevalence of *H. pylori* infection in a cross-section of asymptomatic adult population in Bangladesh. The serum samples were collected from 181 consecutive healthy young male between 20 and 44 yeas of age. Subjects were from different regions of Bangladesh and belonged to average socioeconomic class.

Table-VII shows the baseline data. The mean (±SD) age of the subjects was 30±33 years (range 20 to 44). All were male and came from almost all the districts of Bangladesh. Study subjects were the village people, belonged to average socioeconomic class and cultivation was their sole livelihood.

Table-VII. Baseline data with results

Period of study	August 1995 - November 1995
Sex	All were male
Age (years)	Mean 30.33 (range 20-44)
Socioeconomic status	Average
Test undertaken to detect *H. pylori*	ELISA (EIAgen, ELISA kit Clone System)
Total number of sample	181
Number of subjects found positive	166
Prevalence of *H. pylori*	92%

Table-VIII shows the seroprevalence rates in different age groups. Among the 181 subjects, 166 (92%) had *H. pylori*-specific IgG antibodies and 15 (8%) were seronegative. There was no significant difference ($P < 0.90$) observed in the prevalence of infection among different age groups.

Table-VIII. *H. pylori* seroprevalence rates in different age groups

Age groups (years)	Number of subjects	Number of positive test	*H. pylori* seroprevalence rate (%)
20-24	33	29	88.00
25-29	100	92	92.00
30-34	34	32	94.00
35-39	13	12	92.30
40-44	1	1	100.00
Total	181	166	91.71

5.3. PHASE-III

5.3.1. Phase-IIIa: Comparative efficacy of different dual- and triple-therapy regimens for the treatment of *H. pylori* eradication and duodenal ulcer healing

Rates of ulcer recurrence in patients whose initial ulcers healed during conventional antisecretory therapy range from 60 to 100 percent per year, but ulcers recur in less than 15% of patients in whom the *H. pylori* has been eradicated successfully by antibacterial treatment (Paper IV) (Vandammeet *al.*, 1991; Olsen, 1994; Calam, 1998; Farthing, 1998).

Since the discovery of *H. pylori* in 1983 by Warren and Marshall, many therapeutic regimens have been tried and advocated in different parts of the world. The present multicentric trial was undertaken to see the comparative efficacy of different triple- and dual-therapy regimens, given for 2 weeks in the eradication of *H. pylori* and healing of duodenal ulcer disease. A total of 82 duodenal ulcer patients with *H. pylori* infection confirmed by rapid urease test, were randomly assigned to receive any one of the following four combination therapy regimens for 2 weeks: OTA (omeprazole [O] 20 mg bid + tinidazole [T] 500 mg bid + amoxicillin [A] 500 mg quid); CAM (colloidal bismuth subcitrate [C] 120 mg qid + amoxicillin [A] 500 mg qid + metronidazole [M] 00 mg tid); OA (omeprazole [O] 20 mg bid + amoxicillin [A] 500 mg quid); and RA (ranitidine [R] 150 mg bid + amoxicillin [A] 500 mg quid). Following endoscopies were performed at 6 weeks (4 weeks after completion of 2 weeks therapy).

Table-IX shows the baseline characteristics of the patients. Majority of the patients were male and belonged to middle and lower socioeconomic classes. Of the 82 patients, 14 (1 OTA, 4 CAM, 5 OA and 4 RA groups) patients did not attend follow-up endoscopy at 6-week (Fig. 1). The remaining 68 patients completed the trial.

The dropout rate (patient lost to follow-up) did not differ significantly among different treatment groups. Patients who completed the trial did not complain of any remarkable side-effects.

Table-X shows different treatment outcomes. DU healing and *H. pylori* eradication rates were different in different treatment groups although not statistically significant. Follow-up endoscopy at the end of 6-week showed DU healing in 92.30% (12/13) from OTA, 94.50% (17/18) from CAM, 83.30% (20/24) from OA and 100% (13/13) from RA groups ($P=0.24$). Eradication of *H. pylori* was documented in 85% (11/13) from OTA, 89% (16/18) from CAM, 67% (16/24) from OA and 69.23% (9/13) from RA groups ($P<0.27$). The correlation between *H. pylori* eradication and DU healing showed simultaneous healing of DU and eradication of *H. pylori* in 85% (11/13) of OTA, 83.5% (15/18) of CAM, 58% (14/24) of OA and 69% (9/13) of RA groups ($P=0.20$). Considering healing and eradication simultaneously, the efficacy of triple-therapy regimens are greater than dual-therapy ($P=0.06$).

Ulcer healing without *H. pylori* eradication was observed in 1 of 13 OTA, 2 of 18 CAM, 6 of 24 OA and 4 of 13 RA groups of patients. On the other hand,

Table-IX. Characteristics of 82 patients with duodenal ulcer and *H. pylori* infection assigned to 4 different treatment regimens

Characteristics	OTA group	CAM group	OA group	RA group
Mean age (years) (range)	36.5 (16-72)	39.41 (14-80)	38.55 (19-70)	43.13 (23-60)
Male/Female ratio	11:3	9:2	22:7	11:4
Ever smoker (%)	10 (72)	15 (59)	15 (52)	7 (47)
Socioeconomic condition				
Upper	0	1	1	2
Middle	8	15	15	10
Lower	6	6	13	5

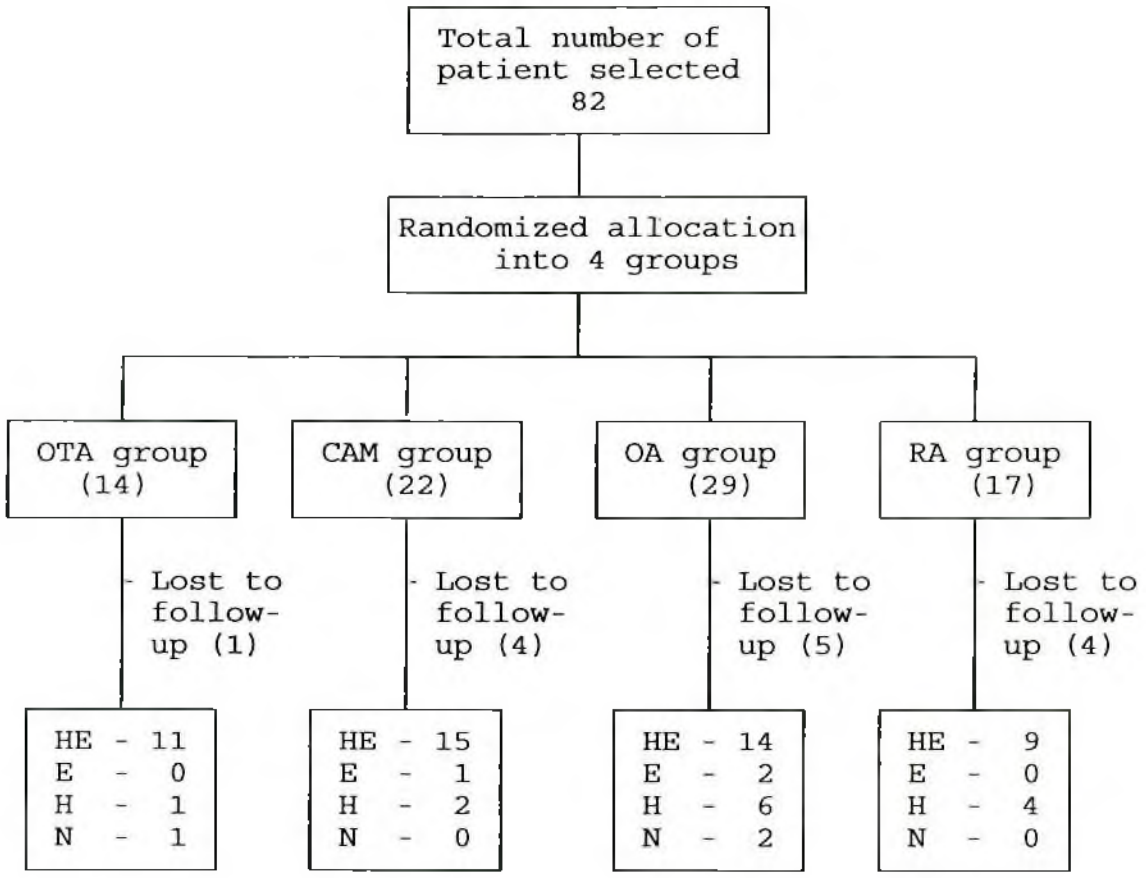

Fig. 1. Flow diagram of the study

A = Amoxicillin
C = Colloid bismuth substrate
E = Eradicated only
H = Healed Only
HE = Healed and eradicated
M = Metronidazole
N = Nothing
O = Omeprazole
R = Ranitidine
T = Tinidazole

Table-X. Study with results at a glance

Categories	Values
Study period	December 1995 to December 1996
Duration of therapy	2 weeks
Place of the study	IPGMR, DMCH, BIRDEM
Inclusion criteria	Endoscpically proven duodenal ulcer and *H. pylori* infection confirmed by rapid urease test
Total No. of the patients included in the trial	82
Total No. of the patients completed the trial	68
Total No. (%) of the groups	4 (OTA 14, CAM 22, OA 29, RA 17)
Total No. (%) of healed cases OA 20 (83%), RA 13 (100%)	OTA 12 (92%), CAM 17 (95%),
Total No. (%) of eradicated cases	OTA 11 (85%), CAM 16 (89%), OA 16 (67%), RA 9 (69%)
Total No. (%) of healed and eradicated cases	OTA 11 (85%), CAM 15 (84%), OA 14 (58%), RA 9 (69%)
Statistical analysis:	
- Healing among the groups (Chi-square test)	$P<0.24$
- Eradication among the groups (Chi-square test)	$P<0.27$
- Both healing and eradication among the groups (Chi-square test)	$P<0.20$
- Comparison between dual- and triple-regimens (Fisher's exact test)	$P<0.06$

O=Omeprazole, T-Tinidazole, A=Amoxicillin, C-Colloid bismuth subcitrate, M=Metronidazole, R=Ranitidine

H. pylori eradication without DU healing was seen in 2 of 24 OA, 1 of 18 CAM and none of the OTA and RA groups of patients.

5.3.2. Phase-IIIb: Comparison between furazolidone-based triple therapy and nitroimidazole-based triple-therapy for the treatment of *H. pylori*-associated duodenal ulcer patients in Bangladesh - a prospective one-year follow-up study

Majority of the *H. pylori* strains isolated from Bangladeshi patients were metronidazole resistant *in vitro*. Overall experience is that metronidazole containing triple-therapy regimens appear to be effective even if *H. pylori* is resistant to metronidazole (Lindkvist*et al.*, 1996; O'Donohoe*et al.*, 1996). Furazolidone, a nitrofurans, has potential cytoprotective effect on gastroduodenal mucosa and antibacterial activity against *H. pylori* (Wotherspoon*et al.*, 1993; McColl*et al.*, 1998; Wotherspoon, 1998). Therefore, in the face of higher nitroimidazole resistance, the clinical study was undertaken to compare the efficacy of furazolidone-based triple-therapy over nitroimidazole-based triple-therapy regimens in the eradication of *H. pylori* infection in Bangladeshi duodenal ulcer patients.

A total of 116 *H. pylori*-associated duodenal ulcer patients were prospectively recruited and randomized to receive omeprazole (O) 20 mg bid, ranitidine (R) 150 mg bid, colloidal bismuth subcitrate (C) 120 mg qid, amoxicillin (A) 500 mg quid, furazolidone (F) 100 mg quid, metronidazole (M) 400 mg tid and tinidazole (T) 500 mg bid in the form of 3 furazolidone-based CAF, OAF and RAF and 2 nitroimidazole-based OAM and OAT triple-therapy regimens for 2 weeks.

Table-XI shows that the baseline characteristics of the patients. Mean age varied from 36 to 37 years (range 20-55 years) and the proportion of men, smokers and lower socioeconomic class in each group varied between treatment groups from 73 to 92%, 44 to 60% and 69 to 75%, respectively. Most of the patients (65%) had more than 5 years of history of duodenal ulcer disease (not shown in table). Twentyone (18%) patients were lost to follow-up, thus there were 116 patients in intention-to-treat (ITT) analysis and 95 in per-protocol analysis (PPA).

Table-XII shows the different treatment outcomes. *H. pylori* eradication rates for ITT analysis were: CAF 85%, OAF 93%, RAF 79%, OAM 79% and OAT 65%.

Overall *H. pylori* cure rates on the basis of ITT were 85% (65/77) in furazolidone group and 67% (26/39) in nitroimidazole group ($P<0.05$); PPA 98% (65/66) and 86% (25/29), respectively ($P<0.05$); and duodenal ulcer healing rates were ITT - 85% (65/77) in furazolidone group and 65% (25/39) in nitroimidazole group ($P<0.05$); PPA - 98% (65/66) and 97% (28/29) ($P>0.50$), respectively. *H. pylori* cure and ulcer healing, difference between ITT and PPA, were: *H. pylori* eradication (ITT [n=116]/PPA [n=95]) 85%/98% for furazolidone group ($P<0.05$), and 67%/86% for nitroimidazole group ($P<0.05$). Duodenal ulcer healing rates were 85%/98% ($P<0.05$) and 65%/97% ($P<0.05$), respectively.

Table-XIII shows the antibiogram profile. Antimicrobial susceptibility study of the 34 of 116 initial *H. pylori* isolates could be done. All 34 isolates were found susceptible to amoxicillin and nitrofurans, but 19 of 34 (54%) pretreatment

Table-XI. Baseline characteristics of patients with duodenal ulcer treated with furazolidone (CAF, OAF, RAF)-based and nitroimidazole (OAM, OAT)-based triple-therapy regimens

Characteristics	Furazolidone-based regimen (n=77)	Nitroimidazole-based regimen (n=39)
Age (years) (mean±SD)	36±14	37±18
Sex (Male/Female)	56/11 (73/27%)	36/3 (92/8%)
Socioeconomic status (Low/Middle)	58/19 (75/25%)	27/12 (69/30%)
Smoking (Yes/No)	46/31 (60/40%)	17/22 (44/56%)

Table-XII. *H. pylori* eradication and duodenal ulcer healing by different anti-*H. pylori* therapy regimen

Drug groups	*H. pylori* cure No. (%)		Duodenal ulcer healing No. (%)	
	ITT (n=116)	PPA (n=95)	ITT (n=116)	PPA (n=95)
Furazolidone-based triple-therapy regimens	65/77 (85)	65/66 (98) P<0.01	65/77 (85)	65/66 (98) P<0.01
CAF:	22/26 (85)		22/26 (85)	
OAF:	26/28 (93)		25/28 (89)	
RAF:	18/23 (79)		18/23 (79)	
Nitroimidazole-based triple-therapy regimens	25/39 (67)	25/29 (86) P<0.05	28/39 (65)	28/29 (97) P<0.05
OAM:	15/19 (79)		18/19 (95)	
OAT:	13/20 (65)		14/20 (70)	
P value	<0.05	<0.05	<0.05	>0.50

Table-XIII. Pretreatment metronidazole susceptibility and *H. pylori* eradication (n=34)

	Metronidazole sensitive (n=15)		Metronidazole resistant (n=19)	
Drug groups	Hp^-	Hp^+	Hp^-	Hp^+
Nitroimidazole	3	2	5	1
Furazolidone	9	1	8	5
Total	12	3	13	6

Hp^- = *H. pylori* eradicated
Hp^+ = *H. pylori* not eradicated

H. pylori were resistant to metronidazole (MRS). Six of these 19 patients harboring MRS *H. pylori* had been randomly assigned to nitroimidazole-based regimen; *H. pylori* was eradicated in 5 (83%) of them despite resistance to metronidazole. On the other hand, 13 of the 19 who received furazolidone-based therapy, 10 had *H. pylori* eradication.

Side-effects: Two patients in the furazolidone group had side-effects during treatment. One patient developed urticaria-type rash one week after initiation and discontinued the therapy, another had anorexia, nausea and vertigo, completed the course. Two patients in the nitroimidazole group had complaints of anorexia, vertigo and metallic taste in the mouth, completed the 2 weeks therapy.

Table-XIV shows the recurrence of *H. pylori*. Of the 95 patients entered in the 12-month follow-up, 16 (16.84%) were shown to have become *H. pylori* reinfected. Examination of the data indicates that, of these 16 patients, 11 (69%) became reinfected within 6 months, 2 at 9 month and 3 at 12 month periods after completion of anti-*H. pylori* therapy. Of the 16 *H. pylori* reinfections, 10 (15%) in furazolidone group and 6 (21%) in nitroimidazole group (P=0.52).

Duodenal ulcer relapse: Seventeen of 95 patients (17.89%) had their ulcer relapse within 12-month period (Table-XIV). Of the 17 patients, 9 were shown to have recurrence of their *H. pylori* infection and the rest 8 relapses were *H. pylori* negative. DU recurrences were 11 (17%) in furazolidone group and 6 (21%) in nitroimidazole group (P=0.74).

Table-XIV. *H. pylori* recurrence and ulcer relapse at 6, 9 and 12 month after treatment

Parameters	Months after termination of treatment							
	6 (n=95)		9 (n=73)		12 (n=66)		Total	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
H. pylori recurrence	11	(11.58)	2	(2.74)	3	(4.55)	16	(16.58)
Ulcer relapse	7	(7.37)	4	(5.48)	6	(9.0)	17	(17.89)

Table-XV shows the correlation between abdominal pain and presence or absence of active ulcer disease. Data on abdominal pain and ulcer disease were available from 66 of 95 follow-up patients. Out of 66 patients, 58 had complete healing of their ulcers, among these, 36 were symptom-free and remaining 22 had abdominal pain, of the 8 active ulcer disease patients, 6 were symptomatic and 2 were asymptomatic. Significantly, majority of the healed DU patients were symptom-free than that of active ulcer and vice-versa.

Table-XVI shows the influence of demographic factors in cure and recurrence of *H. pylori* and DU. Influence of age, sex, socioeconomic status and smoking on cure and recurrences of *H. pylori* and duodenal ulcer were evaluated by logistic regression analysis, have shown that after *H. pylori* eradication, smoking as an independent factor significantly hampered both duodenal ulcer healing and associated with more ulcer relapses.

Table-XV. Correlation between pain and ulcers (n=66)

Duodenal ulcer	Symptomatic No.	(%)	Asymptomatic No.	(%)	P value
Healed	22	(38.0)	36	(62.0)	<0.05
Not healed	6	(75.0)	2	(25.0)	
Total	28	(42.4)	38	(57.6)	

Table-XVI. Influence of age, sex, socioeconomic condition and smoking on cure and recurrence of *H. pylori* infection and duodenal ulcer (logistic regression analysis)

Characteristics	*H. pylori*		Duodenal ulcer	
	Eradication vs non-eradication (Sig)	Reinfection vs no reinfection (Sig)	Healing vs non-healing (Sig)	Relapse vs remission (Sig)
Age (years) (Mean±SD)	36±11 vs 37±11 (0.58)	36±9 vs 37±10 (0.36)	36±11 vs 37±8 (0.94)	38±7 vs 36±10 (0.85)
Sex (Male/Female)	89 vs 82% (0.28)	75 vs 95% (0.13)	91 vs 85% (0.50)	82 vs 96% (0.56)
Socioeconomic status (Lower/Middle)	72 vs 75% (0.76)	81 vs 96% (0.74)	71 vs 85% (0.39)	88 vs 97% (0.29)
Smoking (Yes/No)	52 vs 59% (0.13)	69 vs 52% (0.29)	49 vs 75% (0.07)	76 vs 50% (0.09)

DISCUSSION

6. DISCUSSION

6.1. PHASE-I: Evaluation of tests for diagnosis of *Helicobacter pylori* infection in Bangladesh

Isolation by microbiological culture and histological detection of the organism in gastric mucosal biopsy specimens are still the gold standard for diagnosis of *H. pylori* infection. Noninvasive methods of diagnosis are the urea-breath test and serology. However, standardization of different diagnostic method in our population has not yet been done. Histology allows classification of any gastritis lesion present and the sensitivity of histological techniques for the detection of *H. pylori* is high. The specificity of histological tests like sensitivity depends on the expertise of the pathologist. In a few occasion, it can be difficult to identify *H. pylori* when the few bacteria present are of atypical morphology. In ordinary practice, the sensitivity level of histology is too low to enable it to be used as the sole method for detection of infection (Taylor *et al.*, 1987). In the event of a negative result, another test should be done.

In theory, culture is the most sensitive technique for isolation of *H. pylori* because even one organism in the specimen will multiply to produce a population of billions (i.e. a colony) an thus give a positive result (Van Zwet *et al.*, 1993). The specificity of culture is undoubtedly the best of all of the techniques because, once colonies are established, it is possible to perform all of the tests required for the identification of the bacterium (urease, catalase and oxidase tests) including all of the molecular tests. Culture is essential for research purpose and it is the culture that led to the rediscovery of *H. pylori*.

Considering all these facts, microbiological culture was made 'gold standard' for the diagnosis of *H. pylori* infection in the gastric biopsy specimens.

In this pilot study, we have used various standard methods for detection of *H. pylori* infection in human gastric mucosa. Different detection methods showed different rates of *H. pylori* infection. However, the overall prevalence rates were, respectively, 51%, 55.6%, 82.2% and 93.3% in culture (conventional GasPack system), RUT, histology and serology. But the isolation rate of *H. pylori* by culture in 'candle jar' incubation system was higher than the conventional GasPack system (60% vs 51%) (Morshed *et al.*, 1995). Among the tests used, RUT was highly sensitive (95.5%), specific (82.6%), and had very high positive and negative predictive values (84% and 95%, respectively). The results of RUT agree well with Kawanishi *et al.* (1995) study on significance of RUT for identification of *H. pylori*, where authors found higher correlation between RUT and culture (0.90%) than between histology and culture (0.80%).

Microbiological study (conventional GasPack system) could have yielded more positive results if growth of contamination could have been prevented. Contamination may be due to normal saline used during tissue grinding (at least in 4 to 5 samples), during collection, transport and/or inoculation of biopsy specimens. Inoculation of single antral biopsy specimen in Brucella agar plate could be another possibility for the underestimated culture positivity.

Comparison between microbiological culture study in conventional GasPack and 'candle jar' incubation systems showed that colonies of 0.5 to 1.0 mm diameter

were obtained after 3 days incubation by the conventional GasPack system, whereas in 'candle jar' system, similar colonies were observed after 5 days incubation and about 30% of cells of 'candle jar' turned into coccoid form. However, periodic replacement of lit candles at an interval of 24 hours accelerated the growth and reduced morphological ambiguity. Therefore, we believe that 'candle jar' incubation system may help to isolate *H. pylori* from infected subjects and offer the advantage of testing drug sensitivity where only routine endoscopy and bacteriology facilities are available (Morshed *et al.*, 1995).

H. pylori drug sensitivity study showed that 75% of our clinical isolates were resistant to metronidazole. On the other hand, almost 100% isolates were sensitive to ampicillin, penicillin, ciprofloxacin and nitrofurantoin. Batnavala *et al.* (1994) also observed very high prevalence (90%) of *H. pylori* metronidazole resistance among the Bangladeshi migrants to east London. Previous exposure of patients to metronidazole is responsible for this higher resistance rates. Present results showed the higher sensitivity of histology (95.5%) and serology (100%) in the detection of *H. pylori* infection but their specificities were very low (30.4% and 13.6%, respectively). However, lower specificities for histology and serology in this study are not consistent with that of other authors (Mégraud, 1988; Varobjova *et al.*, 1991; Hu *et al.*, 1992). Higher false negative culture results could be a possibility for these lower specificities of histology and serology.

Among the different symptomatic groups, DU patients have the highest prevalence of culture positivity (64%) than that of the NUD (37.5%) and other

dyspepsia group (25%) of patients. The detection rates of *H. pylori* infection by RUT were, respectively, 64%, 38% and 75% in DU, NUD and other dyspepsia group of patients. Therefore, these findings indicate a good correlation of RUT positivity and culture positivity among DU and NUD patients and consistent with that of the Kawanishi *et al.* (1995) study.

The detection rates of *H. pylori* by histology were, respectively, 80%, 81.5% and 100% in DU, NUD and in other dyspepsia group of patients. A higher histology positivity in NUD patients could be an important predictor of expected greater population prevalence of *H. pylori* infection in Bangladesh. The exact cause of comparatively lowered histology positivity (*H. pylori*) rates in DU patients than that of the other dyspepsia group of patients is not known. Histological examination of single antral biopsy could be a possibility for this underestimated histology positivity in DU patients. About 94% of our symptomatic population including NUD patients (>92%) have *H. pylori* seropositivity. Seventy-five percent of control population (pregnant mothers and their newborns) were *H. pylori* IgG antibody positive. Almost all seropositive mothers had their seropositive child which indicates placental transfer of *H. pylori* IgG antibody from mother to fetus. Nearly 40 to 50% of NUD patients of developed nation known to have *H. pylori* infection (The European *Helicobacter pylori* Study Group, 1997; Talley *et al.*, 1998). However, no causal association between NUD and *H. pylori* is yet to be proved. Higher *H. pylori* seropositivity rates among NUD subjects could be an indication of higher population (endemicity) seropositivity rates. Presence of nearly 80% *H. pylori* infection among our under-five children also suggest higher population *H. pylori* infection.

Gastric antral biopsy from 37 consecutive symptomatic patients of different groups were examined histologically. Evidences of chronic active gastritis were present in 76% DU, 81% NUD and 100% of different dyspepsia group of patients. Though the exact cause of relatively lower incidence of active antral gastritis in DU patients is not known; sampling error could be a possibility for such underestimation. Other histological changes like atrophic gastritis and intestinal metaplasia were seen in a few percent of patients. Four DU, 1 NUD and 2 of the other dyspepsia group of patients had atrophic antral gastritis. Similarly, intestinal metaplasia were detected in 1 DU and 1 gastric cancer patient.

Clinical history of different dyspepsia group of patients were evaluated. Among the various UGI symptoms, periodicity of pain and nocturnal pain were present in a significantly higher proportion of DU patients than that of the NUD patients. Similar observation was seen in a recent study, where evaluation of UGI symptoms were done in a group of ulcer and non-ulcer dyspepsia patients (Ahmad *et al.*, 1995). The data from this series shows significantly higher incidence of nocturnal pain in DU than NUD patients (88% vs 30%) (Ahmad *et al.*, 1995).

Antacid intake gave pain relief in a significantly greater proportion of DU than NUD patients. This observation is consistent with Horrocks and DeDambal (1978) study.

6.2. PHASE-II: Prevalence of *Helicobacter pylori* infection in asymptomatic Bangladeshi adults

Bangladesh is one of the developing country. A recent study has shown more than 90% *H. pylori* seropositivity in both ulcer and non-ulcer dyspepsia patients (Morshed *et al.*, 1997). Seventy-five percent of the asymptomatic pregnant mothers and their newborns were found *H. pylori* seropositive (Morshed *et al.*, unpublished data).

The exact mode of transmission of bacteria is not known. Epidemiological evidences indicate that person-to-person transmission of *H. pylori* may occur either fecal-oral or oral-oral routes (Goodman *et al.*, 1996). Early childhood has been identified as the critical period for acquisition of infection (Mitchell *et al.*, 1992).

However, population serological survey on *H. pylori* infection is not yet undertaken in our setting. Therefore, the present cross-sectional study on these apparently healthy individuals was undertaken to plan a future large-scale seroepidemiological survey on *H. pylori* in different age and socioeconomic groups.

In the testing process, qualitative assay of *H. pylori* antibody level was made. According to information obtained from the manufacturer, the sensitivity and specificity for EIAgen is more than 90% in detecting *H. pylori*-specific antibody.

It is also noticed that by the age 20, all were infected. Almost similar seroprevalence rates were observed in Indian and African asymptomatic adult population (Holcombe *et al.*, 1992; Prasad *et al.*, 1994).

Using a ^{13}C urea-breath test and serologic assay to indicate infection, it was found that 80% Bangladeshi children became infected by3-5 years of age (Albert *et al.*, 1994).

However, the present data cannot be taken as an exact estimate of our population seropositivity rates, because all age, sex and socioeconomic groups were not included in the study. Another important lacking of this assay was that the used *H. pylori* antigen(s) was not derived from Bangladeshi strains or isolates. Therefore, future large-scale seroepidemiological study covering all age, sex and socioeconomic groups and by using our own *H. pylori* strain derived antigen(s) would likely to provide exact status.

6.3. PHASE-III

Epidemiological studies in Bangladesh have shown a point prevalence of about 12% duodenal ulcer and 3.5% gastric ulcer among the individual above the age of 15 years (Hasan *et al.*, 1985). More than 90% *H. pylori* seropositivity were found among asymptomatic adult population (Ahmad *et al.*, 1997).

The present study was a non-blind multicentric trial to see the efficacy of different anti-*H. pylori* drug regimens in the treatment of *H. pylori*-associated DU patients.

6.3.1. Phase-IIIa: Comparative efficacy of different dual- and triple-therapy regimens for the eradication of *Helicobacter pylori* infection and duodenal ulcer healing

H. pylori eradication rates were very high and almost equal by both triple-regimens (85% in OTA and 89% in CAM groups). On the other hand, comparatively lower eradication rates were achieved by the dual-regimens (67% in OA and 69.23% in RA groups). The eradication rates were not significantly different between triple- versus dual-therapy regimens.

Dual therapy refers to the combination of omeprazole or ranitidine bismuth citrate (RBC) and either amoxicillin or clarithromycine. Most of the work dealing with dual-therapy uses omeprazole and amoxicillin is based on small, uncontrolled, non-randomized studies. Eradication with omeprazole 20-40 mg twice daily in combination with amoxicillin 1 g twice daily (500 mg, 4 times daily) for 2 weeks, was between 50-90% (Howden and Hunt, 1998).

In the present study, combination of omeprazole and amoxicillin in similar doses and duration has achieved 67% cure rates. However, recent data from large, double-blind randomized controlled trials of 2 weeks treatment with omeprazole (20 mg or 40 mg twice daily) and amoxicillin (500 mg or 1 g three times daily) reported *H. pylori* eradication rates only between 39-46% (Borody *et al.*, 1994). There is no data available in literature on combination of ranitidine or famotidine and amoxicillin in the treatment of *H. pylori* infections.

In the present study, ranitidine (150 mg twice daily) and amoxicillin (500 mg four times daily) were given to 17 patients for 2 weeks and achieved 69% *H. pylori* eradication. However, definite conclusion cannot be drawn from such small uncontrolled study.

Classical triple-therapy consists of a bismuth compound (colloidal bismuth subcitrate [CBS] or bismuth subsalicylate [BSS]), metronidazole and either amoxicillin or tetracycline. There are wide variations in the dosages and treatment schedules used in these regimens. Achieved eradication rates with these regimens varied from 30 to 95% (Howden and Hunt, 1998). It is difficult to account for these differences, except by invoking the customary factors of dissimilarities in patient population, incidence of metronidazole resistance, degree of compliance with the treatment and the like. Classic triple-therapy is significantly less effective against pretreatment metronidazole resistant strains (MRS) of *H. pylori* with most eradication results falling between 30% and 60% in this group of patients (Goodwin *et al.*, 1988; Glupczynski *et al.*, 1992; Howden and Hunt, 1998).

Metronidazole resistance rates were observed very high (>90%) among the Bangladeshi resident in east London (Batnavala *et al.*, 1994).

In a recent study, Morshed *et al.* (unpublished data) found 75% metronidazole-resistant *H. pylori* strains isolated from gastric biopsy of a group of dyspepsia patients in Bangladesh. It has been observed that metronidazole resistance influences the success of treatment to lower extent than clarithromycine resistance (Glupczynski and Burette, 1992; Mégraud, 1998). Though the

drug-sensitivity profile was not seen in the present study patients, nearly 90% *H. pylori* eradication was achieved by the present triple-therapy consisting of CBS, amoxicillin and metronidazole given for 2 weeks in 18 patients. Despite the high incidence of metronidazole resistance found in our population, the exact cause of such high eradication achieved by metronidazole, CBS and amoxicillin-containing regimen in the present study is unclear. Triple-therapy combination based on omeprazole plus amoxicillin and metronidazole/tinidazole are frequently studied and are effective in 79-83% of cases. Resistance of metronidazole seems to be a weak but negative factor. Significant side-effects are in the range of 5-20%. Substitution of clarithromycine for metronidazole in such a combination achieved more than 90% *H. pylori* eradication. Though significant side-effects are below 5%, but resistance to clarithromycine is a strong negative predictive factor for success (Hopkins, 1997). Metronidazole is a cheap and easily available agent in Bangladesh. In the present study, combination of omeprazole, tinidazole and amoxicillin were given for 14 days and achieved 85% *H. pylori* eradication rate. Despite high incidence of metronidazole resistance in Bangladesh, both nitroimidazole-based triple- therapy regimen (OTA and CAM) have resulted in $\geq$85% *H. pylori* eradication rates in the present study. Therefore, the results indicate that the efficacy of both the triple-therapy regimen (OTA and CAM) were better than the dual-therapy in the eradication of *H. pylori*.

It is noticed that DU healing occurs almost equally by either antibacterial therapy alone or with combination of antimicrobial and anti-ulcer agents. We have investigated the efficacy of four different combination therapy regimens

comprising of both anti-secretory and antimicrobial drugs in the healing of DU with or without associated *H. pylori* eradication. DU healing at 6 weeks (4 weeks after cessation of 2 weeks combination therapy) was seen. Overall ulcer healing rates were between 83-100% achieved by different dual- and triple-therapy regimens. On the other hand, simultaneous *H. pylori* eradication and DU healing rates were significantly higher with both the triple-therapy regimens (85% by OTA and 84% by CAM) than the dual regimens (58% by OA and 69% by RA) (P=0.06). Therefore, observation of good correlation of ulcer healing and *H. pylori* eradication in our patients established the hypothesis of causal association of *H. pylori* to peptic ulcer disease.

The urease tests provide a simple, rapid and cost-effective method for the detection of *H. pylori*. However, the practical value of these tests depends not only on their sensitivity and specificity, but also on their speed, hence making them a practical tool for the endoscopist in decision-making as to whether or not therapy should be prescribed.

Efficacy of RUT is variable, depending on the size and site of the biopsy and method used. Yousfi *et al.* (1996) found that the diagnostic yield for detecting *H. pylori* by the RUT was not adversely affected by the size of the biopsy forceps, while Laine *et al.* (1996) showed that increasing the amount of tissue in CLO test did significantly hasten the development of positive tests. In another study, Woo *et al.* (1996) investigated the best gastric sites for obtaining a positive RUT. They found that biopsies from gastric angulus had the highest sensitivity for the detection of *H. pylori* as compared to the prepyloric and

corpus sites. Interestingly, the median time to positivity was similar with the angulus and prepyloric sites, but it was significantly shorter than for the biopsies from the corpus (60 vs 180 minutes, respectively).

Agar-based test (CLO) and membrane test (Pyloritek) are the two types of RUT which have been used to detect the presence of urease in the biopsy specimen. The Pyloritek test is designed to give a one-hour reading, without requiring special incubation temperatures. However, several studies have shown that the Pyloritek provides a sensitivity (90-99%) and a specificity (95-100%) at least equivalent to those achieved by CLO test or agar-based test after 12-24 hours.

Authors concluded that the sampling of one additional gastric biopsy did not improve significantly the diagnostic efficacy in untreated patients. But it could be required following treatment with proton pump inhibitor since in this case, *H. pylori* is more likely to be found in the body mucosa and not in the antrum.

The drawback of the present study is that both diagnosis and check cure of *H. pylori* infection were done by positive and negative RUT of two antral biopsy specimens, respectively. This might have led to some false negative results in post-treatment PPI-based therapy patients and may be responsible for overestimated *H. pylori* cure rates in these groups of patients (OTA and OA). However, this may not be true for other post-treatment groups of patients (CAM and RA).

6.3.2. Phase-IIIb: Comparison between furazolidone-based triple-therapy regimens and nitroimidazole-based triple-therapy regimens for the treatment of *Helicobacter pylori*-associated duodenal ulcer patients

Until now, the best results in the eradication of *H. pylori* from the gastric mucosa have been achieved by triple-therapy in which nitroimidazoles have been an important component.

Metronidazole resistance is increasing in both the developed and developing countries (Batnavala *et al.*, 1994; Reddy *et al.*, 1996; Weissfeld *et al.*, 1996). Majority of the *H. pylori* strains isolated from Bangladeshi subjects were metronidazole resistant *in vitro* (Batnavala *et al.*, 1994). However, overall experience is that metronidazole-containing triple-therapy regimens appear to be effective even if *H. pylori* is resistant to metronidazole (Goodwin *et al.*, 1988; Glupczynski and Burette, 1992).

Furazolidone, a monoamine oxidase inhibitor, has been reported to be effective in treating peptic ulcer disease and has anti-*H. pylori* activity. Potential ulcer healing mechanisms of furazolidone are cytoprotective effect on gastroduodenal mucosa and antibacterial activity against *H. pylori* (Zheng and Xiang, 1988; Coelho *et al.*, 1992). Furazolidone is a cheaper and easily available agent found in this region of the world.

In the face of higher nitroimidazole resistance, present study was undertaken to compare the efficacy of furazolidone-based triple-therapy over nitroimidazole-based triple-therapy regimens in the eradication of *H. pylori* infection in Bangladeshi duodenal ulcer patients.

High prevalence of *H. pylori* in Bangladesh may result in high reinfection rates (Ahmad *et al.*, 1997). Therefore, the next aim of the present study was to see the rate of *H. pylori* reinfection after its successful eradication.

H. pylori cure rates were significantly higher in the patients treated with triple-therapy regimens comprising of furazolidone, amoxicillin and an anti-ulcer (omeprazole/ranitidine/colloidal bismuth subcitrate) agent (ITT 85%, PPA 95%) than the patients treated with metronidazole/tinidazole, amoxicillin and omeprazole (ITT 67%, PPA 86%) given for 2 weeks. However, high dropout rates (18%) have resulted in significant difference between ITT and PPA. Significantly higher *H. pylori* eradication rates were achieved using omeprazole in combination with amoxicillin 1 g with furazolidone 400 mg than that of the with amoxicillin 1 g and tinidazole 1 g (93% vs 65%) ($P < 0.05$).

To our knowledge, no study with such furazolidone-based triple-therapy combination was used to treat *H. pylori* infection. Coelho *et al.* (1992) showed 60% *H. pylori* eradication in the duodenal ulcer patients when treated with furazolidone, amoxicillin and metronidazole administered for 5 days.

In a randomized controlled trial, using furazolidone, nitrofurantoin and placebo as monotherapy in Peruvian adults with antral gastritis associated with *H. pylori* infection. Morgan *et al.* (1988) have found significantly higher *H. pylori* clearance rates in both furazolidone and nitrofurantoin groups compared with placebo group. Authors also observed higher effectiveness of furazolidone over nitrofurantoin. However, a high percentage of these patients experienced

recolonization by *H. pylori* within 6 weeks after completion of treatment. It has been proved that monotherapy is ineffective due to higher occurrence of recolonization and therefore, obsolete, the use of monotherapy for *H. pylori* eradication. Achievement of better cure rates and experience of minor side-effects with furazolidone-based regimens in the present study favor their use as effective therapy in *H. pylori* eradication.

Resistance to antibiotics is considered as the primary reason for failure of eradication therapies. Resistance to metronidazole is due to lack of reduction of this compound whose genetic basis is still unknown (Mégraud, 1998). Indeed, there are problems with regard to the techniques used to detect this resistance. It has been observed that only minimum inhibitory concentrations (MICs) determined by agar dilution, and not E-test, correlate with the clinical outcome in clinical trials (Lind *et al.*, 1999). Metronidazole resistance influences the success of treatment to a lesser extent than clarithromycine resistance (Mégraud, 1998). Batnavala *et al.* (1994) have observed a very high prevalence (>90%) of metronidazole resistance among the Bangladeshi residents in east London. Morshed *et al.* (unpublished data) have found 75% metronidazole-resistant *H. pylori* among a group of dyspepsia patients in Bangladesh, and 54% (19/34) of the present pretreatment isolates were found resistant to metronidazole.

In the present study, both the treatment regimens were tested in the randomized patients irrespective of their pretreatment metronidazole-resistant *H. pylori* strains status. *H. pylori* cure rates by nitroimidazole-based regimens were: PPA 86% and ITT 67%. Uses of metronidazole-containing triple-therapy in patients

with metronidazole-resistant strains by Glupczynski and Burette (1992) and Rautelin *et al.* (1992) have shown higher cure rates. Glupczynski and Burette (1992) reported in up to 70% *H. pylori* eradication in patients with primary resistance to metronidazole when colloidal bismuth subcitrate was added to combination of amoxicillin and metronidazole. With similar regimen used by Rautelin *et al.* (1992) found 63% *H. pylori* eradication in patients with pretreatment metronidazole-resistant strains.

In the present study, we achieved 83% (5/6) *H. pylori* eradication when omeprazole was added to metronidazole and amoxicillin given for 2 weeks in patients with pretreatment metronidazole-resistant *H. pylori* strains. Uses of omeprazole in place of CBS in our study patients resulted in comparatively higher eradication rates. Similarly, Lind *et al.* (1999) have overcome the impact of primary metronidazole resistance and achieved a very high *H. pylori* eradication rates as well, by using combination of omeprazole, metronidazole and clarithromycine. These findings indicate that *in vitro* metronidazole resistance do not always correlate with resistance of it in multidrug treatment regimens.

RECURRENCE OF *HELICOBACTER PYLORI*

Knowledge of reinfection rate is important for understanding both epidemiology of *H. pylori* infection and patient management. To date, there have been a substantial number of studies in developed countries have examined the *H. pylori* reinfection rate in patients treated for peptic ulcer disease. It now appears that

reinfection is an unusual event in the developed world (Graham *et al.*, , 1987; Seppala *et al.*, 1992; Borody *et al.*, 1994; Parsonnet, 1995).

At the present time, information regarding reinfection rate in the developing countries is limited to small studies giving variable results. Acquisition appears most frequent in persons under 15 years of age and perhaps even in younger children (Batnavala *et al.*, 1995; Pounder and Ng, 1995). New acquisition in adults appears to occur at rates far lower than those in children with major mode of spread related to the geographic area, socioeconomic and family circumstances (Feldman *et al.*, 1998). An increased rate of acquisition was found in those coming to an area of high seropositivity from one of lower seropositivity (Ramirez-Ramos *et al.*, 1993). Based on our own data, the prevalence of *H. pylori* in asymptomatic adults in Bangladesh is more than 90% (Ahmad *et al.*, 1997), which compare with a prevalence of 20-25% in developed countries (Mégraud *et al.*, 1989). In addition, approximately 90% of children in Bangladesh are *H. pylori* infected by 3-5 years of age (Albert *et al.*, 1994).

Because the prevalence of *H. pylori* infection in the developing countries is significantly greater than that in the developed countries and given the living conditions, one might expect the reinfection after treatment may be high (Mégraud *et al.*, 1989; Ahmad *et al.*, 1997). Assessment of *H. pylori* reinfection rates may be affected by the quality and number of detection methods used. For accurate assessment of *H. pylori* eradication, it has been recommended that at least two tests should be used. In the present study, we confirmed *H. pylori* eradication at 1-month after completion of therapy using

rapid urease test (RUT) and culture at endoscopy. Simultaneous serial ^{13}C-urea breath test (UBT) was performed at monthly interval for a period of 12 months from the completion of therapy to detect reinfection. The time interval we selected was 3 months after treatment as the cutoff point for the cure of *H. pylori* infection. Our study shows that 16 of the 95 patients with duodenal ulcer successfully treated for *H. pylori* infection became reinfected during the 12-month period. This represented an overall 17% of annual reinfection rates. Among these 16 reinfections, 11 (69%) occurred within 6 months, indicating probable recrudescence. This observation is consistent with one of the longest follow-up study for 9 years by Bell and Powell (1996), where the authors found 57 reinfections, of them 45 occurred within 6 months of treatment. Authors suggested that most early reinfection in adults are in fact late recrudescence. The major drawback of the present study was that we do not know the molecular typing of our pre-eradication and post-eradication isolates of *H. pylori* of 16 of 95 patients reinfected with *H. pylori*. Therefore, differentiation of recrudescence from reinfection could not be established in our study. Five of the 16 reinfected patients (31%) were found to be reinfected 6 months after completion of treatment. Of these 5 patients, 2 at 9 months and 3 at 12 months were found to have reinfection. *H. pylori* eradication by furazolidone and nitroimidazole-based regimens was found to have no significant role in the subsequent *H. pylori* reinfection. Based on these results, we would suggest that those patients reinfected within the first 6 months after completion of treatment represent recrudescence, whereas patients reinfected at 12 months, most likely represent true reinfections. It has been observed that high prevalence of

H. pylori in the developing countries result in high reinfection rates. Given our interpretation, the true rate of reinfection in this population is low (4.55% per annum), indicating that even where the prevalence of infection is high, reinfection with *H. pylori* is unexpectedly low.

DUODENAL ULCER RECURRENCE

Since the crucial observation made by Warren and Marshall that eradication of *H. pylori* greatly diminishes the recurrence rate of duodenal ulcer. Studies have shown that ulcer relapse is relatively uncommon in patients cured of *H. pylori* (Tytgat, 1995). More recently, Hopkins *et al.* (1996) reviewed several duodenal ulcer eradication studies and noted an overall recurrence rate of 6% following successful eradication of *H. pylori* compared with 67% when the infection remained. In the present study, 17 of 95 patients treated for *H. pylori* and having healed their ulcers had an ulcer relapse within 12-month period. Nine of these patients were reinfected with *H. pylori*. Eight patients had an ulcer relapse despite the fact that they were *H. pylori* negative. Ulcer relapse in these eight patients had no history of consumption of aspirin or nonsteroidal antiinflammatory drugs. Recurrence of ulceration in these patients may have an alternative etiology. Duodenal ulcer disease either related to sustained high acid output or due to specific disease, like tuberculosis and Crohn's disease, can be the probable possibilities considered in these patients. Among the various demographic factors, smoking as an independent factor has significantly hampered ulcer healing and as well as more ulcer relapses after eradication of *H. pylori* infection. However, smoking had no influence over *H. pylori*

eradication and reinfection in these patients. This observation is consistent with that of Cuttler and Schubert (1993) and Graham *et al.* (1991).

CORRELATION BETWEEN PAIN AND ULCERS

A frequent dissociation between the presence of an ulcer crater and symptoms have been found in several recent trials. Ulcer healing does not guarantee disappearance of symptoms, although the majority of patients who are ulcer-free by endoscopy are asymptomatic, 4 to 39% of ulcer-free patients do have persistent symptoms. Conversely, in clinical trials, ulcers may be present without producing symptoms, from 15 to 44% of symptom-free patients have been found to have a DU crater at endoscopy (Peterson *et al.*, 1977; Ippoliti *et al.*, 1978).

In the present study, significantly increased number of DU-free patients were asymptomatic than that of the non-healed DU (62% vs 25%). On the other hand, 38% of ulcer-free and 75% of active DU patients had persistence of symptoms. Therefore, disappearance of symptoms by no means guarantees ulcer healing, nor does the persistence of symptoms necessarily predict a persistent ulcer crater.

CONCLUSIONS

7. CONCLUSIONS

1) Among the diagnostic tests used, rapid urease test was found to be the most effective for the diagnosis of *H. pylori* infection.

2) Fifty to seventy-five percent of *H. pylori* strains isolated from Bangladeshi patients were metronidazole resistant *in vitro*.

3) More than 90% asymptomatic adults in Bangladesh were *H. pylori* seropositive and it was noticed that by the age of 20, all were infected.

4) Triple-therapy regimens consisting of daily omeprazole 20 mg bid, amoxicillin 500 mg qid plus metronidazole 400 mg tid or tinidazole 500 mg bid, given for 2 weeks have achieved 65-85% *H. pylori* eradication and combination regimen comprising of daily colloidal bismuth subcitrate 120 mg qid, amoxicillin 500 mg qid and metronidazole 400 mg tid, given or 2 weeks resulted in 89% *H. pylori* eradication.

5) Though metronidazole resistance plays an important role in the failure to eradicate *H. pylori*, we observed more than 80% *H. pylori* eradication rates with present metronidazole-based triple-therapy regimen (OAT and OAM) in patients with metronidazole-resistant *H. pylori* strains. Number of patients included was small, therefore, definite conclusion cannot be drawn from this data.

6) Furazolidone-based triple-therapy regimens consisting of daily furazolidone 100 mg qid, amoxicillin 500 mg qid plus either of the following: ranitidine 150 mg bid, omeprazole 20 mg bid and colloidal bismuth subcitrate 120 mg qid, given for 2 weeks, have achieved 79 to >90 percent *H. pylori* eradication.

7) Therefore, observation of significantly better efficacy of triple-therapy combination of furazolidone, amoxicillin plus an antiulcer agent over metronidazole/tinidazole and amoxicillin plus antiulcer agent, can be considered as an alternative to conventional triple-therapy regimens in terms of clinical efficacy, tolerance and cost.

8) Correlation between pain and ulcers: It is observed that disappearance of symptoms by no means guarantees ulcer healing, nor does the persistence of symptoms necessarily predict a persistent ulcer crater.

9) Among the various demographic factors, smoking as an independent factor, has significantly hampered ulcer healing and as well as more ulcer relapses after eradication of *H. pylori* infection. However, smoking had no influence over *H. pylori* eradication and reinfection in our patients.

10) Overall, *H. pylori* recurrence rates were >16% (16/95) at one year, and of these more than 60% have occurred before 6 months, indicating probable recrudescence. Five of these 16 reinfected patients (30%) were found to be reinfected 6 months after completion of treatment. Of these 5 patients, 2 at 9 months and 3 at 12 months were found to have reinfection.

Thus, true yearly reinfection rates were near 5 percent (3 of 66 who completed the 1-year follow-up).

11) Finally, observation of comparatively lower *H. pylori* reinfection rates emphasizes the eradication therapy as the choice of treatment for peptic ulcer patients in the developing countries.

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9. REFERENCES

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APPENDICES

APPENDICES

APPENDIX-I: PAPERS

Paper-I
[Published in: Lancet 1995; 346:511]

GROWTH OF *HELICOBACTER PYLORI*

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Helicobacter pylori, a causative agent of gastritis[1,2], was first isolated from a human gastric biopsy specimen in 1983[3]. The slow growth, factitious nature, and special growth requirement have made the culture of *H. pylori* difficult. In developing countries, *H. pylori* related diseases are mostly diagnosed by signs and symptoms of patients or by serological and/or other immunological techniques[4]. Because of difficulties in culture and sensitivity testing, selection of drug regimens against this pathogen depends completely on data provided by laboratories from developed countries.

An *H. pylori* culture system requires microaerobic conditions. A gas pack, which generates microaerobic conditions and is commonly used for the isolation of *Campylobacter jejuni*, is currently being used for isolation of *H. pylori*[4]. IN a preliminary experiment, it has been observed that carbon dioxide (CO_2) is important for the growth of *H. pylori* and successive culture has been possible in

5% CO_2 incubator (M.G. Morshed, unpublished data). It is also known that *C. jejuni* can grow evenly in a candle jar, which has made its culture easier. We attempted to culture *H. pylori* from a biopsy specimen of a gastritis patient with use of a candle jar at high humidity.

First, we established the cultivation system of *H. pylori* in our BIRDEM microbiology laboratory with a commercial gas pack (Campybak Systems, BBL Microbiology System) following standard procedures[5]. Then we extended our study to see whether *H. pylori* can be grown in a candle jar like *C. jejuni*. Candle jar incubation was evaluated against the conventional gas pack system. 10 patients, 6 with duodenal ulcer and 4 with non-ulcer dyspepsia, were selected and endoscopic biopsy sections were collected. In both culture systems, equal volumes of biopsy materials were inoculated onto Skirrows modified agar media and incubated at 37°C following standard techniques[5]. In the candle jar system, a steel anaerobic jar (BTL, UK) was used. To ensure high humidity, tissue papers soaked in sterile water were place in a plastic petri dish with a perforated top. Inoculated plates, the petri dish containing soaked tissue papers, and lit candles were placed in the stool jar carefully and its lid was immediately closed in order to generate at high CO_2 and low oxygen atmosphere. Then the jar was incubated at 37°C. Among the samples, 6 showed profuse growth of characteristic colonies of *H. pylori* at both systems. The isolated bacteria were identified as *H. pylori* on the basis of colony morphology, gram reaction, and urease, catalase, and oxidase tests. It should be noted that colonies of 0.5-1.0 mm diameter were obtained after 3 days incubation by the commercial gas pack, but in a candle jar, similar colonies could be obtained after 5 days and about

30% cells turned to coccoid form. However, periodic replacement of lit candles at an interval of 24 h accelerated the growth and reduced morphological ambiguity.

We believe that this system will help to isolate *H. pylori* from gastritis patients and offers the advantage of testing drug sensitivity where only routine endoscopy and bacteriology facilities are available.

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Paper II

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EVALUATION OF CULTURE, HISTOLOGICAL EXAMINATION, SEROLOGY AND THE RAPID UREASE TEST FOR DIAGNOSIS OF *HELICOBACTER PYLORI* IN PATIENTS WITH DYSPEPSIA IN BANGLADESH

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SUMMARY

Helicobacter pylori, a gram-negative, microaerophilic bacterium, has been established to have a causal association with chronic gastritis, peptic ulcer, gastric adenocarcinoma, and low-grade lymphoma. The present study was undertaken to evaluate the efficacy of culture, histological examination, the rapid urease test, and serology for the diagnosis of *H. pylori* infection. A total of 45 consecutive subjects with various upper gastrointestinal symptoms were included in this study. The rates of diagnosis of *H. pylori* infection was 51.1%, 55.6%, 82.2% and 93.3%, by culture, rapid urease test (RUT), histological examination, and serology, respectively. The sensitivity, specificity, and

positive and negative predictive values were 95.5%, 82.6%, 84.0% and 95.0%, respectively, for RUT; 95.5%, 30.4%, 56.8% and 87.5% for histological examination; 100%, 13.6%, 54.8% and 100% for serology.

INTRODUCTION

Helicobacter pylori, the gram-negative microaerophilic bacterium that is the causative agent of chronic gastritis[1,2], was first isolated from a human gastric biopsy specimen in 1983[1]. The human gastric mucosa[3] and the gastric antrum[4] are the commonest sites of colonization and constitute the only known natural reservoir of *H. pylori*. Etiological association with *H. pylori* infection have been established for type B antral gastritis, peptic ulcer disease, and gastric adenocarcinoma and lymphoma[4-11]. Although its slow growth, fastidious nature, and special growth requirements have made culture of *H. pylori* difficult, isolation by microbiological culture and histological detection of the organism in gastric mucosal biopsy specimens are still the gold standard for diagnosis of *H. pylori* infection[12]. Non-invasive methods of diagnosis are the urea breath test (C^{13} or C^{14}) and serology[13-15]. However, standardization of the diagnostic criteria in our population has not yet been achieved. The present study was undertaken with the aim of establishing bacteriological culture and histopathological facilities for the isolation and detection of *H. pylori* in gastric biopsy specimens and of comparing the efficacy of various diagnostic methods from a Bangladesh perspective.

METHODS AND SUBJECTS

Forty-five consecutive patients with upper gastrointestinal (UGI) symptoms underwent UGI endoscopy were included in this study. They were recruited from the endoscopy clinics of the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) and the Institute of Postgraduate Medicine and Research (IPGM&R). UGI endoscopy was performed after overnight fasting in consenting subjects. Individuals with a history of antibiotic or bismuth salt intake in the preceding 8 weeks or history of gastric surgery were excluded from the study.

A brief case history of each subject was recorded on a preformed questionnaire. Usually four to six antral biopsy specimens were collected from each subject for the rapid urease test (RUT), and bacteriological and histopathological examinations, and 3 to 5 ml of venous blood was drawn from each for the test anti-*H. pylori* antibody test.

Culture

Endoscopic biopsy specimens were collected from the gastric antrum. Each biopsy specimen was immediately transferred to Stewart modified transport medium and brought to the laboratory. The biopsy specimens were homogenized in 200 μl of normal saline with a tissue homogenizer and spread onto BASC (Brucella agar supplemented with 1% heat-inactivated horse serum and 0.1% ß-cyclodextrin) medium for culture[16]. To prevent contamination,

vancomycin 10 μg/ml, amphotericin B 5 μg/ml, trimethoprim 5 μg/ml, and polymyxin B 10 μg/ml were also added to the media[16]. The culture plates were incubated at 37°C in a Campy Pak jar (BBL Microbiology System, Maryland) under microaerophilic (5% O_2, 10% CO_2, 85% N_2) conditions using Campy Pak (Oxoid, Hampshire, UK) for 3-5 days. Characteristic colonies of *H. pylori* were confirmed by oxidase, and urease hippurate hydrolysis tests and by their Gram stain morphology.

Rapid urease test

One of the biopsy specimens was directly inoculated into the RUT tube to detect the urease activity of *H. pylori* as described by Kawanishi *et al.*[17]. Some of the homogenized tissue was also inoculated in RUT tubes for rechecking. A positive result was recorded if there was a color change from yellow to pink within 6 hours.

Histological examination

One biopsy specimen from the gastric antrum was used for histological examination. The specimens were fixed in 10% buffered formalin, processed, and then embedded in paraffin. Four sections 3-4 micron thick were stained with hematoxylin and eosin for histological evaluation and with modified Giemsa stain for identification of *H. pylori*[18-20]. The presence of the characteristic spiral organism was considered *H. pylori* positive, and the specimen was considered negative, if it was not seen in any of the four slides.

Serology

Serum anti-*H. pylori*, IgG was detected by a commercial enzyme immunoassay kit (EIAGEN, Clone System, Italy). The test was performed according to the manufacturer's instructions. This assay system is based on qualitative and quantitative detection of IgG-specific antibodies to *H. pylori*. Antibody levels of >15 AU/ml (arbitrary unit per milliliter) were considered positive.

RESULTS

The study population was comprised of 25 patients with duodenal ulcer (DU), 16 patients with non-ulcer dyspepsia (NUD), one patient with gastric ulcer (GU), and three patients with gastric carcinoma. Mean ages were 43 ± 16 years for the DU patients, 38 ± 13 years for the NUD patients, and 60 ± 17 years for the gastric carcinoma patients. Forty-one of the 45 dyspeptic patients were male. The study population was mostly in the middle socioeconomic class.

The *H. pylori* detection rates by culture, the rapid urease test (RUT), histological examination and serology are shown in Table-I. The overall detection rates of *H. pylori* were 51.1%, 55.6%, 82.2% and 93.3% by culture, RUT, histological examination and serology, respectively. *H. pylori* could be isolated in cultures of biopsy specimens from 16 DU patients, 6 NUD patients and 1 gastric carcinoma patient. The colonies were circular, translucent, and convex, varying from 0.5 mm to 1 mm in diameter. Gram-stained smears of the bacteria revealed long, curved bacilli. *H. pylori* was confirmed by positive oxidase,

catalase and urease tests. Biopsy cultures from 5 DU patients, 7 NUD patients and 1 GU patient yielded no growth at all, whereas cultures from 4 DU patients, 3 NUD patients and 2 gastric carcinoma patients showed growth of contaminants. Among the 45 patients, 16 DU patients, 6 NUD patients, 2 gastric carcinoma patients, and 1 GU patient were positive in the RUT and 25 DU patients, 15 NUD patients, 1 GU patient and 1 gastric carcinoma patient were positive for anti-*H. pylori* IgG (Table-I).

The test results were compared and the diagnostic efficacy of RUT, histological examination and serological studies was measured with that of culture. the sensitivity, specificity, and positive and negative predictive values were, respectively, 95.5%, 82.6%, 84.0% and 95.0% for RUT, 95.5%, 30.4%, 56.8%, and 87.5% for histological examination, and 100%, 13.6%, 54.8% and 100% for serological studies (Table-II).

DISCUSSION

Various standard methods for detection of *H. pylori* infection in human gastric mucosa were evaluated. Different methods yielded different rates of detection of *H. pylori* infection, however, the overall detection rates by culture, RUT, histological examination and serology were 51.1%, 55.6%, 82.2% and 93.3%, respectively. Among the diagnostic tests used, RUT was found to be the most sensitive (95.5%) and specific (82.6%), and had very high positive (84.0%) and negative (95.0%) predictive values when compared with culture results as the gold standard. The results of RUT agreed well with the findings of Kawanishi

Table-I. Detection of *H. pylori* infection in dyspepsia patients by different diagnostic methods

Endoscopic findings	Number of cases	Culture	RUT	Histological examination	Serology
Duodenal ulcer	25 (55.6%)	16	16	20	25
Non-ulcer dyspepsia	16 (35.6%)	6	6	13	15
Other forms of dyspepsia (gastric ulcer and gastric cancer)	4 (4.9%)	1	3	4	3
Total	45	23	25	37	42
Percentage	100	51.1	55.6	82.2	93.3

Table-II. Comparative efficiency of diagnostic tests for *H. pylori*

Test	Culture	RUT	Histological examination	Serology
Positive	23	25	37	42
Negative	22	20	8	3
True positive	-	21	21	23
True negative	-	19	7	3
False positive	-	4	16	19
False negative	-	1	1	0
Sensitivity	-	95.5%	95.5%	100.0%
Specificity	-	82.6%	30.4%	13.6%
PPV	-	84.0%	56.8%	54.8%
NPV	-	95.0%	87.5%	100.0%

PPV = Positive predictive value
NPV = Negative predictive value

et al.[17] who reported a more significant correlation between the results of RUT and culture (0.90) than between histological findings and culture (0.80). Microbiological studies could yield more positive results if the growth of contaminants could be prevented. Inoculation of single antral biopsy specimen for culture might be another possibility for this underestimated positivity.

Our test results showed high sensitivity of histological examination (95.5%) and serology (100%) for detection of *H. pylori* infection, but their specificities were very low (Table-II). These findings are not consistent with those of others[12,18,20]. Higher false negative cultures may be attributable to the low specificity of histological examination and serology. In this study, seropositivity was very high in comparison to culture. A pilot study of the seroprevalence of *H. pylori* in Bangladesh was performed among 226 healthy asymptomatic adult males and yielded 89.8% positivity (data not shown). Among the different dyspepsia groups, the duodenal ulcer patients had higher culture positivity (64.0%) than the non-ulcer dyspepsia patients (37.5%). Similarly, detection of *H. pylori* infection by RUT was 64.0% and 37.5% in the DU and NUD patients, respectively. Thus, the results suggest a good correlation between RUT and culture positivity among DU patients (P=0.0000) and NUD patients (P=0.0002).

The overall detection rates of *H. pylori* infection by histological examination were 82.2% versus 93.3% by serology. Such a high positivity in both ulcer and non-ulcer dyspepsia indicates that the prevalence of *H. pylori* infection in Bangladesh is very high.

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Paper-III
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PREVALENCE OF *HELICOBACTER PYLORI* IN ASYMPTOMATIC POPULATION - A PILOT SEROLOGICAL STUDY IN BANGLADESH

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ABSTRACT

Epidemiological reports reveal that *Helicobacter pylori* is distributed among all population in the world. The present cross-sectional study was undertaken to see the *H. pylori* seroprevalence rates among the asymptomatic adults, as yet reportedly no such data available in Bangladesh. Serum samples were collected from 181 consecutive subjects who attended at the health check-up center of Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, during the period of August to November 1995 for medical check up. The mean age of these subjects was 30.33 years (range 20-44 years). Incidentally all were male and belonged to

average socioeconomic class. *H. pylori*-specific IgG antibody level was assayed by an enzyme immunoassay kit EIAgen (Clone System). Among the 181 subjects, 166 (92%) had *H. pylori*-specific antibodies and 15 (8%) were seronegative. No significant difference ($P < 0.90$) in seroprevalence rates was observed among different age groups. However, the results of higher seroprevalence rates of *H. pylori* infection in these asymptomatic adult population of Bangladesh are consistent with that of Africa and India.

INTRODUCTION

Helicobacter pylori a gram-negative, microaerophilic bacterium, considered as the main etiological agent for chronic gastritis[1] and also an important determinant for ulcerogenesis, especially in the long-term recurrence of duodenal and gastric ulcer disease[2,3]. The evidences are also mounted regarding its association with gastric adenocarcinoma and low-grade primary gastric lymphoma[4-9].

Recent epidemiological reports of *H. pylori* reveal that it is distributed among all population in the world. The prevalence rate in the developed countries is generally lower than that in the developing countries, reflecting socioeconomic status; the poorer a population, the earlier is the age of infecting *H. pylori*, resulting in the higher prevalence rate[10-12].

Since the recognition of role of *H. pylori* in the etiology of various gastroduodenal diseases, a number of methods which can be used for the diagnosis of *H. pylori* infection and are highly accurate but have the drawback of

requiring gastroduodenoscopy. A possible noninvasive alternative is ^{13}C-urea and ^{14}C-urea breath tests, which depends, however, on specialized instrument or radioactive isotopes[15,16]. The simplest noninvasive test is to detect the specific antibodies to *H. pylori* in serum[17-20]. However, the systemic immune response to *H. pylori* infection confers no protection against the organisms and its presence is of diagnostic value only[21].

Bangladesh is one of the developing country having a reported highest point prevalence of duodenal ulcer among the population above the age of 15 years[22], thus an attempt has been made to see the seroprevalence of *H. pylori* in a cross-section of asymptomatic adult population and which will be helpful to plan a future large-scale population survey in different age and socioeconomic groups.

SUBJECTS AND METHODS

The serum samples were collected from 181 consecutive healthy young male individuals who attended during the period of August to November 1995, for medical check-up at Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) Health Care Centre (BHCC) to work as immigrant worker in Malaysia.

Subjects came from different parts of Bangladesh. Information about their age and socioeconomic status were noted.

After separation of serum by centrifugation, samples were stored at -20°C before testing. The serum samples test was based on the ELISA (enzyme-linked immunosorbent assay) technique[23] by using ELISA test kit (EIAgen, Clone System S.P.A., Casalecchio Di Reno, Italy). This assay system was based on qualitative detection of IgG specific antibodies to *H. pylori*. The type of antigen(s) used in EIAgen kit was not detailed in the supplied instructions. The test was performed according to manufacturer's instruction.

H. pylori antigens were immobilized on the wells of micro-well plate. Diluted patients serum was added to the wells. IgG antibodies specific to *H. pylori*, if present, bind to the antigen on the micro-wells. The intensity of the color corresponds directly to the amount of antibodies present. The cutoff values of more than 15 arbitrary unit (AU) per milliliter (ml) was considered as positive test.

Statistical analysis

Statistical comparison were made with JMP software (SAS Institute)[24]. A two-sided P value of 0.05 was the criterion for statistical significance. Chi-square test was done to find any difference in prevalence of different age groups of population.

RESULTS

The baseline data is summarized in Table-I. The mean age of these subjects was 30.33 years (range 20 to 44). All were male and came from almost all the

Table-I. Baseline data with results

Period of study	August 1995 - November 1995
Sex	All were male
Age (years)	Mean 30.33 (range 20-44)
Socioeconomic status	Average
Test undertaken to detect *H. pylori*	ELISA (EIAgen, ELISA kit Clone System)
Total number of sample	181
Number of subjects found positive	166
Prevalence of *H. pylori*	92%

districts of Bangladesh. Study subjects were the village people, belonged to average socioeconomic class and cultivation was their sole livelihood.

Among the 181 subjects, 166 (92%) had *H. pylori*-specific IgG antibodies and 15 (8%) were seronegative (Table-II). There was no significant difference ($P < 0.90$) observed in the prevalence of infection among different age groups.

DISCUSSION

The exact mode of transmission of bacterium is not known. Epidemiological evidences indicate that person-to-person transmission of *H. pylori* may occur either fecal-oral or oral-oral routes[25]. However, at present it is assumed that the most likely mode of spread would be from saliva in childhood[26,27]. Recent molecular typing studies on isolates from the United Kingdom and other countries at the National Collection of Type Cultures (NCTC) showed considerable diversity in strains from different individual, yet multiple isolates from different gastroduodenal sites in an individual are usually identical and similarities of DNA sequence between isolates from family members indicate that some *H. pylori* infection are acquired in households during childhood[28].

Bangladesh is one of the developing countries having a reported highest point prevalence of duodenal ulcer among the population above the age of 15 years[22]. However, population serological survey on *H. pylori* infection is not yet undertaken in our setting. Therefore, the present cross-sectional study on these apparently healthy individuals was undertaken to plan a future large-scale seroepidemiological survey on *H. pylori* in different age and socioeconomic groups.

Table-II. *H. pylori* seroprevalence rates in different age groups

Age groups (years)	Number of subjects	Number of positive test	*H. pylori* seroprevalence rate (%)
20-24	33	29	88
25-29	100	92	92
30-34	34	32	94
35-39	13	12	92.3
40-44	1	1	100
Total	181	166	91.71

In the testing process, qualitative assay of *H. pylori* antibody level was made. According to information obtained from the manufacturer, the sensitivity and specificity for EIAgen is more than 90% in detecting *H. pylori*-specific antibody. It compares favorably with the results of other *H. pylori* ELISAs in which 93.8-100% sensitivity and 79-90% specificity were reported[29,30].

It is also noticed that by the age 20, all were infected. Almost similar seroprevalence rates were observed in Indian and African asymptomatic adult population[32,33].

However, the present data cannot be taken as an exact estimate of our population seropositivity rates, because all age, sex and socioeconomic groups were not included in the study. Another important lacking of this assay was that the used *H. pylori* antigen(s) was not derived from Bangladeshi stains or isolates. Therefore, future large-scale seroepidemiological study covering all age, sex and socioeconomic groups and by using our own *H. pylori* strain derived antigen(s) would likely to provide exact status.

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Paper IV

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TWO WEEKS COMBINATION THERAPY WITH ANTIMICROBIALS AND ANTI-ULCER AGENTS IN DUODENAL ULCER (DU) PATIENTS - A COMPARATIVE STUDY OF DIFFERENT REGIMENS IN THE HEALING OF DU AND ERADICATION OF *HELICOBACTER PYLORI*

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SUMMARY

We compared the efficacy of different regimens given for 2 weeks in the eradication of *Helicobacter pylori* and healing of duodenal ulcer disease. A total of 82 patients with duodenal ulcers and *H. pylori* infection confirmed by endoscopy and rapid urease test, respectively, were randomly assigned to receive any one of the following four combination therapy regimens for two weeks: OTA group (omeprazole 20 mg bid + tinidazole 500 mg bid + amoxicillin 500 mg qid); CAM group (colloidal bismuth subcitrate 120 mg qds + amoxicillin 500 mg qds + metronidazole 400 mg tid); OA group (omeprazole 20 mg bid + amoxicillin 500 mg qid); and RA group (ranitidine 150 mg/bid +

amoxicillin 500 mg qid). Follow-up endoscopies were performed at 6 weeks (4 weeks after completion of 2 weeks therapy). A total of 68 patients completed the trial. *H. pylori* eradication rates were: OTA 84.6%, CAM 88.9%, OA 66.7% and RA 69.2% ($P<0.27$). On the other hand, duodenal ulcer healing rates were: OTA 92.3%, CAM 94.5%, OA 83% and RA 100% ($P<0.24$). Simultaneous DU healing and *H. pylori* eradication were: OTA84.6%, CAM 83.5%, OA 58.3% and RA 69.2% ($P<0.20$). The classical triple therapy (CAM) and the other triple therapy (OTA) have shown almost equal efficacy in eradicating *H. pylori* infection and duodenal ulcer healing ($P<0.92$). Borderline significant differences ($P<0.06$) have been found between triple (OTA+CAM) and dual regimens (OA+RA) regarding simultaneous duodenal ulcer healing and eradication of *H. pylori*.

Therefore, future study with larger sample size using triple and dual therapy regimens may provide more better and scientifically significant results.

INTRODUCTION

Helicobacter pylori is the commonest bacterial pathogen worldwide and has been identified in all countries. The recognition of gastritis due to *H. pylori* has revolutionized the therapeutic approach to peptic ulcer disease[1,2]. The strong relationship of *H. pylori* with peptic ulcer disease is inferential and is largely based upon a plausible hypothesis: the strong association with *H. pylori*-induced gastritis and improved rate of ulcer healing with *H. pylori* suppression[3,4], and the markedly decreased recurrence rates for duodenal ulcer disease after eradication

of the bacteria[5,6]. Rates of recurrence in patients whose initial ulcers healed during conventional antisecretory therapy range from 60 to 100% per year, but ulcers recur in less than 15% of patients in whom the organism has been eradicated by antibacterial treatment[3,7-10].

Since the discovery of *H. pylori* in 1983 by Warren and Marshall[11], many different therapeutic regimens have been advocated in different parts of the world[12-21]. Triple therapy with bismuth compound results in high eradication rates and is the current treatment of choice, although a substantial proportion of patients receiving such therapy have significant side-effects. The results of dual therapy (amoxicillin and omeprazole) are good but still with varied success while the dual therapy comprising clarithromycine and omeprazole can develop resistance to macrolides. However, triple therapy using combinations of omeprazole, metronidazole or tinidazole, and amoxicillin or clarithromycine resulted in a very high cure rates (90%)[22-24]. Tolerability, compliance and cost of such regimens may vary from population to population and also within the same population. Therefore, it is necessary to conduct such clinical trials in as many parts of the world as possible.

Duodenal ulcer disease is a common health problem in Bangladesh. Since epidemiological data from Bangladesh provide convincing evidence about the high incidence of peptic ulcer disease[25] and 92% *H. pylori* seropositivity in asymptomatic population[26], it is imperative that large-scale trials are done here. Therefore, the present multicentric clinical trial was undertaken to see the comparative efficacy of different triple and dual therapy regimens, given for 2 weeks in the eradication of *H. pylori* and healing of duodenal ulcer disease.

PATIENTS AND METHODS

Setting

The study was conducted from December, 1995 through December, 1996. Eighty-two consecutive patients with endoscopically proven duodenal ulcer from endoscopy units of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) and Dhaka Medical College Hospital (DMCH), gave informed consent to be randomly assigned to receive one of the following four treatment group for 2 weeks. Number of patients selected from different centers were 37 from BSMMU, 22 from BIRDEM and 23 from DMCH.

Inclusion criteria

Patients aged from 14 to 80 years had to have a duodenal ulcer with a diameter of 5 mm or more to quality for the study. Patients with gastric ulcer, or who had taken bismuth or antibiotics during the 4 weeks before endoscopy, were not included. Those who were regularly taking nonsteroidal anti-inflammatory drugs or corticosteroids also excluded from this study. Other exclusion criteria were surgical treatment of ulcer-related conditions, pregnancy and penicillin allergy.

Treatment regimens

All treatment regimens were taken in oral route.

OTA group: Omeprazole (20 mg bid), tinidazole (500 mg bid) and amoxicillin (500 mg qid) - 14 patients.

CAM group: Colloidal bismuth subcitrate (120 mg qid), amoxicillin (500 mg qds) and metronidazole (400 mg tid) - 22 patients.

OA group: Omeprazole (20 mg bid) and amoxicillin (500 mg qid) - 29 patients.

RA group: Ranitidine HCl (150 mg bid) and amoxicillin (500 mg qid) - 17 patients.

Endoscopy and patients assignment

An endoscopic examination was performed before treatment to check duodenal ulcer and biopsy specimens were taken from the antrum for rapid urease test (RUT) to find out associated *H. pylori* infection. RUT was performed to see the urease activity and positive test was considered when clear reddening in the sample appeared within 6 hours at room temperature. A positive result of RUT alone was considered as criteria for a patient to be classified as positive for *H. pylori*[27,28]. All patients with duodenal ulcer and positive urease test were randomly assigned to the four treatment groups immediately after endoscopy. Both the patients and their physicians were aware of the treatment assignments.

Before starting treatment, the patients' medical history were recorded. Six weeks after randomization (i.e. four weeks after the completion of therapy) follow-up endoscopy was performed, to check the healing of the ulcers and biopsy specimens were taken for the RUT test to confirm the eradication of *H. pylori*. All patients were asked by research investigator about compliance with the treatment, side-effects and symptoms related to the ulcer. Patients who took more than 80 percent of the supplied drugs were considered compliant.

Statistical analysis

Statistical comparison were made with JMP software (SAS Institute)[29]. A two-sided P value of 0.05 was the criterion for statistical significance. The primary outcome in this study was duodenal ulcer healing, eradication of *H. pylori* and healing plus eradication. The number of patients healed, eradicated, and healed plus eradicated in different groups were compared by Chi-square test separately. The Fisher's exact test was performed to compare the number of healed and eradicated cases between dual and triple regimens.

RESULTS

Most (78%) of the patients were male and belonged to middle and lower socioeconomic classes (Table-I). Among the study subjects, 1 from OTA group, 5 from OA group, 4 from both RA and CAM group did not attend the follow-up endoscopy at 6 weeks (Fig. 1). The remaining 68 patients completed the trial. The dropout rate did not differ significantly among different treatment groups.

Table-I. Characteristics of 82 patients with duodenal ulcer and *H. pylori* infection assigned to 4 different treatment regimens

Characteristics	OTA group	CAM group	OA group	RA group
Mean age (years) (range)	36.5 (16-72)	39.41 (14-80)	38.55 (19-70)	43.13 (23-60)
Male/Female ratio	11:3	9:2	22:7	11:4
Ever smoker (%)	10 (72)	15 (59)	15 (52)	7 (47)
Socioeconomic condition				
Upper	0	1	1	2
Middle	8	15	15	10
Lower	6	6	13	5

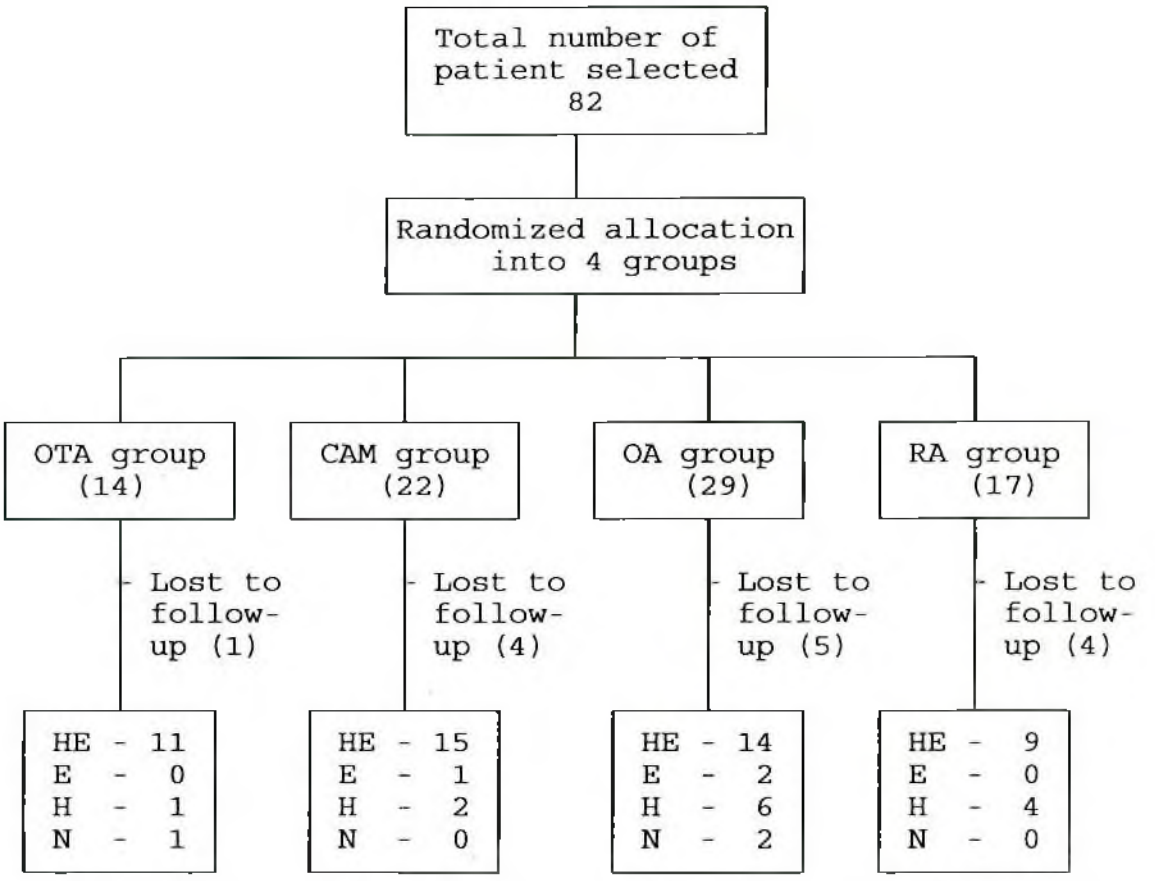

Fig. 1. Flow diagram of the study

A = Amoxicillin
C = Colloid bismuth substrate
E = Eradicated only
H = Healed Only
HE = Healed and eradicated
M = Metronidazole
N = Nothing
O = Omeprazole
R = Ranitidine
T = Tinidazole

Patients who completed the trial did not complain of any remarkable side-effects. Characteristics of the patients of different treatment groups are shown in Table-I. Follow-up endoscopy at the end of 6 weeks showed complete healing of duodenal ulcer in 12 (92.3%) out of 18 from CAM group, 20 (83.3%) out of 24 from OA, and all (100%) out of 13 from RA group ($P<0.24$). Eradication of *H. pylori* was documented in 11 (84.6%) out of 13 from OTA, 16 (88.9%) out of 18 from CAM, 16 (66.7%) out of 24 from OA, and 9 (69.2%) out of 13 from RA group ($P<0.27$). Duodenal ulcer healing and *H. pylori* eradication rates were not different significantly in these groups.

Simultaneous healing of DU and eradication of *H. pylori* were achieved in 11 (85%) out of 13 from OTA, 15 (83.5%) out of 18 from CAM, 14 (58.3%) out of 24 from OA, and 9 (69.2%) out of 13 from RA group ($P<0.20$). Considering simultaneous DU healing and *H. pylori* eradication, the efficacy of triple therapy was greater than dual therapy, although that was borderline significant ($P<0.06$).

DISCUSSION

H. pylori eradication rates were very high and almost equal by both triple regimens, 85% in OTA and 89% in CAM group. On the other hand, comparatively lower eradication rates were achieved by dual regimens, 67% in OA and 69% in RA group. Though the eradication rates were not significantly different between triple versus dual therapy, the results are consistent and comparable with that of the similar trial in different centers of the world[12-21].

Classical triple therapy (comprising colloidal bismuth, metronidazole and amoxicillin or tetracycline) resulted in high eradication rates especially in the organisms sensitive to metronidazole and is the current treatment of choice because of its cost and efficacy[30-32]. The classical triple therapy for 14 days eradicated *H. pylori* in up to 90% of patients[10,33-35]. Eighty to 90 percent eradication are achieved by triple therapy regimens consisting of double antimicrobial therapy plus an antisecretory agent[23-25,36,37]. The combination of metronidazole, amoxicillin and ranitidine given for 12 days resulted in the eradication of *H. pylori* infection in 89% of patients in a well-designed, randomized double blind trial with 50 patients in each arm of the study[7]. The substitution of clarithromycine for metronidazole in such a combination resulted in similar rates of eradication[36,38]. Ranitidine with bismuth compound administered with clarithromycine produced an eradication rate of more than 80% in one small study[37]. However, better results were achieved when omeprazole used in combination with double antimicrobial drugs. Combinations of omeprazole, metronidazole and amoxicillin or clarithromycine resulted in eradication of *H. pylori* in 88 to 90% of patients[22-24]. In the present study, combination of omeprazole, tinidazole and amoxicillin given for 14 days resulted in the eradication of *H. pylori* in 85% of patients. Therefore, cure rate achieved by triple therapy consisting of omeprazole, amoxicillin and tinidazole is comparable to classical triple therapy.

In case of dual regimens, the combination of amoxicillin and omeprazole has been studied most extensively, and the eradication rates varied widely, 37-80% from study to study, depending on the doses and timing of drug treatment[31,32,39].

In our study, 14 days treatment with the combination of omeprazole and amoxicillin in 24 patients resulted in the eradication of *H. pylori* in 67% of patients. The substitution of ranitidine HCl (150 mg bid) for omeprazole (RA group) given in 13 patients for 2 weeks caused almost similar result (69% of eradication). However, definite conclusion cannot be drawn from the observation of such a small study. Therefore, at the moment, it will be inappropriate and unjustified to substitute the combination of an antisecretory and amoxicillin for triple therapy.

Simultaneous *H. pylori* eradication and DU healing rates were comparatively higher in triple therapy regimens (85% by OTA and 84% by CAM) than that of the dual (58% in OA and 69% in RA groups), although borderline significant ($P < 0.06$). But observation of good correlation of ulcer healing and *H. pylori* eradication in our patients again established the causal association of *H. pylori* in peptic ulcer disease.

Therefore, future study with similar triple therapy regimens and by including adequate number of DU patients in each arm may provide more better and scientifically significant results.

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Paper V

(Abstract Published in: Indian J Gastroenterol 2000; 19(Suppl 2):A62-3)

A COMPARISON BETWEEN FURAZOLIDONE-BASED TRIPLE THERAPY AND NITROIMIDAZOLE-BASED TRIPLE THERAPY FOR THE TREATMENT OF *HELICOBACTER PYLORI*-ASSOCIATED DUODENAL ULCER PATIENTS IN BANGLADESH: A PROSPECTIVE RANDOMIZED 1-YEAR FOLLOW-UP STUDY

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SUMMARY

In Bangladesh, the point prevalence of duodenal ulcer (DU) was found near 12% above the age of 15 years and *Helicobacter pylori* prevalence was more than 90% among asymptomatic adults. High prevalence of *H. pylori* in Bangladesh may result in high reinfection rates. Majority of the *H. pylori* strains isolated from Bangladeshi patients were metronidazole resistant *in vitro*. Furazolidone, a nitrofurans, has potential cytoprotective effect on gastroduodenal mucosa and antibacterial activity against *H. pylori*. Furazolidone was thought of an alternative to substitute metronidazole in the triple therapy combinations. The aim of the present study was to evaluate the efficacy of furazolidone-based triple

therapy over nitromidazole-based triple therapy in the cure of *H. pylori* infection and to determine the rates of *H. pylori* reinfection after its successful eradication.

One hundred sixteen patients were prospectively recruited and randomized to receive omeprazole (O) 20 mg bid, ranitidine (R) 150 mg bid, colloidal bismuth subcitrate (C), 120 mg qid, amoxicillin (A) 500 mg qid, furazolidone (F) 100 mg qid, metronidazole (M) 400 mg tid and tinidazole (T) 500 mg bid in the form of three furazolidone-based CAF, OAF, RAF, and two nitroimidazole-based OAM and OAT triple therapy for two weeks. DU at endoscopy and *H. pylori* infection confirmed by any 2 positive tests: RUT, culture and ^{13}C-UBT, RUT and culture were done at 0 week, 8 week, 12 week and at or before 1 year when clinical symptoms reappeared. *H. pylori* positive patients were included irrespective of their *in vitro* metronidazole susceptibility. Simultaneous serial ^{13}C-UBT was performed at 4 weeks interval from 0 week to 1 year to detect reinfection. Cutoff interval used to define cure rate was 12 weeks.

According to intent-to-treat (ITT) analysis after 12 weeks, *H. pylori* cure rates were: furazolidone-based regimen 85% and nitroimidazole-based regimen 67% ($P = < 0.05$); DU healing rates were 85% and 65%, respectively ($P = < 0.05$). On the basis of perprotocol analysis (PPA), the *H. pylori* cure rates were 98% in furazolidone group, 86% in nitroimidazole group ($P < 0.05$), and duodenal ulcer healing rates were 98% and 97%, respectively ($P > 0.5$). Out of 116 patients, 95 were available for monthly follow-up. Sixteen patients were reinfected over 12 months (11 within 6 months, 2 at 9 months, and 3 after 1 year).

Our results have indicated that combination of an antisecretory with furazolidone and amoxicillin can be a better alternative to that of nitroimidazole and amoxicillin in *H. pylori* eradication and ulcer healing. Overall *H. pylori* reinfection rates were >16% at 1 year where majority (69%) were within 6 months indicating probable recrudescence. However, reinfection rates was as slow as 5% (3 out of 66), who completed the 1-year follow-up.

INTRODUCTION

Association of *Helicobacter pylori* with gastroduodenal ulcer diseases is well established. About 95% of patients with duodenal ulcer (DU) and perhaps 80% of gastric ulcer (GU) patients are *H. pylori* infected[1]. In Bangladesh, the point prevalence of duodenal ulcer was found near 12% above the age of 15 yeas[2] and *H. pylori* seroprevalence was more than 90% among asymptomatic adults[3].

Rates of ulcer recurrence in patients whose initial ulcer healed during conventional anti-ulcer agents range from 60 to 100%, whereas eradication of *H. pylori* infection results in a faster healing and markedly decreased annual recurrence rate[4,5].

The National Institute of Health (NIH) Consensus Development Conference in 1994 recommended that all patients with documented DU or GU who have *H. pylori* infection should receive antimicrobial therapy to cure infection. Since then, in successive years, different triple therapy regimens had received approval from the world experts on *H. pylori* for the treatment of patients with

H. pylori-associated peptic ulcer disease (PUD). Recommended regimens are comprised of either a proton-pump inhibitor (PPI) or ranitidine bismuth citrate (RBC) with clarithromycine and either amoxicillin or metronidazole/tinidazole[7]. These recommended regimens have known efficacy of faster ulcer healing and resulted in fewer recurrences.

Given the importance of eradicating *H. pylori* infection in patients with ulcer disease, it is vital that the infection be treated optimally with a combination regimen that has an acceptably high eradication rate. These have been variably defined. Reasonable targets would be $\geq 90\%$ cure rate on perprotocol analysis (PPA) and $\geq 80\%$ cure on intent-to-treat (ITT) analysis[8]. Additional important features to be taken into account when planning treatment include compliance, likelihood of adverse events and the cost, especially for the developing world.

Knowledge of reinfection rates is important for understanding both the epidemiology of *H. pylori* infection and patient management. To date, there have been a substantial number of studies in the developed countries that have examined the *H. pylori* reinfection rate in patients treated for PUD. In the developed countries, reinfection after successful eradication of *H. pylori* appears unusual. At the present time, there have been few studies examining the rate of *H. pylori* reinfection in the developing countries[4,9-11]. Given the higher prevalence of *H. pylori* infection in most developing countries, one might expect the reinfection rate of *H. pylori* to be high. Published studies from the developing countries have included a limited number of subjects and in some cases have only had short follow-up periods[12].

Combination drug regimens are essential to maximize the chance of eradicating the infection and to minimize the risk of promoting antimicrobial resistance. Metronidazole resistance is increasing in both the developed and developing countries[13-15]. Majority of the *H. pylori* strains isolated from Bangladeshi subjects were metronidazole resistant *in vitro*[13]. However, overall experience is that metronidazole-containing triple therapy regimens appear to be effective even if *H. pylori* is resistant to metronidazole[16,17].

Furazolidone, a monoamine oxidase inhibitor, was demonstrated to be effective in the treatment of patients with peptic ulcer disease. Potential ulcer healing mechanisms of furazolidone are cytoprotective effect on gastroduodenal mucosa and antibacterial activity against *H. pylori*[18,19]. Furazolidone is a cheaper and easily available agent found in this region of the world. In the face of higher nitroimidazole resistance, furazolidone was thought of an alternative to substitute metronidazole in the triple therapy combinations.

The present study was undertaken to investigate the efficacy of triple therapy regimens comprising of an anti-ulcer agent plus amoxicillin and furazolidone over that of amoxicillin and nitroimidazole (metronidazole/tinidazole) on the eradication of *H. pylori* infection in Bangladeshi DU patients. High prevalence of *H. pylori* in Bangladesh may result in high reinfection rates. Therefore, the next aim of the present study was to see the rate of *H. pylori* reinfection after its successful eradication.

ETHICS

The study protocol was approved by the institutional ethical committee and written informed consent was obtained from all the patients.

SUBJECTS AND METHODS

A total 116 patients with endoscopically confirmed *H. pylori*-associated duodenal ulcer disease were prospectively recruited and randomized to receive omeprazole (O) 20 mg bid, ranitidine (R) 150 mg bid, colloidal bismuth subcitrate (C) 120 mg qid, amoxicillin (A) 500 mg qid, furazolidone (F) 100 mg qid, metronidazole (M) 400 mg tid and tinidazole (T) 500 mg bid, in the form of 3 furazolidone-based CAF, OAF, RAF and 2 nitroimidazole-based OAM and OAT tripe therapies for 2 weeks.

Patients with a circumscribed break in the duodenal mucosa of apparent depth were covered by an exudate but without relevant cardiovascular, renal, liver disease and other systemic ailment were included. Intake of bismuth compounds or antibiotics, proton-pump inhibitor within the previous month or regular intake of nonsteroidal antiinflammatory drugs within the past two months were exclusion criteria. Pregnant and lactating women and women of childbearing age not taking adequate precautions to avoid pregnancy were also excluded from the study.

Compliance to therapy was evaluated by counting the returned tablets. Any adverse event (sign and symptoms, such as nausea, vomiting, bad taste.

diarrhea, headache, etc.) was recorded on the case record form and classified as mild (awareness of sign or symptoms but easily tolerated), moderate (discomfort sufficient to cause interference with normal activities) or severe (incapacitating, with inability to perform normal activities).

Methods

Endoscopy was performed at the entry visit and was repeated at 8 week, 12 week and at or before 1 year when clinical symptoms reappeared.

Either complete epithelization of the duodenal mucosa or formation of white scar was used as the criterion for ulcer healing. Patients whose ulcer remained unhealed at 8 week, continued the antisecretory drug therapy for an additional 4-week period and reendoscopy done at 12 week.

Two biopsy specimens were taken from antrum. *H. pylori* eradication was defined by the absence of microorganism both at urease test and at microbiological culture, 8 to 12 weeks after therapy had begun. Simultaneous serial ^{13}C-urea breath test was performed at 4-week interval from 0 week to 1 year to detect eradication of infection and reinfection. Cutoff interval used to define cure rate was 12 weeks.

Biopsy specimens

At endoscopy, 2 biopsy specimens were obtained from the antrum, one for culture, another for rapid urease test (RUT).

Culture

Biopsy tissue was transferred to transport medium before inoculation in culture plate. Homogenized specimen was inoculated in Brucella agar (Difco, USA) supplemented with 10% sheep blood and Skirrow's antibiotic (vancomycin 10 mg/ml), polymyxin B (5 μg/ml), trimethoprim (5 μg/ml) and amphotericin B (5 μg/ml) supplement. Growth of *H. pylori* was observed after 3 days. If no growth occurred within 3 days, the plates were reinoculated for 2-4 days. The appearance of an opaque, translucent, convex and circular (0.5-1.0 mm diameter) colonies indicated presumptive evidence of *H. pylori*. Cultures were verified to be *H. pylori* using oxidase, catalase and rapid urease test.

Rapid urease test (RUT)

The second biopsy specimen was placed on the urea agar medium (Difco, USA) inside the vial. The test was recorded as positive if the color changed from yellow to pink within 2 hours at room temperature. Tubes showed no change in color within 2 hours were checked again after 24 hours for the late positive result.

^{13}C-urea breath test

After an overnight fast, an oral dose of urea solution consisting of 100 mg ^{13}C-urea i(99 AP; Mass Trace, Woburn, MA, in 20 ml water was given with a test meal of 200 ml pasteurized full-cream milk. Breath samples were collected

before and 30 minutes after ingestion of the substrate. The ratio of ^{13}C to ^{12}C in the baseline and 30-minute samples was determined by isotope ratio mass spectrometer (Breath MAT^{Plus}; Finnigan, Bremen, Germany). $^{13}Co_2/^{12}Co_2$ ratios were expressed as delta $^{13}Co_2$ values relative to the PDB standard. An increase of the delta $^{13}Co_2$ value at 30 minutes over the baseline value of more than 3.5% was considered positive for *H. pylori*[20].

Statistical analysis

Differences between the different treatment groups in the incidence of duodenal ulcer healing and eradication of *H. pylori* were assessed with Chi-square test. Influence of age, sex, socioeconomic status and smoking on *H. pylori* eradication and ulcer healing were evaluated by logistic regression analysis (SPSS 7.5 for Windows).

RESULTS

The demographic characteristics of the patients in the two major study groups were almost similar (Table-I). Mean age varied from 36 to 37 years (range 20 to 55 years) and the proportion of men, smokers and lower socioeconomic class in each group varied between treatment groups from 73 to 92%, 44 to 60% and 69 to 75%, respectively. Most of the patients (65%) had more than 5 years of history of duodenal ulcer disease (not shown in table). Twenty-one (18%) patients were lost to follow-up, thus there were 116 patients in intent-to-treat (ITT) analysis and 95 in per-protocol analysis (PPA).

Table-I. Baseline characteristics of patients with duodenal ulcer treated with furazolidone (CAF, OAF, RAF)-based and nitroimidazole (OAM, OAT)-based triple-therapy regimens

Characteristics	Furazolidone-based regimen (n=77)	Nitroimidazole-based regimen (n=39)
Age (years) (mean±SD)	36±14	37±18
Sex (Male/Female)	56/11	36/3
Socioeconomic status (Low/Middle)	58/19	27/12
Smoking (Yes/No)	46/31	17/22

***H. pylori* eradication (Table-II):** Cure of *H. pylori* and duodenal ulcer healing rates were analyzed by both intent-to-treat (ITT) and per-protocol (PPA) analysis methods.

***H. pylori* cure rates:** On the basis of ITT, 85% (65/77) in furazolidone group and 67% (26/39) in nitroimidazole group ($P < 0.05$); PPA 98% (65/66) and 86% (25/29), respectively ($P < 0.05$).

Duodenal ulcer healing rate: ITT 85% (65/77) in furazolidone group and 65% (25/39) in nitroimidazole group ($P < 0.05$); PPA 98% *65/66) and 97% (28/29), respectively ($P > 0.50$).

Antibiogram profile (Table-III): Antimicrobial susceptibility study of the 34 of 116 initial *H. pylori* isolates could be done. All 34 isolates were found susceptible to amoxicillin and nitrofurans, but 19 of 34 (54%) pretreatment *H. pylori* were resistant to metronidazole (MRS). Six of these 19 patients harboring MRS *H. pylori* had been randomly assigned to nitroimidazole-based regimen; *H. pylori* was eradicated in 5 (83%) of them despite resistance to metronidazole. On the other hand, 13 of the 19 who received furazolidone-based therapy, 8 had *H. pylori* eradication.

Side-effects: Two patients in the furazolidone group had side-effects during treatment. One patient developed urticaria-type rash one week after initiation and discontinued the therapy, another had anorexia, nausea and vertigo, completed the course. Two patients in the nitroimidazole group had complaints of anorexia, vertigo and metallic taste in the mouth, completed the 2 weeks therapy.

Table-II. *H. pylori* eradication and duodenal ulcer healing by different anti-*H. pylori* therapy regimen

Drug groups	*H. pylori* cure No. (%)		Duodenal ulcer healing No. (%)	
	ITT (n=116)	PPA (n=95)	ITT (n=116)	PPA (n=95)
Furazolidone-based triple-therapy regimens	65/77 (85)	65/66 (98) P<0.05	65/77 (85)	65/66 (98) P<0.05
CAF:	22/26 (85)		22/26 (85)	
OAF:	26/28 (93)		25/28 (89)	
RAF:	18/23 (79)		18/23 (79)	
Nitroimidazole-based triple-therapy regimens	25/39 (67)	25/29 (86) P<0.05	28/39 (65)	28/29 (97) P<0.05
OAM:	15/19 (79)		18/19 (95)	
OAT:	13/20 (65)		14/20 (70)	
P value	<0.05	<0.05	<0.05	>0.50

Table-III. Pretreatment metronidazole susceptibility and *H. pylori* eradication (n=34)

	Metronidazole sensitive (n=15)		Metronidazole resistant (n=19)	
Drug groups	Hp^-	Hp^+	Hp^-	Hp^+
Nitroimidazole	3	2	5	1
Furazolidone	9	1	8	5
Total	12	3	13	6

Hp^- = *H. pylori* eradicated
Hp^+ = *H. pylori* not eradicated

Recurrence of *H. pylori* (Table-IV): Of the 95 patients entered in the 12-month follow-up, 16 (16.84%) were shown to have become *H. pylori* reinfected. Examination of the data indicates that, of these 16 patients, 11 (69%) became reinfected within 6 months, 2 at 9 month and 3 at 12 month periods after completion of anti-*H. pylori* therapy. Of the 16 *H. pylori* reinfections, 10 (15%) in furazolidone group and 6 (21%) in nitroimidazole group (P=0.52).

Duodenal ulcer relapse: Seventeen of 95 patients (17.89%) had their ulcer relapse within 12-month period (Table-IV). Of the 17 patients, 9 were shown to have recurrence of their *H. pylori* infection and the rest 8 relapses were *H. pylori* negative. DU recurrences were 11 (17%) in furazolidone group and 6 (21%) in nitroimidazole group (P=0.74).

Influence of age, sex, socioeconomic status and smoking on cure and recurrences of *H. pylori* and duodenal ulcer were evaluated by logistic regression analysis (Table-V), have shown that after *H. pylori* eradication, smoking as an independent factor significantly hampered both duodenal ulcer healing and associated with more ulcer relapses.

DISCUSSION

Until now, the best results in the eradication of *H. pylori* from the gastric mucosa have been achieved by triple-therapy in which nitroimidazoles have been an important component.

Table-IV. *H. pylori* recurrence and ulcer relapse at 6, 9 and 12 month after treatment

Parameters	Months after termination of treatment						Total	
	6 (n=95)		9 (n=73)		12 (n=66)			
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
H. pylori recurrence	11	(11.58)	2	(2.74)	3	(4.55)	16	(16.58)
Ulcer relapse	7	(7.37)	4	(5.48)	6	(9.0)	17	(17.89)

Table-V. Influence of age, sex, socioeconomic condition and smoking on cure and recurrence of *H. pylori* infection and duodenal ulcer (logistic regression analysis)

Characteristics	*H. pylori*		Duodenal ulcer	
	Eradication vs non-eradication (Sig)	Reinfection vs no reinfection (Sig)	Healing vs non-healing (Sig)	Relapse vs remission (Sig)
Age (years) (Mean±SD)	36±11 vs 37±11 (0.58)	36±9 vs 37±10 (0.36)	36±11 vs 37±8 (0.94)	38±7 vs 36±10 (0.85)
Sex (Male/Female)	89 vs 82% (0.28)	75 vs 95% (0.13)	91 vs 85% (0.50)	82 vs 96% (0.56)
Socioeconomic status (Lower/Middle)	72 vs 75% (0.76)	81 vs 96% (0.74)	71 vs 85% (0.39)	88 vs 97% (0.29)
Smoking (Yes/No)	52 vs 59% (0.13)	69 vs 52% (0.29)	49 vs 75% (0.07)	76 vs 50% (0.09)

Metronidazole resistance is increasing in both the developed and developing countries[13-15]. Majority of the *H. pylori* strains isolated from Bangladeshi subjects were metronidazole resistant *in vitro*[13]. However, overall experience is that metronidazole-containing triple-therapy regimens appear to be effective even if *H. pylori* is resistant to metronidazole[16,17].

Furazolidone, a monoamine oxidase inhibitor, was demonstrated to be effective in the treatment of patients with peptic ulcer disease. Potential ulcer healing mechanics of furazolidone give cytoprotective effect on gastroduodenal mucosa and bacterial activity against *H. pylori*[18,19]. In the face of higher nitroimidazole resistance, present study was undertaken to compare the efficacy of furazolidone-based triple-therapy over nitroimidazole-based triple-therapy regimens in the eradication of *H. pylori* infection in Bangladeshi duodenal ulcer patients.

We found that *H. pylori* cure rates were significantly higher in the patients treated with triple-therapy regimens comprising of furazolidone, amoxicillin and an anti-ulcer (omeprazole/ranitidine/colloidal bismuth subcitrate) agent (ITT 85%, PPA >95%) than the patients treated with metronidazole/tinidazole, amoxicillin and omeprazole (ITT 67%, PPA 86%) for 2 weeks.

To our knowledge, no study with such furazolidone-based triple-therapy combination was used to treat *H. pylori* infection. Coelho *et al.*[19] showed 60% *H. pylori* eradication in the duodenal ulcer patients when treated with furazolidone, amoxicillin and metronidazole administered for 5 days.

In a randomized controlled trial, using furazolidone, nitrofurantoin and placebo as monotherapy in Peruvian adults with antral gastritis associated with *H. pylori* infection, Morgan *et al.*[20] have found significantly higher *H. pylori* clearance rates in both furazolidone and nitrofurantoin groups compared with placebo group. Authors also observed higher effectiveness of furazolidone over nitrofurantoin. However, a high percentage of these patients experienced recolonization by *H. pylori* within 6 weeks after completion of treatment. It has been proved that monotherapy is ineffective due to higher occurrence of recolonization and therefore, obsolete, the use of monotherapy for *H. pylori* eradication. Achievement of better cure rates and experience of minor side-effects with furazolidone-based regimens in the present study favor their use as effective therapy in *H. pylori* eradication.

Resistance to antibiotics is considered as the primary reason for failure of eradication therapies. Resistance to metronidazole is due to lack of reduction of this compound whose genetic basis is still unknown[21]. Indeed, there are problems with regard to the techniques used to detect this resistance. It has been observed that only minimum inhibitory concentrations (MICs) determined by agar dilution, and not E-test, correlate with the clinical outcome in clinical trials[22]. Metronidazole resistance influences the success of treatment to a lesser extent than clarithromycine resistance[21]. Batnavala *et al.*[13] have observed a very high prevalence (>90%) of metronidazole resistance among the Bangladeshi resdents in east London. Morshed *et al.* (unpublished data) have found 75% metronidazole-resistant *H. pylori* among a group of dyspepsia patients in Bangladesh, and 54% (19/34) of the present pretreatment isolates were found resistant to metronidazole.

In the present study, both the treatment regimens were tested in the randomized patients irrespective of their pretreatment metronidazole-resistant *H. pylori* strains status. *H. pylori* cure rates by nitroimidazole-based regimens were: PPA 86% and ITT 67%. Uses of metronidazole-containing triple-therapy in patients with metronidazole-resistant strains by Glupczynski and Burette[17] and Rautelin *et al.*[23] have shown higher cure rates. Glupczynski and Burette[17] reported in up to 70% *H. pylori* eradication in patients with primary resistance to metronidazole when colloidal bismuth subcitrate was added to combination of amoxicillin and metronidazole. With similar regimen used by Rautelin *et al.*[23] found 63% *H. pylori* eradication in patients with pretreatment metronidazole-resistant strains.

In the present study, we achieved 83% (5/6) *H. pylori* eradication when omeprazole was added to metronidazole and amoxicillin given for 2 weeks in patients with pretreatment metronidazole-resistant *H. pylori* strains. Uses of omeprazole in place of CBS in our study patients resulted in comparatively higher eradication rates. This finding indicates that *in vitro* metronidazole resistance does not always correlate with resistance of it in multidrug treatment regimens.

Knowledge of reinfection rate is important for understanding both epidemiology of *H. pylori* infection and patient management. To date, there have been a substantial number of studies in developed countries have examined the *H. pylori* reinfection rate in patients treated for peptic ulcer disease. It now appears that reinfection is an unusual event in the developed world[4,9-11].

At the present time, information regarding reinfection rate in the developing countries is limited to small studies giving variable results. Acquisition appears most frequent in persons under 15 years of age and perhaps even in younger children[24,25]. New acquisition in adults appears to occur at rates far lower than those in children with major mode of spread related to the geographic area, socioeconomic and family circumstances[26]. An increased rate of acquisition was found in those coming to an area of high seropositivity from one of lower seropositivity[27]. Based on our own data, the prevalence of *H. pylori* in asymptomatic adults in Bangladesh is more than 90%, which compare with a prevalence of 20-25% in developed countries[28]. In addition, approximately 90% of children in Bangladesh are *H. pylori* infected by 3-5 years of age[29].

Because the prevalence of *H. pylori* infection in the developing countries is significantly greater than that in the developed countries[3,28] and given the living conditions, one might expect the reinfection after treatment may be high. Assessment of *H. pylori* reinfection rates may be affected by the quality and number of detection methods used. For accurate assessment of *H. pylori* eradication, it has been recommended that at least two tests should be used. In the present study, we confirmed *H. pylori* eradication at 1-month after completion of therapy using rapid urease test (RUT) and culture at endoscopy. Simultaneous serial ^{13}C-urea breath test (UBT) was performed at monthly interval for a period of 12 months from the completion of therapy to detect reinfection. The time interval we selected was 3 months after treatment as the cutoff point for the cure of *H. pylori* infection. Our study shows that 16 of the 95 patients with duodenal ulcer successfully treated for *H. pylori* infection

became reinfected during the 12-month period. This represented an overall 17% of annual reinfection rates. Among these 16 reinfections, 11 (69%) occurred within 6 months, indicating probable recrudescence. This observation is consistent with one of the longest follow-up study for 9 years by Bell and Powell[30], where the authors found 57 reinfections, of them 45 occurred within 6 months of treatment. Authors suggested that most early reinfection in adults are in fact late recrudescence. The major drawback of the present study was that we do not know the molecular typing of our pre-eradication and post-eradication isolates of *H. pylori* of 16 of 95 patients reinfected with *H. pylori*. Therefore, differentiation of recrudescence from reinfection could not be established in our study. Five of the 16 reinfected patients (31%) were found to be reinfected 6 months after completion of treatment. Of these 5 patients, 2 at 9 months and 3 at 12 months were found to have reinfection. *H. pylori* eradication by furazolidone and nitroimidazole-based regimens was found to have no significant role in the subsequent *H. pylori* reinfection. Based on these results, we would suggest that those patients reinfected within the first 6 months after completion of treatment represent recrudescence, whereas patients reinfected at 12 months, most likely represent true reinfections. It has been observed that high prevalence of *H. pylori* in the developing countries result in high reinfection rates. Given our interpretation, the true rate of reinfection in this population is low (4.55% per annum), indicating that even where the prevalence of infection is high, reinfection with *H. pylori* is unexpectedly low. The findings of the present study are showing comparatively lower rate of reinfection at one year despite the presence of higher prevalence of infection.

Several studies have suggested that in the developing countries, *H. pylori* may be transmitted on a communitywide basis via fecal contamination of water caused by inadequate sanitation[31,32]. However, the finding of the present study could not exactly support the significance of route of transmission via fecal-oral over that of oral-oral in our population.

Since the crucial observation made by Warren and Marshall that eradication of *H. pylori* greatly diminishes the recurrence rate of duodenal ulcer. Studies have shown that ulcer relapse is relatively uncommon in patients cured of *H. pylori*[33]. More recently, Hopkins *et al.*[5] reviewed several duodenal ulcer eradication studies and noted an overall recurrence rate of 6% following successful eradication of *H. pylori* compared with 67% when the infection remained. In the present study, 17 of 95 patients treated for *H. pylori* and having healed their ulcers had an ulcer relapse within 12-month period. Nine of these patients were reinfected with *H. pylori*. Eight patients had an ulcer relapse despite the fact that they were *H. pylori* negative. Ulcer relapse in these eight patients had no history of consumption of aspirin or nonsteroidal antiinflammatory drugs. Recurrence of ulceration in these patients may have an alternative etiology. Duodenal ulcer disease either related to sustained high acid output or due to specific disease, like tuberculosis and Crohn's disease, can be the probable possibilities considered in these patients. Among the various demographic factors, smoking as an independent factor has significantly hampered ulcer healing and as well as more ulcer relapses after eradication of *H. pylori* infection. However, smoking had no influence over *H. pylori* eradication and

reinfection in these patients. This observation is consistent with that of Cuttler and Schubert[34] and Graham *et al.*[35].

In conclusion, observation of significantly better efficacy of triple-therapy combination of furazolidone, amoxicillin plus an antiulcer agent over metronidazole/tinidazole, amoxicillin plus antiulcer agent, can be considered as an alternative to conventional triple-therapy regimens in terms of clinical efficacy, tolerance and cost. Though metronidazole resistance plays an important role in the failure to eradicate *H. pylori*, but we observed more than 80% eradication rates with present nitroimidazole-based triple-therapy regimens (OAT and OAM) in patients with metronidazole resistant *H. pylori* strains. Despite *H. pylori* eradication, smoking as an independent factor has significantly hampered ulcer healing and associated with more ulcer relapse. The results of the present study have shown that after *H. pylori* eradication, the reinfection rate at one-year is low, though not as low as developed world.

Finally, observation of comparatively lower *H. pylori* reinfection rate, reemphasizes the eradication therapy as the choice of treatment for peptic ulcer disease in the developing countries.

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APPENDIX-II

FORMULATION OF DIFFERENT MEDIA USED IN THIS STUDY

Stuart transport media (Merck, Germany)

Composition		Gram/Liter
Sodium glycerophosphate	...	10.0
Sodium thioglycolate	...	1.0
Calcium chloride	...	0.1
Methylene blue	...	0.002
Agar-agar	...	8.0

Final volume made up to 1 liter with distilled water

pH adjusted to 7.4 (approximately)

Autoclaved at 121°C for 20 minutes at 15 lbs/cm^2 pressure

Brucella agar (Difco, USA)

Composition		Gram/Liter
Bacto peptone	...	20.0
Bacto dextrose	...	1.0
Bacto yeast extract	...	2.0
Sodium chloride	...	5.0
Sodium bisulfite	...	0.1
Bacto agar	...	15.0

Final volume made up to 1 liter with distilled water

pH adjusted to 7.0 (approximately)

Autoclaved at 121°C for 20 minutes at 15 lbs/cm^2 pressure

Urea agar-base (Difco, USA)

Composition		Gram/Liter
Bacto peptone	...	1.0
Bacto dextrose	...	1.0
Sodium chloride	...	5.0
Potassium phosphate monobasic	...	2.0
Urea	...	20.0
Bacto phenol red	...	0.012

Bacto urea agar-base (29 g) was dissolved in 100 ml distilled water, pH was adjusted to 6.8 (approximately), and then sterilized by membrane filtration using Millipore membrane filter, 0.22 μg pore size (Sigma, USA). Bacto agar (15 g) was suspended in 900 ml distilled water and dissolved completely and autoclaved at 121°C for 20 minutes at 15 lbs/cm^2 pressure. Finally, Bacto urea agar-base (100 ml) was added to Bacto agar (900 ml) at 55°C and mixed well.

Tryptone urea broth (Oxoid, England)
(Soybean-casein digest medium, U.S.P.)

Composition		Gram/Liter
Pancreatic digest of casein	...	17.0
Papain digest of soybean meal	...	3.0
Sodium chloride	...	5.0
Dibasic potassium phosphate	...	2.5
Glucose	...	2.5

Final volume made up to 1 liter with distilled water

pH adjusted to 7.3 (approximately)

Autoclaved at 121°C for 20 minutes at 15 lbs/cm^2 pressure

APPENDIX-III

ANTIBIOTIC SUPPLEMENT PREPARATION FOR USE IN BRUCELLA AGAR MEDIA

Vancomycin solution (Sigma, USA)

Stock solution	...	10 mg/ml
Working solution	...	10 μg/ml

One ml working solution was added to 1 liter media.

Polymyxin B solution (Sigma, USA)

Stock solution	...	5 mg/ml of 0.1 N HCl solution
Working solution	...	5 μg/ml

One ml working solution was added to 1 liter media.

Trimethoprim solution (Sigma, USA)

Stock solution	...	5 mg/ml
Working solution	...	5 μg/ml

One ml working solution was added to 1 liter media.

Amphoterin B solution (Sigma, USA)

Stock solution	...	5 mg/ml
Working solution	...	5 μg/ml

One ml working solution was added to 1 liter media.

APPENDIX-IV

REAGENT PREPARATIONS USED IN THIS STUDY

Crystal violet solution

Composition		Gram/Liter
Crystal violet	...	20.0
Ammonium oxalate	...	9.0
Absolute ethanol	...	95.0

Final volume made up to 1 liter with distilled water

Lugol's iodine solution

Composition		Gram/Liter
Potassium iodide	...	20.0
Iodine	...	10.0

Two of the above reagents were mixed well and properly dissolved in 1 liter distilled water.

Rectified spirit (95.6% ethanol)

Absolute ethanol	...	956 ml
Distilled water	...	44 ml

Safranin solution

1) Stock solution

a)	Safranin O (certified)	...	10 g
b)	Ethanol (95%)	...	200 ml

2) 100 ml of stock solution was added to 900 ml distilled water

Oxidase reagent (Sigma, USA)

Composition		Gram/Liter
N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride	...	100.0

Final volume made up to 1 liter with distilled water

APPENDIX-V

^{13}C-UREA BREATH TEST

Identification

Study Number: Name:

Occasion of ^{13}C-urea breath test

Date:

Pretreatment visit (- week 0) ____

4-weeks-after-treatment visit (= week 9) ____

Follow-up visit (= week 13, 17, etc.) ____

Materials

Test meal: 200 ml of pasteurized Bangladeshi full-cream milk (in bag); store in cold room. This is used to prevent rapid gastric emptying.

Urea solution: Prepared by adding 20 ml of boiled cold water to 100 mg ^{13}C-urea. Tap the water gently to ease the urea to the bottom of the container. After adding the water, replace the lid firmly and swirl to dissolve the urea.

Vacutainer tubes: 15 ml siliconized tubes.

Label tubes: 1 tube: Date, name and number of patient and '0 min'

2 tubes: Date, name and number of patient and '30 min'

Sampling straw (large plastic drinking straw): Do not use to mix urea solution.

How to collect the expired air

The patient is asked to stop breathing for a moment (pCO_2 |). The sampling straw is placed to the bottom of the vacutainer tube. Then, the patient breathes normally down the straw until condensation occur; breathing is continued and the straw is slowly removed from the tube. As soon as the tube clears the tip of the straw, the tube must be closed immediately. The samples can be stored at room temperature.

Time	Real time	Procedure
Before drinking the ^{13}C-urea and test meal		Take 1 basal sample in labeled VACUTAINER TUBE. (Label: Date, & number of patient and '0 min'
0 min	_____	Let the patient drink about half of the TEST MEAL (milk). Then, let the patient drink the UREA SOLUTION and rinse the container 2X with 20 ml of boiled cold water which the patient has to drink also. After that, let the patient drink the rest of the TEST MEAL (milk). The patient has to sit resting during the test without food or drink.
30 min	_____	Take 2 breath samples, each in 1 labeled VACUTAINER TUBE. (Label: Date, name and number of patient and '30 min'.

Result

Date received: Positive/Negative

Remarks

Name of the study coordinator performing the ^{13}C-urea breath test: _____