

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

SEROPREVALENCE OF *HELICOBACTER PYLORI*
INFECTION IN PATIENTS ATTENDING FELEGE
HIWOT HOSPITAL IN BAHIR DAR, NORTH WEST
ETHIOPIA

BY
TESFAHUN TADEGE

JUNE 2003

This dissertation is dedicated to my Grand mother Asayech Agegnehu and my mother Bernesh Kassie, who sacrificed everything in their possession including their life but unfortunate to see me. This is an offer to their relentless and unfailing love.

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By

Tesfahun Tadege

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Department of Biology
Addis Ababa University

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SCHOOL OF GRADUATE STUDIES

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ABBREVIATIONS

BSA	Bovine Serum Albumin
CagA	Cytotoxin Associated protein
CLO	Campylobacter Like Organisms
EIA	Enzyme Immuno Assay
FDA	Food and Drug Administration
GIT	Gastrointestinal Tract
Hsp	Heat Shock Proteins
IB	Immunoblot
Ig	Immuno globulin
LPS	Lipopolysaccharide
MALT	Mucosa-Associated Lymphoid Tissue
Mb	Mega Base
NIH	National Institutes of Health
NSAID	Non-Steroidal Anti-Inflammatory Drug
OMP	Outer Membrane Proteins
PAF	Platelet Activating Factor
PBS	Phosphate Buffered Saline
PPI	Proton Pump Inhibitor
ROS	Reactive Oxygen Species
rRNA	Ribosomal Ribonucleic Acid
TNF	Tumour Necrosis Factor
UBT	Urea Breath Tests
VacA	Vacuolating Associated CytotoxinA

ABSTRACT

Infection with *Helicobacter pylori* occurs worldwide, but the prevalence varies greatly among countries and among population groups within the same country. *H. pylori*, a previously obscure organism, has now been associated with many of the most important diseases involving gastroduodenal tissues. Because dyspepsia is one of the commonest complaints in any Ethiopian Out Patient Department, this study was undertaken to determine the magnitude of *H.pylori* infection in adult patients with and without dyspeptic symptoms. Overall total of 200 [128(64%) males and 72(36%) females], 100 dyspeptic (48 males and 52 females) and 100 non-dyspeptic (80 males and 20 females) patients attending Felege Hiwot Hospital, Bahir Dar, Ethiopia, were investigated for *H.pylori* infection between mid November- mid March 2003. Sera obtained from the blood by centrifugation were tested for presence of IgG antibodies against *H.pylori* antigens using EIA. Of the total 200 studied subjects, 112 (56%) positive, 66 (33%) negative and 22 (11%) were borderlines for *H.pylori* infection by EIA. Of these 68(53.1%) of males and 44 (61.1%) of females were seropositive for *H.pylori* infection. There was no statistically significant difference in *H.pylori* seropositivity between males and females, $p>0.05$. All sera (200) were also tested for detection of IgG antibodies to specific *H.pylori* proteins by immunoblot (IB) analysis to confirm EIA results. All sera yielded positive in EIA were positive in IB assay and all sera that were negative in EIA were also negative in IB assay results. Out of 22 borderline results by EIA, 12 (6 males and 6 females) were confirmed to be positive. Combining EIA and IB, the overall seroprevalence of *H.pylori* in Bahir Dar, Ethiopia was 49- 70% and the seroprevalence of dyspeptics and non-dyspeptics was 63-70% and 49-54%, respectively. There was no statistically significant difference between dyspeptics and non-dyspeptics, $P>$

0.05. In conclusion, there was no significant difference in the rate of *H.pylori* infection between dyspeptic and non-dyspeptic patients, sex, ABO blood groups and presence or absence of rhesus factors. But there is insufficient evidence to confirm or refute the existence of difference in the rate of seroprevalence between dyspeptic and non-dyspeptics. The burden of illness due to dyspepsia with respect to quality of life and economic consequences is of considerable from economic, social, and personal vantage points. For this effective, safe, accessible and consistent control strategy is crucial. This study showed that serological methods (EIA and IB) could be used as diagnostic tools to determine the status of *H. pylori* infection and for epidemiological studies. Because Ethiopia is endowed with different ethnic groups, therefore, serological assays should be evaluated for each particular group and adjusting cutoff value is necessary to use serological tests as a screening tool.

CHAPTER I

INTRODUCTION

CHAPTER I. INTRODUCTION

1.1. Discovery of *Helicobacter pylori*

Spiral bacteria were observed in biopsies from human stomach mucosa and their historical origins of discovery rooted in the later half of the nineteenth century by Kreinitz in 1906 and in animal mucosa even earlier than this. In 1979 Robin Warren, a pathologist in Perth, Western Australia, began to notice that curved bacteria often were present in gastric biopsy specimens submitted for histological examination. Warren and Marshall (1983) isolated the organism by inoculating the biopsy specimens onto selective media used for isolation of Campylobacters and incubated the cultures under microaerobic conditions. Subsequently, the organism due to its resemblance to campylobacters the bacterium was first called campylobacter like organisms and then *Campylobacter pylori*, because of its principal localization in the pyloric parts of the stomach. Later on 16S rRNA sequence analysis showed that the isolates were different from the true campylobacters to be subsequently qualified in to a new genus *Helicobacter* (Romaniuk, *et al.*, 1987), and the species *Helicobacter pylori*, the first member of the new genus *Helicobacter* and constituted a genus of its own (Goodwin *et al.*, 1987). There after more than 25 species have been identified from different hosts (Owen, 1998; Solink and Schauer, 2001).

Many of evidences now indicate that once acquired, *H. pylori* persists usually for life, unless eradicated by antimicrobial therapy (Blaser, 1997). Marshall and Warren noted that *H. pylori* infection was associated with duodenal ulceration (Marshall and Warren, 1984), and this observation too was rapidly confirmed and extended to include gastric ulceration. By 1994, a consensus conference convened by the National Institutes of Health (NIH) concluded that *H. pylori* was a major cause of peptic ulcer disease and recommended

that infected individuals with ulcers be treated to eradicate the organism (NIH 1994). There is now overwhelming evidence that *H. pylori* is linked to gastric adenocarcinoma, the second most common cause of cancer morbidity and mortality worldwide (Huang *et al.*, 1998). Treatment of gastric MALToma patients with antibiotics that eradicate *H. pylori* often leads to regression of the tumor (Walt, 1996). Thus, *H. pylori*, a previously obscure organism, has now been associated with many of the most important diseases involving gastroduodenal tissue.

Dyspepsia is one of the commonest complaints in any Ethiopian outpatient department (Tsega, 1980; 1983; Zein and Kloos, 1988). In a prospective study with endoscopy and gastric mucosal biopsy in patients with and without dyspepsia, histological evidence of intestinal metaplasia was present in (85%) dyspepsia cases and in (81%)-controls. More over, 94% patients with NUD had body superficial and/or ironic atrophic gastritis while 130 (961) had full thickness antral gastritis, all (100%) controls had body superficial and/or chronic atrophic gastritis and 99% had full thickness antral gastritis. *H. pylori* was detected in 65% of patients and 56% asymptomatic controls (Tsega *et al.*, 1996b).

At present there are only a few studies addressing the possibility of an association between *H. pylori* infection and dyspepsia and/or non-dyspepsia in Ethiopian patients (Asrat, 2003; Tsega *et al.*, 1996b). Published informations are also very sparse concerning the different diagnostic methods to detect *H. pylori* from different clinical samples (Asrat, 2003). Because of this fact it is important to conduct further study in the Ethiopian settings in order to ascertain the importance of *H. pylori* infection in dyspeptic and non-dyspeptic patients.

1.2. The Genus *Helicobacter*

The genus *Helicobacter* now consists of different species colonizing the mucosal surfaces of humans, other animals and birds. In general these species can be broadly grouped according to their colonization sites as shown in Table 1.1. These are: -

- i) *Helicobacters* colonizing the gastric mucosa
- ii) *Helicobacters* colonizing the intestinal mucosa
- iii) *Helicobacters* colonizing the liver and biliary tree

Gastric *Helicobacter* species are widely distributed in mammalian hosts. In many cases they cause an inflammatory response resembling that seen with *H. pylori* in humans. Although not usually pathogenic in their natural host, these organisms serve as models of human disease. Enterohepatic *Helicobacter* species are equally diverse groups of organisms that have been identified in the intestinal tract and the liver of humans, the mammals and birds. They have been linked with inflammation and malignant transformation in immunocompetent hosts with more severe clinical disease in immunocompromised humans and animals (Solink and Schauer, 2001).

Table 1.1. The genus *Helicobacter*, their hosts and some morphological characteristics (Adapted from Owen, 1998; Solink and Schauer, 2001).

Species	Main Host/s	cell size (µm)	fla. No.	Fla.dis.	Fla. sheath
<i>Helicobacter</i> Species Colonizing the Gastric mucosa					
<i>H. pylori</i>	Human	2.0-4.0	4-6	Polar	+
<i>H. acinonyx</i>	Cheetah	2.0-5.0	2-5	Polar	+
<i>H. musielae</i>	Ferret	2.0-5.0	4-8	Peritrichous	+
<i>H. nemensirinae</i>	Pig-tailed macaque	2.0-5.0	4-8	Polar	+
<i>H. suis</i>	Pig	1.5-5.2	up to 6	Bipolar	ND
<i>H. heilmannii</i>	Cat, dog, monkey, (human)	3.5-7.5	12	Bipolar	-
<i>H. fells</i>	Cat, dog, (human)	5.0-7.5	14-20	Bipolar	+
<i>H. bizzozeronii</i>	Dog	5.0-10.0	10-20	Bipolar	+
<i>H. salomonis</i>	Dog	5.0-7.0	10-23	Bipolar	+
<i>Helicobacter</i> Species Colonizing the Intestinal Mucosa					
<i>H. cinaedi</i>	Human, hamster	1.5-5.0	2	Bipolar	+
<i>H. fennelliae</i>	Human	1.5-5.0	2	Bipolar	+
<i>H. muridanim</i>	Rat, Mice	3.5-5.0	10-14	Bipolar	+
<i>H. pametensis</i>	Wild birds, pig	1.5	2	Bipolar	+
<i>H. trogontum</i>	Rat	4.0-6.0	5-7	Bipolar	+
" <i>F. rappini</i> "	Sheep, dog, pig	6.5	10-20	Bipolar	+
<i>H. rodentium</i>	Mice	5.0	2	Bipolar	-
<i>Helicobacter</i> Species Colonizing the Liver and Biliary Tree					
<i>H. canis</i>	Dog, (human)	4.0	2	Bipolar	+
<i>H. pullorum</i>	Poultry, human	3.0-4.0	1	Polar	-
<i>H. cholecystus</i>	Hamster	3.0-4.0	1	Polar	+
<i>H. hepaticus</i>	Mice	1.5-5.0	2	Polar	+
<i>H. bilis</i>	Mice	4.0-5.0	3-14	Bipolar	+
<i>H. typhlonicus</i>	Mice	ND	ND	ND	ND
<i>H. Sp. flexispira (Flexispira rappini)</i>	dog, mice, sheep, humans	ND	ND	ND	ND
<i>H. mesocricetorum</i>	Hamsters	ND	ND	ND	-

ND= not determined, Fla = Flagella, Dis = Distribution

1.3. Biology of *Helicobacter pylori*

H. pylori are microaerophilic, and gram-negative spiral shaped bacteria that display bluntly rounded ends in gastric biopsy specimens. However, when cultured on solid medium, the bacteria dominantly assume a rod-like shape; with little or no spiral shapes. After prolonged culture on solid or in liquid medium, coccoid forms typically predominate (Dunn *et al.*, 1997). In gastric biopsy specimens, *H. pylori* organism's equipped with four to six unipolar-sheathed flagella, which are essential for bacterial motility (Goodwin, *et al.*, 1987).

H. pylori, by virtue of its urease enzyme and other products, has become extremely well adapted to its unique niche within the gastric mucus. It also has considerable genetic heterogeneity, and studies have suggested that this diversity may allow each strain to become uniquely adapted to each host to an extent that, for some subjects (Logan and Walker, 2001). An average genome size of *H. pylori* is about 1.67 Mb and G-C content 39% (Tomb *et al.*, 1997). Approximately 40% of *H. pylori* isolates contain plasmids ranging in size, but the plasmids do not contain recognized virulence factors (Kleanthous, *et al.*, 1991). The *H. pylori* genome possesses at least two copies each of the 16S and 23S rRNA genes. The variable location of multiple genes in genomic maps suggests the extensive rearrangement of the *H. pylori* genome (Jiang, *et al.*, 1996). *H. pylori* strains are naturally competent for DNA uptake, which, through recombination, provides a mechanism for the diversity observed (Go *et al.*, 1996).

A variety of putative outer membrane proteins (OMPs) have been identified. Urease and HspB are located strictly within the cytoplasm in early log phase cultures of *H. pylori* (Phadnis, *et al.*, 1996). However, in late-log-phase cultures, urease and HspB become

associated with the bacterial surface in a novel manner: these cytoplasmic proteins are released by bacterial autolysins and become adsorbed to the surface of intact bacteria due to the unique characteristics of the outer membrane (Phadnis, *et al.*, 1996).

H. pylori is fastidious and the organism requires complex basal medium (either solid or liquid) with some form of supplementation such as whole blood, heme, serum, charcoal, cornstarch, or egg yolk emulsion (Henriksen, *et al.*, 1995). *H. pylori* grows poorly, if at all, under anaerobic conditions. Growth in liquid media is typically enhanced by agitation and by incubation in a CO₂ -rich atmosphere (Coudron and Stratton, 1995).

1.4. Epidemiology of *Helicobacter pylori* Infection

a) Prevalence

Infection with *H. pylori* occurs worldwide, but the prevalence varies greatly among countries and among population groups within the same country, with high rates (~90%) of infection being associated with low socioeconomic status and high densities of living (Bardhan, 1997). Confirmation has been provided by recent studies conducted in Northern Italy, Tuscany, Russia, Sri Lanka, France, (Malaty *et al.*, 2001; Bazzoli *et al.*, 2001; Reshetnikov *et al.*, 2001; Fernando *et al.*, 2001; Broutet *et al.*, 2001). Seroprevalence of *H. pylori* among Chilean city-dwellers was ~ 60% in the age group of 15-19 years (Hopkins *et al.*, 1993) and the risk of *H. pylori* seropositivity was related to increasing age, low socioeconomic status, and consumption of vegetables. Recent studies in Ethiopia showed that 69-91% among dyspeptic patients attending Black Lion Hospital using invasive and non-invasive tests (Asrat, 2003) and 89% among blood donors in Addis Ababa using serological methods (Desta *et al.*, 2002a)

b) Incidence

Although the natural history of *H. pylori* infection has not been fully defined, results from serological, therapeutic, and volunteer studies suggest that once infection is acquired it persists for years and possibly for life. As acute *H. pylori* infection passes undetected, the incidence of infection can be determined indirectly from epidemiological studies. Acquisition of *H. pylori* infection appears on the basis of age-specific prevalence constant over time in some populations. Incidence extrapolated from prevalence data of developed countries is estimated to be 0.5%-1.0% of susceptible persons per year; in developing countries the incidence appears to be higher. With industrialization and improvement of sanitation and hygiene, prevalence is gradually shifted from childhood to adulthood, thus translating high childhood infection rate into a high prevalence of adult infection (Parsonnet, 1995).

c) Gender and *Helicobacter pylori*

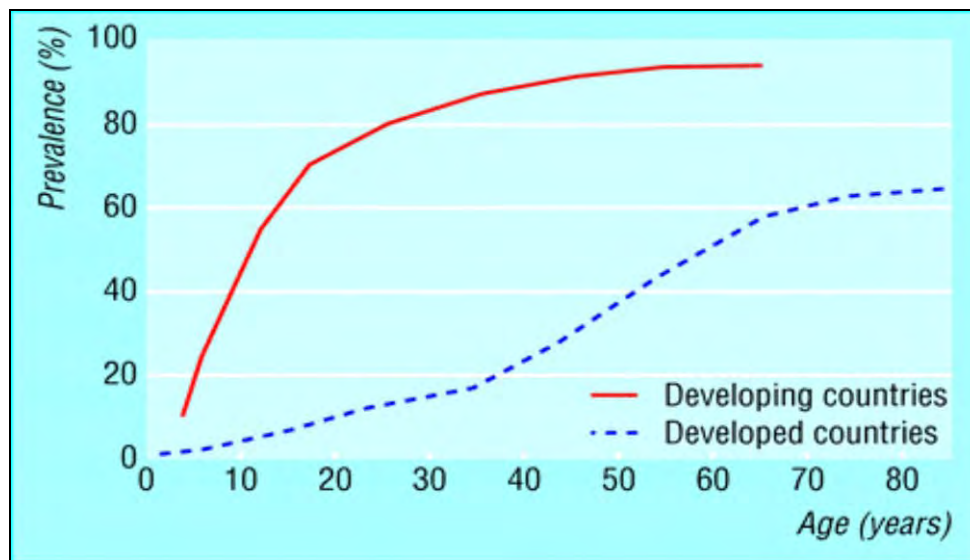
Data available from several developing countries suggest that rates of *H. pylori* infection in men and women are approximately the same (Yilmaz *et al.*, 2002; Kim *et al.*, 2001; Megraud *et al.*, 1989). However, in a large French cross-sectional study, a significantly lower prevalence of *H. pylori* infection was observed in females as compared with males (Broutet *et al.*, 2001). Moreover, in a study among the different adult ethnic groups, the overall prevalence of *H. pylori* infection was higher in males compared to females (Everhart *et al.*, 2000).

d) Age and *Helicobacter pylori*

It is well accepted that higher rate of *H. pylori* infection is acquired in childhood in developing countries, though the age at which the highest rate of acquisition occurs remains

unclear (Figure 1.1.). Generally, *H.pylori* seroprevalence increase steadily with age (Yilmaz *et al.*, 2002). One of the first studies of *H.pylori* prevalence was done in 1989 in developing countries (Megraud *et al.*, 1989). Among Algerian children, 43% were seropositive and rose steadily with age, reaching a peak of 92% between the ages of 40 and 90 years. In a seroprevalence study of *H. pylori* infection in a rural area from Brazil, antibodies to *H. pylori* were detected in the serum of 77.5% children and teenagers and in 84.7% adults and prevalence of infection increased with age (Dutra Souto, *et al.*, 1998). In Ivory Coast (Megraud *et al.*, 1989), the seroprevalence of *H. pylori* in children was 54% rising gradually to 70% -80% throughout adulthood. In Kenya 93% of 14 asymptomatic adults whose age greater than 15 were found to have *H. pylori* during endoscopy (Lachlan *et al.*, 1988).

Figure 1.1. Prevalence of *H.pylori* Infection By Age in Developing and Developed countries (Adapted from Bardhan, 1997)



Among 500 Finnish blood donors (Kosunen *et al.*, 1988), the prevalence increased from 10% (18-25 years) to 60% (56-65 years) and in 1000 patients from France presenting

for health screening, the prevalence increased from 3.5% (0-9 years) to 35% (>60 years). A further study of 260 blood donors aged between 18 and 61 showed an overall rate of seropositivity of 39%, and the prevalence was 21% in those less than 21 years old and 60% in those over 50 years old (Breuer *et al.*, 1996). Peak acquisition is believed to occur in childhood and studies in children from high and low prevalence countries showed that in Ethiopia the peak seroconversion rate occurred in the 2-4 years age group, with 60% positive at age 4 years and 100% positive at age 12 years; whilst in Sweden the peak seroconversion rate was 20% at age 9-10 years. (Lindkvist *et al.*, 1996).

Prevalence of *H. pylori* infection is rapidly decreasing, not only in the Western developed world but also in the Far East. A study from Japan showed decreased prevalence from 73% in 1974 to 39% in 1999 (Fujisawa *et al.*, 1999). A dramatic reduction in the prevalence of *H. pylori* infection has also been reported in Korean children less than 10 years (Kim *et al.*, 2001). The reasons for the decline in the prevalence of *H. pylori* colonization are unknown. The causes of the disappearance of *H. pylori* are largely speculative. Putative reasons include: (i) smaller family sizes; (ii) larger time intervals between the first and the second child; (iii) increased hygiene; and (iv) more common and widespread consumption of antimicrobials during childhood (Blaser, 1999).

e) ***Helicobacter* and Ethnicity, Socioeconomic & Environmental Factors**

Although the exact reasons for the differences in *H. pylori* seropositivity in different ethnic and racial groups are not known, socioeconomic factors, environmental factors, sociocultural practices and genetic predisposition may all contribute toward acquisition of *H. pylori* infection (Megraud *et al.*, 1989).

Marked differences in the seroprevalence of *H. pylori* have been observed between various ethnic and racial groups living in the same area. A study conducted in Singapore among patients with no ulcerative dyspepsia found the seropositivity among Malays, Chinese, and Indians to be 22%, 48% and 57% respectively (Kang *et al.*, 1990). In a study among the different adult age adjusted ethnic groups for *H. pylori* showed that prevalence was substantially higher among non Hispanic blacks (52.7%) and Mexican Americans (61.6%) than non Hispanic whites (26.2%)(Everhart *et al.*, 2000). In Australia, native Aborigines and Caucasian Australians were compared with El Salvadorian and Ethiopian refugees. The seroprevalence in the Australians of Caucasian decent was 15%, compared with 40% in the El Salvadorians and 43% in the Ethiopians. Individuals studied in Vietnam, Algeria and the Ivory Coast also had different seroprevalence rates. The seroprevalence in the 0-9 year age group was much higher in populations from Vietnam (13%), Algeria (45%) and the Ivory Coast (55%), compared with France (3.5%). By the age of 30 years, 75% of all adults in these three countries had serological evidence of colonization by *H. pylori*, compared with 24% of adults in France (Megraud *et al.*, 1989). A lower socioeconomic status is associated with a higher prevalence of *H. pylori* infection (Yilmaz *et al.*, 2002), and this association has been found worldwide, including countries in Asia (Katelaris *et al.*, 1992), South America (Hopkins *et al.*, 1993). Several of these studies have also demonstrated an inverse relationship between *H. pylori* prevalence and the educational level of the population studied. In Saudi Arabia (Al-Moagel *et al.*, 1990) the prevalence of *H. pylori* infection in college graduates and non-graduates was 54% and 77% respectively. Environmental factors, such as general level of hygiene, water supply and sanitation and crowding in the household, have been reported to be linked with *H. pylori* infection (Rocha

et al., 1994). All these factors are interrelated and are linked with the overall standard of socioeconomic development. Social overcrowding has been revealed as a significant risk factor for acquisition of *H. pylori* in other studies (Yilmaz *et al.*, 2002; Choe *et al.*, 2002). A recent seroprevalence study among adult healthy blood donors in Addis Ababa, Ethiopia showed that 89.0% of the sera were positive for *H.pylori* infection (Desta *et al.*, 2002a).

f) Sources of infection

To make public health recommendations & to limit the transmission of a pathogenic microorganism, its source and route of transmission must be well known. However, there are no significant animal or environmental reservoirs for strains infecting humans other than humans. *Helicobacter* like organisms have been isolated from domestic, commercially reared cats (Fox, 1995) and it has been suggested that it might be a zoonotic pathogen with transmission occurring from cats to humans. After adjusting for potential confounders in a study on factory workers in the United Kingdom, there was no association between *H. pylori* seropositivity and cat ownership during childhood (Webb *et al.*, 1996). Although *H. pylori* is believed to be strictly human pathogen, *H. pylori* or similar organisms could be isolated from several nonhuman species, including primates, pigs, and cats (Fox, 1995). Moreover epidemiological studies showed that having cats at home was not found to be a risk factor. In some studies it was even found to be a protective factor, which disappeared after adjustment of socio-economic status (Norris *et al.*, 1999).

g) Routes of Transmission

The mode of transmission of *Helicobacter pylori* is largely unknown. The most likely recognized source for *H. pylori* is the human stomach, although it is not known by

what route the organism is transmitted to the stomach. Evidence suggests close personal contact is important and that acquisition occurs mainly in childhood (Vaira *et al.*, 2001).

i) Fecal-Oral Transmission

Successful isolation of *H. pylori* from feces proved that *H. pylori* could survive in the intestinal tract and shed into the environment along with feces (Saborio *et al.*, 1994), although this does not necessarily imply the presence of viable bacteria. Studies suggested that *H. pylori* infection could be food-borne or water-borne.

ii) Oral-Oral Transmission

The oral cavity as a possible reservoir of *H. pylori* has been suggested (Allaker *et al.*, 2002). Possible oral–oral transmission has been investigated in eating of pre-masticated foods among some ethnic groups, the use of the same spoon by both mother and child, intimate oral–oral contact, and aspiration from vomit (Megraud, 1995). There is no direct evidence for transmission via the last two routes, but possible transmission via intimate oral–oral contact has been suggested indirectly by the fact that spouses and children of individuals infected with *H. pylori* were more often seropositive than spouses and children of non-infected individuals (Megraud, 1995). However, other studies showed that sexual intimacy and cohabitation among adult individuals are not risk factors for *H. pylori* transmission.

iii) The Gastric-Oral Route

It has been suggested that *H. pylori* may be passed more directly from stomach to mouth, without the need for an oral reservoir. Transmission via vomitus has been suggested

as a possibility and has more recently been proposed as the main route of transfer, specifically during epidemic acute *H pylori* infection in childhood. In suggesting this route of transmission, it has also been argued that mucus-rich vomiting caused by acute *H. pylori* infection may be the bacterium's mechanism for promoting survival, by providing a vehicle for transmission to new hosts (Axon, 1995). For the gastric oral route to be feasible, *H pylori* would need to be able to survive in vomitus, but survival of the bacteria in an acidic environment without the presence of urea is limited (Marshall *et al.*, 1990).

1.5. Pathogenesis of Infection

There is now extensive evidence implicating *H. pylori* in the pathogenesis of chronic superficial gastritis. Voluntary ingestion of the bacterium by two human volunteers resulted in acute or chronic gastritis (Dunn *et al.*, 1997). Many virulence factors including mechanisms and their effects have been identified as shown in Table 1.2.

Table 1.2. *Helicobacter pylori* pathogenic mechanisms, virulence factors and their effects(Adapted from Dunn *et al.*, 1997)

Pathogenic Mechanisms	Virulence factors	Effects/properties
I. Gastric colonization	Flagella	Active movements through mucin
	Urease	Neutralization of gastric acid
	Adhesins	Anchoring <i>H. pylori</i> to the gastric epithelium
II. Persistence (Survival)	Superoxide dismutase	Prevent phagocytosis and killing
	Catalase	Prevent phagocytosis and killing

	Coccioid forms	Dormant form
	Heat shock proteins	Sheathing antigen
	Urease	Sheathing antigen
III. Disease inducing (Tissue damaging)	Proteolytic enzymes	Degrades mucin
	CagA	Related to ulcer and gastritis
	VacA	Damage of the epithelium
	Urease	Toxic effect on epithelial cells, disrupting cell tight junction
	Phospholipase A	Digest phospholipids in cell membranes
	NAP*	Leads to neutrophil mediated mucosal injury
	Alcohol dehydrogenase	Gastric mucosal injury
IV. Others	Lipopolysaccharide	Molecular mimicry
	Lewis ^x /Lewis ^y blood group antigen	Autoimmunity

*NAP Neutrophil activating protein

The presence of *H. pylori* in the gastric mucosa is almost always associated with mucosal inflammation due to infiltration by neutrophils and monocytes. The levels of tumour necrosis factor (TNF) alpha, interleukin (IL)-1b, and IL-8 are all raised in the gastric mucosa of patients with *H. pylori* infection compared with those who are not infected. *Helicobacter pylori* causes the release of growth-regulated chemokines, macrophage inflammatory proteins, and monocyte chemotactic activating factor, all of which are involved in the generation of intense inflammatory reaction that occurs in gastric mucosa

(Dunn *et al.*, 1997). *H.pylori* related inflammation may be highly variable, ranging from minimal infiltration to severe dense inflammation with microabscess formation. Concomitantly, there often are degenerative changes of the surface epithelial cells including mucin depletion, cytoplasmic vacuolization, and disorganization of mucosal glands. After eradication of *H. pylori* infection by antimicrobial agents, most of these features disappear rapidly (Dunn *et al.*, 1997).

H. pylori infection is regarded as a slow bacterial infection. All individuals will not develop disease. Symptoms can be related to the actual time of *H.pylori* infection (acute) or they might bear in relation to the late effects of *H.pylori* infection (such as chronic gastritis, ulcer, gastric carcinoma). The clinical course of *H. pylori* infection is highly variable and is influenced by both microbial and host factors. *H. pylori* infection include asymptomatic chronic gastritis, chronic *H. pylori*-associated dyspepsia, peptic ulcer disease, gastric adenocarcinoma and *H. pylori*-associated mucosa-associated lymphoid tissue (MALT) lymphoma (Dunn *et al.*, 1997).

The majority of infected individuals are asymptomatic, despite the presence of chronic inflammation of the gastric mucosa. The prevalence of *H.pylori* and gastritis in asymptomatic subjects from the U.S. and Europe increases with age, rising from about 10% in the 20's to about 50% in the 50's/60's of age. In contrast, *H.pylori* and histologic gastritis occur at an earlier age and with much greater frequency in developing countries; perhaps 80% of people in these areas are infected by the end of childhood (Laine, 1998).

The ultimate outcome of long-standing active chronic inflammation is loss of glands, called atrophy, and ultimately intestinal metaplastic change. The development of atrophy occurs earlier in developing countries compared with developed countries, probably

because of acquisition of *H. pylori* at earlier age in developing countries, but which environmental factors are responsible for accelerated development of atrophy are largely unknown. Studies showed that elimination of *H. pylori* gastritis increases gastric acidity, probably by reducing non-parietal alkaline secretion, and this may facilitate gastroesophageal acid reflux (Tytgat, 2000).

In an international study on 17 populations from 13 countries, it was concluded that there is a roughly six-fold risk of gastric cancer in populations with 100% *H. pylori* infection (The Eurogast Study Group, 1993). However, in Africa, a puzzling feature is that although there is a very high prevalence of the infection, associated complications (including gastric cancer) are very uncommon (Segal *et al.*, 1998).

1.6. Dyspepsia

Although there are many definitions of dyspepsia have been proposed over the last 25 years, it has been defined by the Rome Working Teams as pain or discomfort centered in the upper abdomen associated with a variety of symptoms such as fullness in the upper abdomen, bloating or nausea or vomiting (Talley *et al.*, 1999). Dyspeptic symptoms, not only vary in intensity, stimuli localization and duration, but also in pathophysiology. Multiple pathophysiological processes may be involved in the development of a symptom and more than one symptom may be present as a feature of dyspepsia (Jiwani and Qureshi, 2001; Talley *et al.*, 1999).

Using a commercial enzyme-linked immunosorbent assay (ELISA), studies showed that *H. pylori* infection does not play an important role in overall symptoms of dyspepsia in the community. A study conducted in India comprising of 80 symptomatic and 67

asymptomatic individuals showed that 38 (56.7%) asymptomatic and 49 (61.3%) symptomatic individuals were positive for *Helicobacter pylori* infection, indicating *H.pylori* was not associated with dyspepsia, and was more prevalent in elderly subjects (Singh *et al.*, 2002).

However, one study has indicated that dyspeptics are almost twice as likely to show symptomatic improvement if *H. pylori* is eradicated. Some epidemiological studies have suggested a higher prevalence of *H. pylori* infection in patients with dyspepsia. Unfortunately, there are few high quality therapeutic trials that have specifically investigated whether *H. pylori* infection causes dyspepsia (Jaakkimainen *et al.*, 1999).

In supporting this, the results of some previous studies showed that some patients with dyspepsia who were colonized with *H. pylori* show better responses to antimicrobial therapy than to placebo an effect not seen in patients with nonulcer dyspepsia but not *H. pylori* infection (Kang, *et al.*, 1990). In a recent study (De Giacomo *et al.*, 2002), severe epigastric pain and ulcer-like dyspepsia were significantly associated with *H. pylori* infection, while dyspeptics with recurrent abdominal pain was not. Over all, subtle differences in dyspepsia-scoring systems may also have contributed to the outcome of these differences (Suerbaum and Michetti, 2002).

1.7. Diagnosis of *Helicobacter pylori* Infection

There are numerous tests available to diagnose *H. pylori* (Table 1.2.), but none is perfect. Each has advantages and disadvantages, which will make it more or less, appropriate for different situations. Histological examination of gastric tissue, bacterial culture, rapid urease testing, and PCR analysis, when used to test gastric tissue, all require

endoscopy; therefore they incur expense and a risk, of complication due to the procedure. In contrast, breath tests, serology, and gastric juice PCR, are noninvasive tests that do not require endoscopy. The choice of test used for diagnosis of *H. pylori* infection will depend, in most cases, on the clinical information sought and the local availability and cost of individual tests (Kullavanijaya *et al.*, 2002).

a) Invasive Methods

All biopsy-based methods for detecting *H. pylori* are liable to sampling error because infection is patchy. In addition, after partial effective eradication treatment, low levels of infection can easily be missed by endoscopic biopsy, leading to overestimates of the efficacy of eradication treatment and reinfection rates (Logan and Walker, 2001).

i) Culture: - Culture of *H. pylori* has two major advantages. First, it allows antimicrobial susceptibility testing; second, isolates obtained by culture can be characterized in detail. Although the sensitivity of culture in highly experienced laboratories is greater than 95%, other methods for the diagnosis of *H. pylori* infection are simpler, prone to less variability, and more timely. Positive cultures are usually detected after 3 to 5 days of incubation. *H. pylori* is identified on the basis of colony morphology (translucent colonies varying in size); colonies consist of gram-negative, curved (not usually helical) rods that are urease, catalase, and oxidase positive (Dunn *et al.*, 1997). Although only a few centers routinely offer microbiological isolation of *H. pylori*, the prevalence of multi-resistant strains makes it increasingly likely that culture and antibiotic sensitivity testing may become a prerequisite for patients with persistent infection after initial or repeated treatment failure. Because *H. pylori* is highly fastidious, culturing can lead to false negative results and risks

of overgrowth or contamination make it the least sensitive method of detection, and it is the least readily available test for use (Piccolomini *et al.*, 1997).

ii) Histologic assessment: - *H. pylori* can be visualized at high magnification with conventional hematoxylin and eosin (H & E)-stained sections. Bacteria are located in the mucus adherent to the surface epithelium and are often found deep within the crypts, which makes it less reliable when few bacteria are present. In addition, luminal debris on the surface of the epithelium can be mistaken for *H. pylori* in H & E stained sections. Using special stains such as the Warthin-Starry and modified Giemsa stains facilitates histological identification of bacteria. The distribution of *H. pylori* in the stomach is not uniform, which can give false negative results (Misra *et al.*, 2000). Moreover, histologic identification of bacteria with the characteristic morphology of *H. pylori* is, in part, observer dependent. Factors that influence the ability to correctly identify *H. pylori* include bacterial density, type of stain used, and the enthusiasm and experience of the laboratorian (Laine *et al.*, 1997).

iii) Biopsy urease tests (rapid urease tests): - The early observation that *H. pylori* produces large amounts of urease activity led to the development of methods for the indirect detection of the organism in gastric biopsy tissue. The sensitivity of all urease-based tests for detection of *H. pylori* is dependent upon the bacterial load in the stomach. The Campylobacter like organisms (CLO) test, developed by Marshall, was the first commercially available biopsy urease tests designed specifically for *H. pylori* detection. Phenol red and urea; in the presence of urease, the urea is hydrolyzed, leading to a pH (and hence a color) change of the indicator. The test is interpreted up to 24 h after placement of the gastric biopsy sample onto the agar gel (Cutler *et al.*, 1995).

Table 1.3. Comparative accuracy of tests for *H pylori* infection (Adapted from Logan and Walker, 2001)

Invasive		
Test	Sensitivity	Specificity
Histology	88-95%	90-95%
Culture	80-90%	95-100%
Urease test	90-95%	90-95%
Non-invasive		
C-UBT	86-95%	86-95%
Serology:		
ELISA	80-95%	80-95%
NPT	60-90%	70-85%
Stool antigen	90-95%	90-95%

UBT=urea breath test. NPT=near patient test.

b) Non-invasive Methods

Non-invasive serological tests have been widely used for the diagnosis of *H. pylori* infection, mainly in adults. Cutler *et al.* (1995) concluded that the noninvasive tests (EIA, PCR, and urea-based breath tests) are as accurate as the invasive tests (e.g., culture of gastric

biopsy or histologic examination) at predicting the *H. pylori* infection status of untreated patients.

i) **Serology – EIA:** - Infection of the gastric mucosa with *H. pylori* results in systemic as well as local immune responses, including elevation of specific IgG and IgA levels in serum and elevated levels of secretory IgA and IgM in the stomach, thus allowing the development of serologic tests for detection of the bacteria. A comparative study on the Pyloriset EIA-G and Pyloriset EIA-A showed that Pyloriset EIA-G update is a reliable and accurate test because of its 100% sensitivity, 100% negative predictive value makes it a very useful screening test, hence, conjunctive IgA testing is not necessary. Recently, EIA test sensitivity and specificity has been also reported to have 80-95% and 78-97% respectively (Herbrink and van Doorn, 2000).

It appears to be similar to any other bacterial infection (i.e. after approximately 14 days, IgM is present, and by 21 days, IgG is detectable). IgM declines over the ensuing three months; thus, patients with chronic *H. pylori* infection usually have no IgM, but always have IgG (Kullavanijaya *et al.*, 2002). Serologic methods have proven especially valuable in screening large numbers of individuals in epidemiological studies including retrospective studies to determine the prevalence or incidence of infection. Serologic tests are noninvasive, relatively rapid and simple to perform can be done from stored samples, and much less expensive than tests requiring endoscopic biopsy. Further, serologic tests are less likely to be confounded by suppression of *H. pylori* infection by bismuth compounds, proton pump inhibitors, or antibiotics taken for unrelated conditions than are urease-based tests, which are dependent upon and reflect the current bacterial load. Although a wide variety of serologic methods for detection of *H. pylori* have been described in the literature, most tests available

commercially are enzyme-linked immunosorbent assay methods. The utility of any serologic test for the detection of *H. pylori* specific antibodies is dependent on the antigen preparation used. In general, three types of antigen have been used. These include *crude antigens* such as whole cells and whole-cell sonicates, *cell fractions* such as glycine extracts and heat-stable antigens, and *enriched antigens* such as urease and a 120-kDa antigen. Currently pooled glycine extracted antigens are widely used to give negligible serological cross-reactivity with *Campylobacter jejuni* (Herbrink and van Doorn, 2000).

In the absence of therapeutic intervention, antibody levels remain elevated, perhaps for a lifetime, reflecting the duration of infection. After eradication of *H. pylori*, specific immunoglobulin G (IgG) and IgA levels tend to decrease, typically to approximately half of the pretreatment value within 6 months (Herbrink and van Doorn, 2000). Low levels of specific IgG tend to persist for months even after eradication of *H. pylori*; therefore, using serologic tests to assess the effects of treatment may be problematic unless the pre- and post-treatment sera can be directly compared. Presumably, some individuals with gastric atrophy continue to have serologic evidence of a prior *H. pylori* infection that has “burnt out.” Serology is a “global” method, reflecting infection anywhere in the stomach; it may be more accurate than the “gold standard” biopsy-based methods, which are local (each biopsy specimen represents approximately 0.001% of the surface of the stomach) and subject to a variety of sampling errors. Serologic tests may be positive in patients with gastric atrophy, in which the number of *H. pylori* organisms is so small as to be undetectable by biopsy or breath test-based methods. Therefore, an argument can be made that antibody tests should be used only as screening tests. A negative test predicts a low probability of infection in a patient with a low likelihood of infection, and further evaluation for infection is

unnecessary. Given the same patient, however, confirmation of a positive test may be desirable (Herbrink and van Doorn, 2000).

a) Laboratory Based Serology: - Laboratory-based serological testing has the advantage of requiring very little patient time, since only a venous blood sample is required. It is therefore particularly suitable where large numbers are to be tested, like, for epidemiological surveys. Whilst advantages in terms of convenience and cost are important considerations, the usefulness of serological tests depends largely on their sensitivity and specificity. A wide range of kits for carrying out these tests is available commercially and their accuracy has been shown to vary considerably (Vaira *et al.*, 2002). Most enzyme immuno assays yield quantitative results and setting a local positive/negative cut-off point may also be advantageous. Serum antibodies (IgG) against *H. pylori* were detected using commercial kits and they have 95.8% sensitivity and 95.5% specificity (Megraud, 1997). In another study *H. pylori* IgG EIA was 97.6% sensitive and 94.1% specific (Jaskowski *et al.*, 1997). The most accurate 'blood test' is the one that uses serum antibodies from clotted blood taken at venesection because the dilution of the serum is constant (Vaira *et al.*, 2002).

b) Office-Based or "Nearpatient" Tests: - Antibody tests now commercially available include not only laboratory based tests but also the so-called "office-based" or "nearpatient" tests, which use either serum or fingerprick whole blood. Their performance characteristics to date suggest that they are less accurate than are at least some laboratory-based tests; with sensitivity (68-76%) and specificity (91-97%) (Day and Sherman, 2002). Fingerstick tests are relatively inexpensive and simple to use, and results are available in 5 to 15 min. A disadvantage of the office-based tests is that they are qualitative only, precluding their use for early follow-up testing in assessment of cure after anti-*H. pylori*

treatment and their more widespread use on many patients could have them performed inappropriately or unnecessarily. Patients testing positive would then receive treatment for *H. pylori* infection, which may not have been responsible for their presenting problem. More widespread application of treatment of *H. pylori* infection will lead to an increased occurrence of adverse events and will promote the development of antimicrobial resistance (Howden and Hunt, 1998). In addition, Fingerprick test results can vary when there is difficulty of obtaining blood, particularly squeezing the finger can change the haematocrit by mixing tissue fluid with the blood sample, changing the concentration of antibody in the serum (Vaira *et al.*, 2002). Other antibody tests include the detection of IgA antibody in serum (Cutler *et al.*, 1995) and detection of anti-*H. pylori* IgG antibody in saliva and urine (Fallone *et al.*, 1996). Currently, these tests are in research and development and the accuracy of each of these assays is lower than that of serum IgG antibody tests (Megraud 1997; Jaskowski *et al.*, 1997).

ii) Serology - Immunoblot: - Serum immune response can also be detected by immunoblotting, which is more sensitive as well as more specific than EIA. Good agreement was found between positive culture results and immunoblot-positive sera (97.5%), compared to that found between culture results and EIA results (87.5 to 92.5%), the authors concluded that the immunoblot appears to be a more sensitive assay, especially with sera with low levels of antibodies that were not detected by EIA. Immunoblotting allows for the analysis of the immune response to a number of defined antigens, which EIA does not, and for the differentiation of species-specific and cross-reacting antibodies. It can be used as an additional indicator of antibody response when the outcome of EIA is doubtful. Moreover, it

permits detection of antibody responses to specific antigens, e.g., the cytotoxin-associated CagA protein, VacA; which may have pathological implications (Nilsson *et al.*, 1997).

In this test separated proteins are better exposed allowing antibodies to bind more easily, compared with antibody recognition of protein mixtures coated in microtiter wells, thus increasing the specificity and, especially, the sensitivity of the test (Karvar *et al.*, 1997). Another factor that may contribute to increased accuracy of immunoblotting is the ability to evaluate immune response to a number of defined antigens, which is not the case of ELISA, allowing the differentiation of species-specific and cross-reacting antibodies (Nilsson *et al.*, 1997). Immunoblot showed a higher sensitivity in pediatric population (98.6%) (Oleastro *et al.*, 2002) than that observed in adults (95.6%) (Monteiro *et al.*, 2001).

iii) Urea Breath Test: - The principle of urea breath tests (UBTs) for the diagnosis of *H. pylori* is similar to that of other urease based tests. Urea is provided as a substrate that, in the case of the UBT, is ingested as either [¹³C] or [¹⁴C] urea. *H. pylori* urease hydrolyzes the ingested urea into labeled bicarbonate, which is exhaled as labeled CO₂, which is collected. The ¹⁴C isotope is detected with a scintillation counter, while the ¹³C isotope is detected by mass spectrometry most commonly, although other analytical methods have been developed (Koletzko *et al.*, 1995). Some microorganisms within the oropharynx also may hydrolyze urea; therefore, if urea is presented in liquid form, an early peak in labeled CO₂ may occur during the breath test. Delivery of ¹⁴C urea in tablet form is more advantageous, because it avoids the problem of detecting urease-positive oral bacteria (Piccolomini *et al.*, 1997).

iv) Polymerase Chain Reaction (PCR): - PCR offers great promise as a highly sensitive and specific technique for the detection of *H. pylori*. PCR techniques for the detection of *H. pylori* in gastric biopsy specimens have been described by a number of

laboratories, although the accuracy of such techniques varies widely. Factors affecting test accuracy include the choice of primers and target DNA, contamination, specimen preparation, bacterial density, and technical issues regarding the PCR procedure (el-Zaatari *et al.*, 1995). PCR techniques for the detection of *H. pylori* are still in their infancy and can detect nonviable forms; it is unlikely that such techniques will have widespread use in the initial detection of *H. pylori*, except in the research environment. However, PCR methods hold great promise in the detection of genetic differences between *H. pylori* strains for research and epidemiologic studies. Generally, invasive methods are expensive, complex, time consuming, require highly experienced man power and can lead to false negative result, hence not appropriate for population based studies. The false negative results of biopsy, culture, and rapid urease test are attributed to the patchy nature of *H. pylori* infection, which can lead to endoscopic sampling error (Oleastro *et al.*, 2002; Stone, 1999).

1.8. Treatment

The strongest evidence for the pathogenic role of *H. pylori* is that successful eradication of the organism leads to enhanced ulcer healing and reduces the risk of bleeding and ulcer recurrence (Peura, 1998). With this evidence, the National Institutes of Health Consensus Conference in 1994 recommended that all *H. pylori* infected patients with gastric or duodenal ulcers, whether on first presentation or recurrence, should be treated with antimicrobials (NIH, 1994). However, in asymptomatic *H. pylori* infected patients without ulcers, antimicrobial treatment is not recommended, since there is limited data to support prophylactic antimicrobial therapy to prevent ulceration in the future (NIH, 1994). Furthermore, there is insufficient evidence to support treatment of all *H. pylori* infected

individuals in order to prevent gastric malignancy. Anti-*Helicobacter* therapy should be reserved only for patients who are infected with *H. pylori*, have evidence of peptic ulcers and clinical symptoms or have complications and a history of refractory ulcers (Bourke *et al.*, 1996).

H. pylori can be eradicated with antibiotic therapy; however, to achieve eradication rates of greater than 90%, more than one agent has to be used. Monotherapy results in temporary suppression of *H. pylori* but usually fails to eradicate the organism. *H. pylori*'s location deep within the lumen of gastric glands and pits and between the gastric epithelium, protected by mucus, shelters it from antimicrobial agents. *H. pylori* can readily develop antibiotic resistance with single antibiotic use, especially with metronidazole and clarithromycin. The selection of a treatment regimen should be individualized and based on efficacy, compliance, antibiotic resistance and cost (Salcedo and Al-Kawas, 1998).

i) Triple Therapy

The standard antimicrobial therapy today for successful eradication of *H. pylori* infection is triple therapy. Effective antibiotic-based therapies for eradicating *H. pylori* have been developed in recent years. At this time, the most proven effective treatment is a 2-week course of treatment called triple therapy, which involves the use of two antibiotics and either an acid suppressor or a stomach-lining shield. Two-week triple therapy kills the bacteria, reduces ulcer symptoms, and prevents its recurrence in more than 90% of patients (Soll, 1996).

ii) Quadruple therapy

Quadruple treatment regimens consist of the traditional bismuth-based triple therapy with the addition PPI, to provide symptomatic relief and ulcer healing and to increase the efficacy

Table 1.4. Treatment regimens used to eradicate *Helicobacter pylori* (Adapted from Dunn *et al.*, 1997)

Regimen	Dosage	Length of therapy	Eradication rate (%)
<i>Triple therapy containing bismuth</i>			
Tetracycline HCl plus	500 mg q.i.d.	2 weeks	85
Metronidazole plus	250 mg q.i.d.		
Bismuth subsalicylate	2 tablets q.i.d.		
<i>Dual therapy</i>			
Clarithromycin plus	500 mg t.i.d.	2 weeks	74
Omeprazole	400 mg daily		
<i>or</i>			
Clarithromycin plus	500 mg t.i.d.	2 weeks	82
Rantidine bismuth citrate	400 mg b.i.d.		
<i>Triple therapy regimens</i>			
Metronidazole or	500 mg b.i.d	1 or 2	90
Amoxicillin plus	1 g b.i.d.	weeks	
Clarithromycin plus	500 mg b.i.d		
Omeprazole or	20 mg b.i.d		
Lansoprazole	30 mg b.i.d		
Rantidine bismuth citrate plus	500 mg b.i.d		
Clarithromycin plus	400 mg b.i.d.	2 weeks	90
Amoxicillin	500 mg b.i.d		

of triple regimens (Salcedo and Al-Kawas, 1998). There are many FDA (food and drug administration) approved antisecretory-antimicrobial drug combinations used to treat *H. pylori* infection. Table 1.3. Lists the regimens currently used.

1.9. Vaccine Development

Although significant progress has been made in treating *H. pylori* infection, antimicrobial-based treatments are suboptimal and are not a long-term solution. The participation of several of these genes in pathogenesis has already been confirmed by genetic disruption. It is now well established that urease and VacA protein are virulence factors for this organism. The *cag* PAI genes have also emerged as other well confirmed virulence participants. Further investigation of *H. pylori* virulence factors has provided information that is being used in the development of novel therapies and vaccines to treat and prevent *H. pylori* infection (Ghiara *et al.*, 1997). To date, researchers have identified several *H. pylori* antigens, which confer protection against *H. pylori* infection, or in eradicating an already established infection in the murine models, including purified VacA, urease (and its subunits), CagA (Corthesy-Theulaz *et al.*, 1995), heat shock proteins (HspA and HspB), and catalase. Therapeutic vaccination has also been successful in ferrets and Rhesus monkeys infected with *H. mustelae* and *H. pylori*, respectively (Dubois *et al.*, 1999). Most of these antigens have been given orally in combination with mucosal adjuvants, which improve their low immunoreactivity (Ghiara *et al.*, 1997). However, the inherent toxicity of mucosal adjuvants has been the major limitation for their use as vaccines in humans.

1.10. Objectives of the study

To determine the magnitude of *Helicobacter pylori* infection in adult patients with dyspeptic symptoms and without dyspeptic symptoms attending Felege Hiwot Hospital, Bahir Dar, North West Ethiopia by serological methods.

CHAPTER II
MATERIAL AND
METHODS

CHAPTER II. MATERIAL AND METHODS

2.1. Study area

Bahir Dar is one of the most important urban centers; a lake-port town located in the northwestern part of Ethiopia. It is the central city of Amhara Regional State and a notable tourist center. Geographically it is located at the southern most part of Lake Tana, the source of River Abay (The blue Nile) and situated at altitudes of 1800 meters above sea level. It has a direct access both to the water of lake Tana and to the very upper most course of the River Abay. It has 320 and 490 kms air and ground distances, respectively, from the capital city of the county, Addis Ababa. It had a total population of 150,000 as of 1998, which is growing at alarming rate. With regards to the health services there is only one hospital (Felege Hiwot) and one health center (Personal communication).

Felege Hiwot Hospital has around 179-200 beds and an average of 60-80 patients visit the hospital per day by the year 2000-2001. A study entitled on “drug prescribing patterns for out patients in three hospitals in North West Ethiopia” showed that the two top most recorded causes of morbidity among Felege Hiwot hospital attendants of adult population were respiratory diseases and gastrointestinal diseases (Desta *et al.*, 2002b).

2.2. Study Design

This was a case control study of *H.pylori* infection between dyspeptic and non-dyspeptic patients that considered a randomly selected study subjects from Felege Hiwot Hospital out- patient and in- patient departments. At the time of recruitment the investigator obtained signed consent from subjects after informing them about the objectives, procedures, and treatment of *H. pylori*.

2.3. Study subjects

i) Patients

A total of 100 consecutive informed and consented adult patients (15 or more years of age) with dyspeptic symptoms from Felege Hiwot Hospital were investigated for *H. pylori* infection between mid November 2002-March 2003 (Fig 3.1.).

Dyspepsia has been defined as abdominal pain or discomfort in the central portion of the upper abdomen associated with symptoms such as bloating or nausea or vomiting or fullness of the abdomen (Talley *et al.*, 1999).

ii) Controls

A total of 100 consecutive informed and consented adult patients (15 or more years of age) without dyspeptic symptoms from Felege Hiwot Hospital were included as controls in the study.

All appropriate informations (demographic, clinical, and laboratory data) were recorded on the questionnaire prepared for this study (Appendix 1).

Sample size

a) *Dyspeptic subjects:* - From previous developing countries, 90% prevalence of (*H.pylori*) infection (refer section 1.3) with dyspeptic symptoms 6% effect size and 95% confidence interval.

b) *Non dyspeptic subjects:* - Similarly, 80% prevalence, 8% effect size and 95% confidence interval.

Calculation of sample size was done using the formula

$n = \frac{Z^2 \pi (1 - \pi)}{E^2}$, Where π = population proportion, E = effect size, Z = obtained from table at the given confidence interval.

Exclusion Criteria

Patients and controls that have been taking antibiotics including treatment for *H.pylori*, non-steroidal anti-inflammatory drugs (NSAIDS) or steroids in the past one-month were excluded before they give consent.

2.4. Collection, Handling and Transport of Specimen

Five to ten ml venous blood was collected from each informed and consented adult dyspeptic patients and non-dyspeptic patients as was determined by the physician. The sera were obtained from the blood by centrifugation (3000 RPM for 10 minutes). Sera were aliquoted and immediately stored at -20°C. All sera were transported in icebox to the Bio-Medical Research Training Project Laboratory (BRTP), Faculty of Medicine, Addis Ababa University and stored at -20°C until tested. Blood grouping and Rh factor were determined using commercially prepared antisera by slide agglutination technique.

2.5. Serology

i) Enzyme immuno assay (EIA)

A total of 200 sera collected from patients were examined for the presence of IgG antibodies against *H. pylori* pooled antigens using EIA method as described previously (Asrat, 2003). Briefly, microtiter plates (Maxisorp immunoplate NUNC, Roskilde, Denmark) were coated overnight with acid glycine extracted cell surface proteins from *H. pylori* reference strain CCUG 17874 and incubated at 4°C for 16 h. Plates were washed

three times in washing-buffer (Phosphate buffered saline or PBS, 0.05% and Tween-20, PBS-T). Antigen-coated wells were then blocked with blocking-buffer (3% BSA or bovine serum albumin in PBS-T), covered and incubated for 1h at 22 °C.

One hundred µl of test serum diluted 1:800 in PBS-T was added and incubated for 90 min. at 37 °C. Serum was tested in duplicate and a PBS-T control for each sample tested to monitor non-specific background readings. After repeated washings, 100µl alkaline phosphatase labeled goat antihuman IgG antibodies (Sigma, St Louis, USA) was added and plates incubated for 60 min. at 37 °C. After aspiration and washing, 100 µl of a substrate solution containing p-nitrophenyl phosphate (Sigma), diethanolamine, and MgCl were added and incubated for 30-60 min. 37 °C. The absorbance values were at 405nm reader using a Bio Rad –spectrophotometer. A reference standard, human gammaglobulin (Pharmaca & Upjohn, Stockholm Sweden) was run in parallel throughout the procedure. The absorbance was read by using a microplate reader (Bio Rad) at 405nm and the EIA results were calculated according to the procedure described by (Asrat, 2003) and expressed as relative antibody activity (RAA). RAA values >35 and <25 units were considered as positive and negative, respectively. RAA values between 25 and 35 were regarded as low positive (borderline). A known positive and negative serum for *H. pylori* was run in parallel throughout the procedure as a quality control (provided from Lund University, Sweden).

ii) Immunoblot (IB) assay

All sera were tested with immunoblot to confirm EIA results. The same glycine extract antigens used for EIA was taken for immunoblot. Immuno assay was done according to the procedures described previously (Asrat, 2003). Briefly, immunoblot strips containing *H. pylori* acid glycine extracted cell surface proteins (provided by Ingrid Nilsson, Lund

University, Sweden) were probed with patient sera diluted 1:100 and incubated at 4°C for 16 h under gentle shaking. After repeated washings with washing buffer, strips were incubated with horseradish peroxidase labelled rabbit antihuman IgG antibodies (Dako, A/S, Glostrup, Denmark) diluted 1:600 and incubated at 22°C for 2 h. Membrane bound antibodies were detected by adding a substrate solution containing 50 mM sodium acetate buffer, 1% 3-amino-9-ethylcarbazole (Sigma) and 30% H₂O₂. Strips were incubated in darkness at room temperature for 30 min. The intensity of the band obtained with the patient's sera were compared to that obtained with a strip containing molecular mass markers and the results were interpreted positive for *H. pylori* infection based on the presence of antibody reactivity to high molecular mass CagA proteins (120 KDa) and or at least two to five of the low molecular mass proteins [33,31,30,29,26 KDa] as previously reported (Asrat, 2003).

2.6. Statistical analysis

EPI-INFO Version 2000 (CDC, Atlanta, Georgia, USA) with Yates's correction and Pearson Chi-square was applied to test whether differences between values were significant. P values less than 0.05 were considered as statistically significant.

2.7. Ethical Consideration

The study was approved by Addis Ababa University Science Faculty of Biology Department Ethical Committee. Patients after sample analysis were informed about *H. pylori* infection status and written informed consent was obtained from all study subjects (Appendix II).

CHAPTER III

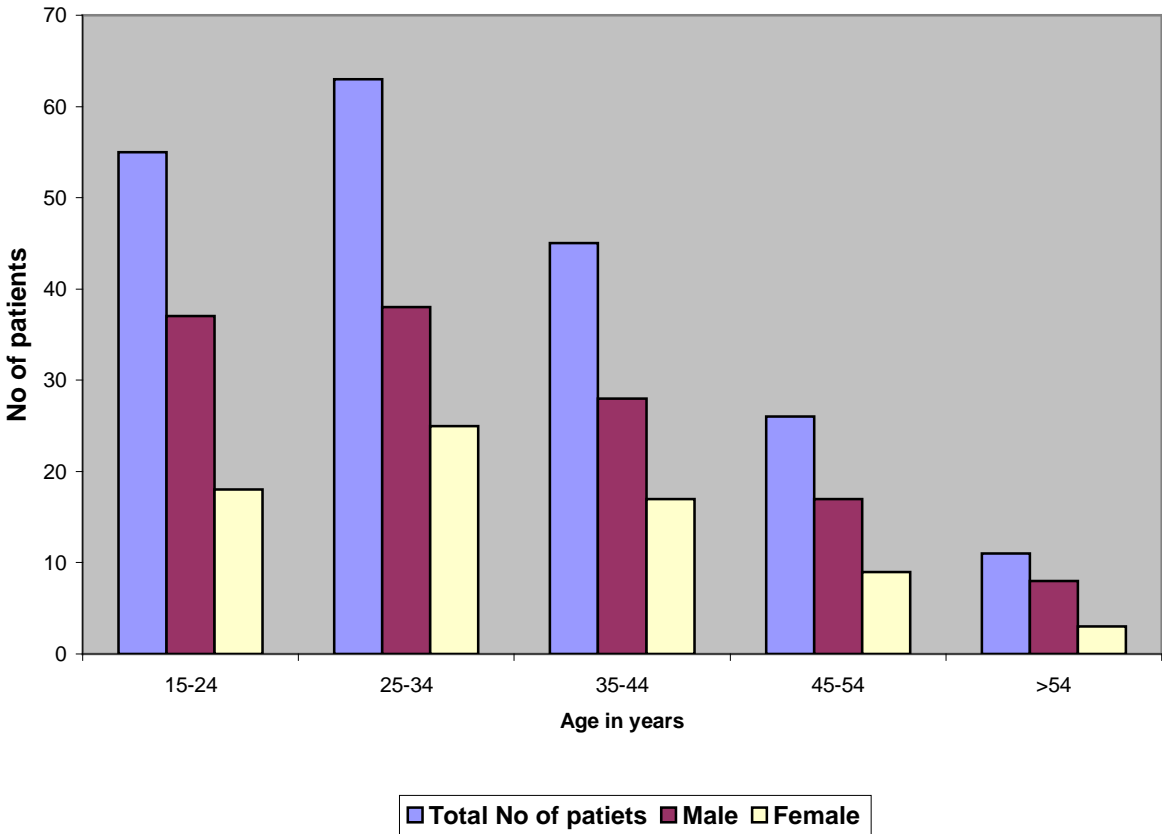
RESULTS

CHAPTER III. RESULTS

3.1. Study subjects

. The age sex distribution of dyspeptic and non-dyspeptic patients are shown in Figure 3.1.

Figure 3.1. Age and sex distribution of adult 200 dyspeptic and non-dyspeptic patients investigated for *Helicobacter pylori* infection



Of the total 200 (dyspeptic and non-dyspeptic) subjects 128 (64%) were males and 72 (36%) females with mean age 32.7, range 15-75, respectively. Subjects between age 15 and 44 comprise 81.5%. The male to female sex ratio was 1.8:1. The mean age of cases and controls was 34.05 and 31.4 respectively. Among the dyspeptics the numbers of males were 48

(48%) and 52 (52%) females. Of the controls 80 (80%) were males and 20 (20%) females (Table 3.1.).

Table 3.1. Sex and mean age in relation to patients with and without dyspepsia

	Males (%)	Females (%)	Mean age
Dyspeptic	48 (48)	52 (52)	34
Non dyspeptic	80 (80)	20 (20)	31.4
Total	128 (64)	72 (36)	32.7

Among the different symptoms of dyspepsia 100 or all (100%), 82% and 51(51%) had complaints of epigastric pain, nausea and vomiting respectively. None of these dyspeptic patients had complaints of upper or lower GI bleeding. The duration of dyspeptic symptoms ranges between 1 month and 25 years, and most (72%) have had dyspeptic symptoms varied between 1- 10 years (Table 3.2.).

Table 3.2. Duration of dyspeptic symptoms in 100 patients

<u>Duration in years</u>	<u>No of subjects tested</u>
<1	22 (22%)
1-3	39 (39%)
4-10	33 (33%)
>10	6 (6%)
<u>Total</u>	<u>100 (100%)</u>

3.2. Detection of *Helicobacter pylori* infection by serology

a) Enzyme Immuno Assay (EIA)

Serum samples from 200 study subjects, 100 dyspeptic (48 males and 52 females) and 100 non-dyspeptic (80 males and 20 females) were analysed for the presence of anti-*H. pylori* IgG antibodies with EIA. Of the total 200 studied subjects, 112 (56%) and 66 (33%), were found positive and negative, respectively. Borderline values measured >25 and < 35 were 22 (11%) (Table 3.3.).

Table 3.3. Frequency of *H. pylori* infection detection in dyspeptic and control patients by serological methods

Serological methods	<i>H.pylori</i> positive (%)	<i>H.pylori</i> negative (%)	Total (%)
I. EIA			
Dyspeptic patients	63 (63)	37 (37) ¹	100 (100)
Non dyspeptic (Controls)	49 (49)	51 (51) ²	100 (100)
II. Immunoblot			
Dyspeptic patients	70 (70)	30 (30)	100 (100)
Controls	54 (54)	46 (46)	100 (100)

¹ Borderline results included (n=13)

² Borderline results included (n=9)

There was no statistically significant difference in seroprevalence of *H.pylori* infection between dyspeptics (63%) and non-dyspeptic (49%) groups, p=0.06.

Table 3.4. Seroprevalence rates of *H.pylori* infection in relation to age and sex

Age group	Total tested	Males (%)	Females (%)	Both (%)	95%CI interval]
15-24	55	15 (27.3) ³	11 (20) ²	26 (47.3) ⁵	[0.33-0.61]
25-34	63	24 (38.1)	15 (23.8) ²	39 (61.9) ²	[0.49-0.74]
35-44	45	15 (33.3)	10 (22.2) ¹	25 (55.5) ¹	[0.4-0.7]
45-54	26	10 (38.5) ²	5 (19.2) ¹	15 (57.7) ³	[0.37-0.77]
>54	11	4 (36.4) ¹	3 (27.3)	7(63.6) ¹	[0.31-0.89]
Total	200	68 (53.1) ⁶	44 (61.1) ⁶	112 (56) ¹²	200

^X Numbers of patients with borderline results in EIA and positive in IB.

Age and sex distribution of all seropositives for *H. pylori* by EIA are shown in Table 3.4. A total of 128 (64%) males and 72 (36%) females were included in the study. Sixty eight (53.1%) of males and 44 (61.1%) of females were seropositive for *H.pylori* infection. There was no statistically significant difference in *H. pylori* seropositivity between males and females, $p>0.05$. The overall male to female seropositivity ratio was 1.54:1. Considering both genders the highest seropositivity rate was recorded in the age group of above 54 (63.6%). Nearly, half of the adolescents (47.3%) in the age group 15-24 and two third (59.7%) of the adults above 25 years of age were infected. There was no statistically significant difference in *H.pylori* infection among the different age categories, $P>0.05$, conveniently grouped in Table 3.4.

b) Immunoblot (IB) assay

All 200 serum samples, from 100 dyspeptic and non-dyspeptic patients were further analysed by immunoblot. *H.pylori* IgG detected in 70 (70%) and 54 (54%) from dyspeptic (n=100) and control (n=100), respectively. Representative immunoblot results are shown in Figure 3.2.

Combining EIA and IB results, there was no statistically significant difference in the frequency of detection of *H.pylori* between dyspeptics (63-70%) and controls (49-54%), $P > 0.05$.

The most prevalent blood type was blood type O (43%) followed by A (23%), B (12.5%) and the least prevalent was AB (11%). More than 57% of the subjects with these blood types were positive for *H.pylori* (Table 3.5.), A (59.6%) B (57.8%) AB (59.1%) and O (66.3%). There was no significant difference in the rate of seroprevalence of infection among the different blood groups, $p > 0.05$.

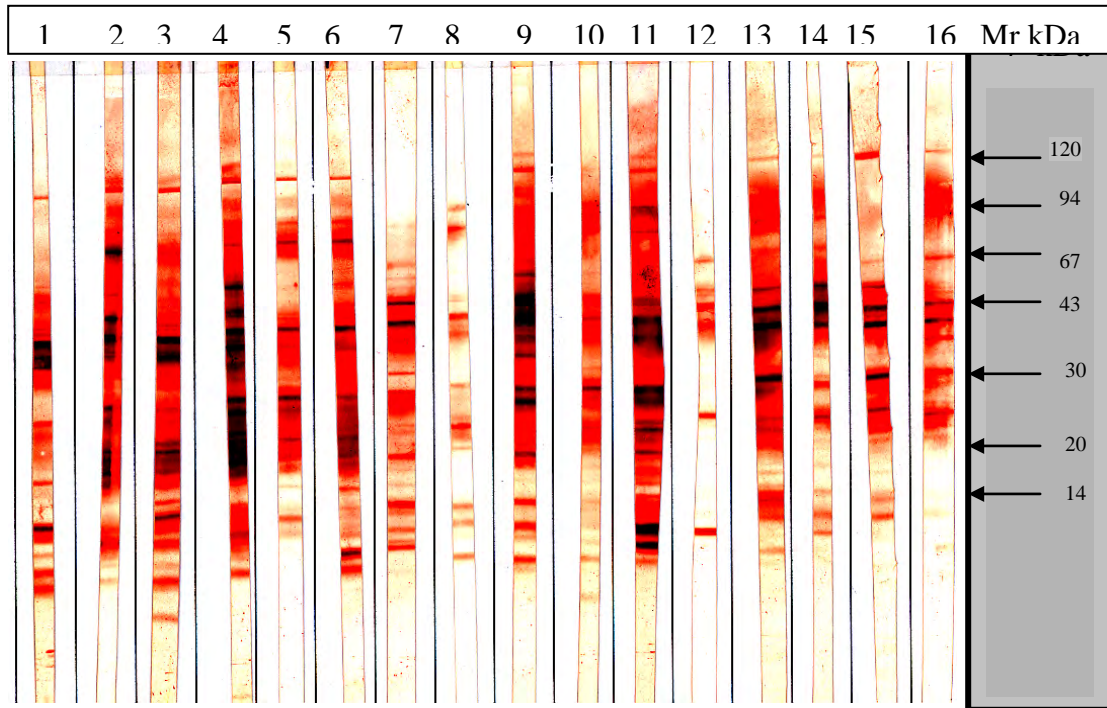


Figure 3.2. Representative immunoblot results of sera from study subjects.

Lanes: 1-6, 9, 11, 13, 15-16 IB positives, Lanes: 7-8, 10, 12 and 14 IB negatives; Molecular mass is shown on the right.

Table 3.5. *Helicobacter pylori* seroprevalence rate in relation to ABO blood group

Blood group	No of infected (%)	Total tested (%)
A	28 (59.6)	47 (23.5)
B	26 (57.8)	45(22.5)
AB	13 (59.1)	22 (11)
O	57 (66.3)	86 (43)

Table 3.6. *Helicobacter pylori* seroprevalence rate in relation to Rh antigen D

Rh antigen D	No of infected (%)	Total tested (%)
Rh positive	118 (61.5)	192 (96)
Rh negative	5 (62.5)	8 (4)

With respect to Rh factors 192 (96%) and 8(4%) of the studied subjects were Rh positive and Rh negative respectively. Of these 61.5% of Rh positives and 62.5% of Rh negatives were seropositive for *H. pylori*. There was no statistically significant difference between Rh positive and Rh negative subjects with respect to seroprevalence of *H.pylori* infection, $P>0.05$ (Table 3.6.).

CHAPTER III

DISCUSSION

CHAPTER III. DISCUSSION

The overall seroprevalence of *H. pylori* infection in Bahir Dar in this study was 49-70%. This is in agreement with that reported in the previous studies in Ethiopia 56- 65% by Tsega *et al.*, (1996a); and not significantly different from the work of Kassa *et al.*, (1996) 70% and some other developing countries e.g. Jamaica (Lindo *et al.*, 1999); Zaire, Costa Rica, Ivory Coast, India, Papua-New Guinea, Jordan, Saudi Arabia, China and Taiwan, with figures between 36-81% in the age between 3-81 years (Bardhan, 1997). However, there are reports from different parts of the world with lower prevalence of *H. pylori* infection e.g. 27% and 15-46% in Kuwait and Gambia, respectively (Bardhan, 1997).

The possible reasons for these variations between this and other studies could be: -

1. Variable methods and criteria used to determine active *H. pylori* infection. These include rapid urease tests, culture, histology, PCR, and urea breath test.
2. The selection (sampling) method used (cluster, systematic, etc.), other studies use highly selected subjects or include patients receiving antibiotics for other infections.
3. Variability in the performance of diagnostic assay between patient populations from different geographic regions, possibly due to differences between antigenic properties of local bacterial strains and those used to prepare antigen for the test.
4. Frequent recombination may be one mechanism that permits *H. pylori* to adapt to individual host organisms and could also facilitate global spread of chromosomal mutations such as those that confer resistance against some antibiotics. It may be that strains belonging to a particular clonal grouping are more efficient in colonizing individuals of a specific

ethnic background. Genetic differences among *Helicobacter pylori* may affect the ability of the different strains to bind to appropriate receptors in the stomach, which could account for these variations between populations

The over all seroprevalence in this study is relatively lower than reports of previous studies in Ethiopia (Asrat, 2003; Desta *et al.*, 2002a). More over, numerous prevalence studies performed and have shown that two patterns of *H. pylori* infection exist. Group I is made of developing countries where the majority of children become infected with *H. pylori* during childhood, and chronic infection persists during adult life (Chile, Algeria, Thailand, Nepal, Peru, South Africa, Vietnam). Group II includes populations with rare acquisition of infection during childhood, but subsequent constant incidence rate (about 0.5-1% of the population per year). These are countries with high socio-economic status: France, Belgium, Great Britain, Finland (Pounder and Ng, 1995; Weill *et al.*, 2002).

The low prevalence in this study may be attributed to differences in study area, subjects, sample size or elimination of *H. pylori* infection as a result of antibiotic treatment in occasion of concomitant diseases (Giardiasis, amoebiasis, respiratory diseases, etc.), as these diseases reported to be more prevalent in the study area (Desta *et al.*, 2002b).

In some studies highly selected subjects were included. In line with this, in a recent study in Ethiopia by Asrat (2003), only dyspeptic patients with severe complaints referred to Black Lion Hospital were investigated for *H. pylori* infection. In addition, both invasive and non-invasive of various detection methods were used. But in this study only EIA and IB assay were used as detection methods and both dyspeptic and non-dyspeptic patients were

investigated for *H. pylori* infection. All these could contribute for the differences in the rates of seroprevalence between this study and the work of Asrat (2003).

With the discovery of *Helicobacter pylori* in 1983, and its subsequent discovery as the cause of peptic ulcer disease, *H. pylori* has also been hypothesized to be causative of dyspepsia as well. *H. pylori* infection results in inflammation (chronic active gastritis); this chronic inflammation of the gastric mucosa may have an effect on gastric mucosal receptors, gastrointestinal motility, or other unknown factors that may contribute to dyspeptic symptoms. Dyspepsia is one of the commonest chronic medical disorders that may be due to a variety of underlying diseases of the upper gastrointestinal tract. The evidence for a role of *H. pylori* infection in the various dyspeptic diseases has been reviewed in various papers (Fennerty, 2002).

The seroprevalence of *Helicobacter pylori* infection in Bahir Dar in this study was 63-70% and 49-54% among dyspeptic and non-dyspeptic (controls), respectively. This is in agreement with that reported in the previous studies, in Ethiopia 65% in patients with dyspepsia and 56% asymptomatic controls by Tsega *et al.*, (1996a). Therefore, there was no significant difference between the rate of *H. pylori* infection among dyspeptic and non-dyspeptic (controls), which is also compatible with the results of other studies (Danesh *et al.*, 2000; Singh *et al.*, 2002; Locke *et al.*, 2000). Some other studies also strengthen these reports in that symptom relief after *H. pylori* cure is minimal, which shows lack of association between dyspepsia and *H. pylori* infection (Talley *et al.*, 1999; Laine *et al.*, 2001).

To the contrary, dyspeptics show two fold symptomatic improvement if *H. pylori* is eradicated, and suggested that *H. pylori* infection prevalence may be higher in dyspeptics

than non-dyspeptics (Kang *et al.*, 1990; Giacomo *et al.*, 2002; Jaakkimainen *et al.*, 1999; Suerbaum and Michetti, 2002). Higher prevalence of *H. pylori* in dyspepsia compared with controls in a Norwegian population-based study reported, 36% of asymptomatic controls while 48% of dyspeptic patients had the infection. (Talley & Quan, 2002).

In addition, performance of blinded, randomized, controlled studies demonstrating relief of symptoms in dyspeptic patients infected with, and treated for, *H. pylori* considered as evidence confirming a causal effect between *H. pylori* and dyspepsia. But numerous studies addressing the possibility of an association between *H. pylori* and prevalence of dyspepsia or specific symptoms have been inconclusive and controversial (Talley & Quan, 2002). Danesh *et al.*, identified 30 observational studies comprising 3392 cases of dyspepsia; while small studies often reported associations between *H. pylori* and dyspepsia, potential confounders were not adequately considered and the results were not usually confirmed in larger, better controlled studies (Danesh *et al.*, 2000).

In general, results of worldwide epidemiological studies have been unconvincing. No clear-cut link has been documented concerning the rate of seroprevalence of *H. pylori* between individuals with and with out dyspeptic symptoms. In the randomized controlled trials, methodological weaknesses may explain in part the conflicting results, but even in the well-conducted trials controversy persists.

The possible reasons for these variations could be, misdiagnosis of patients, differences in dyspepsia scoring systems, sample size, lack of clear-cut definition of dyspepsia, methodological weaknesses, including low study power, a lack of randomization and interplay of various confounding (social, economical and demographic) factors.

There was no significant difference in the overall prevalence of *H. pylori* infection between males (53.1%) and females (61.4%), $p > 0.05$. This finding is compatible with other studies (Asrat, 2003; Singh *et al.*, 2002; Weill *et al.*, 2002; Yilmaz *et al.*, 2002; Kim *et al.*, 2001; Megraud *et al.*, 1989). However, some studies have found a higher prevalence of *H. pylori* infection in males, which may be related to higher exposure of the males to potential environmental sources of contamination including human faeces (Replogle *et al.*, 1995; Broutet *et al.*, 2001).

The distribution of seroprevalence according to age is shown in Table 3.3. Seropositivity of adolescents aged 15-24 was 47.3% and increased to 61.9% at the age of 25-34. The highest seroprevalence was recorded (63.6%) in adults aged more than 54, in agreement with other studies (Kim *et al.*, 2001). There was no significant difference in the rate of seroprevalence among the different age groups. This is in contrary to previous studies (Yilmaz *et al.*, 2002; Megraud *et al.*, 1989; Kosunen *et al.*, 1988; Weill *et al.*, 2002; Reshetnikov *et al.*, 2001). The possible explanation for the differences in the rate of seroprevalence among the different age groups between this study and others could be due to variations in methodology, some use invasive and others use both invasive and non invasive but most use only serological methods and/or randomization.

The rationale for an association between *H. pylori* infection and blood group "A" came from the discovery that one of the adhesins of *H. pylori*, blood group antigen binding A (BabA), may favour *H. pylori* colonization of the stomach (Ilver *et al.*, 1998). However, Boren *et al.* have provided extensive evidence that the Lewis b blood group antigen mediates attachment to mucus cells (Boren *et al.*, 1993). The availability of fucosylated receptors might be reduced in individuals with blood group A and B phenotypes, compared

with blood group O individuals, thus explaining the long-recognized greater risk of peptic ulcer development in blood group O individuals (Boren *et al.*, 1993). In this study although the most prevalent blood group was blood type O (43%), the rate of seroprevalence among the different blood groups was more or less similar with a prevalence of O (66.3%), A (59.6%), B (57.8%) and AB (59.1%), which indicates absence of significant difference among the different blood groups, $p>0.05$. This is in agreement with many studies (Weill *et al.*, 2002; Niv *et al.*, 1996; Klaamas *et al.*, 1997), but contrary to (Peach *et al.*, 1997; Ilver *et al.*, 1998). Although, the majority of subjects (96%) were Rh positives but the seroprevalence of *H. pylori* in Rh positives and Rh negatives found to be 61.5 % and 62.5% respectively. There was no statistically significant difference in the rate of seroprevalence between these blood groups, $p>0.05$, which is in agreement with other studies (Weill *et al.*, 2002).

CONCLUSION AND RECOMMENDATION

There was no significant difference in the rate of *H. pylori* seroprevalence between dyspepsia and non-dyspepsia and there was no significant association with sex, age groups, ABO blood groups and presence or absence of rhesus factors. But there is insufficient evidence to confirm or refute the existence of significant difference in the rate of *H. pylori* infection between dyspeptics and non-dyspeptics.

In Ethiopia the incidence of *Helicobacter pylori* infection and dyspepsia is very common, elimination of it may be cost-effective in the long term, but even eradication is not a guarantee for long-term relief. A number of expert consensus groups have suggested that it is advisable to treat *H. pylori* infection in patients with dyspepsia following full investigation. This study showed that serological methods (EIA and IB) can be used as diagnostic tools to determine the status of *H. pylori* infection and for epidemiological studies, because they are generally simple, reproducible, inexpensive, and can be done on stored samples. Ethiopia is endowed with different ethnic groups, therefore, serological assays should be evaluated for each particular group and adjusting cutoff value is necessary to use serological tests as a screening tool. Though immunoblot assay very labour intensive and expensive, it can also be useful to identify patients with specific antibodies against major bacterial antigens such as CagA. The burden of illness due to dyspepsia with respect to quality of life and economic consequences is of considerable from economic, social, and personal vantage points as dyspepsia is one of the common complaints among Ethiopian patients. More over knowing the possible routes of transmission and risk factors are important in designing control strategies. Thus further investigations along this line is a worth consideration.

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Appendix I

Questionnaire to be administered for investigation of Helicobacter pylori infection in adult patients with or with out dyspeptic symptoms attending OPD of Felege Hiwot Hospital, Bahir Dar.

I. Patient Identification

1. Serial No _____
2. Full name _____
3. Card No _____
4. Blood group _____
5. Sex M/F _____
6. Age _____
7. Address: - Woreda _____ Kebele _____ House no _____
8. Occupation _____
9. Date of collection _____
10. Time of collection _____

II. Clinical data

11. Nausea: yes/ no
12. Vomiting: yes/ no
13. Abdominal pain/discomfort: yes/ no
14. Epigastric pain: yes/ no
15. Upper GI bleeding: yes / no

16. Lower GI bleeding: yes/ no

17. Duration of illness _____

18. Pertinent physical findings_____

III. Serology Result

Blood type _____

EIA RAA value _____

Negative/positive/Borderline

Immunoblot Positive /Negative

APPENDIX II

PATIENT CONSENT FORM

Description: - Stomach inhabiting bacterium, *Helicobacter pylori*, is mainly responsible for gastric related diseases. Most of these diseases can be treated medically.

I. Purpose of the study: - To study the seroprevalence of *Helicobacter pylori* infection among dyspeptics and non dyspeptics.

II. Procedures:

2.1 Filling concent form

2.2 Five to ten ml venous blood will be collected from each informed and consented subjects using sterile syringe

2.3 Serum obtained by centrifugation and aliquoted will be stored at -20°C until tested

2.4 Serological analysis will be done at the Bio-Medical Research Training Project Laboratory (BRTP), Faculty of Medicine, Addis Ababa University.

2.5 Results will be given after serological analysis.

III. Advantage to the patient:

Know the status of *Helicobacter pylori* infection. The patient may have drugs free of charge or to seek treatment if the need arises in the future, as will be determined by the physician.

I _____ here by give my consent for examination of blood specimen to detect only *Helicobacter pylori* infection as the doctors find best for me.

Date: _____

Name: _____

Signature: _____

DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

MSc Candidate:

Tesfahun Tadege, BSC

Signature:

Date and place of submission

04-07-03

Addis Ababa, Ethiopia