SIGNIFICANCE OF *H. PYLORI* IN GASTRIC CARCINOGENESIS

2.1 PRE-HELCOBACTER ERA

In the pre *H. pylori* era both duodenal ulcer and gastric cancer were known to be intimately associated with gastritis (Siurala, *et al.*, 1972). The pattern and severity of the gastritis were also known to differ between the two diseases. Gastritis in duodenal ulcer disease was primarily antral and spared, the gastritis corpus, allowing continued high levels of acid secretion. (Faber. 1927). Since *H. pylori* are found relatively uniform throughout the stomach, the restriction of gastritis to the antrum in duodenal ulcer disease was not because *H. pylori* were somehow restricted to the antrum. (Genta, *et al.*, 1994).

The predominance of inflammation in the antrum in duodenal ulcer disease was known to be reversible because maneuvers that permanently reduced acid secretion, such as vagotomy led to rapid progression of the inflammation into the gastric corpus. (Aukee, *et al.*, 1972). These experiments in nature suggested that gastric acid secretion or some other closely related event, was responsible for keeping *H. pylori* from interacting with the gastric mucosa to cause inflammation (Graham,
(1983), has attracted so much research interest on \textit{H. pylori} as a medically important microbe. Dr. Robin Warren, a pathologist had detected this bacteria in the stomach tissue of patients with digestive complaints. Warren had noted how, almost every patient with stomach inflammation also had the bacteria and this is held true for almost all patients with chronic severe indigestion and peptic ulcers. The breakthrough came in 1983 with the discovery, and isolation and characterization of \textit{Campylobacter pyloridis} from the gastric mucosa of patients with chronic active gastritis (Marshall and Warren, 1983). The organism was originally named \textit{Campylobacter pyloridis} and later to \textit{Campylobacter pylori} because of its structural similarity to other \textit{Campylobacter} species. In 1989, it was given the name \textit{Helicobacter pylori} with a new genus, \textit{Helicobacter}, on the basis of distinct functions and enzymatic properties (Goodwin \textit{et al.}, 1989)

2.3 PREVALENCE OF HELICOBACTER PYLORI

The prevalence of \textit{H. pylori} varies worldwide, with nearly 50% of the population being infected by the age of 10. In the developing countries the situation is more grave with the infection rate reaching nearly 90%. Over 80 million patients are treated for gastric and duodenal diseases annually with anti-ulcer drugs. The anti-ulcer market alone is estimated to be in the excess of 10 billion US$. 

Figure 2. World distribution of *H. pylori* infection and its gastric consequences from common chronic gastritis (100% infected) to cancer (about 2%).

The greatest achievement in gastroenterology in last century
2.4 MODE OF TRANSMISSION

The mode of transmission of *H.pylori* is unknown but, assumed to be similar to other pathogenic bacteria from the gastrointestinal tract. *H.pylori* could be either a strictly human pathogen like *Salmonella typhi*, acquired from the contaminated environment or a zoonotic pathogen for which animal reservoirs can be found. Viable *H.pylori* have never been isolated from the environment except in one study from Peru (Hulten *et al.*, 1996.). No animal reservoirs for *H.pylori* were thought to exist until *H. pylori* was successfully isolated from one group of pathogen-free domestic cats (Fox, 1995). The major modes of transmission of *H.pylori* are still uncertain, but oral-oral gastro-oral and fecal to oral routes could be the possibilities (Goodman and Correa, 1995; Megraud, 1995; Axon, 1996; Cammarota et al., 1996). The possibility of entrogenic transmission has also been documented and transmission at endoscopy due to inadequate sterilization of the endoscope tube has also been reported (Langenberg *et al.*, 1990).

2.5 PATHOPHYSIOLOGY OF INFECTION

The unique pathogen *H.pylori*, is now an undisputed etiological agent inducing gastric inflammation and has been accepted as a major cause of gastro duodenal disease (Dixon, 1996; Stolte, 1992; Foreman, 1993). *H.pylori* infection and chronic acute gastritis are inextricably linked and it also plays a pivotal role in the development of peptic ulcer disease (Kuipers
et al., 1995). *H.pylori* infection is the first bacterial infection involved in carcinogenesis in man (WHO, IARC, 1994). It has also been associated with the development of gastric non-Hodgkin's lymphomas (Parsonnet et al., 1994) and with another lymphoproliferative disorder, gastric mucosa associated lymphoid tissue (MALT) lymphoma (Wotherspoon et al., 1991; Eidt et al., 1994). The involvement of *H.pylori* in developing gastric adenocarcinoma was established in Mongolian gerbil animal model (Watanabe, et al., 1998).

2.6 IMMUNO SURVEILLANCE OF HELICOBACTER PYLORI

The ability of *H.pylori* to produce Lewis antigens which resemble host Lewis structures seems to favour survival in the stomach by escaping the host immune response through molecular mimicry, but has also been implicated in the pathogenesis of atrophic gastritis and gastric cancer.

A high prevalence of anti-Lewis antibodies in *H.pylori* infected patients was reported (Heneghen, et al., 2001), and was strongly associated with bacterial Lewis phenotype. A study from Broutet et al., (2002) additionally included other virulence factors such as CagA and Vac A in order to define strain types associated with atrophic gastritis. They identified a 'cluster' of parameters (SI a /mL/Cag A(+) Le(x) + Le(y) +) and those strains were significantly associated with atrophic gastritis. This finding is of particular importance in the light of the prospective study by Uemura et al., (2001) who found an increased, gastric cancer risk in *H.pylori* infected
patients with atrophic gastritis. A new aspect of Lewis regulation is brought up by Moran et al., (2002) who were able to identify pH as an environmental regulator of Lewis phase variations. Under low pH, Lewis expression was shifted from Le(X) to Le(Y). Since Le(Y) closely resembles the type I determined Le(b), which is predominant in the human gastric mucosa, this mechanism promoted molecular mimicry and thereby adaptation of *H. pylori* to its ecological niche.

### 2.7 BACTERIAL VIRULENCE FACTORS

*H. pylori* infection is a bacterial infection of mucosal surface. Many bacterial diseases can be related to specific virulence factors. The extensive experience with disease – specific virulence factors in other bacterial infection led to an ongoing and intensive search for disease specific virulence factors for *H. pylori*.

**VacA** – The vacuolating cytotoxin is present in most of the *H. pylori* strains, however, only 50% of strains are able to induce vacuolization in epithelial cells *in vitro*.

**CagA** – The cytotoxicity associated gene is a marker for a large 40kb locus containing over 40 genes, which has been referred to as the “pathogenecity island”. The cag locus exhibits inflammation induced activity by contributing to the stimulation of IL-8 production. This pathogenecity island is also thought to encode type IV secretory system that permits the surface expression of proteins interacting with epithelial cells.
Ice A is induced when \textit{H.pylori} contacts with epithelial cells.

Bab A adhesion of \textit{H.pylori}, which binds to fucosylated Lewis B hit-blood group antigen on the epithelium cells.

2.8 WORKING HYPOTHESIS

Infection with \textit{H.pylori} is a risk factor for gastric adenocarcinoma. \textit{H.pylori} infection is the trigger for the sequence of carcinogenesis because there is strong evidence for \textit{H.pylori} infection as a cause of chronic atrophic gastritis and intestinal metaplasia, two possible precancerous lesions. (JIA-Quing Huang \textit{et al.}, 1998). Studies are necessary to establish the prevalence of \textit{H.pylori} in regions where the incidence of gastric cancer is higher, like in Southern parts of India. It is also necessary to find out the status of distribution of \textit{H.pylori} during the course of gastric carcinogenesis because \textit{H.pylori} infection may have major influence on the progression from gastric epithelial cell transformation. The present study was therefore focused on the prevalence of \textit{H.pylori} in the study population and its distribution in different stages of neoplastic progression.

2.9 STUDY DESIGN

This study was carried out in a total study population of 200 patients with different stages of carcinogenesis including 20 age matched controls. The study subjects were classified into 5 groups as mentioned earlier in (Chapter-1), based on Sydney System of histological
classification, in consultation with pathologists. The clinical records of study subjects were also maintained systematically with the help of gastroenterologists. Identification of *H. pylori* was carried out in biopsy and blood samples of the above said study subjects by Rapid Urease Test, ELISA, Histology and by Geimsa Staining.

2.10 METHODOLOGY

2.10.1 Sample Collection

2ml of blood and 5 gastric biopsy samples were collected from each patient after clinical examination by gastroenterologists. Likewise, normal biopsy samples were taken from patients who had complaints other than gastroduodenal diseases. The tissues and blood were processed and stored for further analysis as given in appendix.

2.10.2 Identification of *Helicobacter Pylori*

The patients who were considered *H. pylori* positive were positive for the following three tests, rapid urease test, ELISA for anti *H. pylori* antibody and histology using Geimsa staining.

2.10.3 Rapid Urease Test

Fresh biopsy samples were immediately transferred to 0.5ml of 10M urea solution pH 6.5 with phenol red in a 1.5ml eppendorf tube. The colour change from orange to pink was an indication of the urease activity.
2.10.4 ELISA

The patients sera were analysed for anti *H. pylori* antibody by ELISA using commercial kit (supplied by Magi Well Biotechnologies) as per the manufacturer's protocol.

2.10.5 Histological Examination

Biopsy samples of the gastric mucosa from patients obtained by endoscopy were fixed in 4% para formaldehyde and 5µm thin frozen sections were stained with Haematoxylin and Eosin for histological analysis. The samples of the gastric mucosa were also stained with Geimsa staining for *Helicobacter pylori* detection. All the histological factors were evaluated according to the criteria of the revised Sydney system (Dixon, *et al.*, 1996)

2.11 RESULTS

2.11.1 Histological Status in the Study Population

A total of 200 samples were analysed for *H. pylori* infection using well-established methods. Out of the 200 cases, 143 were positive for *H. pylori* infection (71.5%). The distribution of *H. pylori* infection in relation to the study population (200 cases) is shown in the Fig. 5. Among the gastric pre-cancerous and cancerous samples that were analysed for *H. pylori* infection, only 41 were negative for *H. pylori* infection (41/180) (22%) and remaining were *H. pylori* positive (139/180) (77%). This depicted a clear relationship between *H. pylori* infection and gastric carcinogenesis. These
results suggest that *H. pylori* infection is a major risk factor for gastric carcinogenesis.

2.11.2 **Risk Estimation**

The risk of *H. pylori* in gastric carcinogenesis was calculated by taking the range of *H. pylori* infection between normal and patients. There was a highly significant association between *H. pylori* infection and development of gastric cancer (P< 0.0001) and the Odds Ratio being (51.3) with a relative risk (RR) of 13.56 and the 95% CI being 3.95 to 51.13. Thus we suggest from this result that those with *H. pylori* infection are 13.56 times greater at risk of getting cancer than those without *H. pylori* infection.

2.11.3 **H. Pylori Status in Relation to Histopathological Progression**

Histopathological classification was done using revised Sydney System by haematoxylin and eosin staining of each section (Plate 2.1). Accordingly, 5 groups of study subjects were included. They were normal, gastric mucosa obtained from normal control without gastric disorders (20 cases), chronic gastritis (63 cases), atrophic gastritis (20 cases), intestinal metaplasia (11 cases) and adenocarcinoma (86 cases).

Eighty percent of the normal samples analysed were negative for *H. pylori* infection (16/20) and 4 were positive for *H. pylori*. In chronic gastritis cases, 56 were positive for *H. pylori* infection, 56/63 (88.9%), 7 were negative for *H. pylori* 7/63 (11.1%). In the case of atrophic gastritis, 3
PLATE-2.1

Histopathology of Gastric Carcinogenesis

A. Normal Stomach (20X)
B. Chronic Gastritis (40X)
C. Atrophic Gastritis (40X)
D. Intestinal Metaplasia (40X)
E. Adeno carcinoma (40X)
PLATE-2.1

Histopathological Stages of Gastric Carcinogenesis
PLATE 2.2

Identification of *Helicobacter pylori* in sections using Giemsa Staining

A. *Helicobacter pylori* in gastritis

B. *Helicobacter pylori* in gastritis

C. *Helicobacter pylori* in adeno carcinoma

D. *Helicobacter pylori* in adeno carcinoma
PLATE-2.2

Giemsa Staining of *Helicobacter pylori* in various stages of Gastric Carcinogenesis
Figure 2.2 Distribution of H. pylori in various histopathological grades of Gastric Lesions

Histopathological stages: ■ H. pylori negative  ■ H. pylori positive
were negative for *H. pylori*, 3/20 (15%) remaining 17 were positive for *H. pylori* 17/20 (85%). Interestingly when analysed in 11 cases of intestinal metaplasia all the cases were positive for *H. pylori* infection 11/11 (100%) whereas, in the case of adenocarcinoma, 31 were negative for *H. pylori* 31/86 (36%), 55 cases were positive for *H. pylori* infection 55/86 (64%).

When the data were statistically analysed, there was a highly significant association between the histopathologic grades of gastric preneoplastic and neoplastic tissues and *H. pylori* infection ($\chi^2 = 43.956; p = 0.0001$) Fig.2.1. The results suggest that, the rate of *H. pylori* infection increases as the gastric lesion progresses histopathologically from normal to intestinal metaplasia. Also, the prevalence of *H. pylori* infection was high in preneoplastic lesions such as gastritis, atrophy and metaplasia when compared to normal. The result also showed a decrease in *H. pylori* infection in adenocarcinoma and this may be due to the transformation of epithelial mucosa into hard tumor, unfavourable for the bacterial adhesion and survival.

2.12 DISCUSSION

*H. pylori* appears to be the most frequent cause of gastritis in humans (Kelly, *et al.*, 1998) which in turn appears to be the major risk factor for gastric and duodenal ulcers, gastric lymphomas and gastric cancer (Blaser, *et al.*, 1992, Graham, *et al.*, 1993; Metz, 1999). *H. pylori* was also found to
be the strongest risk determinant of the entire range of gastric carcinogenesis stages as reported previously. (Silva, et al., 1990; Correa et al., 1990).

*H. pylori* infection was detected in 71.5% of the study population. The development of *H. pylori* mediated gastric cancer seems to be closely related to intestinal metaplasia formed in the chronically inflamed mucosa (Satoh et al., 1995) which indicated that *H. pylori* infection is usually associated with antral atrophic gastritis and intestinal metaplasia.

Epidemiological surveys, (Correa, et al., 1970) histopathological examinations (Nakamura, et al., 1968) and biochemical analysis have shown that intestinal metaplasia, especially the sulphomucin secreting type, may be the possible precancerous lesion leading to intestinal type adenocarcinoma in humans(Kawachi, et al., 1976; Tahara, 1994). Intestinal metaplasia has been widely assumed to be linked to chronic gastritis with mucosal atrophy (Silva, et al., 1990). Although the pathogenesis of intestinal metaplasia has not been clarified as yet, Scott et al., (1990) suggested that hyperproliferative state in the inflammed gastric mucosa may be responsible for the progression from normal mucosa to metaplastic epithelium. Moreover Recavarren-Arce et al., (1991) reported that an altered gastric microflora, subsequent to hypochlorhydria due to atrophy increases N-nitroso compounds in the gastric lumen, and these substances may induce intestinal metaplasia. In the present study, we found that 88.9% of chronic gastritis, 85% of atrophic gastritis and 100% of the intestinal metaplasia
patients were infected with *H. pylori*. This suggests a strong association
*H. pylori* with tumour initiation and progression.

Although the pathogenesis of the tumour is unknown, there are
several possible mechanisms by which *H. pylori* infection may play a part in
the carcinogenesis of gastric epithelium. One explanation is increased
epithelial turnover in the inflamed mucosa. Cahill *et al.*, (1994) have stated
that gastric mucosal cell proliferation is significantly higher in patients with
*H. pylori* positive gastritis than in those with *H. pylori* negative gastritis.
Infection with *H. pylori* alters many gastric factors that contribute to the
pathogenesis of gastric cancer such as induction of atrophic gastritis with
intestinal metaplasia and dysplasia, predicted (Correa *et al.*, 1984) long
before the discovery of *H. pylori*.

Watanbe *et al.*, (1998) first reported that when 5-wk-old
Mongolian gerbils were orally inoculated with *H. pylori* induced gastric
carcinomas, which was located in the pyloric region. After the 26th week,
severe active chronic gastritis, ulcers and intestinal metaplasia could be
observed in the infected animals. After the 62nd week, adenocarcinoma had
developed in the pyloric region of 37% (20/27) of the infected animals.
Hence it was found that adenocarcinoma development seems to be closely
related to intestinal metaplasia. After this, 5 week old Mongolian gerbils
were infected with *H. pylori* ATCC-43504 strain by Honda *et al.*, (1998).
Who reported that atrophic gastritis and intestinal metaplasia also appeared
in the lesser curvature of the ventral mucosa 6 months after inoculation.
Eighteen months after *H.pylori* inoculation, 50% (2/5) of the infected Mongolian gerbils showed three well-differentiated gastric cancers. This suggested that early stages of *H.pylori* infection might be one of the risk factors increasing carcinogenesis of gastric cancer. The pathway of "*H.pylori* → atrophic gastritis → intestinal metaplasia → atypical hyperplasia → Intestinal type cancer" (Correa 1992).

Data from our study suggest that *H.pylori* infection is more strongly associated with gastric cancer, with the relative risk of 13.56. A population-based study also has provided epidemiological evidence for the prevalence of *H.pylori*. A thorough knowledge of the prevalence of *H.pylori* may help in devising new preventive and treatment strategies in the management of *H.pylori* and gastric cancer.
Chapter-III