INTRODUCTION

The steadily increasing trend of cancer incidence gains attention on the key step called “Carcinogenesis” for an extensive research. Although research on cancer has been going on for a long time, no solution seems to be appearing because cancer is not a “single disease with a single causative factor”. It exists in more than 100 forms and has many causative agents, from genetic factors to infectious agents. (Morris et al., 1995). No single genetic alteration is sufficient for the induction of cancers, in vivo. A tumor is an abnormal mass of tissue, the growth of which is virtually autonomous and exceeds that of normal tissues. In contrast to non-neoplastic proliferations, the growth of tumors persists after cessation of the stimuli that initiated the change. Malignant tumors are often called cancers and those which arise from epithelial cells are “Carcinomas”.

It has been realized for many years that cancer can be considered as a “genetic disease” and the genetic injury may be acquired in somatic cells by environmental agents or inherited germline. Four classes of genes are the targets of genetic damage: they are growth-promoting proto-oncogenes, growth inhibiting tumor-suppressor genes, genes that regulate apoptosis and genes that regulate DNA repair. It is only in recent years that the involvement of specific proto-oncogenes in the development of cancer
has been demonstrated at the molecular level (Lakshmi et al., 1997, Wang et al., 2002). The proteins encoded by these proto-oncogenes are known to be components of cell cycle and cell signaling pathways (Diebold et al., 1996; Sidranshy and Hollstein, 1996, Yang and Korsmayer, 1996).

Carcinogenesis is a multi step process and malignancy is attributed to invasiveness, excessive growth, escape from the immune system and metastasis which occurs in a stepwise fashion—a process called tumour progression. At the genetic level, progression results from successive mutations or persistent exposure to causative agents (Fearon and Vogelstein, 1990, Uemura et al., 2001) Mounting evidence from current tumour research suggests that multiple oncogenes, tumour suppressor genes and growth factors act in concert through various autocrine loops and cascades to effect complete transformation of the normal cells to metastatic tumour.

1.1 ADENOCARCINOMA AND STOMACH

Over 95% of cases of gastric cancer are adenocarcinoma derived from the mucosal epithelium of the stomach. Two major histological variants of the disease are recognized; the epidemic intestinal or well differentiated type and the diffuse/scirrhous or poorly differentiated type. Intestinal type lesions follow a progressive series of stages from chronic gastritis → gastric atrophy → intestinal metaplasia → dysplasia → early carcinoma → invasive carcinoma → metastasis. Poorly differentiated
cancers lack the well recognized precursor changes seen in the intestinal-type lesions. The etiological factors related to the diffuse – type of stomach cancer are less clear. The real cause of gastric carcinogenesis is not fully understood, however, several environmental factors including *Helicobacter pylori*, excessive intake of salt, bile reflux, N-nitroso compounds and deficiency of antioxidants have been linked with different stages of gastric carcinogenesis (Correa, 1992).

Gastric cancer is a malignant neoplasm of the stomach mucosa. Despite recent advances in early diagnosis and treatment, cancer of the stomach continues to be a major world health problem. Although there has been a significant decline in its incidence in industrial countries since 1930’s, it still remains the second most frequently occurring cancers, with an estimated 670,000 cases per year world wide.

1.2 **EPIDEMIOLOGY**

1.2.1 **International status of Gastric Cancer**

Although gastric cancer rates have been declining in recent decades, it remains one of the most serious health burden throughout the world. Understanding its cause is important for the primary and secondary prevention of the disease. The highest death rates for many decades were registered in Japan, followed by Northern Europe and the Andean populations of Latin America. The recent decline has been more marked in Japan and Northern Europe. (Kurihara *et al.*, 1989). Gastric cancer was the
Figure 1.1 Cancer incidence in males at Chennai, India.
most frequent neoplasm registered in the world until 1980's (Parkin et al., 1988). In the United States, American Indians, Hispanics, Blacks and Immigrants from Northern Europe, Asia and Latin America display risks that are considerably higher than those of native white Americans (Muir et al., 1987). The American Cancer Society estimated 20,000 cases in 1989 and 3,800 in 1995. A sharp increase of carcinoma localized in the gastric cardia has been registered in white males of the United States and Western Europe, thus far unexplained on etiological grounds (Blot, et al., 1991).

1.2.2 National Status of Gastric Cancer

1.2.2.1 Chennai Status

Even though gastric cancer is the second most common cancer of the world, in India gastric cancer occurrence varies across the country. In Northern part of India, the gastric cancer incidence was relatively low when compared to the Southern part. The incidence rate of gastric cancer in India is low when compared to Japan and other Western Countries. Interestingly the gastric cancer incidence in Chennai, is ranking first in India. Fig.1.1 & 1.2). This information prompted us to formulate this study

1.3 CLINICO PATHOLOGY OF GASTRIC CANCER

Gastric carcinoma is divided into two general clinicopathologic patterns; the epidemic intestinal type or well-differentiated carcinoma, and diffuse type or poorly differentiated carcinoma. The etiologies and the molecular alterations leading to these two histochemical patterns may be
Clinicopathology of Stomach

A. Normal stomach
B. Chronic Gastritis
C. Gastric Ulcer
D. Prepyloric Ulcer
E. Early Malignant growth
F. Adeono carcinoma
PLATE-1.1
Endoscopic Pictures of Stomach
significantly different enough from each other to consider them as two separate entities. A series of changes have been identified as precursors to the intestinal type of gastric carcinoma, representing apparently sequential steps in the precancerous process, namely superficial gastritis, chronic gastritis, atrophic gastritis (gland loss), small intestinal metaplasia, colonic metaplasia and dysplasia (Correa, 1990a and 1990b). Poorly differentiated cancers lack the well recognized precursor changes seen in the intestinal type lesions, Plate 1.1.

1.4 HISTOPATHOLOGY OF GASTRIC CANCER

Gastric cancer arises from precursor lesions. The precursor lesions are chronic gastric mucosal inflammation (gastritis), epithelial hyperplasia, dysplasia and metaplasia (Fig.1.3). Pathological changes include elongated, branched and tortuous glands, cystic dilation with crypt abscesses and pseudo stratification up to several cells deep. Multifocal intestinal metaplasia is characterized by epithelial column elongation, microvillous brush borders and single large cytoplasmic round vacuoles. Transition zones from normal to hyperplastic or dysplastic epithelium are usually discernible and occurs in a wave of decreasing severity. (Loss of chief cells in the gland is the characteristic feature of gastric atrophy) Carcinoma \textit{in situ} includes intraglandular folding, papillary and ductular projections and moderate to marked cellular pleomorphism (Dixon, \textit{et al.}, 1996). Cancer cells are undifferentiated in most of the malignant growth and in the mature forms, there is a tendency to develop epithelial pearls.
Fig 1.3 HISTOPATHOLOGICAL STAGES OF GASTRIC CANCER

Normal

Chronic Gastritis

Atrophic Gastritis

Intestinal metaplasia

Dysplasia

Early Carcinoma

Advanced carcinoma
1.4.1 Macroscopic Classification of Gastric Adeno Carcinoma

(Fine G et al., 1985)

i. Fungating or Polyploid

ii. Ulcerating

iii. Superficial Spreading

iv. Diffusely Spreading

1.4.2 Microscopic Classification of gastric Adeno Carcinoma

i. Intestinal

ii. Pylorocardial or (antral)

iii. Signet ringcell

iv. Anaplastic (Undifferentiated)

Other histologies include

i. Papillary adenocarcinoma

ii. Mucinous adenocarcinoma

iii. Adenosquamous carcinoma

iv. Squamous cell carcinoma (or) Mixed adenocarinoma and choricarcinoma (Machara et al., 1992)

1.5 ETIOLOGY

Gastric cancer is a multifactorial disease. The original hypothesis published in 1975 described three major etiological factors, namely excessively salted foods, low intake of ascorbic acid and carotenoids
(Correa, et al., 1975) While the precise etiology is unknown, acknowledged risk factors for gastric cancer include:

i. *Helicobacter pylori* gastric infection

ii. Aging

iii. Male Gender

iv. Diet low in fruits and vegetables

v. Diet high in salt, smoked or preserved foods

vi. Chronic atrophic gastritis

vii. Intestinal metaplasia

viii. Perinicious anemia

ix. Gastric adenomatous polyposis

x. Cigarette smoking

xi. Menetrier’s disease (giant hypertrophic gastritis)

xii. Familial adenomatous polyposis

1.6 SYMPTOMS OF GASTRIC CANCER

Early detection of stomach cancer is quiet difficult because there are no symptoms in the early stages and in many cases, cancer spreads before it is diagnosis when symptoms occur and they are often so vague. Stomach cancer causes;

i. Indigestion or a burning sensation (heart burn)

ii. Discomfort or pain in the abdomen

iii. Nausea and vomiting
iv. Diarrhea or constipation

v. Bloating of the stomach after meals

vi. Loss of appetite

vii. Weakness and fatigue

viii. Vomiting blood or presence of blood in the stool

1.7 SCOPE OF THE STUDY

The cause of cancer is not only a consequence of uncontrolled cell growth but may also be due to loss of growth suppressing activities. One of the most common and fatal malignancies of the world population affecting both genders is gastric cancer. Substantial evidence has been gathered linking gastric cancer with chronic infection of Helicobacter pylori.

This study aims to assess the host factors associated with Helicobacter pylori induced carcinogenesis developed from the normal mucosa to malignant tumour. The association of Helicobacter pylori infection with the progression of gastric neoplasia is now well established (Konturek. et al., 2001) but the exact role of Helicobacter pylori in the development of gastric cancer is poorly understood. Recent evidences suggest that alterations in epithelial cell growth in Helicobacter pylori colonised mucosa is dependant on specific host factors rather than bacterial factors. Nevertheless, the role of the host factors in the pathogenesis of Helicobacter pylori associated disease has been largely ignored. Therefore, an attempt has been made to study the effect of Helicobacter pylori on host
factors and its significant role during the progression of gastric cancer from chronic gastritis to invasive cancer. Lymphocytic invasion and the secretion of proinflammatory cytokines, alteration in the cell adhesion protein expression and the abnormal activation of protooncogenes are involved in the pathomechanism of gastric carcinogenesis. This implies that the *Helicobacter pylori* may mediate these changes on the host factors leading to gastric cancer. An understanding of the mechanism of the interaction between *Helicobacter pylori* and the host factors is still incomplete (Gernerd, et al., 2002) and studies are therefore needed on cellular host factors in relation to *Helicobacter pylori* infection. Therefore, the primary objective of this study was to understand the mechanism of *Helicobacter pylori* associated gastric carcinogenesis and the involvement of host factors in the progression of gastric cancer.

### 1.8 ORGANIZATION OF THE STUDY

#### 1.8.1 Rationale for the present investigation

In India, with change in life styles, the incidence of gastric cancer has been increasing steadily. Particularly in South India, gastric cancer ranks first followed by cervical cancer. The risk factors for gastric cancer are poorly understood, although a number of risk factors have been suspected world-wide. A higher risk of the development of gastric cancer has been reported in subjects with positive serologic tests for *Helicobacter pylori* (Parsonnett, et al., 1991, Nomura, et al., 1991). In 1994, WHO and IARC consensus group announced *Helicobacter pylori* as a class I carcinogen.
Although *Helicobacter pylori* infection can be treated, the organism still infects half of the world population.

The role of this chronic gastric infection has received strong support from four independent cohort studies which found that infection with *Helicobacter pylori* increases the risk of gastric cancer, (Nomura, *et al.*, 1991, Forman, *et al.*, 1991; Sierra, *et al.*, 1992;)

Although recent research developments on gastric cancer have shown *H. pylori* as an etiological factor, the host factors involved in the outcome of the pathogenesis are not yet clearly understood. A number of questions regarding *H. pylori* involvement in human gastric carcinogenesis remain unanswered and new pathogenic mechanisms of *H. pylori* infection must be sought to be addressed. In this context, it is very relevant to look at the involvement of cellular host factors in determining the clinical outcome of *H. pylori* infection. An understanding of this interaction will unravel adaptation processes between bacteria and host, but will also help to identify infected patients who are at higher risk of serious disease.

The association between *H. pylori* infection and development of gastric cancer is not absolute, because the majority of people infected with *H. pylori* do not develop gastric cancer. Peptic ulcer diseases and gastric cancer occur only in a subset of individuals chronically infected with *H. pylori* (Chiou, *et al.*, 2005). Therefore, both bacterial and host factors are presumed to contribute to this differential response.
Recent research has centered on identifying the host genes which are upregulated in association with *H. pylori* infection, determining their involvement in the pathogenesis of gastric tissue (Wong, *et al.*, 1996 and De Risi *et al.*, 1996) A better understanding of the molecular pathology of the disease may provide us with the ability to improve prognosis. This study is focused on the role of three important host factors TNF-α, CD44 and c-H-ras along with *H. pylori* status to have a better understanding of the tumourogenesis at the molecular level. In order to determine the effect of *H. pylori* on these factors in pathogenesis, an attempt has been made to analyse the interrelationship between factors involved in carcinogenesis.

Thus, the objective of the study was to reveal the prevalence of *H. pylori* in the study population. Further, the study was also focussed on the changes in the expression pattern of certain host factors such as TNF-α, CD44 and c-H-ras and their associated signal pathway upon *H. pylori* infection. This may evoke a better understanding of the interlinked complex mechanism involved in gastric carcinogenesis. The findings may help to improve the early detection and prevention of serious consequences of gastric cancer, which in turn, will improve the health care of the patients.

1.9 STUDY DESIGN

The present study was designed to analyse the prevalence of *H. pylori* infection in gastric pathogenesis, and its interactions with specific host factors to understand the mechanism involved in gastric cancer. Few
host factors associated with *H. pylori* influenced gastric carcinogenesis were selected for the study. They are proinflammatory cytokine TNF-α, cell adhesion protein CD44 isoforms and protooncogene c-H-ras p21. In order to identify the mechanism involved in *H. pylori* induced gastric carcinogenesis, the abnormal expression of TNF-α, CD44 isoforms and c-H-ras p21 in various stages of tumor progression in gastric epithelial mucosa were analysed. This study has been carried out in the same study subjects in which the *H. pylori* status has been analysed already. Finally the data obtained in the study of expression of host factors and *H. pylori* status were subjected to correlation analysis to analyse the interaction of these parameters in the sequential evolution of gastric carcinogenesis.

1.10 STUDY GROUP

The study subjects involved both normal subjects with complaints other than gastric diseases as well as with gastric disorders ranging from chronic gastritis, atrophic gastritis, intestinal metaplasia and adenocarcinoma of stomach. The tissue samples for this study were obtained as gastric biopsies from patients in the Medical Gastroenterology OP of the Govt. Stanley Medical College Hospital, Chennai.

Approval for this study was obtained from the Institute’s ethical board and the biopsy samples were collected in three different buffers as per the need of the method. This study was carried out in five study groups
based on revised Sydney system of gastric histopathological classification as given below.

Group I : Age matched normal subjects
Group II : Chronic gastritis
Group III : Atrophic gastritis
Group IV : Intestinal Metaplasia
Group V : Adeno Carcinoma

1.10.1 Parameters Selected for Analysis

1. Presence of *H. pylori* infection in gastritis biopsies

2. Analysis of TNF-α expression

3. Analysis of Cell adhesion protein CD44 and its isoforms

4. Analysis of oncogenes c-H-ras p21 expression

1.10.2 Justification for the choice of Selected Parameters

Eventhough diagnostic and therapeutic advances have been made for cancers in recent years, gastric cancer still ranks as the second most common cancer around the world. Therefore, further development in early diagnostic and prevention strategies are needed to improve the cancer control programme.
In order to achieve this, a clear and concise understanding of the cellular processes involved in the development of gastric cancer is an important prerequisite. Although the association of *H. pylori* with the development of gastric cancer has been proven, the development of gastric cancer varies among individuals in different regions. The prominent parameters involved in *H. pylori* mediated gastric cancers are TNF-α and CD44 which are involved in chronic inflammation and activation of oncogenic signal pathways leading to c-H-ras p21 activation.

1.11 WORKING HYPOTHESIS

The role of *Helicobacter pylori* in the progression of gastric carcinogenesis from chronic gastritis to adenocarcinoma is not yet clear. Therefore, an attempt was made to study the prevalence of *H. pylori* in the development of gastric cancer and its involvement in pathogenesis. *H. pylori* infection appears to be an early event in gastric pathogenesis with additional abnormalities leading to biological transformation (Omar, *et al.*, 2000). The important aspects of the transforming and immortalising activities of *H. pylori* is their association with cellular host factors. Several previous studies have reported the involvement of cellular host factors like proinflammatory cytokine TNF-α, cell adhesion molecule CD44 isoforms and c-H-ras p21 expression in *H. pylori* mediated gastric cancer. (Peek, *et al.*, 1995, Fax, *et al.*, 1996 and Wang *et al.*, 2000). Although various reports have depicted the expression of TNF-α and CD44 isoforms in *H. pylori* infection, the exact role is not clear. It is also important
to note whether an interaction of \textit{H.pylori} and cell surface glycoproteins CD44 molecules occurs and the modification in expression posed by the microbe. The change in the expression of cell adhesion molecule is an important characteristic of malignant growth. However, the role of c-H-\textit{ras} expression in the progression of \textit{H.pylori} induced gastric cancer is still unclear. The hypothetical representation of \textit{H.pylori} induced gastric carcinogenesis is given in (Fig.1.4).

We believe that the documentation of any changes in the above said parameters occurring in the gastric mucosa might have parallel morphological changes at various stages of neoplastic development. These findings may be helpful in diagnosis, prognosis and will aid in the understanding of gastric carcinogenesis which may help to prevent such cancers at an earlier stage.

1.12 OBJECTIVES OF THE STUDY

1. Analysis of the prevalence of \textit{H.pylori} in the sequential evolution of gastric cancer by RUT, ELISA and Histology.

2. The gastric carcinogenesis is a multistep process, the progression of which requires additional cofactors other than \textit{H.pylori} infection. So the present study was also aimed at evaluating the involvement of proinflammatory cytokine TNF-\textit{α}, cell adhesion molecule CD44 and its isoforms and the proto oncogene c-H-\textit{ras} p21 in \textit{H.pylori} induced gastric carcinogenesis.
Stages in the evolution of Gastric cancer

Helicobacter pylori infection

- Inflammatory cell activation and TNF-alpha expression?
- c-H-ras gene activation?
- CD44 isoform expression?
- CD44v6 and CD44v9 expression?

- Normal Mucosa
- Gastritis
- Chronic Gastritis
- Atrophic Gastritis
- Intestinal Metaplasia
- Dysplasia
- Adeno carcinoma
3. This study was also extended to analyse the interactions between these parameters in order to understand the mechanism involved in *H. pylori* induced gastric carcinogenesis by correlation analysis.

4. During the progression of pathogenesis, the alterations in the signal transduction pathway are evident. So a representative population from the study population were analysed for the deviation in signal pathway.

5. Finally to identify the exact role of CD44 in *H. pylori* infection, Co-culturing of Hep-2 cells with *H. pylori* was carried out and the CD44 expression and apoptotsis were studied. Monoclonal antibody blocking was also performed to stress the importance of the role of CD44.

1.13 DATA ANALYSIS

1.13.1 Statistical analysis

1. Univariate analysis of *H. pylori* and expression of markers were studied in relation to clinical and histological diagnosis in each group of study subjects.

2. Assessing the **Odds Ratio** for *H. pylori* was carried out. OR's were calculated as estimates of the relative risk to test for any significant association between the factors studied.

Statistical analysis was done utilizing the SPSS statistical software. Frequency tables were tested for association using Chi-Square and Pearson’s Correlation.
Chapter-II