MOLECULAR SCREENING USING NON RADIOACTIVE DIFFERENTIAL DISPLAY TECHNIQUE IN MALAY KELANTANESE PATIENTS WITH PEPTIC DISEASES

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by

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Dedication

Special thanks are dedicated to my loving parents, Hj Wan Taib Mohamad and Hjh Wan Fatimah Wan Endut for their moral support and prayers. Not forgetting to my dearest husband, Dr. Wan Zulkafli Wan Ibrahim and our three sons, Arif Firdaus, Amiru Solihin and Alif Zulhakimi for their understanding, support and enriching love during my endeavour. I love you all.

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LISTS OF PRESENTATIONS

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Is the Overexpression of Ubiquinol Cytochrome C Reductase in Erosive Gastritis Contributory to Gastric Carcinogenesis?
Malaysian Journal of Biochemistry and Molecular Biology. 2004 Volume10 as Short communications
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ABBREVIATIONS

PUD	: Peptic ulcer disease
H. pylori	: *Helicobacter pylori*
NSAIDs	: Nonsteroidal anti-inflammatory drugs
NOC	: N-nitroso compounds
ASA	: Acetylsalicylic acid
HSV	: Herpes simplex virus
IgA	: Immunoglobulin A
PG	: Pepsinogen
HLA	: Human leukocyte antigen
hTERT	: Human telomerase catalytic subunit
mRNA	: Messenger ribonucleic acid
tRNA	: Transfer ribonucleic acid
rRNA	: Ribosomal ribonucleic acid
cDNA	: Complementary deoxyribonucleic acid
RDA	: Representational difference analysis
SAGE	: Serial analysis of gene expression
DDRT-PCR	: Differential display reverse transcription-polymerase chain reaction
MMLV	: Moloney murine leukemia virus
OGDS	: OesophagoGastroDuodenoScope
PAGE	: Polyacrylamide gel electrophoresis
NIH	: National Institute of Health

RLP27a : Ribosomal protein large 27a

ZES : Zollinger-Ellison syndrome

RFLP : Restriction fragment length polymorphism

kDa : Kilo Dalton

ABSTRACT

Peptic diseases are the most common chronic diseases of adulthood and proven to have a substantial multifactorial inherited components. Genetic influences play some role in the predisposition to both forms of ulcers (gastric and duodenal ulcer). A small proportion of chronic gastric ulcers are susceptible to be transformed into malignancy. The possible somatic mutations that take place have not been extensively studied. The discovery of some genetic changes at the vicinity of the chronic benign inflammatory lesions is important in relation to the elucidation of the carcinogenesis of gastric cancers. The general aims of this study were to screen for differentially expressed genes in peptic diathetic patients and to apply a technique of non radioactive differential display analysis (DDRT-PCR). DDRT-PCR has been shown to be highly effective in identifying sequences that are differentially expressed in various cell types and this technique makes it possible to obtain reproducible result and efficiently identify specific mRNAs. Twenty tissue sample biopsies of gastric mucosa of the antrum were collected from peptic diathetic patients at Endoscopy unit. Total RNAs were extracted by using RNA extraction kit (RNeasy Mini Kit, Qiagen). The DDRT-PCR analysis was performed by a 2- step method which were reverse transcription and polymerase chain reactions (RNAimage Kit 1, GenHunter). Six percent denaturing Polyacrylamide Gel Electrophoresis (PAGE) was carried out in order to obtain the size of separation of cDNA fragments and visualized by silver staining. Once differentially expressed mRNAs were identified, the corresponding cDNAs were eluted from the band of the gel and reamplified. The sequence of cDNAs were determined using an ABI Prism DNA

Sequencer. The sequences were searched for its homology using GenBank databases provided by National Institutes of Health (NIH, USA). Two differentially expressed genes were identified, namely, ubiquinol-cytochrome c reductase complex (Complex III) gene and ribosomal protein L27a gene in gastritis tissue compared to normal gastric tissue. The expressed genes can be analyzed to determine their involvement in the pathogenesis of peptic diathesis. The determination of these genes will be used to study whether similar genetic derangement occur in gastric cancers in the future. This knowledge will enhance the understanding of carcinogenesis of chronic inflammatory lesions.

PENYARINGAN MOLEKULAR MENGGUNAKAN TEKNIK PAPARAN PERBANDINGAN BUKAN RADIOAKTIF DI KALANGAN PESAKIT MELAYU DI KELANTAN BAGI PENYAKIT PEPTIK

ABSTRAK

Penyakit ulser peptik merupakan penyakit kronik yang kerap berlaku pada golongan dewasa dan telah terbukti disebabkan oleh komponen pewarisan pelbagai penyebab. Pengaruh genetik memainkan peranan yang penting di dalam menyumbang kepada kedua-dua jenis ulser (ulser gaster dan ulser duodenum). Sebahagian kecil ulser gaster kronik lebih mudah terubah ke peringkat malignan. Kebarangkalian mutasi somatik yang terlibat masih belum dikaji secara meluas. Penemuan sebarang perubahan genetik pada peringkat lesi inflamasi benigna adalah penting di dalam hubungkait kepada terjadinya fenomena karsinogenesis kanser gaster. Matlamat umum kajian ini adalah untuk mengenalpasti gen yang terekspresi yang berbeza bagi pesakit ulser peptik dan untuk mengaplikasi teknik paparan perbandingan bukan radioaktif ("non-radioactive differential display") atau lebih dikenali sebagai DDRT-PCR. Analisis DDRT-PCR telah terbukti sangat berkesan di dalam pengenalpastian jujukan gen yang terekspresi di dalam pelbagai jenis sel dan teknik ini dapat memberi keputusan yang senang untuk dihasilkan dan pengenalpastian mRNA yang spesifik dengan berkesan. Dua puluh sampel tisu biopsi dari bahagian antrum mukosa gaster dikumpul dari pesakit-pesakit ulser peptik. RNA jumlah diekstrak menggunakan kit ekstrak RNA (RNeasy Mini Kit, Qiagen, USA). Analisis DDRT-PCR merangkumi 2 peringkat teknik iaitu transkripsi terbalik dan tindak balas rantai polimerase (RNAimage Kit 1, GenHunter, USA).

Elektroforesis 6% gel poliakrilimida termusnah (PAGE) dilakukan bagi melihat hasil pemisahan saiz fragmen cDNA setelah diwarnakan oleh pewarnaan perak. Setelah mRNA yang terekspres dikenalpasti, cDNA tersebut dielut dari jalur yang dipotong keluar dari gel dan direamplifikasi. Jujukan gen tersebut ditentukan oleh ABI Prism DNA Sequencer. Jujukan tersebut dibandingkan dengan jujukan homologi yang disediakan di dalam pangkalan GenBank oleh National Institute of Health (NIH, USA). Kami berjaya mengenalpasti dua gen yang terekspresi iaitu gen ubiquinol-cytochrome c reductase (Komplek III) dan gen ribosomal protein L27a pada tisu gastritis berbanding dengan tisu gastrik normal. Gen yang terekspres boleh dianalisa bagi menentukan penglibatannya di dalam patogenesis penyakit ulser peptik. Penentuan jenis gen ini berguna di dalam kajian lanjutan samada ketidakaturan genetik yang sama juga berlaku di dalam kanser gaster. Pengetahuan ini akan mengukuhkan kefahaman proses karsinogenesis bagi lesi inflamasi kronik.

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Peptic ulcer disease (PUD) is a chronic, recurrent disorder that is characterized by lesions in the upper gastrointestinal tract which appears as reddish and inflamed, or as small depressions or excavations in the upper gastro-intestinal tract. An ulcer can form at any area exposed to gastric acid and pepsin, a digestive enzyme instrumental in the breakdown of protein and hence a derivation of a term "peptic ulcer". The areas most commonly affected are the upper part of the duodenum (duodenal ulcer), the stomach itself (gastric ulcer) and less commonly, the esophagus (Greenberger & Thier, 1990). Peptic ulcer diseases affect all age groups, but is rare in children. Men have twice the risk for ulcers as women do. The risk for duodenal ulcers tends to occur first at around age 25 and continues until age 75; gastric ulcers peak in people between the ages of 55 and 65 (Valle, *et al.* 1999).

A series of step-wise precancerous lesions, starting with chronic atrophic gastritis, progressing to intestinal metaplasia, dysplasia, and finally becoming cancer were known

to occur in some cases; this sequence occurs over several decades (Ley, *et al.* 2001). In general, there are various contributory tendencies in carcinogenesis such as people exposed to the risks involving the genetic and environmental factors, prevalence of the lesions in the population, morphological characters of the lesions and their potential evolution from benign to neoplastic lesion. Evidences gathered thus far from several scientific fields has led to the hypothesis that the clinical manifestations of most gastric cancers are only a late event of a biologic phenomenon initiated many years previously during the chronic inflammatory phase.

Clearly, further molecular analysis is needed to identify other alterations that may contribute to gastric carcinogenesis and that may underlie the formation of premalignant lesions of gastric cancer and, thus, may function as markers for an increased risk of developing gastric cancer (Ebert, *et al.* 2000 & Boussioutas *et al.* 2003). To date, multiple genetic and molecular alterations in the multistage processes of gastric carcinogenesis have been reported, including inactivated tumor suppressor genes such as p53 and APC gene and activated oncogenes such as c-met and K-sam, which are frequently amplified and overexpressed in gastric cancer. In addition, microsatellite instability and alteration of adhesion molecule expression were demonstrated in gastric cancer. Identification of more differentially expressed genes in gastric cancer may be needed to elucidate the molecular mechanism of gastric carcinogenesis (Jung, *et al.* 2000).

Incorporation of epidemiological, clinical, histopathological, molecular genetics, microbiological, occupational and behaviour assessments have had a major impact on our understanding of gastric cancer today. In the future, collaboration of scientists from different disciplines will be even more critical because it can lead to the identification of

previously unrecognized factors relevant to gastric carcinogenesis as well as to the further development and subsequent implementation of a successful prevention program (Christian, *et al.* 1999).

Intense research during the past decade has resulted in several discoveries suggesting not only that there are a number of genes that play a relatively minor role in susceptibility of gastric cancer, such as the gene for blood group A, but also that there may be genes that are able to make a major contribution to cancer susceptibility (McConnell, 1983).

It has thus been clear for a number of years that genetic factors predispose to peptic ulcer, but the mode of inheritance of this genetic predisposition has not been resolved. For over a decade, the hypothesis of polygenic inheritance was used to explain the genetics of peptic ulcer. Polygenic or multifactorial inheritance refers to the concept that the hereditary component of a given disorder is due to the contribution of many genes acting together (polygenic), resulting in a continuum of genetic predisposition toward the disorder. Thus, clinical disease would exist when the presence of a sufficient number of genes, perhaps in combination with environmental factors exceeds a threshold level (Rotter & Grossman, 1980). The search for differentially expressed genes in gastric cancer and its premalignant lesions may help to define molecular alterations in the gastric mucosa that may precede the development of gastric cancer.

Differential display technique presents a novel method for the identification of aberrantly expressed genes in various biological states, such as carcinogenesis or developmental process. Generally, this method has proven to be highly effective for the

identification of differentially expressed genes in the process of malignant transformation. Furthermore, compared with other cloning methods, such as subtraction hybridization, this method is advantageous because of its high reproducibility and the identification of mRNAs with a low copy-number per cell (Ebert, *et al.* 2000).

Gastroduodenal ulceration is still poorly understood and changes in gene expression may provide new mechanistic insights (Szabo, *et al.* 2001). Therefore, further molecular analysis is needed to identify other molecular changes that may contribute to gastric carcinogenesis and may function as markers of gastric cancer (Jung, *et al.* 2000). We used this method to search for differentially expressed genes in peptic ulcer diathesis.

1.2 PEPTIC ULCER DISEASE

1.2.1 DEFINITION

Peptic ulcer disease refers to breaks in the mucosa of the stomach and small intestine, principally the proximal duodenum, that are produced by the action of gastric secretions and also contributed by *Helicobacter pylori* infections in many cases. Although peptic ulceration can occur as high as Barret esophagus and as low as Meckel diverticulum with gastric heterotopia, for practical purposes, peptic ulcer disease essentially affects the distal stomach and proximal duodenum (Rubin & Farber, 1999 & Shayne, 2002).

An ulcer is generally thought to occur when there is an imbalance between the aggressiveness forces of acid and pepsin and the less well-defined defensive forces of mucosal resistance and regeneration. The goal of ulcer therapy, both medical and surgical, is to correct this imbalance to promote ulcer healing, relieve symptoms, and

prevent complications and recurrences. Peptic ulcer tends to be an episodic, chronic disorder, characterized by symptomatic periods and pain-free intervals. The natural history of ulcer may be differ as the different diseases leading to an ulcer are delineated by clinical, physiologic and genetic studies (Rotter, *et al.* 1992)

1.2.2 EPIDEMIOLOGY

Peptic ulcer is among the most common of the chronic diseases, occurring in 2% to 10% of a world population, depending on such factors as geography, the specific population, and level of health care. Both gastric and duodenal ulcer rates increase rapidly with age. In the Danish studies the incidence of duodenal ulcer increased almost linearly with age, reaching 0.3% in males over the age of 75 years. For gastric ulcer, the incidence was low before age 40 in males and reached its peak for those aged 60 to 64 years, while in women it increased with advancing age (Rotter, *et al.* 1992). In the United States, approximately 10% of Americans eventually develop peptic ulcer disease (PUD), and about 10% of patients presenting with abdominal pain are diagnosed with peptic ulcer. Prevalence has decreased in the US over the last 30 years. Frequency of PUD is decreasing in the developed world but increasing in developing countries (Shayne, 2002).

Although the rate of incidence of gastric cancer has recently declined, gastric cancer is still one of the most common malignancies worldwide and is the second most common cause of cancer-related deaths (Tahara, *et al.* 1996). The link between gastric ulcers and gastric cancer come from epidemiologic observations in South East Asia, where Bonne and co-workers in 1983 reported that Chinese immigrants had a high frequency of

gastric carcinoma and atrophic gastritis with "goblet cell metaplasia", whereas both lesions were infrequently in native Malays (Correa, 1983).

H. pylori prevalence is high in South East Asia including Malaysia. The background prevalence of the entire Malaysian population is estimated to be around 40%. The previous study in year 2000 carried out in Malaysia found that the incidence was higher in Chinese and Indian communities than in the Malay community. The same trend was also noted in neighbouring Singapore. The reason for this racial differences in incidence is uncertain. The three races in Malaysia have been living in the same country for more than two generations and are exposed to the same environment. Suggested explanations for this finding include genetic differences and transmission and perpetuation of infection within the same ethnic group resulting from varied habits and socio-cultural practices. Another contributory factor may be that the Chinese and Indians being originally immigrants races may have brought the infection over from their home countries. Amjad (2000) reported in his study that 43 (86%) of the 50 index patients had family members sero-positive for *H. pylori* infection. Of the index cases who were Indian all the tested family members were positive, while 90% of the Chinese members tested positive. The incidence of *H. pylori* in the Malay family members was the lowest at 71% (Amjad, 2000).

Kudva from Malaysia reported that peptic ulcer was seen in 21% of 1119 patients while visual evidence of other gastric or duodenal mucosal lesions (gastritis or non-erosive duodenitis) was in 20%. With increasing age, the prevalence of peptic ulcer steadily increased and non-ulcer dyspepsia decreased (Kudva, 1990).

There are over half a million new cases diagnosed each year and up to 4 million people have a flare up of the disease each year. About 1 out of every 10 people will at some time in life have an ulcer.

1.2.3 CLASSIFICATIONS

1.2.3.1 Gastric Ulcer

An acute gastric ulcer is a disease of abrupt or rapid onset and short duration. A focal mucosal defect superficial to the muscularis mucosae heals by epithelial regeneration without scar formation. In a deeper lesion, the amount of fibrosis produced is a reflection of the depth and duration of the lesions. Most acute ulcers therefore heal leaving little fibrotic reaction (Bouchier, *et al.* 1993).

Benign chronic gastric ulcer is a common disorder. A study of self-reported peptic ulcers in the United States found 4.3 million persons ever having had a gastric ulcer, 1.6 million of whom had their ulcers diagnosed in the year before the study. A familiar study from Denmark found a lifetime prevalence of 1.2% for men and 0.6% for women. The incidence and prevalence of gastric ulcers are determined primarily in association with the major causes of the disease: *H.pylori* infection and nonsteroidal anti-inflammatory drug (NSAID) use. Other associated risks, such as smoking, alcohol use and socioeconomic status disappear when these primary causes are taken into account (Yardley, 1990).

1.2.3.2 Duodenal Ulcer

Duodenal ulcer can be defined as erosion in the lining of the duodenum (first part of the small intestine, connecting to the stomach). Duodenal ulcers are commonly associated with the presence of the bacteria *Helicobacter pylori* in the stomach. Risk factors are aspirin and non steroidal anti inflammatory drugs (NSAID) use, cigarette smoking, and older age. Figure 1.1 shows that several factors which contribute to duodenal ulcer disease. A combination of environmental factors, genetic factors and *H.pylori* trigger the clinical sequelae from inflammatory process to ulcer formation. Duodenal ulcer has historically occurred more frequently in men, but more recent data suggest similar rates in both men and women. The lifetime prevalence of a peptic ulcer is 5 to 10% and approaches 10 to 20% in patients who are *H.pylori* positive (http://www.nlm.nih.gov/medlineplus/ency/article/000206.htm).

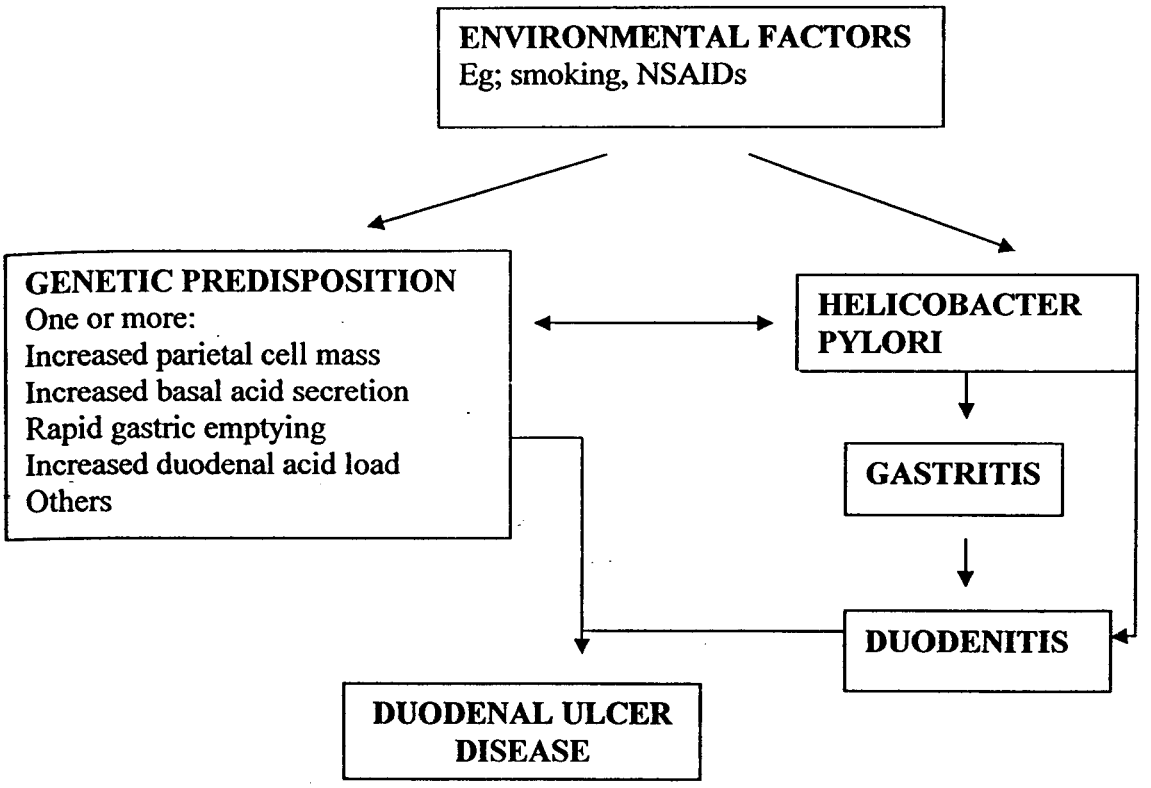

Figure 1.1: A model of the pathogenesis of peptic ulcer and the sequence of genetic predisposition combining with environmental factors to produce duodenal ulcers (adapted from Porro, *et al.* 1999)

1.2.3.3 Gastritis

Gastritis includes a myriad of disorders that involve inflammatory changes in the gastric mucosa, including erosive gastritis caused by a noxious irritant, reflux gastritis from exposure to bile and pancreatic fluids, hemorrhagic gastritis, infectious gastritis and gastric mucosal atrophy (Shayne, 2002).

Acute gastritis, acute ulcer and acute mucosal damage are considered together because they represent the gastric mucosal responses to acute injury. Depending on the causes, they represent varying degrees of mucosal necrosis with subsequent inflammation. Acute gastritis has well-established and consistent clinical associations such as a recent history of drug ingestion, alcohol excess leading to haemorrhagic erosions, shock, sepsis, multi organ failure and so on (Bouchier, *et al.* 1993).

On the contrary, chronic gastritis is a heterogenous group of gastric mucosal disorders characterized by wide spread injury, usually associated with a chronic, mixed acute or chronic inflammatory response. Chronic gastritis can be defined as any diffuse chronic inflammatory process involving the mucosal lining of the stomach. This definition encompasses both specific and non specific subvariants of chronic gastritis. Specific forms of chronic gastritis are associated with distinct disease processes and include various entities such as granulomatous inflammation, eosinophilic infiltrative disorders, Ménétrièr's disease and Zollinger-Ellison syndrome (Bouchier, *et al.* 1993). Chronic atrophic gastritis has been cited as one of the most important precursors of gastric cancer (Nagayo, 1993).

There is currently a resurgence of interest in gastritis. Knowledge about gastritis commenced when a link between the immune system and diffuse gastric mucosal disease was established. The recent rediscovery of the Gram-negative spiral bacterium, *H. pylori*, which exclusively colonizes epithelium of antrum part and the mounting evidence of its causal relationship to the gastritis, has further emphasized the marked heterogeneity of this disorder (Bouchier, *et al.* 1993).

1.2.3.4 Duodenitis

Duodenitis is defined as an inflammatory condition of the proximal duodenum, usually with maximal involvement of the bulb, and often but not invariably associated with dyspeptic symptoms occurring in the absence of a chronic duodenal ulcer. The clinical importance of duodenal inflammation in the absence of chronic ulceration remains unclear. In all probability, *H. pylori* infection is a major cause of chronic duodenitis. Duodenal inflammation may also occur in specific conditions such as tuberculosis, Crohn's disease, coeliac disease, septicaemia, giardiasis and ankylostomiasis (Bouchier, *et al.* 1993).

Inflammatory changes in the first part of the duodenum may occur alone or in association with a peptic duodenal ulcer when the inflammation is most marked in the immediately adjacent mucosa although it can be widespread within the duodenal bulb. Whether non-specific duodenitis always represents a stage which can lead to ulceration or alternatively may follow the healing of an ulcer, or whether it is a distinct entity has not been resolved. The significance of duodenitis and its relationship to duodenal ulceration continues to be debated. The reasons for this include the differing clinical,

endoscopic and histological criteria used to make the diagnosis, the variability of symptoms experienced by patients with "duodenitis" and the usually patchy nature of the inflammation (Cheli & Giacosa,, 1983).

1.2.4 AETIOLOGY

Several factors are suspected to play a role in gastric carcinogenesis, including the effects of diet, exogenous chemicals, intragastric synthesis of carcinogens, genetic factors, infectious agents and pathological conditions in the stomach, such as gastritis. Recent molecular genetic studies have provided evidence that genetic alterations of the human genome play important roles in the multistage process of gastric carcinogenesis (Christian, *et al.* 1999).

1.2.4.1 Diet factors

Coffee, both with and without caffeine, stimulates gastric acid secretion. The evidence linking coffee drinking to ulcers is doubtful. In 1974, Friedman *et al.* did not find any association between alcohol and coffee consumption and the prevalence of peptic ulcer. In contrast, Paffenbarger and co workers (1974) found in college students that ingestion of coffee and other beverages (mainly colas) increased the risk of later development of ulcers. Ingestion of milk decreased the risk. Cigarette smoking was correlated with subsequent development of peptic ulcer. Doll and coworkers found that gastric ulcer healed faster if smoking was stopped (Cooke, 1980). Alcohol (ethanol) also readily causes erosive and hemorrhagic gastritis in both experimental animals and in man, causing changes that are comparable to those seen with nonsteroidal anti-inflammatory

drugs (NSAIDs) and bile acids. Furthermore acute hemorrhagic lesions are frequently found in chronic alcoholics. Even variations in types of alcoholic beverages consumed may be important. For instance, because of low alcohol concentration in beer, inclusion of beer drinkers in a study may reduce correlation between gastritis and alcohol consumption (Yardley, 1990).

Most studies do not implicate type of food as causes of ulcer disease. Dietary treatment, once in vogue, was based on the belief that small, bland meals might reduce the secretion of acid and pepsin, buffer the acid secreted into the stomach, reduce the gastric motor activity and maintain the resistance of the gastric mucosa. Interest has developed in the study of a possible relationship between fiber in the diet and the development of duodenal ulceration, as well as the possible therapeutic effect of high-fiber staple diets. Fiber binds bile acids effectively and may therefore be of potential importance in conditions in which bile reflux is thought to cause mucosal damage. Fiber-enriched wheat bran changes the profile of the postprandial pH curve and reduces pepsin concentrations (Rotter, *et al.* 1992).

The intake of smoked and heavily salted, nitrated and carbohydrated food should be avoided. High salt consumption can cause stomach irritation which can lead to the development of atrophic gastritis. Salt also causes excessive cell replication and increase the mutagenicity of nitrosated foods. Nitrates when reduced to nitrites can lead to subsequent synthesis of carcinogenic N-nitroso compounds (NOC). Smoked fish contain polycyclic aromatic hydrocarbons which when administered in an edible oil vehicle induced forestomach cancer (Christian, *et al.* 1999).

1.2.4.2 Chemical factors

Many non-steroidal anti-inflammatory drugs (NSAIDs) are important causes of acute gastric injury, and of these aspirin (acetylsalicylic acid, ASA) is the best studied. After exposure to aspirin the stomach rapidly develops an acute erosive and hemorrhagic gastritis, seen as multiple petechial hemorrhages and often concentrated in antrum (Yardley, 1990). Chronic acetylsalicylic acid usage is now well established as a cause of gastric ulcer. This is very well documented in the report from the Boston Drug Surveillance Program. In that study there was an association between hospital admissions for newly diagnosed uncomplicated benign gastric ulcer and heavy long-term acetylsalicylic acid ingestion (4 or more days per week). No relationship was found between acetylsalicylic acid usage (heavy or light) and duodenal ulcer. Anti-inflammatory agents such as acetylsalicylic acid, phenylbutazone, indometacin, cortisone and adrenocorticotropin have also been shown to retard the healing of gastric ulcers and favor perforation and hemorrhage making it vulnerable to normal gastric secretions (Cooke, 1980 & Shayne, 2002).

1.2.4.3 Infectious agents

There are various infectious agents which contribute to the development of peptic ulcer. Bacteria such as *Helicobacter pylori, Helicobacter heilmannii* and *Mycoplasma* can tolerate with the acidic environment in stomach. Urease produced by bacteria can neutralize gastric acidity and induce inflammation. Viruses such as Herpes simplex virus and Epstein Barr Virus are thought to cause ulceration to the stomach and duodenum.

1.2.4.3 (a) *Helicobacter pylori*

Some discoveries showed that stomach and duodenal ulcers might be caused by a gram-negative spiral shaped microaerophilic bacterium named *Helicobacter pylori*. *H.pylori* colonizes the antrum in 95% of patients; half of these will also have the organism in the corpus (Bouchier, *et al.*1993 & Disotell, 2003). *Helicobacter pylori* has astonishing ability to colonize the human gastric mucosa, an extraordinary hostile environment, and to persist for decades, despite host inflammatory and immune responses. It is present in practically all human populations. The colonization is about 30-50% in developed countries, while in developing countries it can exceed 80%. Initial colonization occurs predominantly during childhood mainly from other family members. Chronic *H. pylori* colonization is recognized as significant risk factor for gastritis, ulcers and cancers (Matic, 2003 & Amjad, 2000).

H. pylori inhabits exclusively gastric-type epithelium, including gastric metaplasia in the duodenum. The infected epithelium shows degenerative changes comprising intracellular oedema, detachment from the basal lamina and cell necrosis. The prevalence of *H. pylori* infection and chronic gastritis rises with age, in parallel with the age-related increase in the prevalence of gastritis (Bouchier, *et al.*1993).

How *H. pylori* infection produces gastric ulcers is still under intense investigation. Unlike in duodenal ulcers, gastric acid hypersecretion does not occur in gastric ulcers. Ulcerogenesis is caused by tissue damage and loss of normal protective factors. The bacteria penetrates into mucosal layer and attaches to phospholipids, sialylated glycoproteins and Lewis B antigens (in patients with blood group O). Ammonia

generated by *H. pylori* urease damages the gastric mucosa, possibly by depleting α-ketoglutarate, an essential substrate in the tricarboxylic acid cycle.

Helicobacter pylori induces gastric inflammation in virtually all colonized individuals, and such gastritis increases the risk for peptic ulcer disease and distal gastric adenocarcinoma by expressing cytotoxic proteins such as cag A or vag A. However, only a minority of persons carrying *H.pylori* develop clinical sequelae, suggesting that particular bacterial products may contribute to pathogenesis (Peek, *et al.* 2000 & Leung, *et al.* 2002).

Hypothetical steps in ulcer formation by *H. pylori* is described. *H. pylori* uses urease to protect it from acid during transit to the mucin layer. It colonizes the mucin layer and may adhere to the gastric mucosa. Products of the bacteria provoke an inflammatory response that ultimately damages the mucosa. Virulence factors are thought to be involved at each stage (Salyers & Whitt, 1994). *H. pylori* tends to achieve its pathogenetic role by triggering an intense leukocyte infiltration of the gastric mucosa, and neutrophil activation provides a major source of reactive oxygen metabolites which can cause tissue damage mainly in the absence of antioxidants. *H. pylori* virulence factors promote release of a variety of chemoattractants/inflammatory mediators.

Longstanding *H .pylori*-associated gastritis predisposes to gastric carcinogenesis. It is postulated that *H. pylori* causes superficial gastritis which initiates a process that leads, through atrophy, intestinal metaplasia and dysplasia to the development of gastric cancer as shown in Figure 1.2 (Porro, *et al.* 1999). Other interference comes from nutrition defects, abnormalities in antibodies' function and gastrectomy. Various

regimens of reactive oxygen metabolite scavengers appear to have potentials as new treatment strategies for upper gastrointestinal diseases (Kountouras, *et al.* 2001).

It has been postulated that *H. pylori* infection favors back diffusion of hydrogen ions with subsequent breakdown of the mucosal barrier through alteration in the composition of the glycoprotein of the mucus. It has also been postulated that the hyperacidity in duodenal ulcer patients induces gastric metaplasia in the duodenal bulb, which becomes a target for *H. pylori* colonization and ultimately ulcer formation.

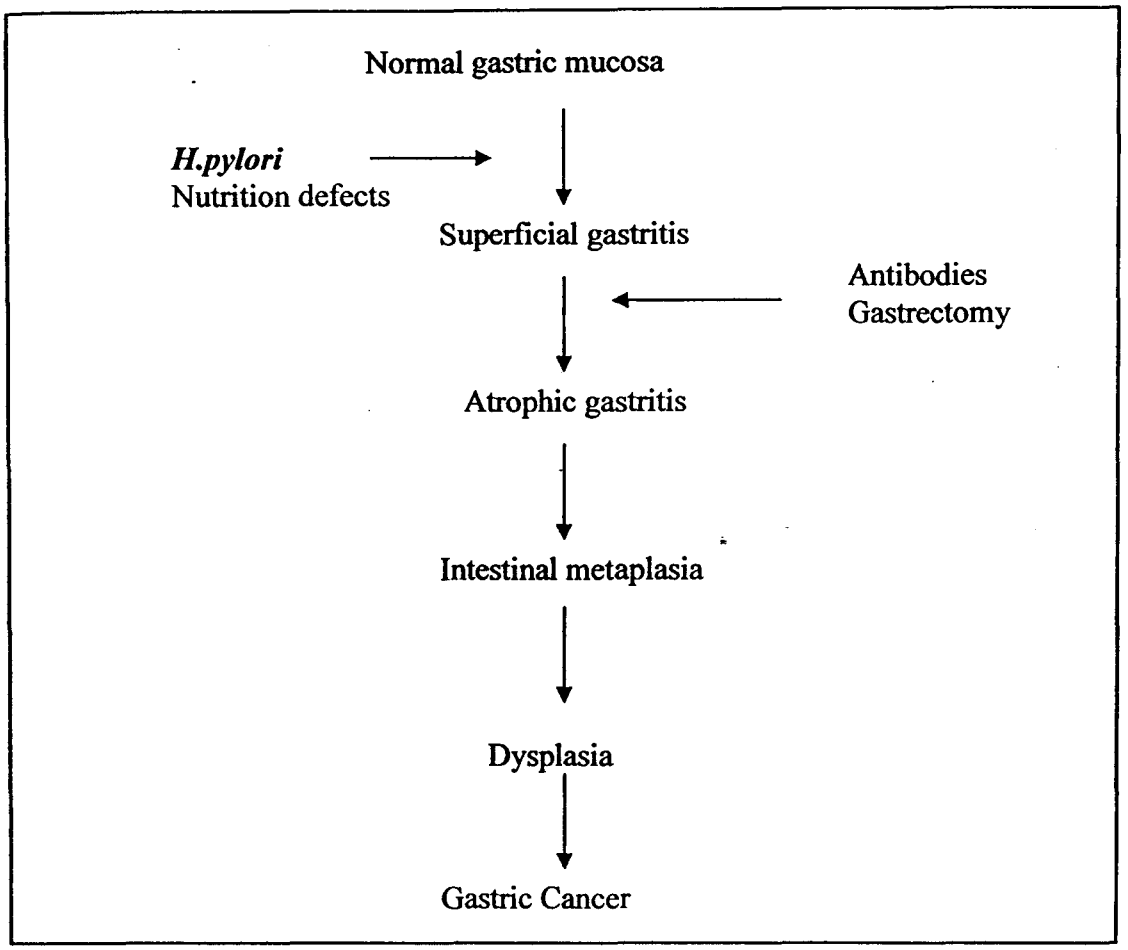

Figure 1.2: The role of *Helicobacter pylori* infection in the development of gastric cancer (adapted from Watters & Kiire, 1995)

1.2.4.3 (b) Herpes viruses

In 1967, Neuman and Knyvett first suggested that herpes simplex virus was an etiologic factor in peptic ulcer. It was subsequently suggested that chronic infection of a vagus nerve by herpes simplex virus (HSV) could provide the mechanism leading to peptic ulcer. Vestergaard and Rune (1980) reported that 94% of recurrent duodenal ulcer patients were seropositive for HSV type 1 compared with 80% of controls. Saliva and duodenal juice were tested for herpes simplex virus type 1 Immunoglobulin A (IgA), and higher levels were found in the ulcer group (Rotter, *et al.* 1992). These findings provide support for an association between active duodenal ulcer and herpes virus infection.

1.2.4.4 Genetic markers

Peptic ulcer disease illustrates the importance of genetic factors and their interaction with environmental mechanisms. The genetic predisposition varies from individual to individuals; all persons are not equally susceptible to peptic ulcer (Grossman, *et al.* 1981). Genes can be used as markers for cell recruitment, activation and mucosal synthesis of immunoregulatory molecules (Dieckgraefe, *et al.* 2000). Several scientists have suggested the role of genetic factors in the pathogenesis of ulcer diseases. The familial basis of duodenal ulcer and its mode of inheritance have thus far been an enigma, resulting in the emergence of the polygenic hypothesis and the concept of genetic heterogeneity (Habibullah, *et al.* 1984).

The precancerous lesions of the gastrointestinal tract can be divided into those determined by single genes and therefore in a more or less simple Mendelian manner and those in which the genetic influence is more complex and due to genes at several loci influencing susceptibility to environmental carcinogenic factors (McConnell, 1983). In the present state of knowledge, it is probably best to assume that an interaction between genetic and environmental factors underlies the great majority of gastrointestinal cancers.

Subsequently, the genetics of chronic gastritis has been extensively investigated by the Helsinki group (1971) and it has been shown that severe atrophic gastritis is largely genetically determined (Varis, 1971). The liability to severe atrophic fundic gastritis was shown to be significantly higher in the first-degree relatives of patients with this type of gastritis. This is a strong probability that this liability to fundic gastritis may be due to a single genetic factor rather than to common environmental factors (McConnell, 1983). Table 1.1 shows a relative risk of genetic factors in contributing to the formation of peptic ulcer disease. Pepsinogen and HLA complex contribute a higher relative risk in ulceration compared to other factors.

Table 1.1: Relative risks with various associated genetic factors in peptic ulceration (adapted from Rotter, *et al.* 1992)

GENETIC MARKER	ALLELE	RELATIVE RISK
ABO blood group	**O**	**1.3**
Secretor status	**Non-secretor**	**1.5**
Rhesus blood group	**Rh positive**	**1.1**
α1-antitrypsin	**Deficiency**	**1.4-3.0**
Pepsinogen (PG)	**Increased urinary PG1**	**2.4**
HLA complex	**B5, B12, Bw35, Bw49**	**2.1-2.9**

The Polygenic hypothesis

The genetics of peptic ulcer cannot be explained by a single, simple autosomal or sex-linked, dominant or recessive defect. Thus, until recently peptic ulcer was considered a polygenic disorder. Polygenic (multifactorial) disorders are thought to be caused by the interaction of several genes with environmental factors. The hereditary component in these illnesses reflects the combined contribution of many genes, resulting in a continuum of genetic predisposition to illness – the more genes, the greater the predisposition. The gene markers, blood group O and secretors status, provided some direct support for the polygenic hypothesis because when they are present together the risk is greater than when they are present separately. Although there may be a polygenic contribution towards peptic ulcer, evidence indicates that the major genetic factors and the differing physiologic observations can be best accounted for by the alternative explanation such as genetic heterogeneity (Grossman, *et al.* 1981 & Mueller & Young, 2001).

1.2.4.4 (a) ABO Blood Group and Secretor Status

The basic concept behind studying blood groups and other gene markers is that if a disease is positively associated with traits that are shown to be inherited in a Mendelian pattern, such as ABΘ blood groups, then these genetic traits form a background of predisposition for the disease (Grossman, *et al.* 1981). In a large number of studies carried out there is an increased proportion of persons with peptic ulceration have been found to have blood group O in a variety of different population groups. These findings do not mean that all persons with blood group O will develop a duodenal ulcer but merely that their risk of having a duodenal ulcer is 30% greater than in persons with

other blood groups. Gastric ulcer is also associated with blood group but the association is not as strong as in the case of duodenal ulcer (Mueller & Young, 1995). The ABO genes are therefore involved in determining liability to the disease and the ABO locus is probably only one of several that play a part (McConnell, 1983). Interestingly, patients with gastric ulcers do not exhibit a greater frequency of blood group O. Associations between certain histocompatibility antigens and peptic ulcers have been claimed but are still debated (Rubin & Farber, 1999).

In Hong Kong, Lam and Ong (1976) grouped their duodenal ulcer patients by age of onset and found that their early-onset group (onset below age 20 years) has a significantly stronger family history, had a frequency of blood group O similar to that of controls, more frequently presented with gastrointestinal bleeding as the first manifestation of the disease and rarely had complications such as perforation, obstruction, intractable pain or secondary gastric ulcer. In contrast, their late-onset group (onset after the age 20 years) had an infrequent family history of ulcer disease, had an increased incidence of blood group O, presented less frequently with gastrointestinal bleeding and had an increased frequency of complications such as perforation, pyloroduodenal stenosis, severe pain, virulent ulcer and secondary gastric ulcer (Rotter & Grossman, 1980).

Peptic ulceration is also associated with the secretor status for the ABO blood system. It has been found that duodenal ulcer and gastric ulcers to a lesser extent, are more common in persons who are non-secretors than in persons who are secretors. In fact, secretor status appears to be more important than a person's blood group in determining the likelihood of developing a peptic ulcer, with persons who are non-secretors being

50% more likely to develop peptic ulceration than the general population. The two factors together have a multiplicative effect, with persons who are non-secretors and blood group O having 2.5 times the risk of developing peptic ulceration compared to the general population (Mueller & Young, 1995 & Ming, 1992). ABH secretor genes determine the ability of individuals to secrete these antigens since they are glycoprotein constituents of gastric mucus. Their absence might alter the ability of mucus to protect the mucosa (Samloff, 1980). These observations on blood group O and nonsecretors have been confirmed by numerous investigators throughout the world and constituted early important evidence for the role of genetic factors in peptic ulcer (Grossman, *et al.* 1981 & Mueller & Young, 2001).

1.2.4.4 (b) Family Studies

The incidence of the disease in relatives of patients is compared with the incidence in the general population to determine familial aggregation or grouping of a disorder. The relatives of index patients share genes in common with the patients in direct proportion to the closeness of their relationship. The multifactorial model thus predicts that such relatives will share some of the disease-predisposition genes and hence will be shifted toward the threshold for disease and have a higher disease frequency than the general population (Rotter & Grossman, 1980). Family studies have consistency shown peptic ulcer disease to be two or three times as frequent in the first-degree relatives of peptic ulcer patients as it is in relatives of control subjects. Because these differences persisted across generations and social classes, genetic factors were presumed to explain these findings (Grossman, *et al.* 1981). First-degree relatives of patients with duodenal ulcers