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# UPPER GASTROINTESTINAL ADENOCARCINOMA; ASSOCIATIONS WITH GASTRIC SECRETORY FUNCTION AND GENDER

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**To:**

**My wife Nasim**

**&**

**My daughter Zahra**

# Preface

Over the past four years, I have had the privilege of working with Professor Kenneth E.L. McColl in the fascinating field of the upper gastrointestinal cancer. His clinical and scientific advice has been invaluable and his personal interest in my career most encouraging. My research in the upper gastrointestinal cancer has taught me important principles of human disease processes as well as giving me insight into effects of such disease on the personal lives and families of afflicted patients.

Much of the work presented in this thesis is the result of fruitful collaboration with many colleagues, all of whom deserve my gratitude.

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**1.** Derakhshan MH, Liptrot S, Paul J, Brown IL, Morrison D, McColl KE. Oesophageal and gastric intestinal-type adenocarcinomas show same male predominance - due to 17 year delayed development in females. Gut. 2008 Oct 6. [Epub ahead of print]

**2.** Derakhshan MH, Liptrot S, Morrison D, Brown IL, McColl KE. A 17-year delay of development of intestinal type adenocarcinoma in females explains male predominance of upper gastrointestinal cancer. GASTROENTEROLOGY 2008; 134 (4, Suppl. 1 ): A306-A307 (abstract)

**3.** Derakhshan MH, Liptrot S, Paul J, Brown IL, McColl KE. Male predominance of adenocarcinoma of upper gastrointestinal tract is related to intestinal histological subtype not tumour location. GASTROENTEROLOGY 2008; 134 (4, Suppl. 1 ): A611 (abstract)

**4.** Derakhshan MH, Malekzadeh R, Watabe H, Yazdanbod A, Fyfe V, Kazemi A, Rakhshani N, Didevar R, Sotoudeh M, Zolfeghari AA, McColl KE. Combination of gastric atrophy, reflux symptoms and histological subtype indicates two distinct aetiologies of gastric cardia cancer. Gut. 2008 Mar;57(3):298-305.

**5.** Hansen S, Vollset SE, Derakhshan MH, Fyfe V, Melby KK, Aase S, Jellum E, McColl KE. Two distinct aetiologies of cardia cancer; evidence from premorbid serological markers of gastric atrophy and Helicobacter pylori status. Gut. 2007 Jul; 56(7):918-25.

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**7.** Gastric phenotype and GERD symptoms indicate two distinct aetiologies of cardia cancer. Derakhshan, MH; Malekzadeh, R; Fyfe, V, et al. GASTROENTEROLOGY. 2007; 132: A618-A618 Supplement: 2 (abstract).

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**9.** Influence of gender on precancerous changes leading to intestinal type upper gastrointestinal cancer. Derakhshan, MH; Malekzadeh, R; Fyfe, V, et al. GASTROENTEROLOGY. 2006; 130: A420-A420 Supplement: 2 (abstract).

## SUMMARY

Gastric and oesophageal cancers were responsible for more than one million deaths in 2002. Although global incidence of gastric cancer is decreasing, this malignancy is still the fourth most common cause of cancer worldwide. The incidence of oesophageal adenocarcinoma is rising rapidly, three-fold in the last two decades. The incidence of adenocarcinoma of gastric cardia is stable.

In the pathogenesis of both gastric and oesophageal adenocarcinomas, the state of the gastric mucosa and its secretory function plays a central role. Non-cardia adenocarcinoma develops in subjects with H.pylori associated atrophic gastritis and hypochlorhydria. Little is known about the gastric phenotype in patients with adenocarcinoma of the cardia and gastroesophageal junction.

Another important but poorly understood risk factor for upper GI adenocarcinoma is male gender.

**In the first study** we aimed to investigate the association between the pattern of H.pylori gastritis and gastric secretory function in 255 H.pylori-infected patients with dyspepsia showing normal endoscopy. Our findings showed that maximal acid output correlates inversely with severity of corpus gastritis, corpus atrophy, and positively related to male gender and serum pepsinogen I.

**In the second study** we compared cancers at the cardia and non-cardia subsites with respect to pre-morbid gastric mucosal atrophy and acid secretion. In a nested case-control study comprising 101,601 men and women enrolled in the Norwegian JANUS cohort, 230 cases of gastric cancer were identified. 173 cases including 144 non-cardia and 44 cardia cancer were enrolled to study. Three controls were matched to each case. Serum pepsinogen I, pepsinogen II, anti-H.pylori IgG antibody and gastrin were measured using serums which had been collected a median of 11.9 years before cancer diagnosis radioimmunoassay method.

Non-cardia cancer was positively associated with H.pylori and gastric atrophy. The diffuse and intestinal histological subtypes of non-cardia cancer were of similar proportions and both showed a positive association with H.pylori and atrophy. Cardia cancer was negatively associated with H.pylori, but H.pylori positive cardia cancer showed a positive association with gastric atrophy. The predominant histological subtype of cardia cancer was intestinal and it was not associated with gastric atrophy

compared to the diffuse subtype. Cardia cancer in atrophic patients had an intestinal: diffuse ratio similar to non-cardia cancer, whereas cardia cancers in persons without atrophy were predominantly intestinal.

These findings indicate two aetiologies of cardia cancer, one associated with H.pylori atrophic gastritis, resembling non-cardia cancer, and the other associated with non-atrophic gastric mucosa, resembling oesophageal adenocarcinoma. Serological markers of gastric atrophy may provide the key to determining gastric versus oesophageal origin of cardia cancer.

**In the third study** we extended our investigation of the aetiology of cardia cancer by examining the association of both serological evidences of gastric atrophy and gastroesophageal reflux disease (GORD) symptoms with adenocarcinoma of the oesophagus, cardia and non-cardia regions of the stomach. This has been performed for the different histological subtypes of the cancer. We have also included H.pylori status and smoking history which are other well established risk factors for upper GI cancer. This has been undertaken in a population in Northwest Iran with a high incidence of upper gastrointestinal cancer. Serum pepsinogen I/II was used as a marker of atrophic gastritis and categorised to five quintiles. History of GORD symptoms, smoking and H.pylori infection was incorporated in logistic regression analysis. Lauren classification was used to subtype gastric and oesophageal adenocarcinoma.

Non-cardia cancer was associated with atrophic gastritis but not with GORD symptoms; 55% of these cancers were intestinal subtype. Oesophageal adenocarcinoma was associated with GORD symptoms, but not with atrophic gastritis; 84% were intestinal subtype. Cardia cancer was positively associated with both severe gastric atrophy and with frequent GORD symptoms though the latter was only apparent in the non-atrophic subgroup and in the intestinal subtype. The association of cardia cancer with atrophy was stronger for the diffuse versus intestinal subtype and this was the converse of the association observed with non-cardia cancer.

These findings indicate two distinct aetiologies of cardia cancer, one arising from severe atrophic gastritis and being of intestinal or diffuse subtype similar to non-cardia cancer, and one related to GORD and intestinal in subtype, similar to oesophageal adenocarcinoma. Gastric atrophy, GORD

symptoms and histological subtype may distinguish between gastric versus oesophageal origin of cardia cancer.

**In the fourth study** we investigated the relationship between gender and upper gastrointestinal adenocarcinoma. Male gender is a well-established risk factor for oesophageal adenocarcinoma. Male predominance of gastric cancer is related to the histological subtype of the tumour being more marked in the intestinal versus diffuse histological subtype. In addition, global data suggests that the male predominance of upper gastrointestinal cancer is related to the anatomical location, being higher for proximal and lower for distal tumours. However, the proportion of the intestinal histological subtype differs according to anatomical site and it is unclear whether it is the anatomical site or the histological subtype which is associated with the gender phenomenon. We have conducted a population-based study to investigate this.

The study was based upon 3270 gastric and oesophageal cancers recorded in West of Scotland Cancer Registry between 1998 and 2002. The Lauren subtype of adenocarcinoma was determined by reviewing 1204 reports and 3241 slides in a sample of 812 cases. Logistic regression models were used to estimate relationship between male predominance and histological subtype, tumour location and age.

We found that the crude incidence rate of intestinal subtype was higher in males (23.86/ $10^5$/ year) versus females (9.00/ $10^5$/ year), giving M/F of 2.65. M/F ratio of intestinal subtype cancer was 3.41 at age <50, reached a peak of 7.86 at age 50-59, and then showed a progressive decrease throughout the life. In contrast, the incidence rate of diffuse subtype adenocarcinoma was similar in both sexes (5.58 vs. 5.20 /$10^5$/ year) yielding M/F of 1.07. Multivariate analyses including histological subtype, tumour location and age indicated that the male predominance was related to the histological type rather than anatomical location. Intestinal type tumour showed similar male predominance of incidence irrespective of its anatomical location (OR, 95% CI: 2.6, 1.78 – 3.9). Further analysis of the age-specific incidence curves indicated that the male predominance of intestinal subtype was due to a 17.2-year delay of development of this cancer in females.

# Chapter 1 Gastric Secretion in Health

## LOOKING AHEAD

## 1. 1. Structure of Oxyntic Mucosa

The largest surface area of stomach is covered by oxyntic mucosa. The luminal surface of the oxyntic mucosa contains numerous orifices that lead to tubular invaginations called foveolae or pits, at the bottom of which open one or more glands. The pit is depicted in continuity with a single gland composed of isthmus, neck, and base, but the pit could be in continuity with two or more glands (Fig. 1). The pit is lined by pit cells. Six types of epithelial cells are distributed along the pit and gland regions [1-4]. The principle cell type of gland is the oxyntic cell, or **parietal cell,** which secretes hydrochloric acid and scattered through the gland. The second numerous cell type is the pepsinogen-secreting **chief cell** that lines the base. Two remaining of these cells are mucus secreting, namely, the surface mucous cell (**MSC**) or pit cell that lines the pit and the mucous neck cell (**MNC**) that lines the neck (Fig.1). Other cell lining the branched tubular glands includes **D cell,** which synthesize and secretes somatostatin [5]. Enterochromaffin-like cells (**ECL**) are located in the lamina properia of the gastric gland and synthesize and secrete histamine [6].

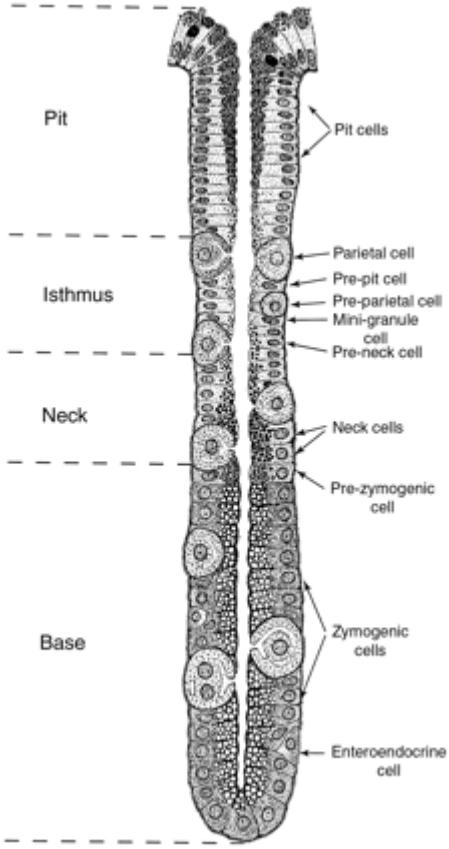



**Fig 1.1:** A typical gastric gland in oxyntic mucosa

## 1.2. Function of the Oxyntic Mucosa

### 1.2.1. Acid Secretegogaues and Inhibitors

Three main stimulating natural mediators are Histamine, Gastrin and Acetylcholine. Histamine is produced by decarboxylation of L-histidine. Most histamine in the body is stored in mast cells and basophil leukocytes, although some is found also in eosinophils and platelets. In the gastric mucosa, histamine occurs mainly in ECL cells and mast cells. The role of mast cell histamine probably reflects the pathophysiological role of mast cells in immune reactions. The ECL cells are rich in histidine decarboxylase (HDC) and are actively producing and releasing histamine [7-10]. Among different type of histamine receptors, H2 receptors are main type, responsible for acid secretory function of histamine.

Gastrin is the key hormonal inducer of acid secretion which is released from endocrine G cells in the gastric antrum. Gastrin can be secreted in response to eating a meal; circulating gastrin stimulates acid secretion by binding to $CCK_B$ receptors on parietal cell and Enterochromaffin-like cells in the corpus of the stomach. Thus gastrin stimulation of acid secretion from parietal cell includes direct activation [11] as well as indirect stimulation via release of the potent acid secretagogue histamine from ECL cells [12-14]. Cholecystokinin type B ($CCK_B$) is the responsible receptor for gastric effect [15].

Acetylcholine is a general neurotransimter mainly secreted by parasympathetic nervous system. Acetylcholine released from vagus nerves stimulate gastric acid secretion via the muscarinic receptor type 3 (M3) on the parietal cell [16, 17]. Cholinergic system can also control the acid secretion by stimulating of the histamine release indirectly [18].

There are numerous peptides and chemicals are thought to have direct and indirect inhibitory effects on gastric acid secretion, including somatostatin, Cholecystokinin, adrenomodulin, calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-

activating peptide (PACAP), antral natriuretic peptide (ANP), pancreatic polypeptide (PP), secretin, polypeptide YY, entroglucagon, serotonin, vasoactive intestinal peptide (VIP) and prostaglandins [19-21]. The most potent physiological inhibitor of oxyntic cells and G cells is 14- or 28- amino acid somatostatin, which is released by the D cell present in close proximity of the G cells in the antrum and of the oxyntic cells in fundus to inhibit these cells by paracrine mechanisms. It is released by the action of H+ on the receptors of the D cells and since its major effect is the inhibition of gastrin release and gastric acid secretion, it may be considered as a typical feedback controller of gastric secretion [22]. Its inhibitory effect is also mediated by the inhibition of histamine release from ECL cells through activation of specific membrane receptors called SSTR2 [23]. Calcitonin gene related peptide (CGRP) is present mainly at the sensory nerve ends in the oxyntic and antral gland area [24, 25] and plays a role in the axonal inhibition of gastric acid secretion. Secretin, produced in duodenum and jejunum may affect acid secretion by direct action on parietal cells and also indirectly through prostaglandins [26]. Two other family members of secretin, VIP and PACAP, are present in enteric neurons. VIP may have an indirect action through release of somastatin [21]. PACAP inhibits histamine release from the ECL cells and somatostatin from the D cells [27], leading to the inhibition of acid secretion [28, 29].

### 1. 2. 2. Bicarbonate and its regulation

The earliest medical literature about bicarbonate ($HCO_3^-$) refers to 1892 by the Danish physiologist Schierbeck [30] followed by Pavlov, who proposed that “alkaline mucus lining the gastric mucosa” neutralized luminal acid [31]. Secretion of $HCO_3^-$ into the surface mucus layer provides a first line of mucosal protection against acid in the stomach. Alkaline secretion by the stomach has been demonstrated in humans and laboratory animals. Bicarbonate secretion by frog [32] and mammalian [33, 34] fundic and antral mucosae mounted in an in vitro chamber was inhibited by anoxia, cyanide, or 2:4 dinitrophenol, indicating its dependence on tissue metabolism. Secretion in vitro was stimulated by cyclic GMP, but not cyclic AMP and cholinergic stimuli, which increased

gastric alkaline secretion in vitro [35] and in vivo [36, 37] while elevating mucosal cyclic GMP concentrations in the canine fundus and antrum [38]. The gastric surface epithelial cells contained high concentration of cyclic GMP diestrase [39], and the antral mucosa was composed mainly of this cell type. Furthermore, gastric antral and gastric fundic alkaline secretions display very similar properties, including almost identical sensitivity to stimulants and inhibitors [40, 41]. The overall conclusion of these studies strongly suggests that metabolic dependent secretion of bicarbonate is a property of the gastric surface epithelial cells [42].

## 1.3. Regulation of Acid Secretion

### 1.3.1. The parietal cell and its receptors

The earliest concept as to how the stomach handls food was enunciated by Hippocrates in the 5$^{th}$ century BC. His idea was that the stomach cooked food. It was not until more than 2000 years later, in the 18$^{th}$ century, that the French physicist Reamur suggested that the stomach digests the dissolves food [42]. Prout discovered gastric hydrochloric acid in 1823, and Beaumont initiated physiology by studying the regulation of acid secretion in a man with gastric cutaneous fistula in 1826 [43]. This event was followed by the identification of oxyntic gland parietal cell as natural secretors of gastric acid by Heidenhain and Golgi in 1875 and 1893, respectively [44].

In healthy adults, 1 billion parietal cells can be found within the acid secreting mucosa in the body and fundus of the stomach. Forty million parietal cells secrete 1 mEq acid per hour resulting in a maximal acid output (MAO) of 20-26 mEq/hr in healthy humans [45]. Hydrochloric acid is produced by the $H^+$, $K^+$-ATPase enzymes in parietal cells. The enzyme transports hydrogen ion into the parietal cell canaliculus in exchange for potassium ion. In the resting parietal, cell the $H^+$, $K^+$-ATPase is inactive and present largely in tubular vesicles in the cytoplasm of the cell. Stimulation of the parietal cell occurs via the acetylcholine ($M_3$), gastrin ($CCK_B$) and /or histamine ($H_2$) receptors on the

basolateral membrane via second messengers. This causes the movement of $H^+$, $K^+$-ATPase to the apical membrane of the cell, where it can exchange H+ for K+. Chloride ions enter the secretory canaliculi from the cytoplasm by a passive transport mechanism, resulting in the secretion of HCL [46-48].

### 1.3.2. Gastric Acid Secretion Pathways; Cephalic Phase

Central stimulation of the vagus nerve by thought, sight, smell, taste and swallowing of food results in acid secretion [49-51]. Early studies suggested that vagal activity during the cephalic phase induces acid secretion directly by stimulation of fundus, and indirectly by stimulation of antrum [52]. Proximal vagotomy decreases the acid response to sham feeding in humans [53]. The acid response to vagal stimulation is controlled by cholinergic system because small doses of atropine eliminates the acid response to intravenous insulin and 2-deoxyglucose which are both chemical stimulants of vagus [54].

Gastrin plays a physiological role in the cephalic phase acid secretion in humans. Modified sham feeding causes small but significant rises in serum gastrin concentrations[55]. However, circulating gastrin responses cannot fully explain the entire cephalic phase acid response to modified sham feeding. Combined cephalic and gastric stimulation in humans enhances acid secretion without a significant rise in circulating gastrin over that produced by gastric phase stimulation alone [56]. The preceding experiments indicated that during the cephalic phase of secretion, vagus nerve stimulation causes both stimulation and inhibition of gastrin release. Vagal cholinergic muscarinic stimulation of the fundus increases acid secretion but inhibits gastrin release. Vagal cholinergic muscarinic and non-cholinergic stimulation of the antrum increases the gastrin release. The net effect of inhibitory and stimulatory vagal pathways during the cephalic phase is a modest increase in circulating gastrin [57]. Based on of Konturek and colleagues' recent studies vagal stimulation by various techniques including classic sham-feeding as well as that induced by insulin hypoglycaemia, the highest response to vagal

stimulation does not exceed 50% of that attained with exogenous stimulus such as histamine or gastrin applied in a dose inducing maximal gastric acid secretory response[58].

There are a few centrally acting inhibitory pathways of gastric acid secretion during cephalic phase. In animal models, injection of calcitonin, CGRP [59], nourotensin [60], bombesin [61], interleukin 1 [62], corticotrophin-releasing factor (CRF) [63], neuropeptide Y [64] and prostaglandins [65] to different parts of central nervous system has been shown to inhibit gastric acid secretion [62, 64, 65]. Most of the pathways through which central acting molecules exert acid inhibition involve vagal and sympathetic nervous system. The hypothalamus also appears to be an important site of action of many peptide inhibitors pf acid secretion. IL-1 and bombesin are examples of gastric acid secretion inhibitors which affect hypothalamus [62, 66]. Peptide YY (PYY), which is structurally and functionally related to neuropeptide Y and released postprandially from the ileum and colon, displays a potent inhibition of gastric acid secretion during cephalic and gastric phases [67].

### 1.3.3. Gastric Acid Secretion Pathways; Gastric Phase

During gastric phase of acid secretion, gastrin plays central regulatory role. Two major pathways of gastrin-related gastric phase are mechanical distension and chemical stimulation by luminal nutrients.

Distension of stomach produces approximately 20% of maximal acid output in humans [68]. Stimulated gastrin and capsaicin-sensitive vagal afferent pathways are two main mechanisms involved in distension-related gastric acid secretion. Marked distension of the stomach induces a rapid rise of gastrin, and this rise is not inhibited by low pH of antrum [69]. The elevated gastrin is only modest and cannot fully account for the total acid response to the distension. A capsaicin-sensitive vagal afferent pathway accounts for 40% of the total acid response to gastric distension, which may or may not involve gastrin [70].

Experiments with anesthetized males suggest that vagus nerves mediate acid secretion by mechanical and chemical stimulation, and that gastrin mediates acid secretion partly by chemical stimulation but not by mechanical stimulation [71].

The strongest stimulants of gastric acid secretion during gastric phase are luminal nutrients. Peptides, amino acids, carbohydrates and fats stimulate acid secretion 2-4 times above that caused by mechanical stimulation [72]. Some components of coffee [73], wine [74] and the carbohydrate metabolites of fermented beer [75] also stimulate acid secretion.

The most essential regulator of acid secretion in gastric phase is gastrin which accounts for most of the acid response to graded increase in intra gastric concentration of peptone [68]. Gastrin was discovered by Edkins in 1905 [76]. In the 1960's Gregory and his colleagues purified gastrin from antral mucosal extract and from a tumour of Zollinger-Ellison syndrome (gastrinoma) and revealed the chemical structure of gastrins [77, 78]. The predominant form of gastrin in the antral mucosa and the circulating blood is 17-amino acid gastrin and 34-amino acid peptide with identical C-terminal sequence including Trp-Met-Asp-72 Phe-NH2, which by itself has similar acid stimulatory activity to the whole gastrin molecule. In the beginning of the 1970's, Yalow and Berson [79, 80] detected another form of gastrin composed of larger molecule and named it, "big big gastrin".

Besides the classic routes of activation through acetyl choline, gastrin, and histamine, a number of metabolic factors (including serum calcium and protein) or amino acid-rich diets influence gastric acid secretion via only partly characterized pathways. After the identification of Calcium Sensing Receptors (CaSR) in gastric tissue and its localization at the basolateral membrane of parietal cells [77], Dufner et al demonstrated the presence of a functional CaSR in the human stomach and showed that this receptor may modulate the activity of acid-secreting H_-K_- ATPase in parietal cells [78]. Stimulation of CaSR with divalent cations or the potent agonist Gd(3+) leads to the activation of the H(+)/K(+)-ATPase and subsequently to gastric acid secretion. Remy et al showed that in the human

gastric parietal cell, the CaSR is coupled to pertussis toxin sensitive heterotrimeric G-Proteins and requires calcium to enhance the activity of the proton-pump [79].

### 1.3.4. Gastric Acid Secretion Pathways; Intestinal Phase

The intestinal phase of gastric acid secretion contributes a small percentage (only 5%) of total acid response to meal. When a meal reaches the proximal part of the duodenum, this phase of gastric secretion occurs. In the 1940's, Gregory and Ivy [80] proved the humoral nature of this phase. Inhibition of gastric secretion by presence of acid in duodenum has been showed by studies dated back to one century ago [81]. This phenomenon was further investigated by Konturek and Grossman [82] in 1965. They found that the upper duodenum is involved in the inhibition of gastric secretion and that the excision of consecutive parts of duodenum leads to a gradual increase in acid production by the stomach. A total excision of the duodenum completely eliminates the inhibitory effect of acid in the intestines on gastric secretion. Subsequently, Konturek and Johnson explained this mechanism by inhibitory intramural and vago-vagal duodenogastric reflexes [83].

The mucosa of proximal small intestine contains significant amounts of gastrin, but only modest increases in circulating gastrin were measured during intestinal perfusion with peptone [84]. There is another factor, so called entero-oxyntin, secreted by small intestine mucosa that enhances the response to exogenous gastrin [85]. The gastrin potentiating the effect of the entero-oxyntin may be the most important contribution of the intestinal phase of secretion to the total acid response to a meal.

During the intestinal phase, absorbed amino acids also stimulate gastric acid secretion. Luminal amino acids appear to stimulate gastric acid secretion through a gastrin dependent pathway, whereas the effect exerted by the absorbed amino acids is mainly gastrin-independent [86].

In contrast to many different mechanisms involved in stimulation of gastric acid secretion during intestinal phase, inhibition of acid secretion is elicited mainly by three factors: intra-luminal fat, acid and hyperosmolar liquids [87]. Intra-luminal fat presumably causes the release of numerous peptide and chemical inhibitors of acid secretion such as secretin, somatostatin, peptide YY, nourotensin, VIP, GIP and enteroglucagon, but the inhibitory mechanism of none of them has been completely determined. Circulating secretin concentration increases in response to oleic acid perfusion of the upper small intestine [88] and by acidification of the duodenal lumen [89, 90]. The release of secretin by duodenal acidification pathway is mediated by a secretin releasing peptide (SRP). The release and action of SRP are neurally mediated depending on vagal afferent pathways [91]. In addition to direct effect of secretion on acid secretion, it can reduce acid secretion by increasing of somatostatin, PGE-2 and finally stimulation of secretion of PACAP-27 [92].

## 1.4. Gastric Secretion of Pepsins and Pepsinogens

### 1.4.1. Discovery of pepsins and other gastric proteinases

Studies on gastric digestion during 1820-1840 led to the discovery of pepsin by Schwann as the agent which, in the presence of stomach acid, causes the dissolution of nutrients such as meat or coagulated egg white [(93)]. Soon afterward it was shown that these protein nutrients were cleaved by pepsin to diffusible products named peptones. Efforts to isolate and purify pepsin were spurred by its widespread adoption for the treatment of digestive disorders, and highly active preparations were available by the end of the nineteenth century. In 1930, Northrop crystallized swine pepsin. The availability of this purified pepsin during the 1930s led to the discovery of the first synthetic peptide substrates for pepsin, thus providing needed evidence for the peptide structure of native proteins, a matter of debate at that time. In 1941, the effect of crystalised pepsin on anti-pneumococcal antibody was studied by Petermann et al [(94)]. After 1945, with the introduction of new separation methods, notably chromatography and electrophoresis, and the availability of specific proteinases, the amino acid sequences of many proteins, including pepsin and its precursor pepsinogen, were determined. In 1952, Janowitz and Hollander measured basal pepsin secretion from gastric content of healthy and peptic ulcer patients. After 1975, the three-dimensional structures of pepsin and many of its relatives were determined by means of x-ray diffraction techniques, greatly extending our insight into the mechanism of the catalytic action of these enzymes [(95)].

### 1.4.2. Classification and Function of Pepsins

The gastric proteinases have been divided into three main groups: 1) pepsins, including pepsin A and pepsin C, 2) chymosin, and 3) cathepsin E. Chymosin is the predominant enzyme secreted by neonates while adults' stomachs secret primarily pepsin A and pepsin C [(96)]. Chymosin is particularly effective in digesting milk proteins and this property accounts for its long standing use in the production of cheese as well as providing a rational for its predominance in neonates. A recently updated classification and nomenclature has been introduced by Gritti et al based on International Union of

Biochemists (IUB) rules [97], (Table 1.1). Gastric pepsins A and C originally were differentiated on the basis of electrophoretic mobility [97] and immunoreactivity. Salmoff et al identified seven isozymes, or isozymogens in the case of proenzymes, in human gastric mucosa, which were designated as pepsinogens 1-7 according to decreasing electronegativity. Based on immunoreactivity, pepsinogen 1-5 were classified as pepsinogen A (or pepsinogen I) while pepsinogen 6 and 7 correspond to pepsinogen C (or pepsinogen II). Recent studies have confirmed and extended the distinction between pepsin A and pepsin C by localising the genes encoding these enzymes to different chromosomes in man. Thus pepsin A genes are found on chromosome 11 [98] while pepsin C is localised to chromosome 6 [99].

In general gastric zymogens have two main roles in humans: 1) facilitating digestion of food proteins and 2) providing an antibacterial barrier. The gastric zymogens, including pepsinogen I and pepsinogen II, each contain a prosegment that serves to stabilize the inactive form and prevent entry of the substrate to the active site. Upon ingestion of food, each of the zymogens is released into the gastric lumen and undergoes conversion into active enzyme in the acidic gastric juice. This activation reaction is initiated by the disruption of electrostatic interactions between the prosegment and the active enzyme moiety at acidic pH values. The conversion of the zymogen into its active form is a complex process, involving a series of conformational changes and bond cleavage steps that lead to the unveiling of the active site and ultimately the removal and dissociation of the prosegment from the active centre of the enzyme [100].

**Table 1.1: Aspartic proteinases and cod numbers designated on the basis of IUB's Enzyme Nomenclature rules. (Modified from Gritti et al [127])**

| Common Name | Alternative Names | IUB Code |
|---|---|---|
| Gastric proteinases | | |
| Pepsin A | Pepsin I, corresponding to zymogen PGA | EC 3.4.23.1 |
| Pepsin B | Cathepsin E, slow-moving proteinase | EC 3.4.23.2 |
| Pepsin C | Pepsin II, fastricsin, corresponding to zymogen PGC | EC.3.4.23.3 |
| Chymosin | | EC.3.4.23.4 |
| Cathepsin D | | EC.3.4.23.5 |
| Other proteinases | | |
| Microbial aspartic proteinases (from fungi and HIV-1) | | EC.3.4.23.6 |
| Renin | | EC.3.4.23.15 |

Although it has been long believed that an optimal pH of around 2 allows pepsin to operate in its natural acidic environment, and at neutral pH the protein is denatured, recently, an inactive pepsin conformation has been identified that accumulates at mildly acidic pH. Campos et al showed that pepsin adopts, in the 6.5-4.0 pH interval, a native-like, although catalytically inactive, conformation [(101)].

The gastric lumen represents a bactericidal barrier, whose major components are an acidic pH and pepsin. In a recent study, pepsin has been shown to affect the motility of the bacteria, one of its most important virulence factors. It showed that the antibacterial effect of pepsin occurs in two phases: rapid loss of motility and subsequent destruction. In another study, investigators used the rapid pepsin-induced bacterial immobilization as a marker of antibacterial efficiency. The proteolytic activity of different pepsins was normalized to values between 2 and 200 U/ml in the hemoglobin degradation test of Anson, performed at pH 2 and 5. They found that pepsin C completely inactivates H.

pylori at proteolytic activities of 2 (pH 5) and 20 (pH 2) U/ml. In contrast, the activities of pepsin A and chymosin required to affect Helicobacter motility were ten times higher [102]. In another study, the susceptibility of Escherichia coli and Helicobacter pylori to pH and the effect of pepsin-mediated proteolysis were investigated. Survival of bacteria was diminished at pHs of less than 3.5, whereas killing required a pH of less than 2.5. Pre-incubation with pig pepsin at 0.5, 1.0 and 2.0 mg ml-1 at pH 3.5 reduced viable counts by 100% for E. coli 690 and E. coli K-12 after 100 min incubation. With H. pylori, the viable counts decreased to 50% of the control after 20 min incubation in 1 mg pepsin ml-1 at pH 2.5, 3.0 and 3.5. The gastric juices showed bactericidal activity at pH 3.5, and the rate of killing was juice dependent, with complete death of E. coli 690 occurring between 5 and 40 min post-incubation. Thus, killing of E. coli and H. pylori occurs optimally at pHs of less than 2.5. At pH 3.5, little effect is observed, whereas addition of pepsin alone or in gastric juice causes a marked increase in bacterial susceptibility, suggesting an important role for proteolysis in the killing of bacteria [103].

### 1.4.3. Regulation of Pepsinogens Secretion in Health

### 1.4.3.1. Stimulation of Pepsinogen Secretion

#### 1.4.3.1.1. Cholinergic System

Acetylcholine and cholinergic analogues have been shown to be potent stimuli for pepsinogen secretion. The secretion of pepsin can be stimulated directly by vagus nerves stimulatory signals or indirectly by one or more following mechanisms: insulin-induced hypoglycaemia, 2- deoxyglucose, alcohol, sham feeding, hyperventilation, vago-vagal reflexes resulting from the distension of the stomach, psychic stimuli and by depression of the cerebral function. All these stimuli are ineffective after vagotomy and can be blocked by administration of suitable doses of atropine [104]. Results from earlier studies have been demonstrated that acetylcholine and cholinergic agents increase pepsinogen secretion through both M1 and M2 muscarinic receptors. The blockade of pepsinogen secretion by different anticholinergic agents suggested a stronger role of M1 compared to M2 receptors. This has been well shown by experiments demonstrating that pirenzepine is a powerful inhibitor of pepsinogen secretion through the M1 receptor. On the contrary, M2 sub-type receptors can be selectively stimulated only in isolated parietal cells and gastric mucosa membranes [105, 106]. Recent studies using antagonists suggested that the M3 receptor subtype plays a prominent role in mediating pepsinogen secretion, but in situ hybridization indicated expression of M1 receptor in rat chief cells. Xie et al developed a murine secretory model of transgenic mice to investigate the regulation of pepsinogen secretion. Their results indicate that, in gastric chief cells, a mixture of M1 and M3 receptors mediates cholinergic stimulation of pepsinogen secretion and that no other muscarinic receptor subtypes are involved in this activity [107].

The results of studies by Blandizzi et al indicate that the activation of muscarinic receptors by vagally released acetylcholine is not sufficient by itself to stimulate pepsinogen secretion and that a facilitatory action mediated by acid secretion is

necessary to allow an increment of peptic output in response to vagal cholinergic stimuli. It is suggested that such facilitatory input is driven to chief cells by local intramural reflexes that involve capsaicin-insensitive intrinsic nerves [108].

#### 1.4.3.1.2. Adrenergic System

In isolated glands, the β-adrenergic agonist, isoproterenol, is able to stimulate pepsinogen secretion [109]. As this stimulation is inhibited by propranolol but not by atropine or H2 blockers, it seems to be a direct effect. Since isoproterenol is unable to stimulate acid secretion from the parietal cells, it seems possible that pepsinogen secretion triggered by isoproterenol infusion is specific. Further experiments on the gastric gland have shown that the β2-selective antagonist ICI-118551 is 100- times more potent than a β1-selective antagonist betaxolol for inhibition of pepsinogen secretion [110-112].

#### 1.4.3.1.3. Cholecystokinin

Octapeptide cholecystokinin (CCK-8) and other related peptides were found to induce pepsinogen secretion in gastric glands [113]. CCK-8 appears to have a direct effect on the chief cells. This stimulation is inhibited by the cGMP analogue, and it seems that the potency of this stimulation depends on the presence of a sulphated tyrosine residue in the molecule. Central administration of CCK-8s stimulates pepsinogen secretion through the activation of peripheral CCKa and CCKb receptors. Also it was observed that CCK-8s stimulus is mainly mediated by CCKa receptors subtype [114, 115]. According to the findings of Blandizzi et al, under in vivo conditions, the stimulant actions of CCK-like peptides on pepsinogen secretion are mediated, at least in part, by an increase in NO generation [116]. Further work of the same team emphasized that increase in pepsinogen output evoked by centrally applied cholecystokinin-8S does not depend on interaction with central nervous sites. Following central or parenteral injection of cholecystokinin-8S, the increase in peptic secretion would result from activation of both peripheral CCKa and CCKb receptors presumably located at the level of gastric mucosa [117].

#### 1.4.3.1.4. Gastrin

Gastrin and pentagastrin stimulate pepsinogen secretion in intact animals, but no direct stimulation of the chief cells has been demonstrated and so far there is no evidence for a direct physiological secretion [118, 119].

#### 1.4.3.1.5. Histamine

There are controversial findings about histamine related pepsinogen secretion. In different in vitru models, histamine is reported to have no effect on pepsinogen secretion [120, 121] or a small stimulatory effect [122]. Histamine can either induce or, in high doses, inhibit pepsinogen secretion [118, 123]. Therefore, it was suggested that these responses are mediated by H2 receptors and that the acidification of the gastric lumen can stimulate histamine secretion which, in its turn, by activating a local cholinergic reflex, can stimulate pepsinogen secretion [124-126]. On the other hand, in vitro studies on isolated gastric glands suggest that acid and pepsinogen secretion are regulated independently, although in vivo the two processes seem to react to the same stimuli. These results indicate that histamine cannot be considered a direct physiological regulator of pepsinogen secretion by the chief cells.

#### 1.4.3.1.6. Other Peptides and Macromolecules

**Secretin:** Secretin has been reported to stimulate pepsinogen secretions *in* vivo, but results of *in vitro* studies showed no clear stimulatory effect of secretin on pepsinogen secretion [127-129].

**Vasoactive Intestinal Peptide (VIP):** VIP has been reported to stimulate pepsinogen secretion through the same receptor mechanism as secretin [129-131].

**Bombesin**: Bombesin also has been shown to stimulate pepsinogen secretion through direct action on gland cells and indirectly by modulating gastrin release [119, 109, 132].

**Epidermal Growth Factor (EGF):** Although epidermal growth factor (EGF) inhibits gastric acid secretion, it stimulates pepsinogen secretion by activating eicosanoid generation, tyrosine kinases, MAP kinases, Ca2+, NO, and guanosine 3',5'-cyclic monophosphate [133]. The EGF dose dependently increases basal pepsinogen secretion and a mitogenic concentration (0.1 nM) of EGF induces submaximal stimulation. Similar effects have been observed with transforming growth factor alpha. EGF effects on pepsinogen secretion are in addition to that induced by CCK-8 and db-cAMP stimulated pepsinogen secretion. EGF-induced pepsinogen secretion is completely inhibited by a human immunospecific EGF receptor antibody and reduced by both genistein and tyrphostin-25, two different tyrosine kinase inhibitors [134].

**Protease Activated Receptors:** Protease-activated receptor-2 (PAR-2) is abundantly expressed in gastric mucosal chief cells, facilitating pepsinogen secretion. According to Kawao et al, PAR-1, a thrombin receptor, like PAR-2, might function to facilitate pepsinogen secretion, suggesting a role of the thrombin-PAR-1-pathway in the stomach [135]. Another study indicates that the activation of PAR-2 causes a Ca2+-ERK-dependent stimulation of pepsinogen secretion [136].

**Na-K-2Cl cotransporter-1 (NKCC)**: NKCC has been detected at exceptionally high levels in the gastric mucosa of several species, prompting speculation that it plays important roles in gastric secretion. NKCC contributes to secretions of Na+, K+, Cl-, fluid, and pepsinogen by the gastric mucosa through a process that is electrogenic in character and independent of acid secretion. The probable source of the NKCC-dependent non acidic electrogenic fluid secretion is the parietal cell. The observed dependence of pepsinogen secretion on NKCC supports the concept that a non acidic secretory stream elaborated from parietal cells facilitates flushing of the proenzyme from the gastric gland lumen [137].

# Chapter 2 Gastric Secretion in Disease

## LOOKING AHEAD

## 2.1. Introduction

The key role of gastric acid secretion in benign and malignant disease of upper gastrointestinal tract has been known since late 19th century. Alterations of acid secretion in the form of hypersecretion and hyposecretion has been a challenging field for many scientists and clinicians who were interested to provide the best evidence to understand pathophysiological pathways of peptic ulcer disease and gastric cancer.

The gastric acid hypersecretion in patients with duodenal ulcer has been studied adequately before *H.pylori* era [1-8]. Also, the development of gastric ulcer has been shown to appear more common in subjects with less acid secretion [9, 10]. Impaired mucosal defence against bile and acid and duodeno-gastric reflux of bile were suggested as main pathological background of gastric ulcer [11, 12]. Traditionally, severe hypochlorhydria or achlorhydria has been known as a characteristic feature of gastric cancer. Achlorhydria, determined by the augmented histamine test, is the functional expression of the most severe atrophic gastritis and is followed by a 3 to 6 fold increased risk of gastric cancer [13, 14].

The Introduction of *H.pylori* by Marshall and Warren in 1984 [15] was a remarkable evolution in the understanding of upper gastrointestinal pathophysiology. *H.pylori*-induced superficial gastritis was introduced as an essential and triggering factor in duodenal and gastric ulcers. The recovery of normal acid secretion following eradication therapy revealed strong association between *H.pylori* infection and gastric alterations of gastric acid secretion in duodenal ulcer. Successful healing of gastric ulcers after *H.pylori* eradication also showed its critical role in the pathogenesis of peptic ulcer disease. Moreover, due to accumulated evidence on the critical role of *H.pylori* in the development of gastric cancer, IARC introduced it as the first degree carcinogen for gastric cancer on 1994 [16].

A short overview of historical and current evidence on the gastric secretory state in general and acid secretion in particular, seems to represent acid as central core of pathophysiology of gastroesophageal malignancies. In this chapter, I will review the alterations of gastric acid secretion in peptic ulcer disease in the first instance. Secondly, the alterations of acid and pepsinogens in gastric cancer and its precancerous lesions will be discussed. Finally, the results of our own study on predictors of maximal acid output in *H.pylori* infected patients will be presented.

## 2.2. Alterations of Gastric Acid Secretion in Duodenal Ulcer Disease

Duodenal ulcer is one of the common upper gastrointestinal diseases in humans. Except for a minor percentage of cases, almost all of the subjects who develop duodenal ulceration have *H.pylori* infection [17, 18]. Eradicating the infection usually cures the ulcer disease [19]. There is now substantial evidence that the infection causes the duodenal ulceration by stimulating increased gastrin release and increased acid secretion ([20-22]).

In duodenal ulcer patients, the *H.pylori* gastritis is mainly confined to the antral mucosa with little inflammation of the acid secreting body mucosa. The inflammation of the antral mucosa results in increased gastrin release and this is apparent, both under basal conditions and following stimulation by food or by gastrin releasing peptide [23-25]. Eradication of the infection results in a fall in serum gastrin with the values returning to normal within 2 to 14 days of commencing anti-*H.pylori* therapy [26, 27].

The hypergastrinaemia associated with *H.pylori* infection in duodenal ulcer patients appears to be due to the inflammation depleting antral somatostatin concentrations and thus disrupting the acid-mediated inhibitory control of gastrin release [28-31]. In the healthy stomach, high acid concentrations in the gastric lumen stimulate the release of somatostatin by the antral D cells, and this inhibits gastrin release by the G cells, thus providing a negative feedback control to prevent excessive gastric acid secretion. This control mechanism is disrupted by *H.pylori* infection. The mechanism by which the infection depletes the somatostatin concentration is unclear. It may be related to the ammonia produced by *H.pylori* elevating antral pH and thus removing the physiological trophic effect that gastric acid exerts on the antral D cells [25]. An observation by De Francesco et al suggests that lymphocyte density in the antral mucosa could play a role in the increased gastrin production occurring in patients with *H.pylori*-induced duodenal ulcer [32]. It is possible that *H.pylori* antral gastritis might affect G and D cell function by the stimulation of low production non-specific cytokines [33, 34]. Other *in vitro* studies have

shown that certain cytokines affect gastrin somatostatin release, though it is difficult to know whether this can be extrapolated to the *in vivo* situation [35].

The important point about the increased gastrin release stimulated by *H.pylori* infection in duodenal ulcer patients is that it leads to increased acid secretion [36, 37]. Such subjects have increased basal acid output and increased maximal acid output stimulated by gastrin-releasing peptide. The excess basal acid output of these patients can be mainly attributed to the *H.pylori*-induced hypergastrinaemia as eradicating the infection results in the normalization of acid secretion and the disappearance of the hypergastrinaemia [23, 24, 35, 38].

There are some dark areas regarding *H.pylori* infection and altered acid secretion in patients with duodenal ulcer versus healthy or non-ulcer dyspeptics. Increased acid secretion is a characteristic of duodenal ulcer patients and not seen in the great majority of subjects infected with *H.pylori* without duodenal ulcer. Gillen et al investigated the physiological explanation for the fact that *H.pylori* infection results in increased acid secretion in duodenal ulcer patients but not in non-ulcer subjects [39]. The degree of increase in serum gastrin concentration was similar in *H.pylori*-infected duodenal ulcer patients and in *H.pylori*-infected non-ulcer subjects. However, the two groups varied markedly in their acid response to the increased gastrin. The duodenal ulcer patients showed a normal or increased acid response to gastrin. This phenomenon is more likely due to the fact that they have a high parietal cell mass, together with the fact that the sensitivity of their parietal cells to gastrin stimulation is normal. This explanation was supported partly by Jacobson et al [40]. In contrast, *H.pylori* infected non-ulcer subjects do not have an increased parietal cell mass, and, in addition, their parietal cells are abnormally insensitive to gastrin stimulation. The difference in parietal cell mass between these two groups may be a genetic factor. The impaired sensitivity to gastrin characteristic of the non-ulcer subjects is due to the fact that in these subjects the *H.pylori*

gastritis involves the body mucosa and impairs its function, whereas in the duodenal ulcer subjects, the acid secreting mucosa is not affected by the *H.pylori* gastritis and its function, therefore, is unimpaired. The reason why the inflammation involves the body mucosa in non-ulcer subjects may be due to the fact that they have a lower genetically determined parietal cell mass as a high acid output protects the oxyntic mucosa from *H.pylori* gastritis.

In summary, several different observations support the hypothesis that the disruption of normal physiological control of gastric acid secretion by *H.pylori* infection develops duodenal ulcer by producing an increased duodenal acid load. Eradication of *H.pylori* infection normalises acid secretion and this is associated with resolution of the ulcer disease. It should be emphasised that this hypothesis applies only to duodenal ulcer disease which is the main complication of the infection. *H.pylori* infection can also result in gastric ulceration and this complication is likely to be related to the bacterium directly damaging the gastric mucosa.

## 2.3. Alterations in Gastric Function Predisposing to Gastric Cancer

Historically, achlorhydria was one of the characteristic features of gastric cancer, which was explained by the presence of severe atrophic gastritis in the mucousa adjacent to the tumour. The carcinogenic process, starting from superficial chronic gastritis, progress to atrophic gastritis, intestinal metaplasia and finally glandular dysplasia and cancer, was well described by Correa in pre *H.pylori* era [41]. Soon after *H.pylori* was recognized as the cause of chronic gastritis, it became apparent that the epidemiological paradigms that linked atrophic gastritis and gastric cancer could be applied to *H.pylori* infection [42-44]. How these inflammatory and precancerous steps progress to cancer and their risk factors are not our main topic in this chapter, only alterations in gastric acid secretion will be discussed here.

In contrast to patients with duodenal ulcer, chronic *H.pylori* infection results in marked hypochlorhydria or achlorhydria in a subgroup of subjects, including whom with asymptomatic infection, non-ulcer dyspeptics, and patients with gastric cancer [45]. In these subjects, the inflammation predominantly involves the body mucosa and this markedly impairs the ability of the parietal cells to secrete acid. The majority of these subjects also have evidence of atrophy of the antral and/or body mucosa and this contributes to the hypochlorhydria. Eradication of the infection results in various degrees of recovery of gastric acid secretion [46]. In subjects with little or no evidence of atrophy, eradication of the infection may result in the complete recovery of normal levels of acid secretion. Regression of gastric atrophy and intestinal metaplasia following *H.pylori* eradication has been shown in experimental animal models [47-49]. In several human studies, gastric atrophy and intestinal metaplasia did not progress [50, 51] and even regressed after *H.pylori* eradication [52-55].

The reason why this subgroup of subjects develops a corpus predominant gastritis and low acid secretion is less clear. However, it may be related to a pre-morbid low acid

output which would allow the gastritis to develop in the body mucosa [56]. As other explanation, it may be related to antigenic mimicry between the parietal cells and the organism with the immune response impairing the function of the parietal cells which would then lead to the development of body gastritis [57]. Alternatively, it may be related to dietary factors such as high salt intake or low Vitamin C intake which will predispose patients to the atrophy of the antrum or body mucosa. Atrophy of either region (mostly that of the body) will impair the ability of the stomach to secrete acid and this will again allow the gastritis to become established in the body mucosa and further inhibit acid secretion.

### 2.3.1. Factors influencing progression to gastric cancer

There has been considerable interest in the factors associated with particular histological and physiological phenotype induced in the stomach by *H.pylori* infection. Studies have been focused on three groups of potential influencing factors, namely (I) different strains of *H.pylori* infection, (II) environmental co-factors, and (III) host genetic factors:

I) Patients infected with the more virulent CagA positive strain, have more intense inflammatory infiltrate both in their antral and body mucousa of the stomach [58]. CagA positive infection is also associated with an increased prevalence of atrophy [59]. However, the distribution of the inflammation between the antral and body region of the stomach and the associated disturbance in gastric secretion is unrelated to the strain of the infection [58]. Infection with the more virulent CagA positive strain of *H.pylori* infection thus increases the risk of developing either gastric cancer or duodenal ulcer disease but the infection does not in itself determine which of these two outcomes more likely are [60].

II) Many environmental factors have been known to be associated with increased risk of gastric cancer. Recent studies have confirmed that *H.pylori*-infected subjects with a low intake of fruit and vitamin C have an increased risk of developing gastric cancer

compared to subjects with *H.pylori* infection and a high intake of fruit and vitamin C [61, 62]. In addition, tobacco smoking is a risk factor for gastric cancer [63, 64]. *H.pylori* infected subjects who smoke have a three-fold increased risk of developing gastric cancer compared to *H.pylori*-infected subjects who do not smoke [61]. Both the diet deficient in antioxidants and the free radicals in cigarette smoke are likely to promote the development of atrophic gastritis in *H.pylori* infected subjects. High intake of salt also has been shown to be able to increase risk of gastric cancer in *H.pylori* infected population, particularly in high risk area such as Japan [65, 66]. According to Lee et al, subjects with *H.pylori* infection and a high salty preference had a 10-fold higher risk of early gastric cancer than subjects without *H.pylori* infection and with a low salty preference [62]. Recently, it has been shown that concurrent helminthic infection modulates the response to Helicobacter felis in mice and reduces the risk of developing atrophy [67]. This appears to be due to down-regulation of the TH1 cytokine response. This observation might explain the relatively low incidence of gastric cancer in African countries, despite the high prevalence of *H.pylori* infection.

III) The role of host genetic factors in determining the response to *H.pylori* infection and subsequent outcome has received considerable attention over the last decade. Our colleagues recently studied 100 first-degree relatives of patients with non-cardia gastric cancer and compared them with 100 control subjects with family history of gastric cancer [68]. The *H.pylori* status, mucosal histology and gastric secretory function of each subject were determined. These studies showed that there is a very high incidence of gastritis, atrophy and hypochlorhydria in *H.pylori*-infected subjects with a family history of gastric cancer. In contrast, prevalence of this phenotype was very rare in the subjects with a family history of gastric cancer but with no evidence of *H.pylori* infection and in *H.pylori*-uninfected subjects with the or without a history of gastric cancer. This observation was consistent with interaction between the infection and a host genetic factor, resulting in a histological and physiological phenotype known to lead to gastric cancer.

The above findings raised many questions regarding the presence of a potential host genetic factor which could interact with the infection and result in gastric cancer. The interleukin-1 gene was the first candidate. It is known that if the infection induces increased production of interleukin-1 by the gastric mucosa and that this cytokine is a powerful inhibitor of acid secretion, it could therefore induce hypochlorhydria in response to the infection [69, 70]. The group found that the pro-inflammatory genotypes were twelve times more common in *H.pylori*-infected subjects with hypochlorhydria and achlorhydria than in infected subjects without this phenotype. In further studies they were able to show that subjects with the pro-inflammatory interleukin-1 genotype had an increased risk of going on to develop gastric cancer [71]. In other side, it is also known that the pattern of gastritis is influenced by gastric acid secretory status. For example, long-term suppression of gastric acid secretion by proton pump inhibitors will transform an antral predominant non-atrophic gastritis to a body predominant gastritis and will also accelerate the development of atrophy [72]. Consequently, the reduction in acid secretion induced by the increased interleukin-1 production will also lead to pangastritis or body predominant gastritis and atrophy.

### 2.3.2. Hypochlorhydria or Atrophic Gastritis?

It is now well established that the subjects in whom *H.pylori* infection induces corpus-predominant gastritis have the greatest risk of gastric cancer [73]. There are also a number of observations indicating that the low acid secretion itself may be an important factor in the carcinogenic process. These are discussed below:

I) For many years it has been recognised that gastric cancer develops against a background of chronic hypochlorhydria [13, 74].

II) Epidemiological studies have indicated that *H.pylori*-infected duodenal ulcer patients have a very low risk of gastric cancer [75]. These subjects have dense colonisation of their antral mucosa with the bacterium and the associated severe antral gastritis. The fact that they do not develop gastric cancer indicates that the infection and inflammation

themselves are not sufficient to induce cancer and that other factors must be involved. The duodenal ulcer patients as discussed above are characterised by high acid output, and this may protect them from processes involved in carcinogenesis.

III) El-Omar et al tested the hypothesis that *H.pylori*-induced hypochlorhydria is associated with an increased risk of gastric cancer by studying acid secretion in first degree relatives of patients with cancer of the mid or distal stomach [(76)]. In that study they discovered that 50% of first degree relatives of gastric cancer patients with *H.pylori* infection had gastric acid hyposecretion compared with only 5% of *H.pylori* infected subjects without family history of gastric cancer. This indicates that there is a close association between the risks of gastric cancer and the development of gastric acid hypochlorhydria in response to *H.pylori* infection.

IV) Hansen et al in a collaboration with our team have also tested the hypothesis that gastric acid hyposecretion predisposes patients to the cancer in a large Norwegian cohort study in which serum samples were stored prior to development of the cancer [(77)]. Subjects with *H.pylori* infection had an overall 3-fold increased risk of developing gastric cancer of the mid or distal stomach. The study was further analysed by measuring serum gastrin concentrations in the stored serum as a surrogate marker of *H.pylori*-induced hypochlorhydria. This indicated that subjects with a high serum gastrin level indicative of hypochlorhydria and *H.pylori* infection had a seven-fold increased risk of developing cancer compared to subjects with the infection and a normal gastrin level.

The original Correa hypothesis for gastric cancer proposed that subjects with gastritis developed atrophy and that the atrophy resulted in hypochlorhydria [(41)]. At present, there is no doubt about relationship between atrophic gastritis and gastric cancer [(76-81)]. In other side, there is a close association between atrophic gastritis and hypochlorhydria. As a consequence, it is unclear whether it is the atrophy itself that predisposes patients to cancer or whether it is the acid hyposecretion present in patients with atrophy that predisposes them to gastric cancer. The hypochlorhydria is then a key factor in the carcinogenic process in that it allows colonisation of the gastric lumen with bacteria able

to synthesise carcinogenic nitrosamines which would induce progressive damage to the gastric mucosa, leading to more severe atrophy, dysplasia and carcinoma. Sobala et al showed that subjects with *H.pylori*-induced hypochlorhydria have in addition to bacterial colonisation, also increased intragastric nitrite [82] concentration and profound depletion of gastric juice ascorbic acid [82, 83] which will also facilitate the formation of nitrosamines [84].

According to El-Omar et al, *H.pylori* gastritis can cause chronic hypochlorhydria or achlorhydria in the absence of atrophy [45]. It is therefore possible that it is the hypochlorhydria that predisposes patients not only to the development of cancer but also to development of the atrophy as a result of epithelial damage by nitrosamines formed in the neutral stomach.

Recent support for this concept that *H.pylori*-induced hypochlorhydria may lead to atrophy comes from the observations in which *H.pylori*-infected subjects have been treated with proton pump inhibitory therapy [85]. These studies have indicated that *H.pylori*-infected subjects with hypochlorhydria who have undergone proton pump inhibitory therapy develop corpus atrophy, which is not seen in *H.pylori*-infected subjects who were not treated with these drugs and whose intragastric pH, therefore, remains acidic. This again supports the concept that hypochlorhydria is a key factor in determining the development of atrophy in response to *H.pylori* infection.

### 2.3.3. Summary and Conclusion

Gastric acid secretion represents a key role in the most benign and malignant upper gastrointestinal disorders. *H.pylori* infection as the most common bacterial infection worldwide exerts variable effects on gastric acid secretion. In some subjects it stimulates increased acid secretion, and this appears to be the key mechanism by which it results in duodenal ulcer disease. In other subjects, it results in profound inhibition of gastric acid secretion, and this is associated with an increased risk of gastric cancer. It can be seen that the development of gastric cancer is a complex, multistage and multifactorial process.

The various stages involve the progression from *H.pylori* superficial gastritis to atrophic gastritis with intestinal metaplasia and hypochlorhydria and then further progression to the development of dysplasia and cancer. Numerous co-factors are involved in promoting or inhibiting this progression. There is increasing evidence that the hypochlorhydria *per se* may interact with the *H.pylori* gastritis in such a way as to facilitate carcinogenesis. Finally, it should be mentioned that in the majority of subjects, *H.pylori* infection results in no overall change in gastric acid secretion and these subjects rarely develop a clinical disease.

## Section 2.4

# Gastric Histology, Serological Markers and Age as Predictors of Gastric Acid Secretion in *H.pylori* Infected Subjects

## 2.4.1. INTRODUCTION

There is a close association between the level of gastric acid secretion and the type of disease affecting the upper gastrointestinal tract. Duodenal ulceration occurs in subjects with high acid secretion [86-90], gastro-oesophageal reflux disease and its complications in subjects with normal [91] or high levels of acid secretion [92-94], gastric ulceration in subjects with moderately reduced secretion [95] and gastric cancer in patients with profoundly reduced or absent acid secretion [96-99].

The level of acid secretion is thought to play a role in the aetiology of these common upper gastrointestinal diseases. Increased gastric acid secretion and duodenal acid load predispose to duodenal ulceration as demonstrated by the Zollinger-Ellison syndrome [100, 101]. Absence of gastric acid secretion is thought to predispose to gastric cancer by allowing colonization of the stomach by carcinogen synthesizing bacteria and also by the reflex hypergastrinaemia stimulating cell proliferation [102-104]. The role of acid in the aetiology of gastro-oesophageal reflux disease is demonstrated by the therapeutic efficacy of acid inhibitory medication.

There has been a marked change in the pattern of upper gastrointestinal disease in the western world over the past century. The incidence of gastric cancer has progressively fallen [105-107], that of duodenal ulceration risen and then fallen and the incidence of reflux disease and its malignant complications has increased [107, 108]. These changes in the incidence of the disease are thought to be due in part to a rise in gastric acid secretion. There are also marked geographical variations in incidence of upper gastrointestinal disease. Reflux disease and its complications are more common in the western versus eastern world and the opposite trend is seen for gastric cancer. Again, these variations are thought to be related in part to regional variations in levels of gastric acid secretion.

A major factor known to influence gastric acid secretion is *Helicobacter pylori* infection which colonises the gastric mucosa of more than 50% of the world's populations. The effect of the infection on acid secretion is related to the pattern of gastritis which it induces in the stomach. Previous studies have examined acid secretion and *H.pylori* gastritis in specific diseases e.g. duodenal ulceration, gastric ulceration and gastric cancer [109-111]. In the present study, we have examined the relationship between the pattern of *H.pylori* gastritis and gastric acid secretion in a large number of subjects without specific upper gastrointestinal disease. For various reasons, acid secretion is now rarely measured and we have therefore also assessed the ability to predict the level of acid secretion from gastric histology and serological markers.

The aim of this study is assessment of the value of gastric histology, serological markers and other characteristics of patients as predictors of gastric acid secretion in *H.pylori* infected subjects.

## 2.4.2. METHODS AND MATERIALS

### 2.4.2.1. Study Population

The study involved 255 *H.pylori*-positive dyspeptic patients with normal upper gastrointestinal endoscopy (i.e., no evidence of mucosal ulcerations, erosions, or neoplastic changes) who had undergone acid secretory studies in our unit over the past 10 years. Subjects were not included if they had taken proton pump inhibitor therapy in the past year or H2 blocker therapy within the previous 3 weeks. Their *H.pylori* infection was confirmed by positive results in at least one of two methods, $C^{14}$ urea breath test and

gastric histology. 123 (48.2%) of them were male and 132 (51.8 %) were female. Mean age was 43.4 years with standard deviation of 13.2 and range of 18 to 84 years. Patients' median (± interquartile range) height, weight and BMI were 167 (± 16) cm, 70 (± 19) Kg and 25 (± 5) Kg/m2, respectively.

### 2.4.2.2. Histologic Assessment

Endoscopic biopsies of both the antrum and body region of the stomach were available in 175 of the patients. The age and sex distributions of this group were not statistically different from rest of cases. The processed biopsies were stained with hematoxylin-eosin and *H.pylori* –specific Cresyl fast violet stain. All slides were assessed by a single pathologist for *H.pylori* density as well as type and severity of gastritis. The histological criteria applied to the evaluation of gastritis were based on the Updated Sydney Classification of Gastritis as follows [(112)]: *Gastritis:* Presence of inflammatory cells of any type in the lamina propria. *Grade of inflammation:* Severity of infiltration of lymphocytes and plasma cells. *Activity of inflammation:* Presence of neutrophils in the inflammatory infiltrate. The sum of the grade and activity was expressed as *combined inflammatory score* (CIS) for each participant with maximum possible score of 6 [(113)]. *Mucosal atrophy* was defined as the separation of the mucosal glands and a decrease in the thickness of mucosa, greater in severity than that seen in the inflammation of the lamina propria and usually associated with an increase in the stromal matrix. *Intestinal metaplasia:* Presence of goblet cells with or without other cellular elements of intestinal mucosal and glandular epithelium.

### 2.4.2.3. Acid Output Measurement:

After a 12-hour fast, an orogastric tube (Anderson Inc., New York, NY) was swallowed, and its position in the dependent part of the stomach was checked using the water recovery test [(114)]. Intermittent suction was then applied using an intermittent suction unit (Omeda, Columbia, MD) that applies suction for 20 seconds in each 32-second cycle. An

intravenous infusion of Pentagastrin (Peptavlon; ICI, Cheshire, England) was started at a rate of 0.6 µg.kg-1.h-1 to stimulate maximal acid secretion. Gastric juice was collected for 1 hour in 15- minute aliquots.

The volume of each 15-minute gastric juice collection was recorded, and its hydrogen ion concentration was measured by titration with 0.1 mol/L NaOH to pH 7 using an autotitrator (Radiometer ETS 822; Copenhagen, Denmark). The acid output per 15-minute period was then calculated by multiplying the volume by the hydrogen ion concentration. Maximal acid output in response to Pentagastrin (MAO) was calculated by taking the mean of two highest consecutive 15-minute collections and expressed as millimoles per hour.

#### 2.4.2.4. Serologic Assay

Blood was obtained from each patient during the visit to the endoscopy unit. Separated serum samples were stored at -70°C until analyzed. Serum pepsinogen I (PG I) and pepsinogen II (PG II) were assayed with enzyme immuno-sorbant assay (ELISA) methods using monoclonal antibodies to pepsinogen I and II (BIOHIT diagnostics, Biohit LTD, UK). Serum gastrin-17 was measured using ELISA kit from the same supplier. All procedures were done according to the manufacture's instructions and results of PG I and PG II reported in µg/L and pmol/L for gastrin-17. PG I/II ratio was calculated and reported in fraction. *H.pylori* cag-A antibodies were determined using a polyclonal EIA method, as previously described [(115)].

#### 2.4.2.5. Statistical Analysis

Except for MAO values, all other variables had relatively non symmetric distribution, so a non-parametric test of Spearman's-rho was used for bivarate correlations. All results were presented with two-sided significance level. Partial correlation analysis with zero-orders was used to estimate possible confounders. For testing the differences of mean MAO in different levels of gastritis and atrophy we used oneway ANOVA and post hoc

Dunnett test, if appropriate. Mann- Whitney U test was used for comparing age of male and female patients.

Stepwise linear regression was applied for determining histologic and serologic predictors of MAO. Body atrophy score, antral atrophy score, serum PG I, serum PG I/II ratio, serum gastrin, age, *H.pylori* density of antrum, body, or *H.pylori* in one of two sites, and body combined inflammatory score were selected as independent variables and MAO as the dependent variable. The main criteria for the probability of F to enter and F to remove of independent variables were <=0.05 and >=0.10, respectively. In addition to standardized coefficients, co-llinearity statistics were presented for each of variables. For most statistical analysis we used SPSS v12.0 software.

### 2.4.2.6. Ethical Approval

This study was approved by the western infirmary ethics committee.

## 2.4.3. RESULTS

### 2.4.3.1. Acid output and *H.pylori* related gastritis

The density of *H.pylori* colonization of the antral mucosa was positively associated with the acid output with a correlation coefficient of (CC) 0.31 ($p<0.01$), but the *H.pylori* density of the body was not correlated significantly with the acid secretion (CC= -0.11, $p=0.19$). Recalculation of this relationship using mean score of *H.pylori* density at any site showed a positive correlation with acid output (CC=0.17, $p<0.05$).

Chronic inflammation of the antrum was positively associated (cc=0.27, $p<0.01$) with acid output, while there was a more potent negative association between the chronic inflammation of the body mucosa and acid output (cc= -0.41, $p<0.01$) (fig 2.1). Active inflammation in the antrum did not show any significant relationship with the acid output but in the body it showed a negative correlation (CC= -0.28 and $p<0.01$) with the acid output. Combined (i.e. active plus chronic) inflammatory scores of the antrum showed a positive correlation with the acid output, reaching a peak of 29.3mmol/hr at histological score 4. However, from score 4 to 6 we found slight decrease in the acid output with the lowest value of 24.2mmol/hr at score 6. Combined inflammatory score of the body after a peak of 33.7mmol/hr at score 2 showed persistent downward trend with minimal acid output of 9.5 at score 6. Statistical tests with ANOVA showed significant difference between mean the acid output among patients with different combined inflammation scores ($F=6.9$, $p<0.5$). Also significant inverse correlation of body combined inflammation with acid output was apparent (CC= -0.50 and $p<0.01$) (fig 2.2).

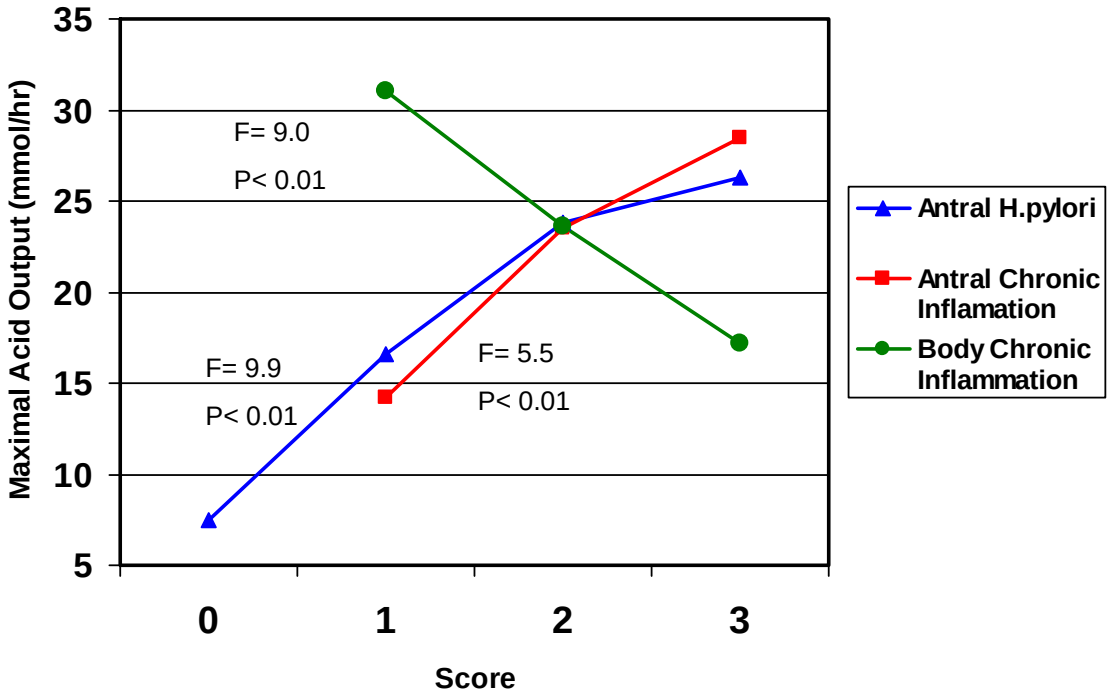



**Fig 2.1:** Relationships of H.pylori infection, antral and body chronic inflammations with maximal acid output

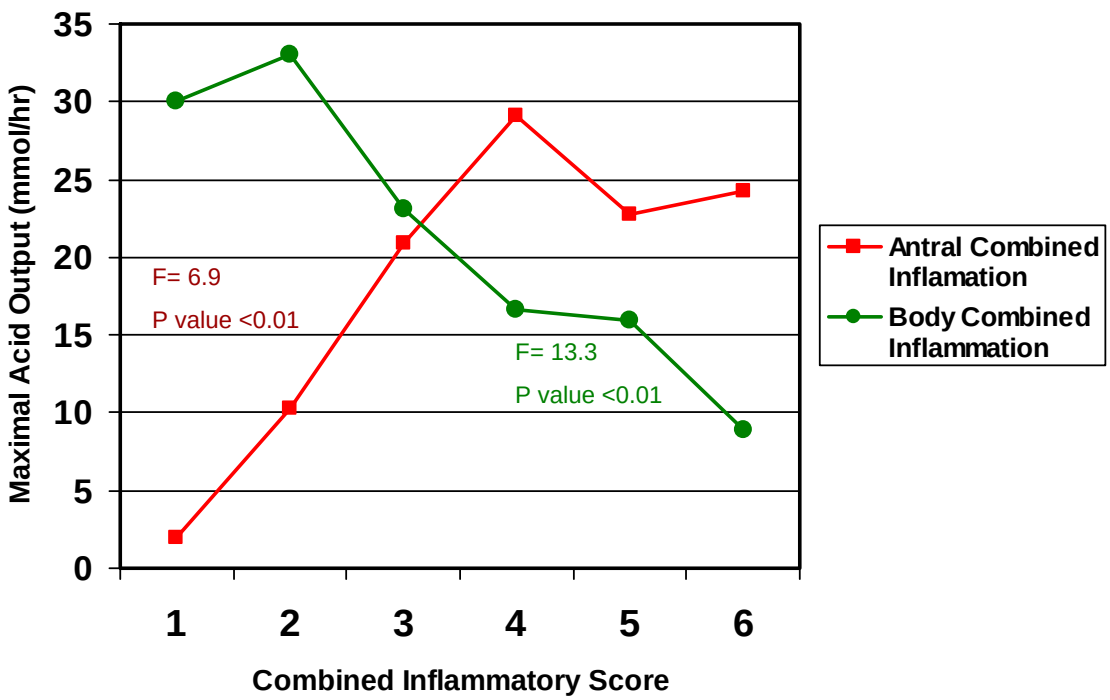



**Fig 2.2:** Relationship of antral and body combined inflammations with maximal acid output

Two other indices, body/antral chronic inflammation ratio and body/antral combined inflammation ratio, were tested for their relationship with the acid output. Both of them showed significant inverse correlation with the acid output, with -0.49 ($p<0.01$). As shown in Fig 2.3, the body / antrum ratio of active inflammation had a similar inverse correlation with the acid output but with a relatively weak significance (CC= -0.17, $p=0.07$ and $F=3.0$, $p= 0.05$).

The density of *H.pylori* per each score of combined inflammation also was evaluated regarding relationship with the acid output. Those indices at both antral and body sites were positively correlated with the acid output with correlation coefficients of 0.29 ($p<0.01$) and 0.38 ($p<0.01$), respectively.

### 2.4.3.2. Acid output and gastric atrophy

Antral-confined atrophy was detected in 19 (12.6%) and atrophy limited to body in 27 (17.9%). Only 11 patients (7.3%) had multifocal atrophy. Mean (± sd) acid output in non atrophic patients was 29.1 (±12.2) mmol/hr. In patients with antral atrophy only, the acid output was 19.5 (±11.1), in subjects with body atrophy only and multifocal atrophy, the acid output was reduced at 11.9 (±7.2) and 11.5 (±10.1)mmol/hr, respectively. Using oneway ANOVA, there were significant differences between groups regarding acid output ($F=22.0$, $p<0.01$). Dunnett t test showed that acid output in each of the groups with atrophy was statistical different compared with non-atrophic group (all $p<0.05$) (Fig 2.4).

As presented in Fig 2.5, both antral and body atrophy scores had significant inverse correlation with acid output. The CC for antral atrophy was -0.30 ($p<0.01$) and for body atrophy it was -0.59 ($p<0.01$). The ratio of body/antral atrophy also showed prominent reverse relationship with acid output from 27.4 mmol/hr with ratio <1 to 10.1 mmol/hr with ratio equal or greater than one (Fig 2.6).

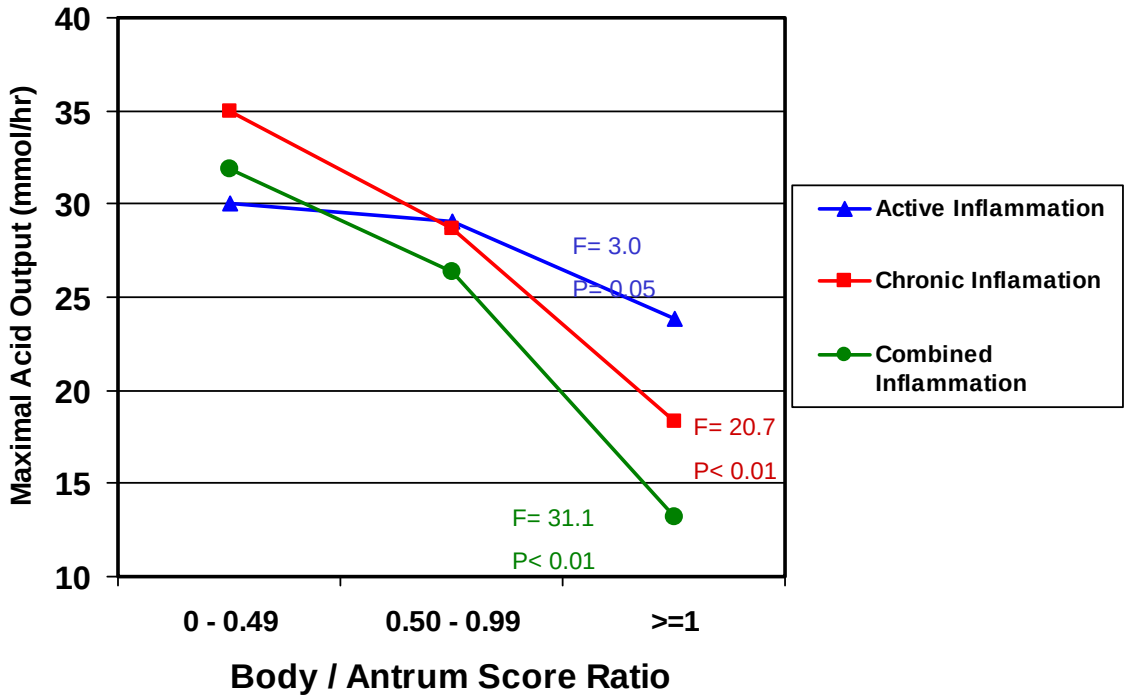



**Fig 2.3:** Relationships of body / antrum ratios of active, chronic and combined inflammation with maximal acid output

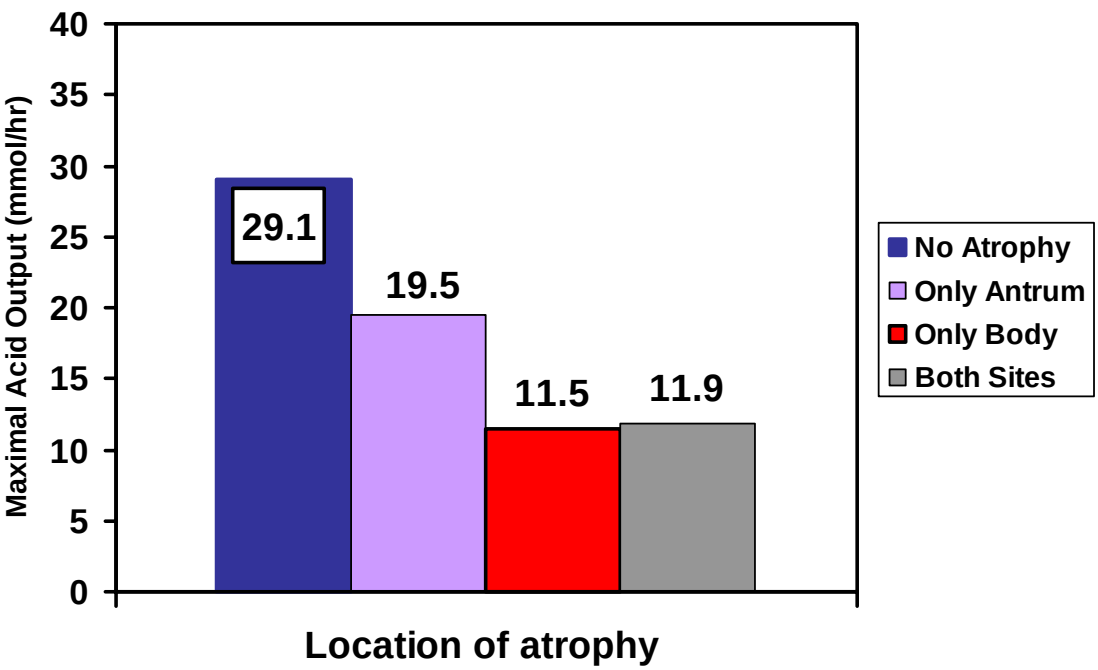



**Fig 2.4:** Mean values of maximal acid output in patients with atrophy at different locations

Zero-order partial correlation showed that the relationship between body atrophy and acid output could be exaggerated with age and body combined inflammation. After controlling the age, the correlation co-efficient dropped form -0.54 to -0.48 (11% decrease). Controlling body combined inflammation also decreased the correlation of body atrophy score and acid output from -0.54 to -0.40 (26% fall). By controlling both variables, the correlation co-efficient dropped from -0.54 to -0.47 (31%).

Intestinal metaplasia at both antral and body sites had negative relationship with acid output with cc= 0.23 ($p<0.01$) and cc= 0.20 ($p<0.05$), respectively.

### 2.4.3.3. Acid output and PG I, PG I/II ratio, gastrin-17

The median serum PG I and PGII levels of all participants were 146.9 µg/L (Interquartile range 97.0) and 9.6 µg/L (Interquartile range 8.0), respectively. Calculated median PG I/II ratio was 15.1 (Interquartile range 11.0). Serum PG I was correlated positively with the acid output at CC= 0.38 ($p<0.01$). There was no relationship between the acid output and serum PG II, but the ratio of PG I/II had a positive CC=0.30($p<0.01$) with the acid output (Figs 2.7 and 2.8).

Median serum gastrin-17 of enrolled patients was 2.2 pmol/L. There was significant correlation between the acid output and serum gastrin-17.

### 2.4.3.4. Cag-A serology; relationship with acid output and gastric histology

In 177 patents who had enough serum for anti Cag A serology, the mean acid output was similar in patients with positive versus negative Cag-A serology with 28.3 (±11.2) and 26.7 (±12.3) mmol/hr, respectively. However re-observation of the correlation between anti Cag A antibody titre and inflammatory histologic parameters revealed its significant relationship with antral active inflammation [cc= 0.35, p value<0.01], body active inflammation [cc=0.33, p value<0.01] and body chronic inflammation [cc=0.24, p value<0.05]. There was no strong and significant relationship between anti Cag A antibody and atrophic or metaplastic changes in gastric mucosa.

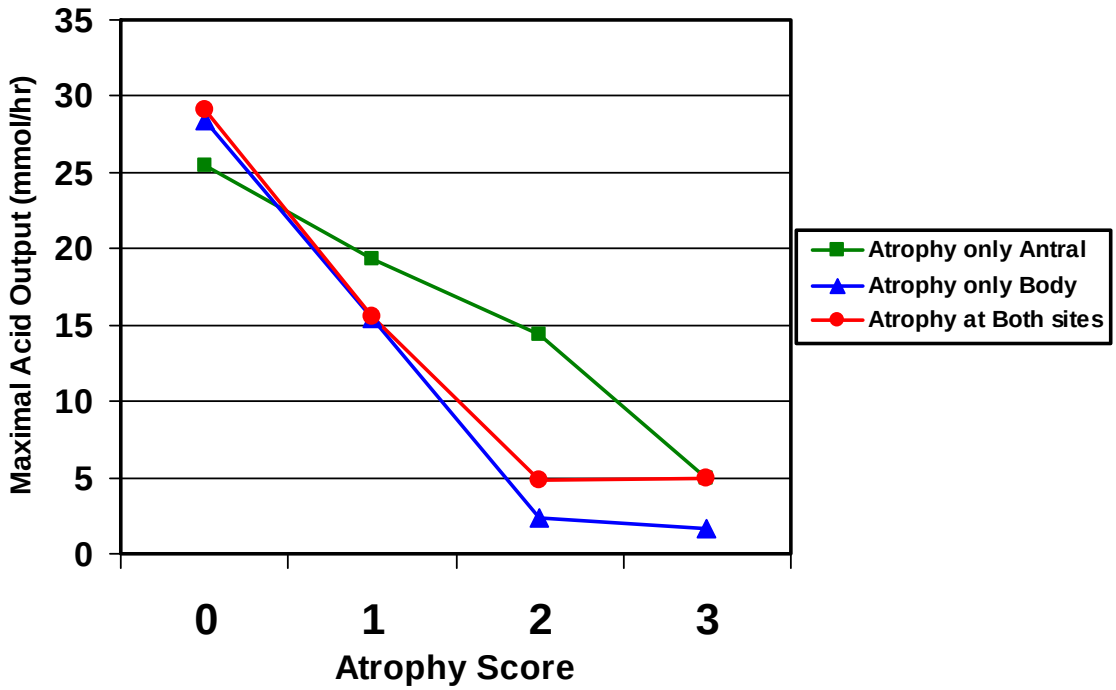



**Fig 2.5:** Relationships of antral and body atrophy with maximal acid output

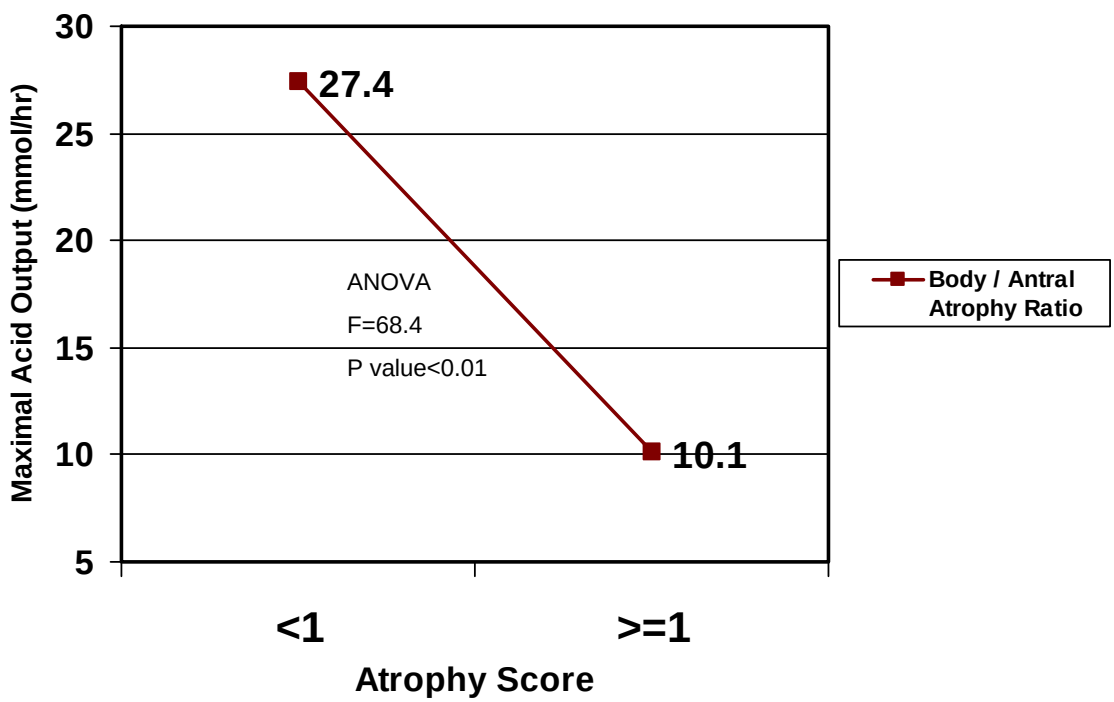



**Fig 2.6:** Relationship of body / antral atrophy ratio with maximal acid output

### 2.4.3.5. Other factors associated with acid output

Age had inverse relationship with acid output (CC: -0.44 and p<0.01) (Fig 2.9). Calculation of relationship between acid output and age in male and female separately, revealed slightly greater inverse relationship among males than females (-0.41 vs. -0.38).

Regarding gender, male patients had mean acid output 28.5(±13.2) mmol/hr and females 21.4 (±10.4) mmol/hr. Statistically, the difference with independent t test was significant (p<0.01). It should be mentioned that female patients were slightly older than males (median age 44 vs. 40, Mann-Whitney U test: p<0.05). Two other variables, body combined inflammatory score and body atrophy were evaluated in the both sexes, and we found no significant difference between males and females.

Smokers, who smoked 3 or more cigarettes per day had mean acid output=29.9(± 9.1) mmol/hr compared with non-smokers who had mean acid output of 26.5 (±12.6) mmol/hr. The difference between the two groups was at borderline significance (p= 0.05). The major histological changes such as antral combined inflammation, body combined inflammation and body atrophy were not statistically different between smoker and non smoker groups, but serum level of PG I was significantly higher in smokers than non-smokers [250.8 (Interquartile range:117) Vs. ( 190.5 (Interquartile range: 138) p<0.05].

The height of patients showed a positive significant correlation with acid output, with CC= 0.43 and P<0.01. Weight also had positive but less potent relationship (CC=0.26, p<0.01) with acid output but BMI did not show any significant relationship (CC=0.02, p=0.84). Gender subgroups were not different regarding relationship of acid output and these variables.

### 2.4.3.6. Best predictors of acid output

In order to determine the best predictors of acid output, all variables which had obvious and significant linear relationship with acid, were selected. Body atrophy score, antral atrophy score, serum PG I, serum PG I/II ratio, serum gastrin, age, *H.pylori* density of

antrum, body, or *H.pylori* in one of two sites, and body combined inflammatory score were entered as independent variables and acid output as dependent variable. The stepwise model produced four models. In the final model, according to standardized coefficients, body combined inflammation with Beta of -0.34, body atrophy (Beta= -0.30), serum PG I (Beta= 0.26) and age (Beta= -0.26) were the most independent predictors of acid output. All other variables were excluded from model according to enter and remove criteria. The R and R square in the final model were 0.79 and 0.62 respectively (table 2.1).

**Table 2.1: Summary of stepwise linear regression analysis for predictors of maximal acid output**

| **Predictors** | **Unstandardized Coefficients** | | ***Standardized Coefficients*** | **P value** | **Co-linearity Statistics** | |
|---|---|---|---|---|---|---|
| | B | SE | *Beta* | | Tolerance % ♣ | VIF ¶ |
| Intercept | 42.38 | 7.00 | | 0.00 | | |
| Body Combined Inflammation | -2.56 | 0.79 | ***-0.34*** | 0.00 | 67 | 1.50 |
| Body Atrophy | -5.44 | 1.94 | ***-0.30*** | 0.01 | 63 | 1.60 |
| Age | -0.33 | 0.11 | ***-0.26*** | 0.00 | 97 | 1.04 |
| Serum PG I | 0.04 | 0.01 | ***0.26*** | 0.01 | 86 | 1.16 |

Dependent variable: Maximal acid output

R= 0.79, R square= 0.62, Standard error of the estimate= 8.43

♣ Tolerance is the percentage of the variance in a given predictor that cannot be explained by other predictors.

¶ A variance inflation factor greater than 2 is usually considered problematic regarding co-linearity by other predictors.

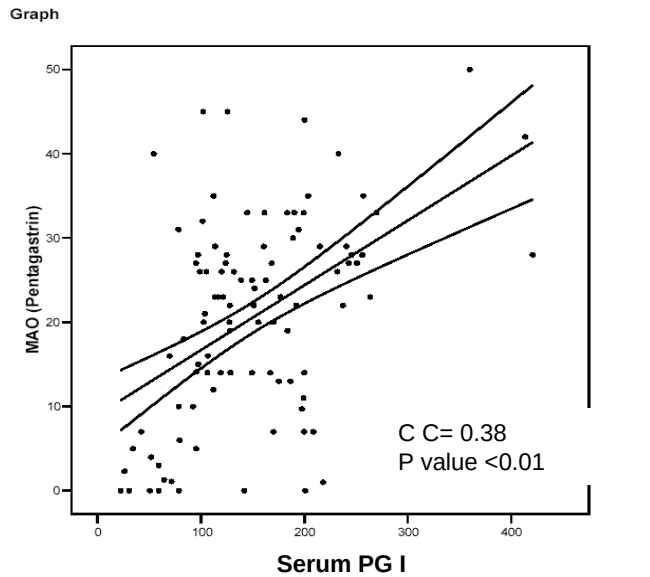



**Fig 2.7:** Relationship of serum pepsinogen I with maximal acid output

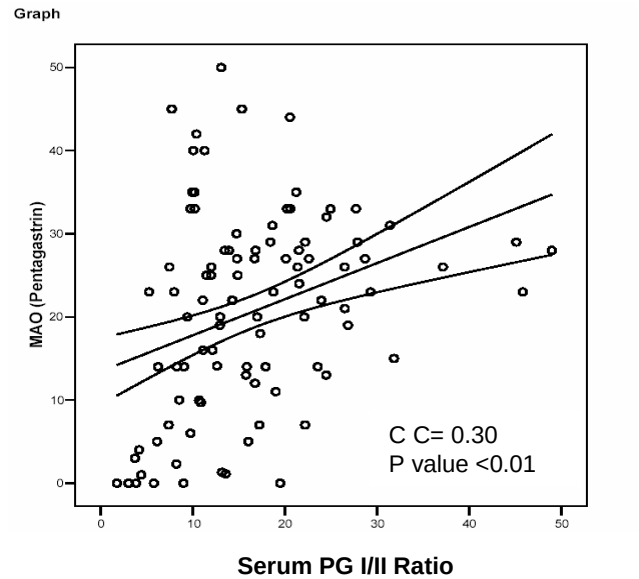



**Fig 2.8:** Relationship of serum pepsinogen I/II ratio with maximal acid output

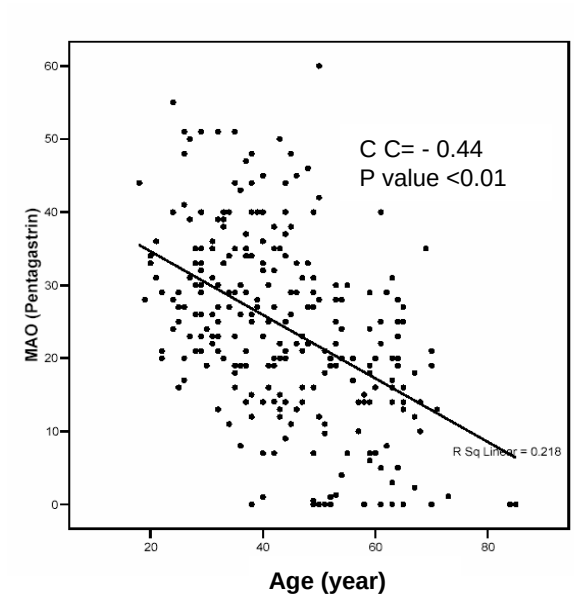



**Fig 2.9:** Relationship of age with maximal acid output

## 2.4.4. DISCUSSION

*H.pylori* infection produces a wide variety of pattern of gastritis [116-125]. The pattern of gastritis varies in the extent to which it involves the antrum versus body region of the stomach and in the extent to which it produces acute inflammation, chronic inflammation, intestinal metaplasia and atrophy. Our current study indicates that these different patterns of gastritis are associated with different levels of gastric acid secretion.

The antrum and body region of the stomach both play an important role in regulating gastric acid secretion. The antral mucosa contains the G cells, which release the gastrin. This hormone is released in response to ingestion of protein-containing foodstuffs and it stimulates the oxyntic mucosa to secrete acid [126]. The hormone acts on the gastrin receptors on the ECL cells of the oxyntic mucosa causing them to release histamine, which acts in a paracrine fashion, binding to the $H_2$ receptors on the parietal cells and thereby stimulating to secrete acid [127-129]. Gastrin also exerts a trophic influence of the ECL cells and parietal cells of the oxyntic mucosa [130-131].

*H.pylori* gastritis influences the function of the antral mucosa, stimulating increased release of gastrin by the G cells [132-136]. It is unclear whether this is due to the influence of inflammatory cytokines [137-139] or due to the ammonia produced by the bacterium's urease activity raising antral surface pH and thus blocking the physiological inhibition of gastrin release by luminal acid [140]. *H.pylori* antral gastritis will thus tend to increase gastric acid secretion by increasing gastrin release. If the infection produces atrophy of the antral mucosa, the rise in gastrin will be moderated due to loss of G cells [141-143]. As gastrin is released by ingestion of food, the main influence of the antrum is in meal-stimulating acid secretion which is technically difficult to measure. We assessed acid secretion in response to stimulation by exogenous gastrin, which acts directly on the oxyntic mucosa.

Our study will therefore not have detected changes in meal stimulated acid secretion due to disturbances in antral function.

The current study demonstrates the strong correlation between *H.pylori* body gastritis and reduced gastric acid secretion. Both inflammation and atrophy of the body mucosa were strongly associated with reduced gastric acid secretion. The effect of the atrophy is clearly explained by loss of acid secreting parietal cells. The inflammation of the body mucosa is thought to produce functional inhibition of acid secretion. We, and others, have shown that eradicating *H.pylori* in patients with low acid secretion produces recovery of secretion, which is associated with resolution of the body gastritis without any change in atrophy [143, 144]. The mechanism by which *H.pylori*-induced inflammation impairs the function of the oxyntic mucosa is not clear. However, the infection stimulates production of interleukin-1 beta, which is a powerful inhibitor of acid secretion, blocking the release of histamine from the ECL cells and also inhibiting the function of the parietal cells [145, 146]. The current study clearly shows that atrophy and inflammation are both independently associated with low acid secretion.

We were able to investigate the relationship of maximal acid output and anti Cag A antibody. While there was a significant correlation between the severities of antral and corpus active inflammation and anti Cag A antibody, the mean acid output was similar in patients with positive versus negative Cag A serology. A more intense inflammatory infiltrate both in gastric antral and body mucosae has been shown previously [58]. Cag A positive infection is also associated with an increased prevalence of atrophy [59]. However, the distribution of the inflammation between the antral and body region of the stomach and the associated disturbance in gastric secretion is unrelated to the strain of the infection [58]. Infection with the more virulent Cag A positive strain of *H.pylori* infection thus increases the risk of developing either gastric cancer or duodenal ulcer disease but the infection does not in itself determine which of these two outcomes are more likely [60].

The serum concentrations of pepsinogens are indicators of gastric atrophy and consequently of gastric secretory function. Pepsinogen I originate from gastric fundic

glands [147] whereas pepsinogen II is secreted by glands of the body, and antral region of the stomach as well as proximal duodenum [148]. We found pepsinogen I the most useful predictor of the disturbance of acid secretion in our *H.pylori* infected subjects. This is consistent with our histological studies confirming that atrophy of the body mucosa was the most powerful predictor of low acid secretion as pepsinogen I is a strong predictor of body atrophy.

We were also able to examine the influence of a variety of demographic characteristics of the patients on acid secretion. Our data showed that smokers who smoked 3 or more cigarettes per day had greater acid secretion compared to non-smokers. There was no significant relationship between cigarette smoking and major histological factors but our data revealed higher level of PG I among smokers. Chronic smoking is associated with higher gastric acid secretion in both ulcer and non-ulcer subjects [149-152]. In addition there is a significant positive association between the number of cigarette smoked and the magnitude of acid secretion [153]. Nicotine administrated intravenously stimulates acid secretion in man [154]. Chronic cigarette smoking stimulates the vagus and activates the parietal cells to enhance acid output [153]. Increased serum PG I in smokers was another finding of our study which could be related to their higher acid secretion with parallel increased chief cell secretory capacity [150].

Our data indicate a marked decline in acid secretion with increasing age in both male and female *H.pylori* infected subjects. The median acid output in 20 year-olds was 35 mmol/h and fell to 15 mmol/h in 70 year olds. This negative association between acid secretion and age in *H.pylori* infected subjects has been previously observed [155]. In contrast, acid output has been reported to remain steady or increase with age in *H.pylori* uninfected subjects [156]. The lower acid secretion in the older *H.pylori* infected subjects can be explained partly (but not fully) by more severe body gastritis and higher prevalence of atrophy [155, 157, 159]. It is unclear to what extent the higher prevalence of low acid secretion and higher prevalence of body gastritis and atrophy in older subjects is due to increasing age or a cohort effect where those born at an earlier time have increased

predisposition to develop these abnormalities. In some patients with severe body atrophy, autoimmune gastritis may be an aetiologic factor for hypochlorhydria in advanced age. That may results as a consequence of interaction between *H.pylori* infection and host response to the organism's antigens, and to gastric auto-antigens including gastric $H^+/K^+$ ATPase [160].

Our study showed that male patients have greater acid output than females. This finding is consistent with previous work [158, 161] but its biologic explanation has not been clearly defined. In females, smaller gastric surface area and corresponding smaller parietal cell mass or lower parietal cell reactivity or both may be responsible [161, 162]. The height of our patients positively correlated with acid output, and this effect was similar in both sexes, suggesting body surface could explain more acid secretion in males. Iijima et al reported that in *H.pylori* infected subjects, there was a more marked fall in acid output with increasing age in males versus females [155]. This is consistent with our current finding.

Due to the number and variety of factors affecting acid secretion and the potential for interaction between them, we performed stepwise linear regression analysis. This indicates four independent factors: 1) body inflammation, 2) body atrophy, 3) serum pepsinogen I and 4) age are powerful predictors of acid secretion. Combination of these factors had strong relationship with acid output and could be used for prediction of acid output in individual patients.

It should be emphasized that this study shows the association between the patterns of gastritis and gastric acid secretion but does not in itself indicate the nature of the association. Previous studies have shown that eradication of *H.pylori* infection causes recovery of gastric acid secretion in patients with body predominant atrophic gastritis thereby providing evidence that this histological pattern of gastritis can cause inhibition in acid secretion [143, 144, 164, 165]. However, it is important to recognise also that acid secretion may affect the pattern of gastritis. This is demonstrated by the fact that inhibition of acid secretion by proton pump inhibitors causes the pattern of gastritis to change from an

antral predominant gastritis to a body predominant gastritis and may also cause acceleration of the atrophic process [166, 167].

Irrespective of the complex nature of the association between the pattern of gastritis and acid secretion, our current study does provide data allowing the prediction of acid secretion based on gastric histology, serological markers and patient characteristics. This may be useful in estimating levels of acid secretion in different populations as well as in individual patients.

# Chapter 3 Upper Gastrointestinal Cancer; Epidemiology & Risk Factors

## LOOKING AHEAD

This chapter deals with the upper gastrointestinal tract adenocarcinoma comprising adenocarcinoma located in distal oesophagus and stomach. Although tumours of proximal part of duodenum are anatomically classified as upper gastrointestinal tract, this topic is not discussed in this overview because of rarity of its malignant tumours.

It should be mentioned that the term of gastric cancer in almost all of the medical literature refers to gastric adenocarcinoma, the most common histological type of gastric cancer. Also, anatomical classification of gastric cancer to proximal and distal commonly refers to tumours located at cardia and non-cardia regions of the stomach, unless alternative definitions provided.

## 3.1. Epidemiology of Upper Gastrointestinal Adenocarcinoma

### 3.1.1. Gastric Cancer

Gastric adenocarcinoma, the main histological type of gastric cancer was said to be the commonest primary cancer in the world in 1980, with almost 670,000 new cases in that year [(1)]. By 1990, it was overtaken by carcinoma of the lung, partly because of the rising incidence of lung cancer, but also because gastric cancer is one of the tumours whose frequency is falling worldwide [(1, 2).]

According to GLOBOCAN 2002, there were an estimated 930,000 new incident and 700,000 mortalities from gastric cancer worldwide [(3)]. Overall age-standardised incidence rates were 22.0 and 10.3 per 100,000 per year in males and females, respectively, with corresponding mortality rates being 14.3 and 8.3 per 100,000 [(3)]. Gastric cancer was the third

most common cause of male cancer after cancers of lung and prostate and the second most common cause of male cancer death (after lung cancer). It was the fifth most common cause of female cancer (after cancers of breast, cervix, large bowel and lung cancers and the fourth most common cause of female cancer death [3].

There are remarkable geographic and ethnic variations in gastric cancer incidence in the world and in its trends for each population through time. The lowest rates have been reported from North America, Western Europe and North Africa and the highest in East Asia, South America and Eastern Europe (Fig 3.1). According to most recent edition of Cancer Incidence in Five Continents (vol IX) [4], reporting incidence rates from population-based cancer registries worldwide, some Japanese populations with an age-standardised rate as high as 80, still have a very high risk of gastric cancer. The incidence rates of gastric cancer in Gharbiah of Egypt (ASR: 3.3 in males and 2.0 in females) and Mumbai of India (4.6 in males and 2.3 in females) are clear examples of low risk areas of North Africa and South Asia, respectively. The wide variation of gastric cancer incidence rate shows more than 15-fold difference between lowest and highest risk populations.

One feature of gastric cancer however, that is consistently observed in all populations (reflected in a comparison of rates by gender in Figure 3.1) is the approximately doubling of the age-standardised risk in males compared to females. In general, female incidence rates at a given age are equivalent to male rates at an age 10-15 years younger. The consistency of this difference has never been adequately explained [5]. The male predominance of gastric and also oesophageal adenocarcinoma appears to be related to the different time of onset rather than the different rate of cancer development, and this topic has been investigated deeply in chapter 6 of this thesis.

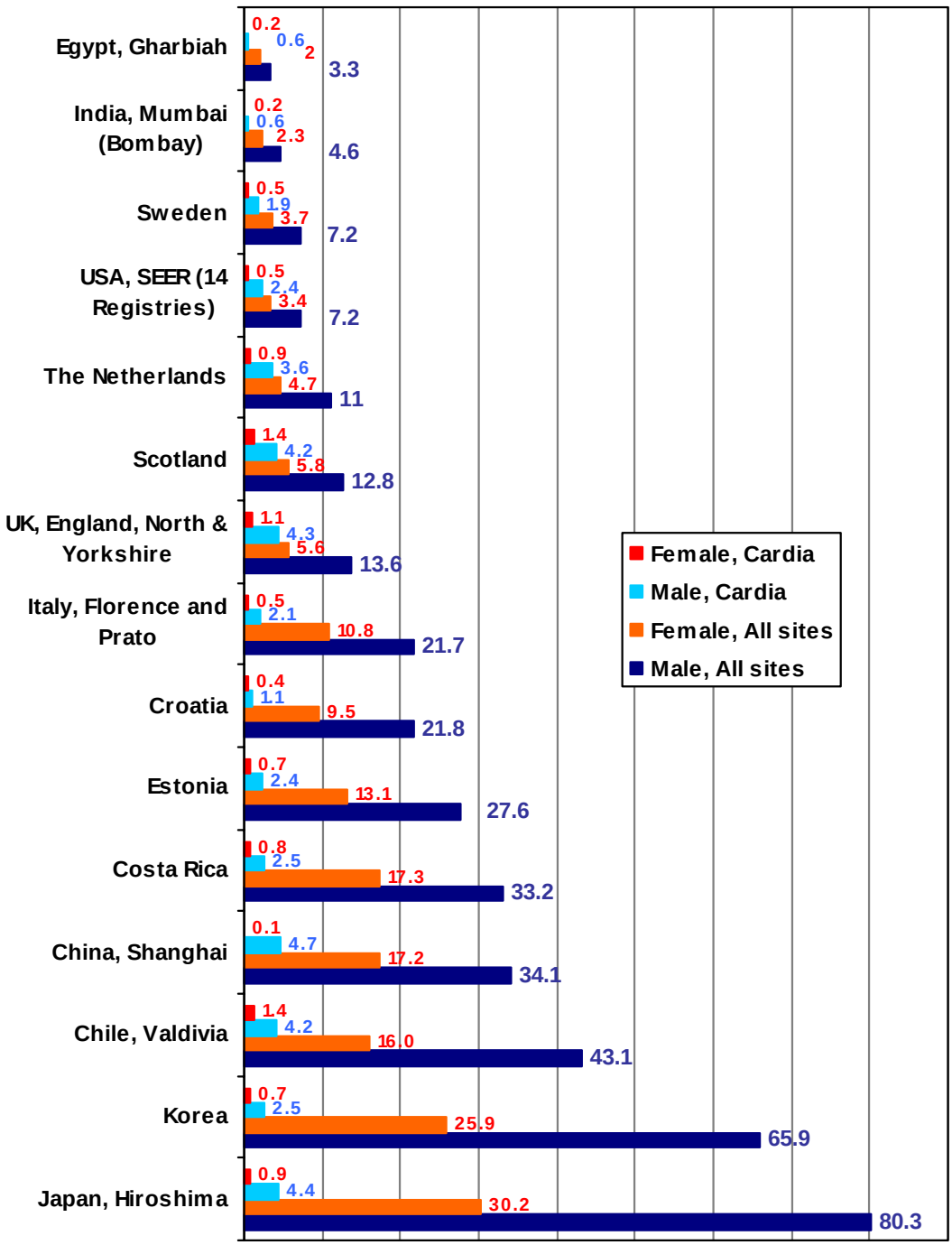



**Fig 3.1.** Incidence rates of gastric cancer per 100,000 person-years for selected cancer registries world wide by sex. Data are presented in format of age-standardised rate by world standard population of all sites and cardia site in separate bars. Source: Cancer Incidence in Five Continents Vol. IX, IARC 2007.

### 3.1.2. Gastric Cancer; Cardia versus Non-Cardia

Despite the small size of cardia relative to the remaining parts of stomach, cancer of this area represents distinct etio-pathological features. Until recent decades, almost all epidemiological findings related to gastric cancer considered as a single entity. In fact, in 1991, a novel observation reported that the incidences of adenocarcinomas of the oesophagus and the gastric cardia were increasing more rapidly than those of any other cancers in the United States. The observation repeated the same result in an updated report [6,7]. Although the reliability of these reports was challenged after a decade by Corley et al [8] who showed that at least a part of the phenomenon could be explained by sub-site misclassification, the reports attract an increasing number of studies focused on distinctive pathogenesis of cardia cancer.

Currently, according to global cancer data, less than 10% of all gastric cancers are located in cardia, but there are very wide variations in reported proportions from different cancer registries. While, the incidence of gastric cancer is low in North America and Western European countries (Fig 3.1), the regions have showed much larger proportion of cardia cancer compared to other populations. Based on the most recent data from IARC [4], cardia cancer accounts for more than 54% of male gastric cancer cases in Montana, USA and the overall reported figures of the other populations of the same country are more than 30%. In Europe, Denmark (43%), Switzerland and UK (overall more than 30%) have largest recorded proportion of cardia cancer. In contrast, in East Asia, Central and South America which have been recorded as high risk areas for gastric cancer, the proportion of cardia cancer is almost always less than 10%.

In spite of considerable worldwide variations in proportion of cardia sub-site to all gastric cancers, the actual incidence rate of cardia cancer appears to have less disparity throughout different populations. The highest registry-based report from Europe was from Scotland with WASR (standardized with world population) of 4.2 and 1.4 in males and females, respectively. The overall incidence rate (WASR) of Cardia cancer in North America is less than 3.0 in males and 0.7 in females. Interestingly, while contribution of cardia cancer to the incidence rate of gastric cancer in Japan is considerably lower than in North American and European populations, the actual incidence rate of cardia cancer is similar or even higher than the latter populations. The highest population-based incidence rate of gastric cardia cancer was reported from China (Nangang), being 13.3 and 5.7 in males and females, respectively [(4)].

### 3.1.3. Oesophageal Adenocarcinoma

Oesophageal cancer, comprising all histologic types, is the eighth most common cancer worldwide, responsible for 462,000 new cases in 2002 (4.2% of the total), and sixth most common cause of death from cancer with 386,000 deaths (5.7% of the total) [(9)]. Worldwide, most oesophageal cancers are squamous cell carcinomas, arising in the middle and lower third of the oesophagus. Recently, there appears to be an increase in western countries in relative and absolute numbers of adenocarcinomas of the lower third of the oesophagus [(10)]. Before the mid 1970s, the incidence of adenocarcinoma of the oesophagus seems to have represented less than 5% of all oesophageal cancers. Thereafter, the incidence of adenocarcinoma of the oesophagus has increased rapidly in the USA [(11-14)] and in the most parts of Europe [(15-17)], and is still rising [(18, 19)]. This increasing trend cannot be explained by changes in the classification of the tumours located near or in the gastro-oesophageal junction (the tumours classified as oesophageal instead of cardia) because the increasing incidence is evident both in adenocarcinoma of the oesophagus and adenocarcinoma of the gastric cardia. The reasons for the increasing incidence are still unknown [(19)].

## 3.2. Histologic Classification of Gastric Adenocarcinoma

As there are marked differences in structure and differentiation, not only between separate carcinomas, but also within individual tumours of gastric cancer, several histological classifications have been used in the practical and academic medicine. This section will introduce each classification briefly and will discuss the Lauren classification and its implications in aetiology and the pathogenesis of gastric adenocarcinoma.

### 3.2.1. The Lauren Classification

Lauren introduced the classification system in 1965. According to the original definition, gastric adenocarcinoma can be divided into three categories: intestinal, diffuse and mixed types [(20)]. In general, intestinal type tumours have a glandular pattern usually accompanied by papillary formations or solid components. The glandular epithelium consists of large pleomorphic cells with large hyperchromatic nuclei often with numerous mitoses. They are usually fairly well polarized columnar cells, sometimes with a prominent brush border and goblet cells. Mucin secretion is variable and can be seen focally in the cytoplasm of scattered cells or extracellulrly in the lamina of neoplastic glands. By contrast, diffuse type adenocarcinomas are predominantly composed of poorly cohesive and widely infiltrating small tumour cells with indistinct cytoplasm and regular, only faintly hyperchromatic though often pyknotic nuclei without many mitoses. Gland formation is unremarkable, except sometimes in the superficial part of the tumour. Mucin secretion is common and extensive throughout the tumour. Signet ring cells are common, and there may be extracellular mucin in the stroma . Connective tissue proliferation is more marked and inflammatory cell infiltration less prominent than in intestinal cancers [(20, 21)].

In general, the mean age of patients with intestinal type tumours is higher than those with diffuse type [22-26]. There is also a considerable gender-based difference in the incidence and age of the onset of intestinal type cancer. While the incidence of diffuse type adenocarcinoma is equal in males and females, intestinal type shows a significant male predominance [25-28].

The overall proportion of intestinal type is higher than that of diffuse type gastric cancer in most populations [20, 24], but this proportion varies with anatomical location of the tumour. In cardia and the proximal part of stomach, a great majority of adenocarcinomas are intestinal type, but in non-cardia cancer, this predominance is less significant [23, 26, 29].

The different carcinogenesis pathways leading to intestinal or diffuse type adenocarcinoma have received much more attention. The intestinal subtype of gastric adenocarcinoma arises due to the progression of chronic superficial gastritis to atrophic gastritis to intestinal metaplasia to dysplasia and finally cancer [30]. The strong relationship of intestinal type adenocarcinoma with each step of this cascade, *H.pylori* infection, atrophic gastritis and intestinal metaplasia has been documented [31-37]. The diffuse type has a similar association with *H.pylori* infection [31-33], but it represents little relationship with severe atrophic gastritis and intestinal metaplasia [34-37]. Diffuse type gastric cancer is also seen in inherited gastric cancer due to germline E-cadherin mutation [38, 39].

### 3.2.2. World Health Organization (WHO) Classification

This system categorizes gastric adenocarcinoma into four categories, namely papillary, mucinous, tubular, and signet ring cell type. Papillary adenocarcinoma is composed of pointed or blunt finger like epithelial processes with fibrous core. This tumour usually makes a polypoid mass into the lumen of the stomach. Tubular adenocarcinoma consists of branching glands embedded in, or surrounded by, a fibrous stroma. Mucinous carcinoma contains large amounts of extracellular mucin in more than 50% of the tumour. In some cases, the cells from glands are lined by columnar mucus-secreting mucosa. In others there are disaggregated

clusters of cells which appear to be floating in lakes of mucin. Signet ring cell carcinomas do not form tubules. They are composed largely of mucin-secreting signet ring cells but also contain cells with no mucin and cells with eosinophilic granular cytoplasm containing neutral mucin. Tumours composed predominantly of signet ring cells are more common in younger patients and in the distal stomach [21, 40, 41]. All of the above types, with the exception of signet ring cell carcinoma, may be graded as well, moderately or poorly differentiated [21]. In spite of excellent reproducibility of the WHO classification, it has poor prognostic value in clinical practice compared to other classification systems [42-44].

### 3.2.3. Other Classifications

**Ming Classification:** This classification was introduced by Ming in 1977. This divides tumours into an expanding type (67%) and an infiltrative type (33%). In the expanding type, the tumour cells are surrounded by small amounts of fibrous tissue with a variable inflammatory response. It is very often accompanied by intestinal metaplasia in the adjacent mucosa. This compares roughly to the intestinal type of Lauren classification. The infiltrative type is ill-defined and contains widely infiltrative tumour cells with poor inflammatory cell response. Accompanying intestinal metaplasia is less common [45]. Again, descriptive features of this type are very similar to the diffuse type in the Lauren classification. Therefore, this system appears to have no significant advantages to the original Lauren classification nor did it show any prognostic value [46, 47].

**Mulligan and Rember Classification:** This classifies gastric adenocarcinoma into mucus cell type, intestinal type and pylorocardiac gland type. It is only the latter variant that distinguishes this classification from that of Lauren and, as the name suggests, it occurs predominantly in the cardia or pylorus. Pylorocardia carcinomas are well-demarcated oxophytic tumours, frequently with surface ulceration. These tumours are commoner in men and are

characterized microscopically by varying-sized glands lined by stratified or singly oriented cylindrical cells that often show striking vacuolation or clear cell change and stain brilliantly with the periodic acid-Schiff reaction [21, 48].

**The Goseki Classification:** This classification was introduced by Japanese pathologist, Goseki in 1992. It attempts to encompass undifferentiated carcinoma as well as the more common types. It is based on tumour histology and includes four grades, based on tubular differentiation and intracellular mucin production. Group I consists of well differentiated tubules with poor intracellular mucin production; group II consists of well differentiated tubules and plentiful intracellular mucin; group III has poorly differentiated tubules and poor intracellular mucin; and group IV tumours are made up of poorly differentiated tubules and plentiful intracellular mucin [49].

**The Carneiro Classification:** Carneiro proposed a new classification in 1997 that is again based on four histological types. These includes glandular and isolated cell carcinomas that are roughly equivalent to the intestinal and diffuse carcinomas of the Lauren classification, a solid variety composed of sheets, trabeculae or islands of undifferentiated cells with no glandular formation, and a mixed type that consists of a mixture of glandular and isolated cell types. Glandular tumours were commonest in the Portuguese population studied, followed by mixed, solid and isolated cell types in descending order [21, 50].

## 3.3. Risk Factors of Gastric and Oesophageal Adenocarcinoma

Development of gastric cancer is result of a multistage process which starts with a superficial non-atrophic gastritis. Except in a small number of cases, the progression of *H.pylori*-induced chronic gastritis to adenocarcinoma is the main carcinogenic pathway. Many environmental, endogenous and microbial factors are involved in this multifactorial process. Oesophageal adenocarcinoma also develops against an inflammatory background mainly induced by the gastroesophageal reflux of acid and bile. In this section, I will review briefly the most common risk factors of the upper gastrointestinal adenocarcinomas of gastric cardia, non-cardia and oesophagus.

### 3.3.1. Helicobacter pylori Infection

After the discovery of *H.pylori* by Marshall and Warren in 1984 [(51)], findings of the most of investigations introduced the bacterium as the main cause of chronic gastritis and peptic ulcer disease. Until 1988 there was no direct evidence of the involvement of *H.pylori* in gastric cancer [(52)]. Now the relationship between gastric cancer and *H.pylori* infection is one of the most investigated area in medical literature with more than 2500 scientific paper indexed by Index Medicus.

The association of *H.pylori* with gastric cancer has been evaluated extensively and I believe that summarising all of the published studies in this limited space is impossible, so a review of a few meta- analysis results may cover most of the high quality investigations. Huang et al [(53)] identified 19 qualified studies and reported a summary odds ratio for gastric cancer in all sub-sites of 1.9 (95% CI: 1.32 – 2.78) [(54-72)]. Cancer of gastric cardia showed a weaker relationship with *H.pylori* than those on non-cardia location (1.23 vs. 3.08; P=0.003).

As expected, association of early gastric cancer with *H.pylori* was more prominent than advanced tumours (6.35 vs. 2.13; P=0.01).

At the same time, Eslick et al reviewed 42 studies including most of publications covered by previous meta-analysis and found an overall odds ratio of 2.04 (95% CI: 1.69-2.45). This study showed no difference between the location of tumour and the power of association [(73)].

Early gastric cancer is considered as an early stage in the development of gastric cancer, and it seems to be more a reliable model for the assessment of relationship between gastric cancer and *H.pylori* infection. In a new meta-analysis conducted by Wang et al [(74)], they summarised results of 15 studies on early gastric cancer and showed that the prevalence of *H.pylori* infection was significantly higher in patients with early gastric cancer (87.3%) than in non-neoplasm controls (61.4%) (OR 3.38, 95% CI: 2.15–5.33) [(75-89)]. In six studies the prevalence of *H.pylori* infection in early gastric cancer was significantly higher than in advanced gastric cancer (OR 2.13, 95% CI: 1.75–2.59) [(75, 77, 78, 80, 86, 87)]. No significant difference in the prevalence of *H.pylori* infection was seen between the patients with intestinal type and those with diffuses type early gastric cancer [(75, 78, 79, 71, 75, 88, 90-92)].

#### 3.3.1.1. *H.pylori* Virulence and Gastric Cancer

Although about half of the world's adult population is infected with H. pylori, only a small minority develop gastric cancer. The results of a sophisticated study by Uemura et al showed that only 2.9% of 1246 *H.pylori*–infected patients developed gastric cancer after a mean endoscopic follow-up period of 7.8 years [(93)]. Variation in many environmental and host factors and their interactions can explain the different outcomes, and the *H.pylori* strain variability may play a role. Carriage of strains with the cag pathogenicity island, a large chromosomal region that encodes virulence genes, including the cag A gene, is associated with an increased risk of the development of peptic ulcer disease as well as gastric precancerous lesions and gastric adenocarcinoma [(94-97)]. A meta analysis by Huang et al

showed that among *H.pylori* infected populations, infection with cag A-positive strain further increased the risk of gastric cancer by 1.64 fold overall and 2.01 fold for non-cardia cancer. Gastric cardia cancer was not affected [98]. In Asian populations, there in no consistency in the results of studies, while some previous authors found no association [99, 100], newer reports showed an increased risk of gastric cancer [101, 102]. In addition, vac A- and bab A-positive *H.pylori* strains are also associated with an increased gastric cancer risk [103-105].

### 3.3.2. Atrophic Gastritis and Intestinal Metaplasia in Gastric non-Cancer

Gastric atrophy was first recognized in 1870 in the post mortem samples of a patient with pernicious anaemia [106]. Atrophic gastritis has been defined as the loss of specialized glandular tissue, including loss of the oxyntic glands containing parietal cells in the gastric corpus [107]. Also, intestinal metaplasia which is often associated with atrophic gastritis is defined as the replacement of original gastric glands with straight tubular crypts lined by absorptive and goblet cells similar to the mucosa of small intestine (complete) or colon (incomplete) and accompanied by inflammatory infiltrates in the lamina propria [108]. Chronic atrophic gastritis, as an intermediate step of the carcinogenesis cascade of gastric cancer has showed a strong relationship with gastric adenocarcinoma, particularly intestinal subtype [109-112]. *H.pylori* infection induces superficial non-atrophic gastritis which progresses to atrophic gastritis with loss of acid secretion and then intestinal metaplasia to dysplasia and cancer. A variety of bacterial, host and environmental factors are known to contribute to the progress through these different pre-cancerous stages [113]. Traditionally, the relationship between atrophic gastritis and gastric cancer has been defined for non-cardia cancer, but our recent studies showed that even adenocarcinomas located at the cardia region may demonstrate a relationship with atrophic gastritis, particularly those with least relationship with gastroesophageal reflux disease [114, 115].

There is a considerable correlation between the geographic distributions of atrophic gastritis and of gastric cancer. Many studies have showed high prevalence of atrophic gastritis in high risk area for gastric cancer; i.e. Japan with a histologic detection rate of atrophic gastritis in 53%, Estonia, in up to 64% of studied population, in Finland 27 - 44%, and in Columbia with 45% in comparable age groups [116-120]. In contrast, results of investigations using the same histologic methods for the diagnosis of atrophic gastritis in populations with low risk of cancer revealed much lower prevalence of this precancerous change, e.g. 28% in Sweden and 22% in Australia [121, 122]. Cancer risk rises exponentially with grade and the extent of atrophic gastritis, and is found approximately 45 to 90-fold in patients with severe atrophic gastritis compared with the cancer risk in subjects with healthy stomach [111, 123].

Atrophic gastritis is generally present as either a multifocal or a diffuse pattern in gastric mucosa and usually is followed by and/ or associated with the appearance of metaplastic glands. A form of mucous metaplasia that has been termed pseudopyloric metaplasia, also known as spasmolytic polypeptide-expressing metaplasia (SPEM), is more strongly associated with gastric cancer than intestinal metaplasia and might be the precursor to the cancerous lesion [124, 125]. The increased risk of cancer following SPEM has also been documented in some mouse model studies [126, 127].

The loss of normal mucosal glands leads to a failure in normal secretory functions of the gastric mucosa. Atrophy in the corpus results in low output of acid, whereas atrophy in the antrum results in impairments in the output of gastrin-17. In atrophic gastritis, the feedback loop controlling acid and pepsin secretions via the gastrin link is broken, resulting in varying degrees of hypochlorhydria, or even achlorhydria, and in hypo- or hypergastrinaemia, depending upon whether the antrum is atrophic or not [111]. The histological grade of atrophic gastritis and the accompanied chronic gastritis of corpus mucosa have a strong negative correlation with acid output, and also with serum/plasma levels of pepsinogen I (or PG I/II ratio) as discussed in the previous chapter. Advanced atrophic corpus gastritis and loss of the

feedback inhibition of antral G cells by low intragastric acidity result in hypergastrinaemia, and the gastrin-17 levels in serum may even be some hundreds of pmols per litre in some subjects if the antrum is normal. The associated metaplastic glands also can not secrete acid or gastrin-17 [111]. With the progression of atrophy, the metaplastic glands and epithelium may appear more and more immature, which is shown as a shift from IM of the complete (''small-bowel type'') type to IM of immature and incomplete types (''colon type''). This shift is considered to reflect an increasing risk of cancer in atrophic gastritis [128]. The hypochlorhydria or achlorhydria in the stomach allows colonization of bacteria other than *H.pylori*, some of which may produce mutagenic and carcinogenic substances [119, 129].

### 3.3.3. Atrophic gastritis and Intestinal Metaplasia in Cardia Cancer

Carditis and intestinal metaplasia of cardia are rather common findings in routine practice and also in populations with high risk of gastric cancer [130, 131]. Gerson et al found that 25% of asymptomatic subjects undergoing screening programme had evidence of intestinal metaplasia at the gastrooesophageal junction [132]. This can be explained by the fact that this anatomical region is subject to damaging effects of both *H.pylori* infection and gastro-oesophageal reflux. This chronic damage contributes to the metaplastic formation and extension of apparent cardia mucosa and is also likely to be key factor in the development of neoplasia at this site [133].

Association of atrophic gastritis with cardia cancer is a new topic and has received less attention in medical literature. In general, atrophic gastritis has not been defined for cardia mucosa; therefore most reported associations of cardia cancer with atrophic gastritis measure the presence and /or severity of atrophy in all gastric mucosa other than cardia.

Unlike the well recognized association of atrophic gastritis with non-cardia cancer, atrophic gastritis has a divergent relationship with cardia cancer. In our study on patients with cardia cancer from Norway which will be presented in next chapter, we found a significant negative

association between *H.pylori* infection and cardia cancer. However, we found that in subjects who were *H.pylori* seropositive, their risk of cardia cancer was markedly increased if they also had evidence of atrophic gastritis [134]. In another study, the nature of association of two types of cardia cancer with atrophic gastritis and gastroesophageal reflux disease was examined on cardia cancer patients from Iran. Cardia cancer was positively associated with both severe gastric atrophy and with frequent GORD symptoms, though the latter association was only apparent in the non-atrophic subgroup and in the intestinal subtype. The association of cardia cancer with atrophy was stronger for the diffuse versus intestinal subtype, and this was the converse of the association observed with non-cardia cancer [115]. These findings indicate two distinct aetiologies of cardia cancer, one arising from severe atrophic gastritis and being of intestinal or diffuse subtype similar to non-cardia cancer, and another related to GORD and intestinal in subtype, similar to oesophageal adenocarcinoma.

### 3.3.4. Smoking and Gastro-oesophageal Adenocarcinoma

According to recent IARC Monograph on Tobacco Smoke and Involuntary Smoking the results from both cohort and case -control studies are consistent with a causal role of tobacco smoking in the development of gastric cancer [135]. In a large population-based cohort of 669570 Korean men [136], yielding 127 cardia and 2409 non cardia cancer, a moderate association was found between smoking and cardia (RR: 2.2; 95% CI: 1.4-3.5) and non-cardia cancers (RR: 1.4; 95% CI:1.3-1.6). In another large study in USA [137], current smokers were at increased risk of cardia cancer (HR: 2.9, 95% CI:1.7- 4.7) and gastric non-cardia cancer (HR: 2.0, 95% CI:1.3 - 3.2). In a study by Sjodahl et al in Norway, the risk of gastric cancer was almost twice as high in daily smokers (HR: 1.9, CI 95%: 1.3 -2.7) as in never smokers. Attributable risk (AR) of gastric cancer was 18.4% [138]. This was similar to the results of European prospective study (EPIC) [139], with an estimated 17.6% of the risk of

gastric cancer attributable to smoking and somewhat higher than the worldwide estimated 11% attributable risk derived from a 1997 meta-analysis of smoking and stomach cancer [140].

With regard to anatomic subsite of gastric cancer and smoking, there is considerable discrepancy in reported associations. While many studies found a significant increase in the risk of cardia cancer [141-146], other studies observed a significant increase in the risk of non-cardia cancer [147-151]. Results of a pooled analysis of two large prospective studies from Japan [152] and also our study on Iranian patients revealed no association of smoking with gastric cardia cancer [115].

There have been controversial results regarding the role of tobacco smoking as a risk factor for oesophageal adenocarcinoma. While there is some evidence of the lack of significant association between oesophageal adenocarcinoma and smoking [115, 144, 153], other studies suggest weak to intermediate associations. A case-control study nested in General Practitioner Research Database in the UK by Lindblad et al showed a weak association (OR= 1.5, 95% CI: 1.1 – 2.0) between current smoking and oesophageal adenocarcinoma [154]. Two large population-based studies also revealed weak to moderate increased risk of oesophageal adenocarcinoma by smoking [143, 155]. This result has been supported recently by two case–control studies with considerable association between oesophageal adenocarcinoma and current smoking [137, 156].

### 3.3.5. Gastroesophageal Reflux Disease (GORD)

#### 3.3.5.1. GORD and Oesophageal Adenocarcinoma

Gastroesophageal reflux disease is the well-established risk factor of oesophageal adenocarcinoma. One of the best pieces of evidence of the association of GORD symptoms with oesophageal adenocarcinoma was introduced by Lagergren et al in 1999 [157]. They showed that among persons with recurrent symptoms of reflux, as compared with persons

without such symptoms, the odds ratio was 7.7 (95% CI, 5.3 -11.4) for oesophageal adenocarcinoma. There was also significant positive correlation between the frequency, severity, and duration of reflux symptoms and the risk of cancer. Among persons with long-standing and severe symptoms of reflux, the odds ratios was 43.5 (95% CI: 18.3 - 103.5) for oesophageal adenocarcinoma. This study was preceded by one study [158] and followed by many other studies supporting the strong association of reflux symptoms with increased risk of cancer [159-161]. Two of these studies found that both frequent GORD symptoms and a history of hiatus hernia were associated with increased risk for oesophageal adenocarcinoma [159, 160].

Barrett's oesophagus or columnar-lined metaplasia of oesophagus is a consequence of long-term GORD. Barrett's oesophagus is a precancerous lesion of oesophageal adenocarcinoma [162]. Although it is not completely established that Barrett's oesophagus is an obligatory step in the development of cancer, there is common belief that it is an intermediate stage between GORD and adenocarcinoma [163]. The cancer risk in patients with Barrett's oesophagus is much higher than those without it. In a recent large prospective study, the incidence of oesophageal adenocarcinoma in patients with Barrett's oesophagus who were under surveillance endoscopy was 1/220 patient–years follow up, equal to 0.45% per year [164]. This rate is obviously higher than 0.023% correspondent expected rate in patients with GORD [161]. In large sample-sized studies, the excess risk of oesophageal adenocarcinoma in patients with Barrett's has been estimated to be 30 to 60-fold relative to the risk of the general population [165-168].

### 3.3.5.2. GORD and Cardia Cancer

Surprisingly the association of gastric cardia cancer with GORD appears to be a topic of review articles rather than original researches. Review of a few available studies showed that unlike the oesophageal adenocarcinoma which represents a strong relationship with GORD, in the case of cardia cancer, this relationship is less marked [157, 169, 170] or even null [171].

A proportion of adenocarcinomas arising at the cardia may have a similar aetiology and pathogenesis to oesophageal adenocarcinoma. This could arise if only the most distal part of the oesophageal squamous mucosa is subject to chronic exposure to gastric juice. In all of the above mentioned studies, the association of cardia cancer with GORD is much weaker than that between reflux and oesophageal adenocarcinoma. This would therefore suggest that only a relatively small proportion of cardia cancers might be attributed to gastro-oesophageal reflux. However, recent studies have demonstrated that the most distal oesophageal mucosa is frequently exposed to acidic gastric juice in healthy volunteers without reflux symptoms [172]. This phenomenon has been referred to as short-segment reflux and it appears to be due to the opening of the distal oesophageal sphincter allowing the gastric acid to reach the first millimetre or two of the oesophageal squamous mucosa but not allowing the acid to reflux past the sphincter into the main body of the oesophagus. The proportion of cardia cancers arising from this short-segment reflux may therefore be substantially higher than the association between these cancers and symptoms of reflux would suggest. In our study, which will be presented in chapter 5 of this thesis, we showed that cardia cancer was positively associated with both severe gastric atrophy [OR, 95% CI: 3.92 (1.77 to 8.67)] and with frequent GORD symptoms [OR, 95% CI: 10.08 (2.29 to 44.36)] although the latter was only apparent in the non-atrophic subgroup and in the intestinal subtype. The association of cardia cancer with atrophy was stronger for the diffuse versus intestinal subtype, and this was the converse of the association observed with non-cardia

cancer. These findings indicate two distinct aetiologies of cardia cancer, one arising from severe atrophic gastritis and being of intestinal or diffuse subtype similar to non-cardia cancer, and another related to GORD and intestinal in subtype, similar to oesophageal adenocarcinoma [115].

### 3.3.5. Male Gender

A remarkable and unexplained characteristic of upper gastrointestinal adenocarcinoma is its male predominance. The higher incidence of these cancers in males has been observed in all parts of the world [3].

The male predominance of gastric cancer is related to the histological subtype of the tumour. Gastric adenocarcinoma may be of the intestinal or diffuse histological subtype as described by Lauren [20]. The gender phenomenon is more marked in gastric cancer of the intestinal versus diffuse histological subtype and this has been described well by Sipponen and colleagues [173].

Oesophageal adenocarcinoma also demonstrates a marked male predominance and tends to present at a younger age in males [174].

Global data from cancer registries suggests that the male predominance of upper gastrointestinal cancer is related to the anatomical location, which is higher for adenocarcinoma of the oesophagus and lower for adenocarcinoma of the distal stomach [175]. The male to female ratio of age-standardised incidence rates for oesophageal adenocarcinoma in Scotland is 4.5:1, for adenocarcinoma of the gastric cardia or gastro-oesophageal junction is 3.5:1 and for non-cardia cancer it is 2.0:1 [176]. However, the proportion of the intestinal subtype differs according to the anatomical site and it was unclear whether it is the anatomical site, or the histological subtype which is associated with the male gender predominance. In chapter 6, we will present results of our population-based study conducted on a randomised sample of all incident adenocarcinoma of the gastric cardia, non-

cardia and oesophagus, 1998-2002 of West of Scotland. Our findings indicate that the intestinal subtype has the greatest impact on the gender ratio, and this is unrelated to whether the carcinoma has developed in the oesophagus or distal stomach. Our study also indicates that the gender phenomenon is due to the development of the intestinal subtype of cancer being delayed by 17 years in females.

Chapter 4

# Gastric Phenotype in Gastric Cancer; Cardia Vs. Non-Cardia

## LOOKING AHEAD

## 4.1. INTRODUCTION

Several observations indicate that cancers of the cardia region of the stomach are etiologically different from those of the rest of the stomach. Cancers of the mid and distal stomach (non-cardia cancers) show a strong positive association with *H.pylori* infection whereas cardia cancer has been reported to have negative, positive or no association with *H.pylori* infection [(1)]. The incidence time trends of cardia and non-cardia cancer also differ with the latter falling while the former is remaining static or increasing [(2-5)].

Substantial advance has been made in our understanding of the aetiology of non-cardia cancer and, in particular, of the role of *H.pylori* infection. The highest risk of non-cardia cancer is in subjects in whom the infection has induced atrophic gastritis and low or absent acid secretion [(6)]. Non-cardia cancer is considered to be the result of progression from *H.pylori* superficial gastritis to atrophic gastritis and hypochlorhydria to dysplasia and finally to cancer [(7)].

The aetiology of cancer of the gastric cardia region remains poorly understood. One reason for this may be the anatomical complexity of the cardia. Cardia mucosa

extends from the oxyntic mucosa of the body of the stomach to the squamous mucosa of the distal oesophagus. It consists of columnar mucosa resembling that of the gastric antrum. In neonates the cardia mucosa is only a few millimetres in length [8]. In adults, the cardia mucosa may be larger and this expansion may occur by metaplasia of the adjacent mucosa into cardia-like mucosa [9-12]. Proximal extension of cardia mucosa can occur by metaplastic transformation of the squamous mucosa of the distal oesophagus – a phenomenon that may be induced by acidic gastro-oesophageal reflux. Distal extension of cardia mucosa may arise from atrophic gastritis of oxyntic mucosa with loss of specialized cells and most commonly induced by *H.pylori* infection [9, 13]. When patients present with adenocarcinoma involving the gastric cardia, it is usually impossible to determine whether the tumour has arisen from metaplasia of the distal oesophageal squamous epithelium, from metaplasia of gastric oxyntic mucosa or from original cardia mucosa.

In a large nested case-control study in the Norwegian population, we observed that *H.pylori* infection was associated with an increased risk of non-cardia cancer but with a reduced risk of cardia cancer [14]. The current study was undertaken to compare cancers at those two sites with respect to premorbid gastric mucosal atrophy and acid secretion.

**AIM**

The aim of this study was to examine the relationship between the state of the gastric mucosa and the risk of subsequently developing cardia versus non-cardia gastric cancer.

## 4.2. MATERIALS AND METHODS

### 4.2.1. Study Setting

This was a nested case-control study. It comprised 101,601 men and women enrolled in the Norwegian JANUS [15] cohort as blood donors in Oslo 1973-1986, as participants in the Oslo Study of Cardiovascular Disease 1972-1973[17], and as participants in the Norwegian Counties Study 1974-1978 carried out by the National Health Screening Service in the three counties Oppland, Sogn og Fjordane, and Finnmark [17,18]. All solid gastric cancers diagnosed among the cohort members through 1992 were identified in The Cancer Registry of Norway. Cases were limited to individuals with available historical serum from whom primary tumour tissue could be verified as gastric adenocarcinoma with unequivocal non-cardia or cardia origin. Of 230 identified gastric cancer cases, fifty-seven were excluded from the study because of no remaining or wasted serum (7), no tissue (2) or metastatic tumour tissue only (10) available for histologic examination, histological type diagnosed or suspected to be other than adenocarcinoma (6), doubt about the stomach being the primary site of the tumour (2), gastric resection prior to cancer diagnosis (16), or disseminated tumour growth precluding determination of cardia/non-cardia subsite origin (14). To each cancer case, controls were matched according to gender, date of birth (within 54 months, 97% within 12 months, median deviation 3 months), date of serum sampling (within 17 months, 99% within 12 months, median deviation 3 months) , and serum source (blood donors, the Oslo Study of Cardiovascular Disease, or county within Norwegian Counties Study). Three controls were matched to 162 cases, and two controls were matched to 11 cases.

### 4.2.2. Cancer Subsites and Subtypes

The gastric adenocarcinoma cases were subsite classified as tumours of the cardia, fundus, body, antrum, or pylorus in accordance with ICD-O2 [19] after review of all clinical, biopsy, and resection reports submitted to the Cancer Registry. When needed, endoscopy and radiology reports were consulted. Cardia cancers were defined as tumours whose centre was judged to be within 2cm distal to the gastro-oesophageal junction. Adenocarcinomas largely or entirely located within the distal oesophagus were excluded. All adenocarcinoma diagnoses were verified on new pathology slides of biopsy or resection specimens and subtype classified according to Laurén [20] as intestinal, diffuse, or other. The *other* subgroup closely corresponds to *mixed* in newer terminology [21]. Five cases were subtype-unclassifiable due to insufficient biopsy size.

### 4.2.3. Serologic Tests

Serum had been collected from each cohort member at start of follow-up and thereafter kept frozen at –25$^{o}$C. Time elapsed between last meal and serum sampling was categorised as <1hour, 1-2 hours, 2-4 hours, 4-8 hours, and >8 hours. Serum anti-*H.pylori* IgG antibody concentration (average of two readings) was measured using the Pyloriset$^{TM}$ EIA-G test kit (Orion Diagnostica, Espoo, Finland). For detection of current or previous *H.pylori* infection (ever infection), we chose a cut-off of 250 U/l, which is lower than the cut-off of 500 U/l recommended by the manufacturer for detection of current infection [14]. The results of the *H.pylori* serology have been previously published [14]. Serum gastrin concentration was measured using antibody R98 which detects both Gastrin 17 and Gastrin 34[22]. Serum pepsinogen I and pepsinogen II concentrations were measured using radioimmunoassay kits (Sorin Biomedica Diagnostics, Saluggia, Italy).

We validated the ability of serum pepsinogen I to pepsinogen II ratio (PGI/II) to detect atrophy. This was performed using stored serum from 175 *H.pylori* positive patients with non-ulcer dyspepsia who had undergone endoscopy with antral and body biopsies. Atrophy of body and antrum was graded as absent, mild, moderate or severe according to updated Sydney classification of gastritis [(23)]. Low PG I/II was valuable in detecting atrophy involving the gastric body. At cut off point = 2.5, sensitivity and specificity of PG I/II was 71% and 67%, respectively for body atrophy of any severity. The area under the ROC curve was 0.84 (95% CI: 0.69-0.99) for body atrophy of any severity. The median (± interquartile range) of PG I/II in patients with none, mild, moderate and severe atrophy was 5.93 (± 3.5), 4.73 (± 3.9), 3.11 (±3.5) and 1.85 (± 0.7) respectively. The non-parametric test showed that values of PG I/II in patients with moderate ($p<0.05$) and severe ($p<0.01$) atrophy were significantly lower than those without atrophy.

### 4.2.4. Statistical Analyses

Relative risks of cancer between groups of *H.pylori* serostatus, PG I/II and gastrin concentrations were estimated as odds ratios (ORs) with associated 95% confidence intervals (CIs) using conditional logistic regression [(24)]. By exploiting the algorithmic equivalence of proportional hazards regression and conditional logistic regression, asymptotic odds ratios were computed using the Cox module of the SPSS statistical computer software package with each matched set as a separate stratum [(25).] For separate analyses of *H.pylori* seropositive and *H.pylori* seronegative cases and controls, we used unconditional logistic regression with adjustment for the matching variables in the original study design. Unconditional logistic regression produced estimates very similar to comparable conditional regression analyses. Tests of contrasts in odds ratios between subgroups of subjects were performed by including an interaction term in the statistical model. For tests of linear trend, the categorized

variable was treated as a continuous variable, and for tests of homogeneity the variable was represented with indicator variables. Two-sided p values <0.05 were considered statistically significant.

## 4.3. RESULTS

We studied 131 (76%) male and 42 (24%) female cases with 390 and 118 matched controls, respectively. In the non-cardia subsites, there were 91 male and 38 female cases. In the cardia, male predominance was much more pronounced with 40 male vs. 4 female cases. Serum sampling took place between 1972 and 1986 with 98% of the samples collected between 1972 and 1977. Median age at serum sampling was 45.6 (range 23.6-63.4) years and median follow-up time to cancer diagnosis was 11.9 (range 0.3-20.3) years in the cases. Median age at cardia cancer diagnosis was 57.5 (range 43.6-63.3) years, and median age at non-cardia cancer diagnosis was 55.8 (range 34.3-68.2) years. The time span over which the serum samples were obtained preceded the introduction of proton pump inhibitor medication to Norway.

As previously reported, the association between *H.pylori* seropositivity and cancer was highly dependent upon the gastric subsite [14] (Table 4.1). *H.pylori* seropositivity was found in 90% (116/129) of the non-cardia cases and in 43% (19/44) of the cardia cases, as compared to 66% (247/376) and 71% (93/132) in the respective control groups. There was a negative association between the infection and cancer of the cardia (OR 0.27, 95% CI 0.12-0.59). This contrasted with the positive association in the non-cardia subsites collectively (OR 4.75, 95% CI 2.56-8.81) (p<0.0005). The odds ratio in the antrum and pylorus combined (OR 7.95, 95% CI 3.07-20.6) was non-significantly higher (p=0.09) than in the fundus and body combined (OR 2.67, 95% CI 1.14-6.25).

**Table 4.1:** Risk of gastric adenocarcinoma (estimated by odds ratio with associated 95% confidence interval) for *H. pylori* serostaus, quintiles of serum pepsinogen I/II and quintiles of serum gastrin concentration according to different gastric subsites and adenocarcinoma subtypes.

| Risk factor | Cardia [44 cases, 132 controls] | Non-cardia overall [129 cases, 376 controls]¤ | Non-cardia, intestinal [59cases, 173 controls] | Non-cardia, diffuse [35 cases, 100 controls] | Non-cardia, mixed [31cases, 91 controls] |
|---|---|---|---|---|---|
| *H. pylori* serostatus | | | | | |
| Negative | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Positive | 0.27 (0.12-0.59) | 4.75 (2.56-8.81) | 3.96 (1.69-9.27) | 3.90 (1.11-13.7) | 5.51 (1.52-20.0) |
| p-value | 0.001 | <0.0005 | 0.002 | 0.034 | 0.010 |
| Serum pepsinogen I/II quintiles | | | | | |
| 5th: 6.060 – 30.973 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| 4th: 4.803 – 6.055 | 0.24 (0.07-0.75) | 1.21 (0.48-3.05) | 1.07 (0.30-3.74) | 0.31 (0.03-3.33) | 4.35 (0.44-43.4) |
| 3rd: 3.777 – 4.795 | 0.68 (0.27-1.74) | 2.49 (1.07-5.78) | 1.50 (0.47-4.82) | 2.02 (0.46-8.86) | 13.4 (1.20-150) |
| 2nd: 2.691 – 3.774 | 0.14 (0.03-0.71) | 5.35 (2.35-12.2) | 2.96 (0.94-9.29) | 7.82 (1.87-32.6) | 14.6 (1.35-157) |
| 1st: 0.323 – 2.688 | 0.78 (0.26-2.39) | 11.6 (4.91-27.5) | 12.5 (3.57-43.9) | 6.29 (1.45-27.3) | 24.9 (2.28-272) |
| p-value for trend | 0.391 | <0.0005 | <0.0005 | <0.0005 | 0.002 |
| Serum gastrin quintiles | | | | | |
| 1st: 2-20 ng/L | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) |
| 2nd: 25-30 ng/L | 1.25 (0.44-3.55) | 1.99 (0.82-4.83) | 1.56 (0.44-5.52) | 8.19 (0.80-84.2) | 1.75 (0.30-10.2) |
| 3rd: 35-55 ng/L | 1.58 (0.58-4.28) | 2.45 (1.13-5.35) | 1.82 (0.59-5.58) | 10.6 (1.13-99.0) | 1.66 (0.37-7.41) |
| 4th: 60-90 ng/L | 0.71 (0.19-2.66) | 3.82 (1.77-8.23) | 3.66 (1.23-10.9) | 8.42 (0.97-73.2) | 3.44 (0.82-14.5) |
| 5th: 95-462ng/L | 1.63 (0.48-5.48) | 8.99 (3.96-20.4) | 12.8 (3.83-42.6) | 21.8 (2.29-208) | 3.68 (0.77-17.7) |
| p-value for trend | 0.702 | <0.0005 | <0.0005 | 0.007 | 0.042 |

¤ Including four histologically unclassifiable cases and 12 corresponding controls

### 4.3.1. SERUM PEPSINOGEN I / II

The individual PG I/II results are presented in Fig. 4.1. In non-cardia subsites collectively, there was a strong association between the PG I/II and subsequent development of cancer. The risk of non-cardia cancer increased monotonously with decreasing quintiles of PG I/II (Table 4.1). A PG I/II below 2.69 (lowest quintile) conferred an overall 11.6 (95% CI: 4.91-27.5) times higher non-cardia cancer risk than a ratio above 6.06 (highest quintile) (p for trend over quintiles <0.0005). The associations were similar for the proximal and distal non-cardia subsites (data not shown). With PG I/II values dichotomized, PG I/II <2.5 were associated with a 4.47 (95% CI: 2.71-7.37) times higher risk of non-cardia cancer than PG I/II >2.5 (Table 4.2). A statistically significant association between low PG I/II and non-cardia cancer was found in both *H.pylori* positive and negative subjects when analyzed separately (Table 4.2).

In the cardia, quintiles of PG I/II showed no linear association with cancer risk (p for trend=0.391) (Table 4.1). However, when PG I/II was dichotomized and *H.pylori* seropositive cases and controls were analyzed separately, an association between atrophy and cardia cancer appeared. H.pylori positive individuals with a PG I/II <2.5 had a 3.33 (95% CI: 1.06-10.5) times higher risk of developing cardia cancer relative to subjects with PG I/II >2.5 (Table 4.2). There were no *H.pylori* negative cases or controls with PG I/II < 2.5 precluding an analogous analysis of *H.pylori* negative subjects.

**Table 4.2:** Risk of adenocarcinoma (estimated by odds ratio with associated 95% confidence interval in an unconditional logistic regression model with adjustment for the matching variables in the original study design) for serum pepsinogen I/II <2.5 (relative to serum pepsinogen I/II >2.5) and serum gastrin concentration ≥60 ng/L (relative to serum gastrin concentration <60 ng/L) according to gastric subsites and *H. pylori* serostatus.

| ***H. pylori* serostatus** | **Overall** | **Seropositive** | **Seronegative** |
|---|---|---|---|
| Gastric subsite<br>Risk factor | | | |
| Non-cardia | [129 cases, 376 controls] | [116 cases, 247 controls] | [13 cases, 129 controls] |
| Pepsinogen I/II >2.5 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Pepsinogen I/II <2.5 | 4.47 (2.71-7.37) | 3.45 (2.01-5.91) | 12.6 (2.25-70.7)¤ |
| p-value | <0.0005 | <0.0005 | 0.004 |
| Gastrin <60 ng/L | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Gastrin ≥60 ng/L | 3.18 (2.03-4.99) | 2.77 (1.69-4.54) | 3.05 (0.71-13.1) |
| p-value | <0.0005 | <0.0005 | 0.133 |
| Cardia | [44 cases, 132 controls] | [19 cases, 93 controls] | [25 cases, 39 controls] |
| Pepsinogen I/II >2.5 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Pepsinogen I/II <2.5 | 1.60 (0.62-4.14) | 3.33 (1.06-10.5) | a |
| p-value | 0.333 | 0.039 | |
| Gastrin <60 ng/L | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Gastrin ≥60 ng/L | 0.88 (0.37-2.06) | 2.23 (0.63-7.87) | 0.38 (0.05-3.03) |
| p-value | 0.761 | 0.213 | 0.362 |

a There were no *H. pylori* seronegative cardia cases or controls with pepsinogen I/II <2.5

¤ Unconditional logistic regression analysis without adjustment for the matching variables (only three cases with pepsinogen I/II <2.5 precluded adjustment)

### 4.3.2. SERUM GASTRIN

The individual serum gastrin results are presented in Fig. 4.2. In non-cardia subsites collectively, a monotonously increasing risk with increasing serum gastrin quintiles was observed, the risk being 8.99 (95% CI: 3.96-20.4) times higher for gastrin values ≥95 ng/L than for values ≤20 ng/L (Table 4.1). The pattern was similar in proximal and distal non-cardia subsites, with ORs tending to be somewhat higher in the distal subsites (data not shown). Length of time between last meal and serum sampling was known for 120 (93%) of these the non-cardia cases and 352 (95%) of corresponding controls. In this subgroup of subjects the association between serum gastrin concentration and cancer was estimated before and after adjustment for time since last meal. Overall, the adjustment did not materially alter the point estimates for cardia or non-cardia subsites. However, the adjustment increased the odds ratios in the distal non-cardia subsites by approximately 30% within each of the serum gastrin concentration quintiles. In an unconditional logistic regression model with adjustment for the variables used for matching in the study design, dichotomizing serum gastrin values at ≥60 ng/L was most discriminating, conferring a 3.18 (95% CI: 2.03-4.99) times increased risk of non-cardia cancer relative to gastrin concentrations <60 ng/L. This association was apparent and of the same order of magnitude in *H.pylori* positive (OR 2.77, 95% CI: 1.69 – 4.54) and *H.pylori* negative (OR 3.05, 95% CI: 0.71 – 13.1) individuals analyzed separately (Table 4.2).

In the cardia, there was no linear association between serum gastrin concentration tertiles (p for trend 1.00), quartiles (p for trend 0.41), or quintiles (p for trend 0.70) (Table 1) and later development of cancer, nor did gastrin dichotomized at various cut-off values show any association with cardia cancer. However, separate analyses for *H.pylori* positive and *H.pylori* negative cases and controls suggested associations

going in opposite directions. In an unconditional logistic regression model, hypergastrinaemia (cut-off at 60 ng/L) was positively associated with cardia cancer in *H.pylori* seropositive persons (OR 2.23, 95% CI: 0.63-7.87) but negatively associated in *H.pylori* seronegative persons (OR 0.38, 95% CI (0.05-3.03), a test for homogeneity between ORs gave p=0.093). Adjustment for time since last meal did not weaken the associations in the subset of subjects for whom this information was available.

### 4.3.3. HISTOLOGICAL SUBTYPES

The cardia and non-cardia cancers had different distributions of histological subtypes. Of the 129 non-cardia cancers, 59 (46%) were intestinal, 35 (27%) diffuse and 31 (24%) mixed and 4 (3%) of unclassifiable histological subtype. In contrast, the 44 cardia cancers comprised 31 (71%) intestinal, 7 (16%) diffuse, 5 (11%) mixed, and 1 (2%) unclassifiable histological subtypes. The proportion of intestinal to diffuse subtype was significantly higher in the cardia versus non-cardia subsite ($p<0.05$).

In the non-cardia region the risk of each of the three histological subtypes increased similarly with decreasing quintiles of PG I/II and increasing quintiles of serum gastrin (Table 4.1).

In the cardia, however, the different adenocarcinoma subtypes showed different associations with both PG I/II and gastrin. For these analyses, the variables were dichotomized because of the limited number of cardia cancers and controls. And for comparison, analogous analyses based on dichotomized variables were done for the non-cardia subsites (Table 4.3). PG I/II <2.5 (relative to PG I/II >2.5) was associated with an increased risk of the diffuse subtype at both non-cardia (OR 3.21, 95% CI: 1.27-8.13) and cardia subsites (OR 3.46, 95% CI: 0.32-37.5). Similarly, there was an association between serum gastrin ≥60 ng/L (relative to serum gastrin <60 ng/L) and the diffuse subtype in both the non-cardia (OR 2.11, 95% CI: 0.92-4.86) and the

cardia (OR 5.30, 95% CI: 0.52-54.6). With respect to the intestinal subtype, both low PG I/II and high serum gastrin showed disparate effects between the two subsites. Low PG I/II was positively associated with the intestinal subtype at non-cardia sites (OR=6.68, 95% CI: 2.79-16.0), but not at the cardia (OR=0.72, 95% CI: 0.19-2.79) (Table 4.3). The contrast in ORs was statistically highly significant (p=0.007). Likewise, hypergastrinaemia was positively associated with the intestinal subtype in non-cardia subsites (OR 4.04, 95% CI: 2.05-7.96), but not at the cardia (OR 0.59, 95% CI 0.22-1.58), (Table 4.3). This difference in ORs was statistically highly significant (p=0.002).

The eight cardia cancers occurring in atrophic (PG I/II <2.5) stomachs were all *H.pylori* positive (Table 4.4). Three of these cancers were of intestinal, three were of diffuse and two were of mixed histological subtype, and this distribution of histological subtypes was similar to that of the *H.pylori* positive non-cardia cancers. The 36 cardia cancers occurring in non-atrophic (PG I/II >2.5) stomachs were predominantly *H.pylori* negative (69%) and had a distribution of histological subtypes which was different from the non-cardia cancers, the intestinal subtype being more prevalent in the cardia (78%) than in the non-cardia (46%).

**Table 4.3**: Risk of adenocarcinoma (estimated by odds ratio with associated 95% confidence interval) for serum pepsinogen I/II <2.5 (relative to serum pepsinogen I/II >2.5) and serum gastrin concentration ≥60 ng/L (relative to serum gastrin concentration <60 ng/L) according to gastric subsites and adenocarcinoma subtypes.

| Histology<br>Gastric subsite<br>Risk factor | Overall¤ | Intestinal | Diffuse | Mixed |
|---|---|---|---|---|
| Non-cardia | [129 cases, 376 controls] | [59 cases, 173 controls] | [35 cases, 100 controls] | [31 cases, 91 controls] |
| Pepsinogen I/II >2.5 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Pepsinogen I/II <2.5 | 4.32 (2.58-7.24) | 6.68 (2.79-16.0) | 3.21 (1.27-8.13) | 2.32 (0.86-6.25) |
| p-value | <0.0005 | <0.0005 | 0.014 | 0.097 |
| Gastrin <60 ng/L | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Gastrin ≥60 ng/L | 2.97 (1.92-4.61) | 4.04 (2.05-7.96) | 2.11 (0.92-4.86) | 2.40 (1.06-5.44) |
| p-value | <0.0005 | <0.0005 | 0.079 | 0.036 |
| Cardia | [44 cases, 132 controls] | [31 cases, 93 controls] | [7 cases, 21 controls] | [5 cases, 15 controls] |
| Pepsinogen I/II >2.5 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Pepsinogen I/II <2.5 | 1.56 (0.60-4.09) | 0.72 (0.19-2.79) | 3.46 (0.32-37.5) | - |
| p-value | 0.365 | 0.632 | 0.307 | |
| Gastrin <60 ng/L | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Gastrin ≥60 ng/L | 0.85 (0.38-1.88) | 0.59 (0.22-1.58) | 5.30 (0.52-54.6) | 0.69 (0.06-8.04) |
| p-value | 0.689 | 0.295 | 0.161 | 0.764 |

¤ Including four histologically unclassifiable cases and 12 corresponding controls

**Table 4.4:** Pictorial representation of two main subgroups of cardia cancer based upon premorbid gastric mucosal atrophy and *H. pylori* status and histological subtype of tumour (numerals indicate numbers of cases in the respective groups).

| **Gastric subsite** | **Cardia 44** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gastric mucosa** | **Atrophic 8** | | | | | | **Non –Atrophic 36** | | | | | |
| ***H. pylori* serostatus** | **Positive 8** | | | Negative 0 | | | Positive 11 | | | **Negative 25** | | |
| **Adenocarcinoma subtype** | **Intestinal 3** | **Mixed 2** | **Diffuse 3** | Intestinal 0 | Mixed 0 | Diffuse 0 | Diffuse 2 | Mixed 2 | Intestinal 7 | Diffuse 2 | Mixed 2 | **Intestinal 21** |

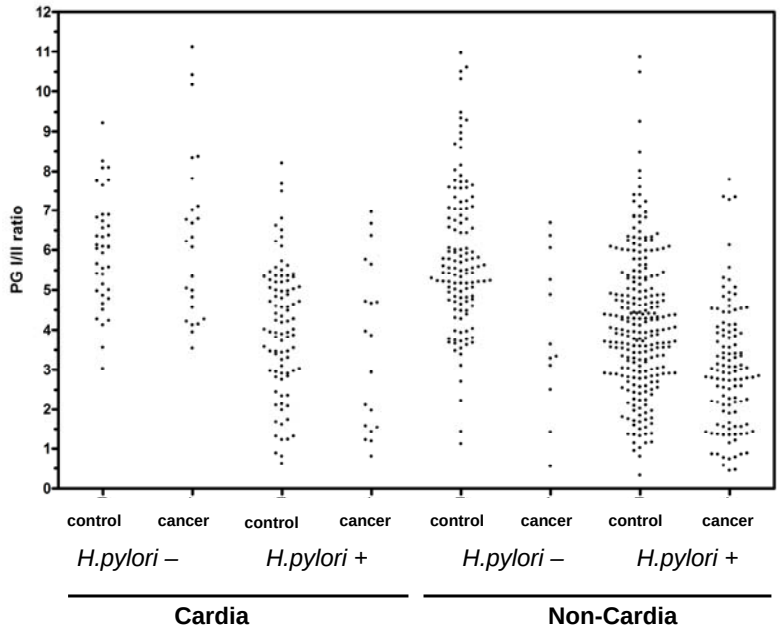



**Fig 4.1:** Pepsinogen I/II in cancer patients and their controls by subsite and *H. pylori* status

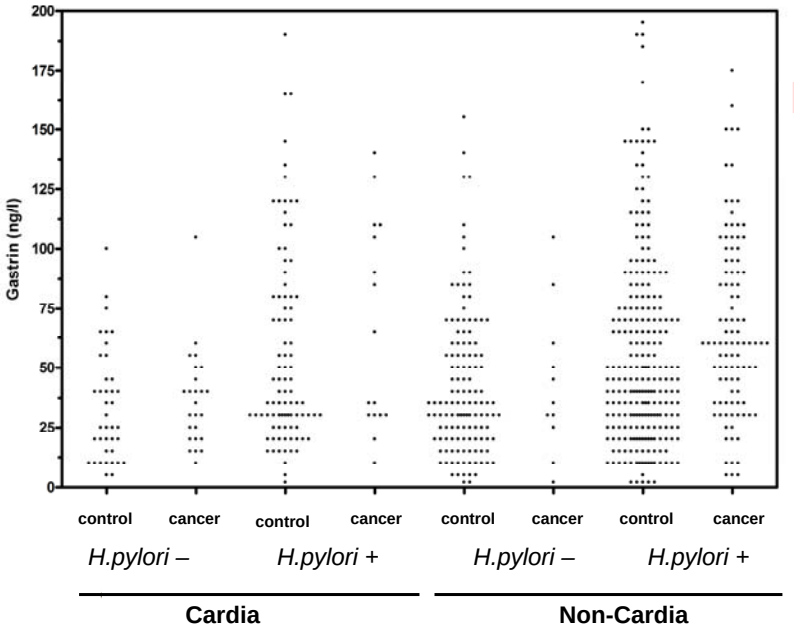



**Fig 4.2:** Serum gastrin in cancer patients and their controls by subsite and *H. pylori* status

## 4.4. DISCUSSION

This study demonstrates the association between the premorbid state of the gastric mucosa and the location and histological subtypes of gastric adenocarcinoma presenting over subsequent years. Cancers of the mid and distal stomach of all histological subtypes were positively associated with *H.pylori* infection, atrophy and hypochlorhydria. Cardia cancer was more complex; it was negatively associated with *H.pylori* infection and the predominant intestinal subtype of cardia cancer was not associated with gastric atrophy. However, in subjects with *H.pylori* infection cardia cancer was positively associated with atrophy and hypochlorhydria. These findings can be explained by cardia cancer being of two distinct aetiologies, some cases being similar to non-cardia cancer and others having a different aetiology.

The findings with non-cardia cancer are consistent with current wisdom that its development is a multistage process [(7)]. *H.pylori* infection induces superficial gastritis which progresses to atrophic gastritis with loss of acid secretion and then to dysplasia and cancer. A variety of bacterial, host and environmental factors are known to contribute to the progress through these different pre-cancerous stages [(26)].

The multistage process of non-cardia cancer development has been traditionally more strongly linked to the development of the intestinal histological subtype of cancer as the diffuse type may develop in subjects with normal gastric mucosa [(27)]. In the latter cases, there is often a strong hereditary predisposition with inherited germ-line

mutations [28]. Our current study, however, along with that of Uemura et al indicates that atrophy and hypochlorhydria are associated with increased risk of the diffuse and mixed histological subtypes as well as intestinal gastric cancer [6].

The association between atrophy and non-cardia cancer was apparent in both the *H.pylori* seropositive and seronegative subjects, the association being statistically non significantly stronger in the *H.pylori* seronegative ones. *H.pylori* seronegativity may in (some of) these individuals be explained by atrophy and hypochlorhydria causing loss of *H.pylori* infection and seropositivity [29-31]. Previous studies have shown that subjects with evidence of atrophy and no evidence of *H.pylori* have the highest cancer risk [31]. Some of the non-cardia cancers may have arisen from gastric atrophy due to causes other than *H.pylori* e.g. autoimmune atrophic gastritis.

In addition to employing the PG I/II as a marker of atrophy we used serum gastrin as a marker of atrophy and hypochlorhydria. Atrophic gastritis markedly impairs the ability of the stomach to secrete acid and the latter stimulates a rise in the circulating concentration of the hormone gastrin. Serum gastrin has been shown to be an independent predictor of atrophic gastritis in subgroups with and without *H.pylori* infection [32]. Similar to PGI/II, serum gastrin is most sensitive to atrophic gastritis affecting the gastric body where the acid secreting parietal cells are located [33]. Serum gastrin increases linearly with an increase in grade of atrophy of the body mucosa and exponentially with a decrease in peak acid output from normal (>10meq/h) to zero [34]. In subjects with achlorhydria or severe hypochlorhydria (peak acid output <1.1meq/h), the degree of accompanying hypergastrinaemia decreases linearly with increasing grade of antral atrophy [34]. This moderating influence of antral atrophy is thought to be due to loss of antral G cells and thus inability to produce the very high rate of gastrin secretion stimulated by profound hypochlorhydria [34].

There was a particularly strong association between elevation of serum gastrin and subsequent risk of non-cardia cancer. It is possible that the rise in serum gastrin associated with atrophy and low acidity may promote the carcinogenic process [35]. The hypergastrinaemic mouse model develops invasive gastric cancer, an effect which is markedly accelerated by *H.pylori* infection and inhibited by gastrin receptor antagonism [36-38].

The state and function of the gastric mucosa associated with cardia cancer is more complex than that associated with non-cardia cancer. In contrast to non-cardia cancer, there was a negative association between *H.pylori* infection and cancer of the cardia. As *H.pylori* infection causes atrophy and hypochlorhydria, we expected to find a lower prevalence of atrophy and hypochlorhydria in the cardia cancers than controls due to the lower prevalence of *H.pylori* in the former (Table 1). Such a finding would be consistent with *H.pylori* protecting from cardia cancer by the same mechanism by which it predisposes to non-cardia cancer, i.e. by reducing gastric acidity. However, despite the significantly lower prevalence of *H.pylori* infection in the cardia cancer patients (43% vs. control 71%) the prevalence of atrophy was at least as high in the cases (18%) as in the controls (13%). The reason for this unexpected finding was that the prevalence of atrophy within the *H.pylori* positive cardia cancer patients was significantly higher than the *H.pylori* positive controls. Cardia cancer patients were thus characterised by having a significantly lower prevalence of *H.pylori* infection but higher prevalence of atrophy in those with the infection as compared to the controls.

What is the explanation for the complex association between the premorbid state of the gastric mucosa and cardia cancer? The lower prevalence of *H.pylori* infection is consistent with *H.pylori* having some protective effect. However, the high prevalence of atrophic gastritis within the *H.pylori* infected subjects suggests that

atrophic gastritis due to *H.pylori* predisposes to cardia cancer. The most plausible explanation for our findings is that cancer of the cardia region is of heterogeneous aetiology and arising by two different pathways with *H.pylori* exerting an opposite influence on the two pathways.

The positive association with atrophic gastritis in the *H.pylori* infected cardia cancer patients is consistent with a subgroup of cardia cancers having a similar aetiology to non-cardia cancer, i.e. being due to *H.pylori* infection progressing to atrophic gastritis and cancer. The serological markers of atrophy detect that mostly involving the body mucosa [(33, 39, 40)]. Body atrophy induced by *H.pylori* gastritis causes distal regression of the apparent cardia-oxyntic junction due to loss of specialised cells [(13)]. Our finding is consistent with a proportion of the cardia cancers having arisen from this process and thus being of similar aetiology to non-cardia cancer. Ye et al recently reported that cardia cancer was not associated with *H.pylori* infection but was associated with gastric atrophy and their observation is thus also consistent with atrophy being involved in a subgroup of cardia cancers [(41)].

The lower prevalence of *H.pylori* infection in the cardia cancer patients supports an additional aetiological pathway in which *H.pylori* may exert *a* protective influence. Several studies have reported a negative association between *H.pylori* infection and oesophageal adenocarcinoma [(41-42)]. A subgroup of the cardia cancers may have similar aetiology to oesophageal adenocarcinoma and be subject to an *H.pylori* protective influence. Proximal expansion of the cardia mucosa can arise by metaplasia of oesophageal mucosa which the same process is thought to lead to oesophageal adenocarcinoma and to be induced by reflux of gastric acid [(9-12)]. The mechanism by which *H.pylori* infection may protect from this process is unclear but may be due to the fact that it causes a fall in acid output with advancing years due to development of atrophy [(43, 44)].

The analyses of the histological subtypes provide further evidence of two distinct aetiologies of cardia cancer. Atrophy increased the risk of the diffuse subtype of cardia cancer to a similar extent to which it increased the diffuse subtype of non-cardia cancer. This was apparent using either PG I/II or gastrin as the marker of atrophy. However, atrophy did not increase the risk of intestinal-type cardia cancer, which was in contrast to the increased risk of the intestinal-type in the non-cardia region. The contrast in associations between atrophy and the intestinal-type in the cardia versus non-cardia regions was statistically highly significant using either low PG I/II ($p=0.007$) or high gastrin ($p=0.002$) as risk indicators. The diffuse type cancers at the cardia thus appear etiologically similar to diffuse non-cardia cancers whereas the intestinal-type cancers at the cardia (or at least the majority of them) are etiologically distinct from intestinal-type cancers in the non-cardia region (Fig 4.3).

The cardia cancers which occurred in the patients with atrophic gastritis (all of whom were *H.pylori* positive) had similar proportions of intestinal and diffuse histological subtypes as occurred in the non-cardia cancers. This is consistent with them being of similar aetiology to non-cardia cancer arising from *H.pylori*-induced atrophic gastritis (Table 4.4). However, the cardia cancers occurring in non-atrophic stomachs (69% of which were *H.pylori* seronegative) had a much higher proportion of intestinal versus diffuse histological subtype (7:1). This predominant intestinal histological subtype is similar to that observed in oesophageal adenocarcinoma [(45)] and is consistent with this subgroup being of similar aetiology to oesophageal adenocarcinoma.

Our observations are relevant to the hypothesis that *H.pylori* infection may protect from oesophageal adenocarcinoma as well as predispose to gastric cancer and that both effects are mediated by gastric atrophy. In order to demonstrate a possible protective effect of *H.pylori* infection on oesophageal adenocarcinoma via gastric atrophy it will be essential to study only oesophageal adenocarcinomas well clear of

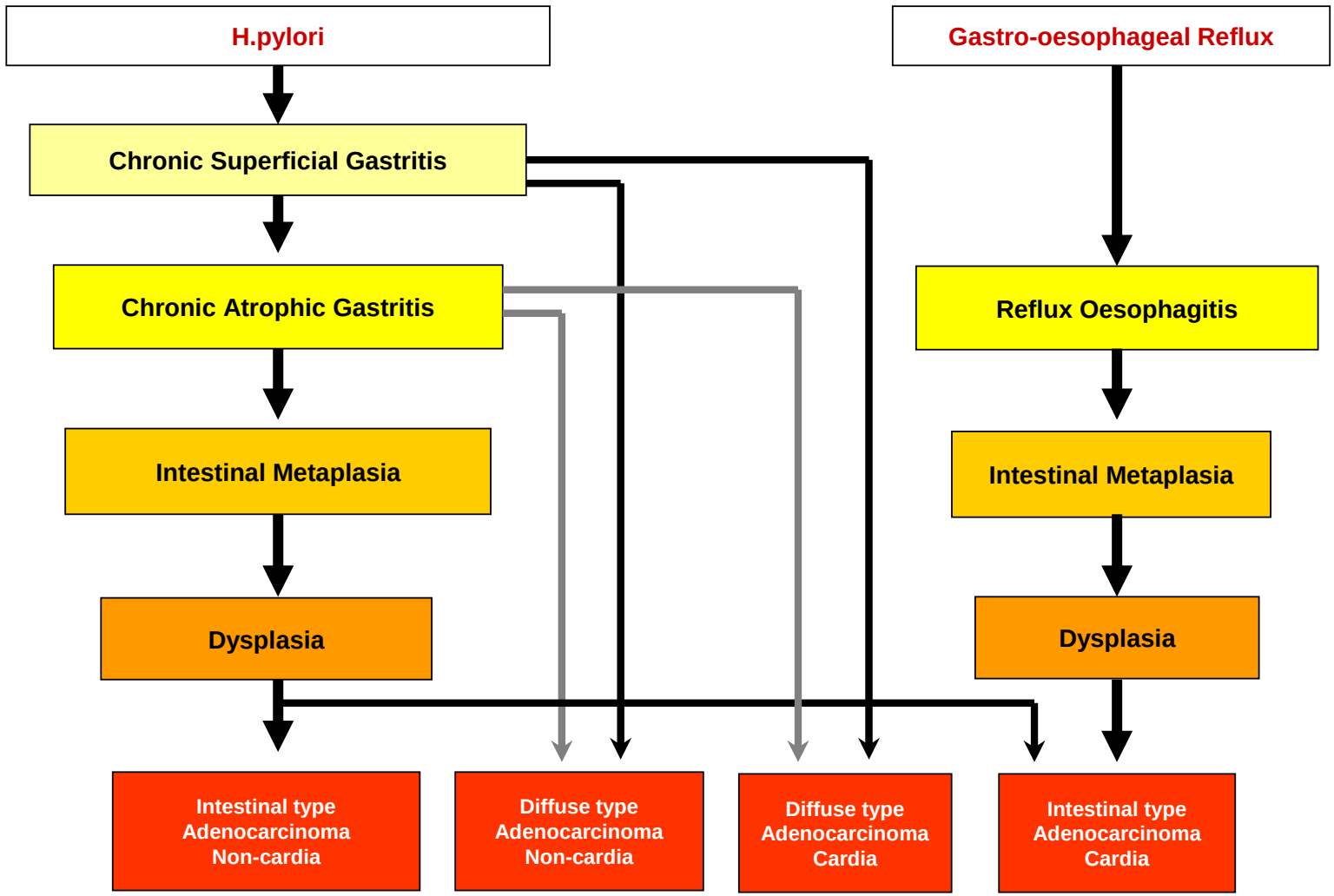



**Fig 4.3:** Histological cascade proposed for carcinogenesis of gastric cancer in non-cardia versus cardia locations. Note two types of cardia cancer, one group related to GORD, is mainly intestinal subype and other group is mixture of intestinal and diffuse subtypes and related to H.pylori induced gastritis.

the gastro-oesophageal junction. Inclusion of any cardia cancers will obscure a possible protective effect of atrophy due to the subgroup of cardia cancers associated with gastric atrophy.

Our finding may also be relevant to the conflicting reports regarding the association between *H.pylori* infection and cardia cancer [1, 46]. In general, the association has tended to be negative in studies originating from the West and positive in studies from the East [1, 46]. Our observation of some cardia cancers aetiologically resembling oesophageal adenocarcinoma and others resembling non-cardia adenocarcinoma may explain the conflicting associations of cardia cancer with *H.pylori* . In parts of the world where oesophageal adenocarcinoma is relatively common, most cardia cancers will be aetiologically similar to oesophageal adenocarcinoma and a protective effect of *H.pylori* infection and associated atrophy will be apparent. In contrast, in parts of the world such as the East where oesophageal adenocarcinoma is rare and non-cardia gastric cancer common, then the predominant aetiological type of cardia cancer will resemble non-cardia cancer and show a positive association with *H.pylori* and atrophic gastritis.

One practical implication of our findings is that the state of the gastric mucosa may provide a key to determining the origin of cardia cancer. As already discussed, it is usually impossible to determine the origin of such cancers by examining them grossly or microscopically. However, if examination of the stomach well clear of the cancerous process reveals atrophic gastritis, then cardia cancer of the intestinal histological subtype is likely to be etiologically similar to non-cardia cancer and have arisen from original gastric mucosa. In contrast, if the patient has a healthy non-atrophic gastric mucosa then cardia cancer of the intestinal histological subtype is likely to be of similar aetiology to oesophageal adenocarcinoma and have arisen from metaplastic oesophageal mucosa produced by gastroesophageal reflux. We have

recently proposed that cardia cancers of the intestinal histological subtype arising in patients with evidence of gastric atrophy should be termed type A and those arising in patients without gastric atrophy termed type B [47]. Cardia cancers of the diffuse histological subtype are likely to be gastric in origin.

In conclusion, our studies indicate that cardia cancers probably comprise two distinct aetiological subtypes, one resembling non-cardia gastric cancer and positively associated with *H.pylori*-induced atrophic gastritis and the other resembling oesophageal adenocarcinoma and negatively associated with *H.pylori* atrophic gastritis. Further studies with larger numbers of cancers are required to determine whether the state of the gastric mucosa will indeed provide the key to differentiate between gastric versus oesophageal origin of cardia cancers.

Chapter 5

# Two Types of Cardia Cancer; Differentiating Role of Atrophic Gastritis, GORD Symptoms, and Histological Subtypes

## LOOKING AHEAD

## 5.1. INTRODUCTION

There has been substantial progress in our understanding of the aetiology of adenocarcinoma of the stomach and oesophagus over recent decades. Most cancers of the mid and distal stomach are a long-term complication of *H.pylori*-induced superficial gastritis. They arise in the subgroup of subjects in whom the superficial gastritis progresses to atrophic gastritis and intestinal metaplasia accompanied with loss of gastric acid secreting capacity [1, 2]. *H.pylori*-induced atrophic gastritis and hypochlorhydria are strong risk factors for both the intestinal and diffuse histological subtype of gastric cancer [2, 3-6]. Another important independent risk factor for gastric cancer is smoking [7-9]. The fall in incidence of adenocarcinoma of the stomach in the Western world over recent decades may be attributable in part to a falling incidence of both *H. pylori* infection and smoking [6, 10].

A major risk factor for adenocarcinoma of the oesophagus is gastro-oesophageal reflux disease [11]. The risk of oesophageal adenocarcinoma increases with both the frequency and duration of reflux symptoms [11-13]. Frequent reflux of gastric juice containing acid, pepsin and bile is thought to induce columnar and intestinal metaplasia of the squamous mucosa of the distal oesophagus [14, 15]. This metaplastic or Barrett's oesophagus has a markedly increased risk of progressing to adenocarcinoma of the intestinal histological subtype [16]. In contrast to adenocarcinoma of the mid and distal stomach, that of the oesophagus is negatively associated with *H.pylori* infection [17]. The mechanism of this negative association is unclear but might be related to a healthy acid secreting stomach being required to provide a refluxate of sufficient acidity to induce oesophageal damage [18].

The aetiology of adenocarcinoma of the cardia and gastro-oesophageal junction is unclear and controversial. Understanding its aetiology is important as most adenocarcinomas of the upper gastrointestinal tract in the western world and in Northwest Iran involve the cardia and GE junction [19, 20]. The association of cardia cancer with *H.pylori* infection is confusing with some studies showing a negative association, some a positive and some no association [21-27]. Some studies indicate that reflux symptoms are a risk factor for cardia cancer but a weaker risk factor than for oesophageal adenocarcinoma [28]. A number of studies demonstrate smoking to be a risk factor for cardia cancer [9, 29].

As discussed in last chapter, we recently studied the association between cancer of the cardia and serological evidence of both *H.pylori* infection and atrophic gastritis [30]. This was performed in a nested case control study. We observed a negative association with *H.pylori* infection but a positive association between atrophic gastritis and cardia cancer in those with the infection. We interpreted this as indicating dual aetiology of cardia cancer with some cases being due to *H.pylori*-induced atrophic gastritis and aetiologically resembling adenocarcinoma of the mid and distal stomach and others being of a different aetiology and associated with a non-atrophic stomach. This latter group might be aetiologically similar to oesophageal adenocarcinoma.

In the current study we extended our investigation of the aetiology of cardia cancer by examining the association of both serological evidence of gastric atrophy and reflux symptoms with adenocarcinoma of the oesophagus, cardia and non-cardia regions of the stomach. This has been performed for the different histological subtypes of the cancer. We have also included *H.pylori* status and smoking history which are other well established risk factors for upper GI cancer. This has been undertaken in a population in Northwest Iran with a high incidence of upper gastrointestinal cancer [20,

[31]. Our studies examining the association with both atrophic gastritis and reflux symptoms provide substantial support for cardia cancer being of two distinct aetiological subtypes, one similar to non-cardia cancer and the other similar to oesophageal adenocarcinoma.

## 5.2. METHOD & MATERIALS

### 5.2.1. Study Setting

This was a case-control study, conducted in Aras Clinic in Ardabil province in Northwest Iran. The area is a well-known high risk region for gastric cancer in general and gastric cardia cancer in particular. Aras Clinic is a referral centre for delivery of investigational, therapeutic and preventative services to all patients with upper gastrointestinal tract disease throughout the Ardabil province. It is specifically equipped and staffed through government funding to conduct research into the aetiology of upper GI cancer. According to the latest estimates from the Ardabil Cancer Registry [(32)], approximately half of all incident upper gastrointestinal cancers diagnosed in Ardabil province are recorded and evaluated in this Centre. The present study has been conducted by collaboration between the University of Glasgow (UK), Digestive Disease Research Centre (DDRC) of University of Tehran and Ardabil University of Medical Science.

### 5.2.2. Cancer and Control Groups

In total, 157 consecutive eligible patients with gastric or oesophageal adenocarcinoma were identified. We excluded 19 eligible patients for the following reasons: very advanced disease that did not allow us to determine exact location of tumour (n= 5), poor co-operation of patient due to severity of the illness (n=4), patient

refusal (n=3) and insufficient or inappropriate serum or histologic samples (n=7). Finally, 138 patients with gastric and oesophageal adenocarcinoma were enrolled into the study including 66 non-cardia, 53 cardia and 19 oesophageal adenocarcinoma. Diagnosis of cancer was made by microscopic verification of multiple endoscopic biopsies and all histologic slides were studied by two certified pathologists (N.R and R.D) and reviewed by third pathologist (M.S) to ensure meeting the protocol requirements in accordance with ICD-O-2 [33]. In controversial cases, diagnosis of cancer was made only after joint agreement of all three pathologists. Cardia cancer was defined as tumours whose main bulk was within 2 cm distal to the gastro-oesophageal junction. Tumours located completely above the gastro-oesophageal junction were considered to be oesophageal in origin. Tumours located anywhere in the stomach other than the cardia were called non-cardia gastric cancer. The histological subtypes according to the Lauren classification were also recorded [34].

Prior to endoscopy, each patient had a standardised interview and details recorded regarding symptoms of reflux and smoking. The average frequency of heartburn and/or acid regurgitation over the five years prior to presentation excluding those of last one year before diagnosis of cancer was recorded. History of smoking was recorded as number of cigarettes per day and duration of smoking in years. Alcohol consumption is extremely rare in this region. The questionnaire employed was validated in a pilot study [35]. A fasting blood sample was collected from each patient before endoscopy and serum stored at –70C for later serologic assessment.

In format of frequency-matched case control design, one control for each case of non-cardia and cardia cancer and two controls for oesophageal adenocarcinoma patients were selected randomly from dyspeptic patients. They were attending the same Centre and their endoscopy had shown no evidence of peptic ulcer or tumours.

The controls were sex and age matched within 4 years. The controls had undergone a similar interview to the cases and had also had serum stored prior to their endoscopy.

### 5.2.3. Serologic Studies

Serum pepsinogen I (PG I) and pepsinogen II (PG II) were assayed with enzyme immuno-sorbant assay (ELISA) methods using monoclonal antibodies to pepsinogen I and II (BIOHIT diagnostics, Biohit LTD, UK). All procedures were done according to manufacture's instructions and results of PG I and PG II reported in µg/L. PG I/II ratio was calculated and reported in fraction. We used serum PG I/II less than 2.5 as a serologic marker of atrophy as previously reported [30].

*H.pylori* infection was assessed by a serologic test using anti *H.pylori* Ig G antibody, supplied by the same manufacturer. A response titre more than 30 enzyme immuno units (EIU) was considered as positive for *H.pylori* infection.

### 5.2.4. Statistical Analysis

Serum PG I/II as serologic marker of atrophy were presented in quintiles. The PG I/II data of each control group were used to make quintiles. Using binary logistic regression, relationship of PG I/II quintiles with each cancer was estimated as odds ratio (OR) with their 95% confidence interval and related p values. PG I/II quintiles were treated as a categorical variable and 5$^{th}$ quintile was used as referent. Smoking, GORD symptoms and *H.pylori* serology were used as possible risk factors of cancer in univariate logistic regression. These variables were also used in multivariate model along with PG I/II quintiles. Smoking was presented as a dichotomous variable (1= Smoker: ≥ 10 cigarettes per day for at least 10 years and no more than 5 years passed since stopping smoking, and 0=non-smoker including never smokers and those who smoked less than limits stated above). GORD symptoms were categorised

as 0= never or less than one time per week, 1= one to two times per week, and 2= more than two times per week. In order to evaluate association of gastric atrophy with risk of different histological subtypes of upper GI adenocarcinoma, we used the serum PG I/II less than 2.5 as a serologic marker of atrophy. Two sided p values less than 0.05 were considered statistically significant. The SPSS statistical software version 14.0 was used for most analysis [36].

## 5.3. RESULTS

### 5.3.1. Gastric non-cardia cancer

A total of 66 patients (49 male and 17 female, mean age 65.9 + 6.5) with non-cardia cancer and similar number of controls were studied (Table 5.1). A monotonous decreasing risk of cancer was observed from the lowest to the highest quintiles of PG I/II (Fig 5.1a). In univariate analysis, the risk was maximal in patients with PG I/II ≤ 2.01 with OR=15.76 (3.92 – 63.43). Smoking also increased the risk of non-cardia cancer with OR=2.22 (1.11 - 4.46) (table 5.2). GORD symptoms in both frequency levels showed a negative relationship with non-cardia cancer, but the association was only statistically significant in patients with GORD symptoms occurring 1-2 times per week. *H.pylori* seropositivity was detected in 93.9% of cases and 74.2% of controls and increased the risk of non-cardia in univariate analysis with OR=2.22 (1.11 - 4.46).

In multivariate analysis including smoking, GORD symptoms and *H.pylori* sero-status, first and second lowest PG I/II quintiles increased the risk of cancer with ORs (95% CI): 21.47 (2.90 – 158.76) and 9.08 (1.10 – 75.29), respectively. Smoking showed a more potent relationship with risk of non-cardia cancer, with OR (95% CI): 5.83 (2.11 – 16.11), GORD symptoms 1-2 times per week continued to show an inverse relationship, with OR (95% CI): 0.31 (0.11 – 0.85), The positive relationship between *H.pylori* infection and non-cardia cancer no longer reached statistical significance OR (95% CI): 1.53 (0.57 -4.14).

**Table 5.1:** Frequency of risk factors of adenocarcinomas of non-cardia, oesophageal and cardia sub-sites, with matched controls

| | **Non-Cardia** | | **Oesophageal** | | **Cardia** | |
|---|---|---|---|---|---|---|
| | Case (66) | Control (66) | Case (19) | Control (38) | Case (53) | Control (53) |
| **PG I/II** [mean (SD)] | 2.01 (1.01) | 3.46 (1.73) | 4.76 (2.00) | 3.43 (1.92) | 3.39 (2.23) | 4.19 (2.46) |
| **Smoking** | | | | | | |
| Ever smoker | 28 (42.4 %) | 15 (22.7 %) | 10 (52.6%) | 9 (23.7 %) | 19 (35.8 %) | 12 (22.6 %) |
| Non smoker | 38 (57.6 %) | 51 (77.3 %) | 9 (47.4%) | 29 (76.3 %) | 34 (64.2 %) | 41 (77.4 %) |
| **GORD symptoms** | | | | | | |
| <1 time per week | 50 (75.8 %) | 33 (50.0 %) | 2 (10.5 %) | 19 (50.0 %) | 25 (47.2 %) | 32 (60.4 %) |
| 1 – 2 times per week | 13 (19.7 %) | 23 (34.8 %) | 6 (31.6 %) | 15 (39.5%) | 14 (26.4 %) | 17 (32.1 %) |
| >2 times per week | 3 (4.5 %) | 10 (15.2 %) | 11 (57.9 %) | 4 (10.5 %) | 14 (26.4 %) | 4 (7.5 %) |
| ***H.pylori* Sero-status** | | | | | | |
| Positive | 62 (93.9 %) | 49 (74.2 %) | 9 (47.4 %) | 28 (73.7%) | 44 (83.0 %) | 39 (73.6 %) |
| Negative | 4 (6.1 %) | 17 (25.8 %) | 10 (52.6 %) | 10 (26.3%) | 9 (17.0 %) | 14 (26.4 %) |
| ***Histological Subtype*** | | | | | | |
| Intestinal | 36 (54.5 %) | n/a | 16 (84.2 %) | n/a | 34 (64.2 %) | n/a |
| Diffuse | 25 (37.9 %) | n/a | 1 (5.3 %) | n/a | 16 (30.2 %) | n/a |
| Mixed / Unclassifiable | 5 (7.6 %) | n/a | 2 (10.5 %) | n/a | 3 (5.7 %) | n/a |

According to the Lauren histological sub-classification of the non-cardia cancers, 36 (54.5%) were intestinal subtype, 25 (37.9%) diffuse, and 5 (7.6%) cases mixed or unclassifiable. The intestinal subtype adenocarcinoma showed strong positive association with gastric atrophy (defined as PGI/II < 2.5) with OR (95% CI): 13.02 (4.39 – 38.61) in multivariate analysis. The diffuse subtype cancer was also associated less strongly with gastric atrophy with OR (95% CI): 3.07 (1.23 – 7.67).

### 5.3.2. Oesophageal adenocarcinoma

19 cases of oesophageal adenocarcinoma (12 male and 7 female, mean age 63.9 + 4.7) were compared with double the number of controls (Table 5.1). In univariate analysis, GORD symptoms, in category of >2 times per week increased the risk of cancer with OR (95% CI) of 28.05 (4.74 – 165.91). In multivariate analysis, involving PG I/II, smoking and *H.pylori* sero-status, this relationship showed a decrease as OR (95% CI): 12.46 (1.80 – 86.47), (Table 5.3). Smoking also showed a positive relationship with the cancer, with OR (95% CI): 4.56 (1.01 – 20.68) which was not affected by other risk factors. There was no association between oesophageal adenocarcinoma and atrophy. Frequency of *H.pylori* infection in patients with oesophageal adenocarcinoma was lower than their matched controls (47.4% vs. 73.7%). While inverse relationship between *H.pylori* and oesophageal adenocarcinoma was evident by univariate analysis (OR; 95% CI: 0.25; 0.08 – 0.75), this negative relationship lost its statistical significance in multivariate analysis (OR; 95% CI: 0.43; 0.10 – 1.91). By the Lauren histologic classification, 16 (84.2%) of the 19 oesophageal adenocarcinomas were intestinal subtype.

**Table 5.2:** Relationship between risk of non-cardia gastric cancer and pepsinogen I /II, smoking, GORD symptoms and *H.pylori* sero-status

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | **Odds Ratio (95% CI)** | **P Value** | **Odds Ratio (95% CI)** | **P Value** |
| **PG Ratio quintiles** | | | | |
| 5th: 5.125 – 7.445 | 1.00 | | 1.00 | |
| 4th: 3.607 – 4.793 | 3.11 (0.62 - 15.58) | 0.168 | 3.50 (0.45 – 27.37) | 0.233 |
| 3rd: 2.707 – 3.560 | 4.77 (1.07 - 21.21) | 0.040 | 6.48 (0.78 – 54.01) | 0.084 |
| 2nd: 2.092 – 2.701 | 7.75 (1.81 - 33.16) | 0.006 | 9.08 (1.10 – 75.29) | 0.041 |
| 1st: 0.189 – 2.011 | 15.76 (3.92 - 63.43) | 0.000 | 21.47 (2.90 – 158.76) | 0.003 |
| **Smoking** | | | | |
| Non smoker | 1.00 | | 1.00 | |
| Ever smoker | 2.22 (1.11 - 4.46) | 0.025 | 5.83 (2.11 – 16.11) | 0.001 |
| **GORD symptoms** | | | | |
| <1 time per week | 1.00 | | 1.00 | |
| 1 – 2 times per week | 0.44 (0.20 - 0.96) | 0.039 | 0.31 (0.11 - 0.85) | 0.023 |
| >2 times per week | 0.44 (0.17 - 1.10) | 0.079 | 0.91 (0.18 – 4.64) | 0.913 |
| ***H.pylori* Sero-status** | | | | |
| Negative | 1.00 | | 1.00 | |
| Positive | 2.22 (1.11 – 4.46) | 0.025 | 1.53 (0.57 – 4.14) | 0.401 |

### 5.3.3. Gastric cardia cancer

We studied 53 cases of cardia cancer (37 male and 16 female, mean age 63.8 + 7.1) and the same number of controls (Table 5.1). A relationship between lowest quintile of PG I/II (≤ 2.37) and cardia cancer was noted in multivariate analysis [OR (95% CI): 3.92 (1.77–8.67)], (Table 5.4). However, there was a heterogenic relationship between atrophy and risk of cardia cancer with a relatively quadratic trend of risk of cardia cancer against different quintiles of PG I/II (Fig. 5.1b). This contrasted with the linear association of non-cardia cancer with atrophy (Fig 5.1a). There was also a positive association between cardia cancer and GORD symptoms at the level of >2 times per week having an OR (95% CI): 10.08 (2.29 – 44.36). No significant effect of smoking was detected in our patients [OR (95% CI):1.40 (0.56-3.51)]. While serologic *H.pylori* infection was more frequent in cases than controls (83.0% vs. 73.6%), there was no significant relationship between cardia cancer and *H.pylori* infection [(OR (95% CI): 2.42 (0.84–7.02)].

We further investigated the nature of the dual association of cardia cancer with atrophy and GORD using the dichotomised values. The association between risk of cardia cancer and atrophy based on dichotomised definition PG I/II < 2.5, showed a significant relationship with OR (95% CI): 3.05 (1.32–7.06). GORD symptoms dichotomised into >2 times/week versus 0-2 times /week also showed a positive relationship with risk of cardia cancer with OR (95% CI): 4.40 (1.34–14.43). In order to further evaluate the relationship between atrophy, GORD and risk of cardia cancer, we recalculated the association of GORD and cardia cancer risk separately in atrophic and non-atrophic subgroups. This showed that the risk of cardia cancer was increased by GORD symptoms in non-atrophic patients, OR (95% CI):8.02 (2.25–28.58)], but not in atrophic patients (Fisher exact test p value= 1.00). (Table 5.5, Fig 5.2).

**Table 5.3:** Relationship between risk of oesophageal adenocarcinoma and pepsinogen I /II, smoking, GORD symptoms and *H.pylori* sero-status

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | **Odds Ratio (95% CI)** | **P Value** | **Odds Ratio (95% CI)** | **P Value** |
| **PG Ratio quintiles** | | | | |
| 5th: 4.482 – 9.409 | 1.00 | | 1.00 | |
| 4th: 3.395 – 4.266 | 0.35 (0.07 – 1.72) | 0.197 | 0.40 (0.05 – 3.29) | 0.397 |
| 3rd: 2.611 – 3.271 | 0.35 (0.07 – 1.72) | 0.197 | 0.26 (0.03 – 2.22) | 0.217 |
| 2nd: 1.978 – 2.483 | 0.29 (0.06 – 1.46) | 0.132 | 0.57 (0.08 – 4.11) | 0.573 |
| 1st: 1.155 – 1.959 | 0.26 (0.05 – 1.49) | 0.131 | 0.41 (0.05 – 3.69) | 0.427 |
| **Smoking** | | | | |
| Non smoker | 1.00 | | 1.00 | |
| Ever smoker | 4.70 (1.54 – 14.34) | 0.007 | 4.56 (1.01 – 20.68) | 0.049 |
| **GORD symptoms** | | | | |
| <1 time per week | 1.00 | | 1.00 | |
| 1 – 2 times per week | 2.60 (0.65 – 10.36) | 0.175 | 1.73 (0.33 – 9.11) | 0.520 |
| >2 more times per week | 28.05 (4.74 – 165.91) | 0.001 | 12.46 (1.80 – 86.47) | 0.011 |
| ***H.pylori* Sero-status** | | | | |
| Negative | 1.00 | | 1.00 | |
| Positive | 0.25 (0.08 – 0.75) | 0.014 | 0.43 (0.10 – 1.91) | 0.268 |

In the cardia, 34 (64.2%) of tumours were classified histologically as intestinal subtype, 16 (30.2%) were diffuse subtype and only 3 cases were mixed subtype or unclassifiable. Both the intestinal subtype and diffuse subtype were associated with gastric atrophy, OR (95% CI):3.64 (1.33–9.97), and OR (95% CI):17.71 (3.66–85.76) respectively.

The association of cardia cancer with GORD symptoms was also related to the histological subtype. The intestinal subtype cardia cancer showed significant relationship with presence of GORD symptoms >2 times per week with OR (95% CI): 5.86 (1.68–20.39). In contrast, the association between GORD symptoms and diffuse subtype statistically was not significant [OR (95% CI): 2.83 (0.56–14.24)].

**Table 5.4:** Relationship between risk of gastric cardia cancer and pepsinogen I /II, smoking, GORD symptoms and *H.pylori* sero-status

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | **Odds Ratio (95% CI)** | **P Value** | **Odds Ratio (95% CI)** | **P Value** |
| **PG Ratio quintiles** | | | | |
| 5th: 6.008 – 11.586 | 1.00 | | 1.00 | |
| 4th: 3.848 – 6.004 | 1.10 (0.50 – 2.40) | 0.817 | 0.92 (0.37 - 2.26) | 0.852 |
| 3rd: 3.062 – 3.734 | 0.50 (0.20 – 1.25) | 0.138 | 0.62 (0.23- 1.71) | 0.355 |
| 2nd: 2.378 – 3.017 | 0.50 (0.20 – 1.25) | 0.138 | 0.49 (0.18 - 1.38) | 0.177 |
| 1st: 0.479 – 2.370 | 2.77 (1.36 – 5.63) | 0.005 | 3.92 (1.77 – 8.67) | 0.001 |
| **Smoking** | | | | |
| Non smoker | 1.00 | | 1.00 | |
| Ever smoker | 1.70 (0.79 – 3.67) | 0.175 | 1.40 (0.56 – 3.51) | 0.476 |
| **GORD symptoms** | | | | |
| <1 time per week | 1.00 | | 1.00 | |
| 1 – 2 times per week | 0.95 (0.40 – 2.29) | 0.915 | 1.47 (0.54 – 4.00) | 0.451 |
| >2 more times per week | 3.15 (1.17 – 8.49) | 0.024 | 10.08 (2.29 – 44.36) | 0.002 |
| ***H.pylori* Sero-status** | | | | |
| Negative | 1.00 | | 1.00 | |
| Positive | 1.46 (0.68 – 3.14) | 0.332 | 2.42 (0.84 – 7.02) | 0.103 |

**Table 5.5:** Relationship between GORD symptoms and risk of gastric cardia cancer in atrophic versus non-atrophic subjects

| PG I/II | GORD symptoms | Cardia cancer | | Fisher's Exact test | OR |
|---|---|---|---|---|---|
| | | Case | Control | P value (two-sided) | (95% CI) |
| Atrophic | > 2 / week | 1 | 0 | 1.000 | NA |
| | 0-2 / week | 24 | 12 | | |
| | Total | 25 | 12 | | |
| Non-atrophic | > 2 / week | 13 | 4 | 0.001 | 8.02 (2.25 – 28.58) |
| | 0-2 / week | 15 | 37 | | |
| | Total | 28 | 41 | | |

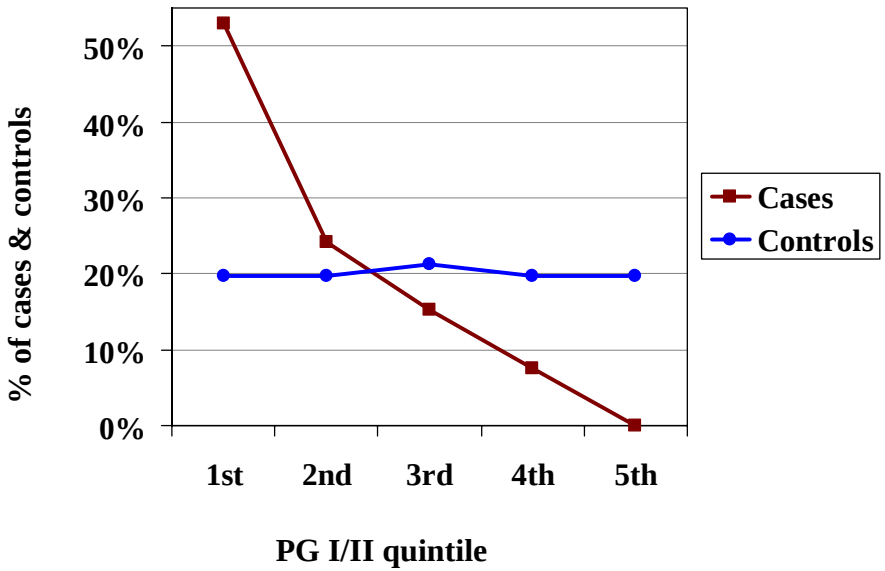



Fig 5.1a: Gastric non-cardia cancer

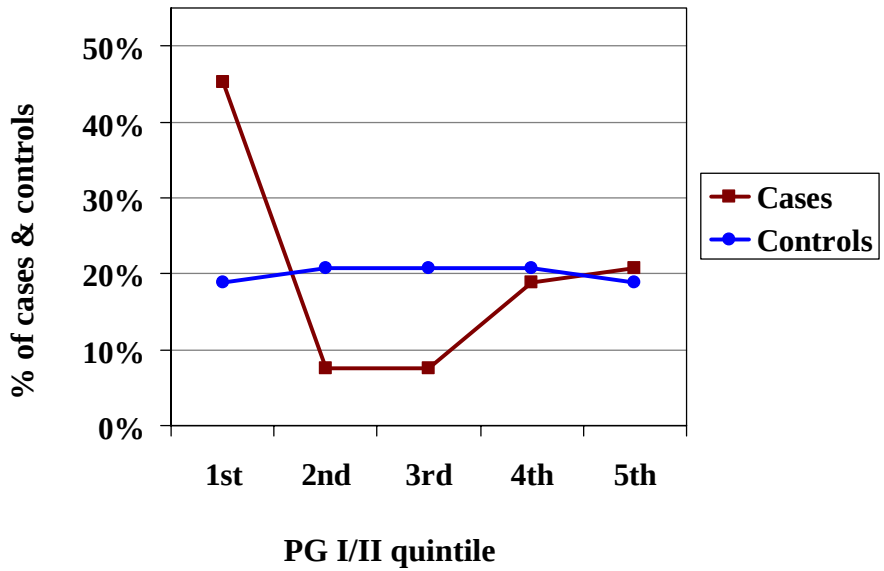



Fig 5.1b: Gastric cardia cancer

Fig 5.1: Relationship between severity of atrophic gastritis, expressed by serum PG I/II and risk of gastric cancer at non-cardia (A) and cardia subsites (B). The first quintile of PG I/II indicates greatest degree of atrophy and 5th quintile least atrophy.

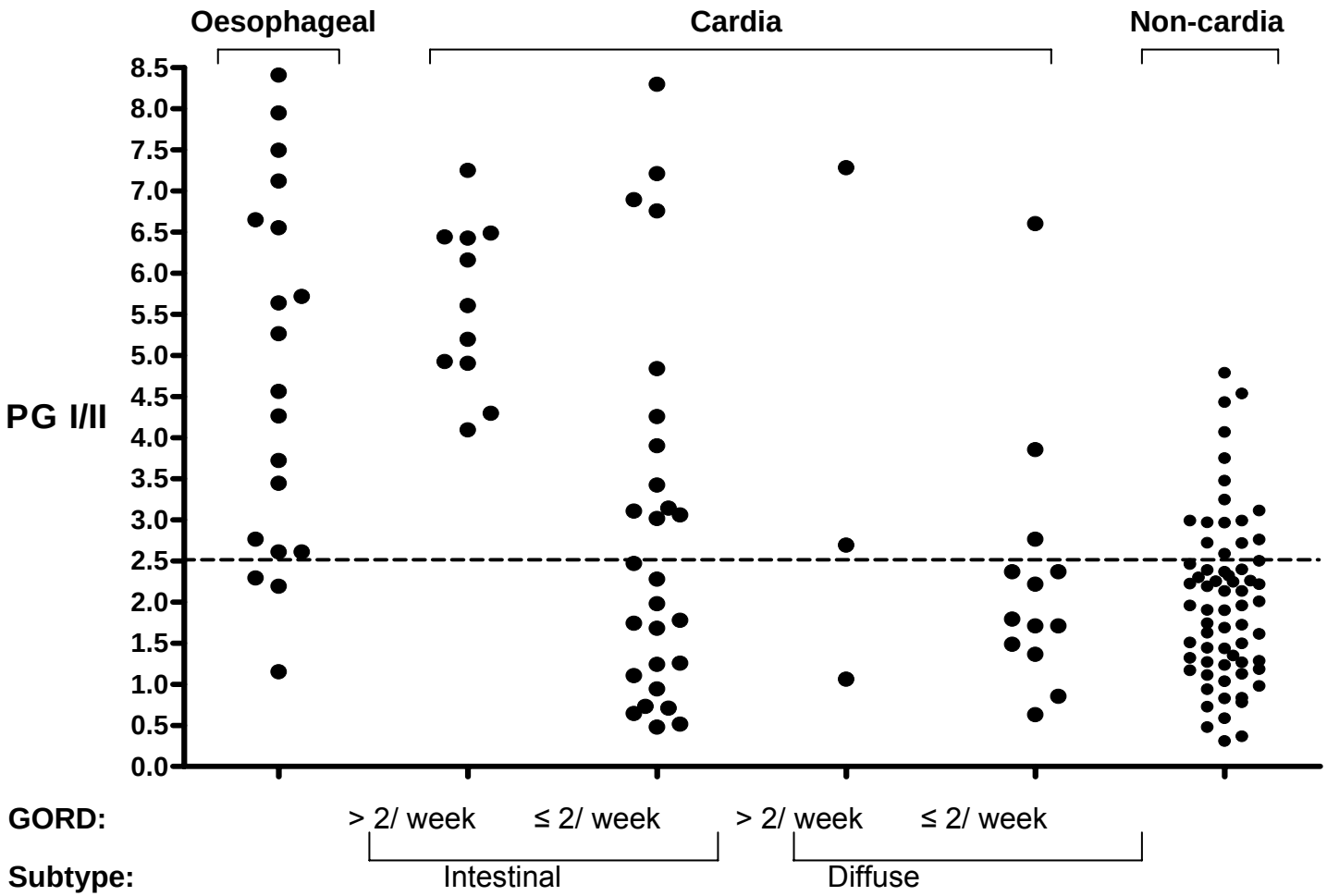



**Fig 5.2:** This presents the PG I/II values in the individual patients with oesophageal, cardia and non-cardia cancers. The cardia cancers are grouped according to histological subtype and frequency of GORD symptoms. Atrophy is indicated by PG I/II values of <2.5 (broken line).

## 5.4. DISCUSSION

In our subjects with non-cardia gastric cancer, we found a strong association between serological evidence of gastric atrophy and risk of cancer and this is consistent with many previous studies [37, 38]. Atrophic gastritis was associated with increased risk of both the intestinal and diffuse histological subtypes of non-cardia cancer but the association was stronger for the former as previously reported [37, 39, 40]. Whereas, the intestinal subtype is nearly always a consequence of atrophic gastritis and intestinal metaplasia, the diffuse histological subtype sometimes develops in a non-atrophic stomach with a strong genetic predisposition being an important factor in some of these cases [41, 42].

An association between *H.pylori* infection and non-cardia cancer was present in the univariate analysis consistent with previous reports [2]. However, this association was lost in multivariate analysis when atrophy and lifestyle factors were included. This is consistent with *H.pylori*-induced atrophic gastritis being the pre-cancerous lesion rather than *H.pylori* infection itself. High prevalence of *H.pylori* infection in the background population shown in the current and previous studies can explain its weak relationship with gastric cancer risk [43].

There was no significant association between frequent GORD symptoms (>2 times/week) and non-cardia cancer in our study. However, the negative association between lees frequent GORD and non-cardia cancer can be explained by the atrophic gastritis protects against GORD symptoms, but it difficult to understand why this would not also protect against more frequent GORD.

We found that smoking was also a risk factor for non-cardia cancer as previously reported. The extent of the association in univariate analysis [OR (95% CI): 2.22 (1.11-4.46)] is consistent with most previous reports, suggesting smoking as a mild to moderate risk factor of non-cardia gastric cancer [8, 44, 45]. Incorporating atrophy, *H. pylori* status and GORD symptoms into multivariate analysis enhanced the effect of smoking [OR (95%): 5.83 (2.11–16.11)]. This indicates that the effect of smoking is not mediated through induction of atrophy but acts independent of the atrophic process.

In contrast to non-cardia cancer, oesophageal adenocarcinoma was positively associated with reflux symptoms. This is consistent with previous reports and the currently accepted hypothesis that gastro-oesophageal reflux causes columnar and intestinal metaplasia which then progresses to intestinal subtype adenocarcinoma [11, 46]. Consistent with this, the great majority of oesophageal adenocarcinomas in our study were of the intestinal histological subtype. There was also a positive association with smoking as previously reported [44, 47]. There was no association with gastric atrophy.

The main purpose of our study was to investigate the aetiology of cardia cancer and its relation to that of non-cardia and oesophageal adenocarcinoma. Cardia cancer showed a complex relationship with gastric atrophy. Severe gastric atrophy indicated by the lowest pepsinogen I/II quintile of <2.37 was associated with an increased risk of cardia cancer. However, unlike non-cardia cancer, there was no evidence of a progressive rise in cancer incidence with falling pepsinogen I/II ratio. Rather, the relationship between pepsinogen I/II ratio and cancer risk showed a quadratic pattern with the risk of cardia cancer being highest for the lowest and highest pepsinogen I/II ratios and lowest for the intermediate ratios. A plausible

explanation for this complex association between cardia cancer and atrophic gastritis is that there are two distinct aetiologies of cardia cancer, one subgroup being associated with severe atrophic gastritis and resembling non-cardia cancer and the other subgroup unassociated or negatively associated with atrophic gastritis and aetiologically resembling oesophageal adenocarcinoma.

Reflux symptoms were also found to be a risk factor for cardia cancer with GORD symptoms of >2 time per week increasing the risk of cardia cancer with OR (95% CI):10.08 (2.29–44.36). Reflux symptoms have been reported previously to be a risk factor for cardia cancer but not as strong a risk factor as for oesophageal adenocarcinoma [(28)]. In our study, we were able to investigate the interaction of reflux symptoms and atrophy in the aetiology of cardia cancer. This showed that reflux symptoms were associated with cardia cancer only in non-atrophic subjects, with a powerful OR (95% CI): 8.02 (2.25–28.58). This is again consistent with two distinct aetiologies of cardia cancer, one being associated with atrophic gastritis and resembling non-cardia cancer and one associated with reflux and resembling oesophageal adenocarcinoma.

Further evidence of two distinct aetiologies of cardia cancer was apparent on examining the atrophy-cancer and GORD-cancer associations separately in the two histological subtypes. The association between atrophy and intestinal subtype adenocarcinoma was weaker in the cardia than in the non-cardia region of the stomach. This is consistent with the intestinal subtype cardia cancer being a mixture of tumours positively associated with atrophy (similar to non-cardia intestinal subtype adenocarcinomas) and tumours unassociated or negatively associated with atrophy (similar to oesophageal intestinal subtype adenocarcinoma).

The association of atrophy with diffuse cancer was stronger in the cardia than in the non-cardia region of the stomach. This difference may be related to the different topographic distribution and extent of atrophy required to produce cancer at those two sites and the ability of PGI/II to detect the atrophy associated with cancer at these two sites. Atrophy tends to start in the distal stomach at the junction between the antrum and body mucosa and progress proximally [48, 49]. Cancers tend to develop within atrophic mucosa and thus cancers of the distal stomach may develop in subjects with less extensive atrophy than would be required to produce cancer up at the cardia region. Furthermore, PGI/II is a reliable marker for detecting extensive atrophy involving the body mucosa but a poor marker for detecting early atrophy or that confined to the antral mucosa [50, 51].

The association between GORD symptoms and cardia adenocarcinoma was also related to the histological subtype. GORD symptoms were strongly associated with the intestinal subtype cancers at the cardia and this relationship was similar to that for oesophageal adenocarcinoma. This association with GORD symptoms and intestinal subtype adenocarcinoma at the cardia is consistent with some of these cancers occurring by the same mechanism as oesophageal adenocarcinoma which is also of the intestinal subtype; the reflux of gastric juice leading to columnar intestinal metaplasia, dysplasia and adenocarcinoma. In contrast, there was no relationship between GORD symptoms and diffuse subtype adenocarcinomas at the cardia.

Our findings thus support two distinct aetiologies of cardia cancer. One subtype is associated with atrophic gastritis and may be of the intestinal, diffuse or mixed histological subtype. It resembles non-cardia cancer and is likely to have arisen by the same process i.e. *H. pylori*-induced atrophic gastritis. The other subtype is associated with GORD and is of the intestinal histological subtype. It is likely to have

a similar aetiology to oesophageal adenocarcinoma and to have arisen from acid reflux induced columnar intestinal metaplasia of original oesophageal squamous epithelium.

The above observations imply that there are not only two distinct aetiologies of cardia cancers but that the structural and functional state of the stomach associated with them is profoundly different. One type is associated with a non-atrophic healthy gastric mucosa producing sufficient acid and pepsin to damage the mucosa of the gastro-oesophageal junction and lead to columnar intestinal metaplasia and intestinal subtype cancer. The other is associated with atrophic gastritis of sufficient severity and extent to involve the proximal stomach leading to the development of intestinal or diffuse subtype cancer from the atrophic gastric mucosa.

It is very difficult pre-operatively, during surgery or even at post-mortem examination to determine whether cancer of the cardia has arisen from original gastric or oesophageal mucosa. Our study points to three factors likely to be useful in determining the origin of the cancer: (i) the histological subtype of the tumours, (ii) the state of the gastric mucosa distant from the tumour and (iii) the frequency of GORD symptoms (Fig. 2). Diffuse histological tumour subtype strongly indicates gastric origin. Intestinal subtype tumours with non-atrophic gastric mucosa and frequent GORD symptoms are highly likely to be of oesophageal origin. Intestinal subtype tumours with atrophic gastric mucosa and less frequent GORD symptoms are likely to be gastric in origin. It is difficult to classify a proportion of the intestinal type cardia cancer. This might be improved by more precise means of assessment of GORD and more accurate determination of the presence/absence of gastric atrophy, i.e. by histology of gastric mucosal biopsies.

One of the limitations of this study is the relatively small numbers of recruited patients (which predominantly refers to patients with oesophageal adenocarcinoma). Application of strict eligibility criteria in general and a low actual incidence of oesophageal adenocarcinoma in the population in particular were the main problems in the performance of the study. The other point to be considered in interpretation of our results is the high proportion of gastric cardia to non-cardia cancer in the target population. Although the results of the current study have been supported by the findings of the previous work of ourselves, further large-scale investigations should be carried out in other populations with different proportions of cardia to non-cardia cancer, i.e. South East Asian countries to further validate the present results.

Chapter 6

# Gender and Upper Gastrointestinal Cancer

## LOOKING AHEAD

## 6.1. Cancer and Gender

In humans, the incidence of most cancers is higher in males than in females. A review of medical literature of the current and last centuries shows a marked male predominance of overall cancer incidence worldwide [1-4]. According to historical evidence, excess incidence and mortality of cancer in males could be a phenomenon of the current and last centuries. William Roger Williams, a late-19th-century writer, wrote; "want of proper exercise and excess of food" leaving men subject to "women's diseases" [5]. The perception that cancer was a "female" disease was based on the observation that females were especially liable to cancer of the breast and uterus, therefore overall cancer mortality due to cancer in females was remarkably greater than that of males [6, 7].

The influence of gender on cancer incidence varies by location and histological types of tumours. Although the overall male to female ratio (M/F) of cancer occurrence of all sites is approximately 1.30, this ratio shows great variation (Table 6.1). Regardless of histological type, cancer of the larynx with a M/F ratio of 8.5 and those located in the bladder with M/F over 4 are strongly male-predominant. Malignant tumours of the thyroid and cancer of the gall bladder occur unusually frequently in females [8]. Almost all other cancers are male-predominant and show a M/F ratio between 1 to 3. Cancers confined to the lungs, oesophagus, stomach, liver, pancreas and kidneys all are major examples of this group.

The gender disparity in cancer incidence may vary by histological type (or subtype) of the tumour in every site. Hepatocellular cell carcinoma shows a remarkable male-predominance compared to other histological types of liver cancers [9]. Lung adenocarcinoma has the least tendency to male gender than other histological types of lung cancer [10-12]. The most common histological type of bladder cancer,

transitional cell carcinoma, also expresses the largest M/F ratio compared to any other types [4].

**Table 6.1: Male to female ratios of cancer incidence in different sites worldwide, estimates of 2002, modified from Parkin et al (1)**

| Site | Males | | Females | | M/F Ratio |
|---|---|---|---|---|---|
| | Cases | ASR (World) | Cases | ASR (World) | |
| Oral cavity | 175,916 | 6.3 | 98,373 | 3.2 | 1.97 |
| Nasopharynx | 55,796 | 1.9 | 24,247 | 0.8 | 2.38 |
| Other pharynx | 106,219 | 3.8 | 24,077 | 0.8 | 4.75 |
| Oesophagus | 315,394 | 11.5 | 146,723 | 4.7 | 2.45 |
| Stomach | 603,419 | 22.0 | 330,518 | 10.3 | 2.14 |
| Colon, Rectum | 550,465 | 20.1 | 472,687 | 14.6 | 1.38 |
| Liver | 442,119 | 15.7 | 184,043 | 5.8 | 2.71 |
| Pancreas | 124,841 | 4.6 | 107,465 | 3.3 | 1.39 |
| Larynx | 139,230 | 5.1 | 20,011 | 0.6 | 8.50 |
| Lung | 965,241 | 35.5 | 386,891 | 12.1 | 2.93 |
| Melanoma of skin | 79,043 | 2.8 | 81,134 | 2.6 | 1.08 |
| Kidney | 129,223 | 4.7 | 79,257 | 2.5 | 1.88 |
| Bladder | 273,858 | 10.1 | 82,699 | 2.5 | 4.04 |
| Brain & CNS | 108,221 | 3.7 | 81,264 | 2.6 | 1.42 |
| Thyroid | 37,424 | 1.3 | 103,589 | 3.3 | 0.39 |
| N.H. lymphoma | 175,123 | 6.1 | 125,448 | 3.9 | 1.56 |
| Hodgkin disease | 38,218 | 1.2 | 24,111 | 0.8 | 1.50 |
| Multiple myeloma | 46,512 | 1.7 | 39,192 | 1.2 | 1.42 |
| Leukemia | 171,037 | 5.9 | 129,485 | 4.1 | 1.44 |
| **All sites but skin** | 5,801,839 | 209.6 | 5,060,657 | 161.5 | 1.30 |

Upper gastroesophageal cancers are common malignancies worldwide. Male predominance in this group of cancers varies by histological types and location of tumour. While oesophageal squamous cell carcinoma shows a relatively mild male predominance, adenocarcinoma of the oesophagus has a strong tendency to male gender [4, 13]. Indeed male gender is an important risk factor for oesophageal adenocarcinoma [14]. Adenocarcinoma of stomach, as the main histological form of gastric cancer, is also a male-predominant cancer. It has been shown that influence of

gender on gastric adenocarcinoma varies by location of tumour, i.e. tumours located in the cardia have a greater M/F ration compared to those in the non-cardia region [15]. Also, the intestinal subtype of the Lauren classification of gastric adenocarcinoma has been shown to express more M/F ratio in comparison to diffuse subtype [16]. In spite of great and invaluable studies regarding the association of tumour location and histological types of upper gastroesophageal cancers, there are many unanswered questions regarding the role of the mentioned factors and the nature of male-predominance in adenocarcinoma of the oesophagus and the stomach.

In this chapter, after a brief introduction to cancers with a prominent gender disparity, including hepatocellular cell carcinoma of the liver, lung cancer and bladder cancer, I will present the results of our study of factors of male predominance of upper gastroesophageal adenocarcinoma. In this study we have investigated the role of histological subtype and location of tumour in the male tendency of adenocarcinoma of the oesophagus, gastric cardia and gastric non-cardia subsites. In addition, we have modelled the age-specific incidence curves of intestinal and diffuse subtypes of upper gastroesophageal adenocarcinoma to compare the lag of cancer development between males and females. In order to produce reliable, unbiased and accurate data, we have studied a randomly-selected sample of all incidences of oesophageal and gastric adenocarcinoma recorded in the West of Scotland Cancer Registry, 1998-2002. In a further complementary study we have tried to answer a new question; in the multistage carcinogenesis cascade of intestinal subtype gastric cancer, where is gender acting? Again, a population-based study of gastric inflammatory and precancerous lesions was performed by randomly selecting adult residents of Ardabil, a high risk area for gastric cancer in Northwest Iran. Prevalence of each inflammatory and precancerous lesion was determined in males and females separately. I will discuss all findings at the end of the chapter.

## 6.2. Major Cancers with Marked Male Predominance

### 6.2.1. Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and accounts for approximately 60-90% of primary liver cancer [9]. Global data suggest that more than 500,000 new cases of primary liver cancer develop each year, equating to an age-adjusted worldwide incidence of 14.97 per 100,000 men and 5.51 per 100,000 women per year [17]. The incidence of HCC shows a huge geographical variation. The age standardised rate of HCC is highest in East Asian countries, i.e. South Korea, Japan and China with approximately 30 per 100,000 and 8 per 100,000 in men and women, respectively. Low incidence area are regions with less than three per 100,000 annually: the United Kingdom, Netherlands, Norway, Sweden and Finland. Most populations fall in the intermediate risk group and have an incidence rate of between three and 30 per 100,000. This cancer obviously involves men rather than women and there is an un-ignorable variation on M/F ratios, from 5: 1 in high risk areas to 2: 1 in low risk areas [9]. HCC usually occurs in individuals with chronic liver disease: in fact, the risk of developing HCC per year in cirrhotics ranges from 2 to 8%, depending on the different aetiologies of the underlying cirrhosis [18, 19].

Chronic hepatitis, caused by the hepatitis B virus or an hepatitis C virus infection, is the major risk factor for the development of HCC. There is considerable evidence for the involvement of various chemical carcinogens such as aflatoxin, cigarette smoking or heavy alcohol consumption. Also, it is well known that HCC develops more frequently in male cirrhotic patients than in females, and this striking male predominance led to the introduction of male gender as a risk factor. Not only do males develop HCC more

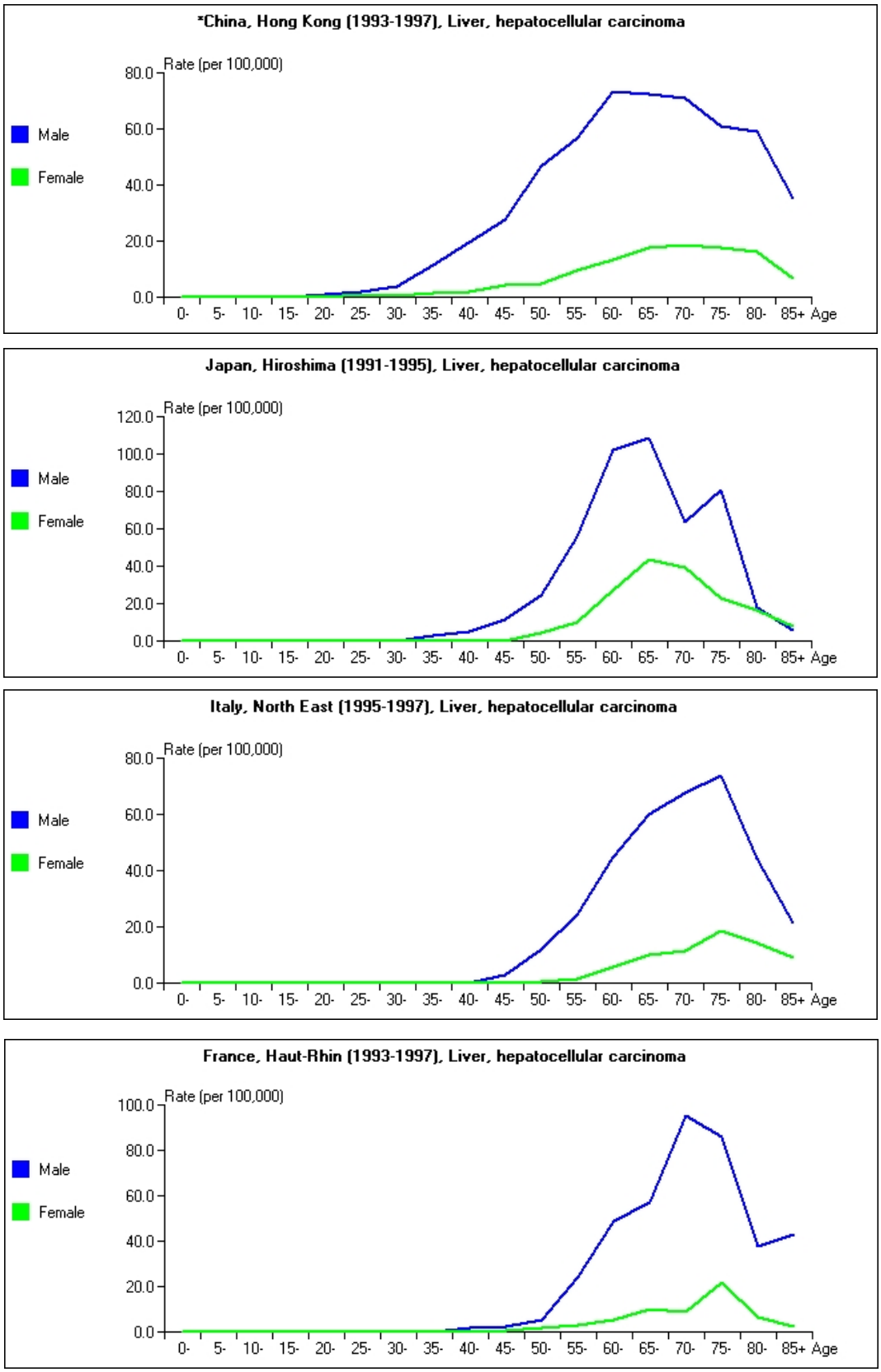



**Fig 6.1:** Gender difference in age specific incidence curves of hepatocellular carcinoma in populations with different risk of cancer
(Plotted from Parkin et al: Cancer in five continents Vol. VIII 2002)

often than females, but once they develop the cancer, it is also more likely that the disease recurs, and the survival period is shorter [20].

Many investigators have focused on the possible importance of sex hormones in determining such preference for the male gender. In fact, HCC occurs more often in males with chronic liver disease. Males are under the constant influence of androgens throughout their life, but due to the presence of the underlying liver disease they also present a characteristic 'feminization' of their phenotype due to a relative hyper-estrogenic state. As a result, both the presence of male sex hormones and the effect of the cirrhosis-induced feminization have been blamed as responsible, at least in part, for the development of HCC [21]. On the other side, all these hormonal effects on the carcinogenic pathway interact with the host immune system, which play a critical role in response to viral (or even non-viral) infection.

#### 6.2.1.1. The role of Androgens

Association of androgens with an increased risk of liver neoplasm has been known for a long time. Agnew et al in 1952 reported an increased development of liver tumours in different strains of mice after chronic exposure to androgens [22]. This finding was supported by later studies which showed excess susceptibility of rodents to chemical and viral induced carcinogenesis [23-26]. In in vitro studies, the growth and proliferation of a hepatic normal or tumour cell line has been shown to be increased by dehydrotestosterone (DHT) and testosterone. In clinical practice, the use of androgenic steroids is associated with an increased risk of liver neoplasm including HCC [27-30]. In a study on H-ras12V transgenic mice by Wang et al, orchidectomy significantly reduced the incidence of hepatotumorigenesis in males. However, no significant difference was detected in the incidence of tumorigenesis between ovariectomized and non-ovariectomized females. Molecular biochemical experiments

showed that the sex organ-related factors significantly influenced transgene expression, which contributed to activation of the MAPK signaling pathway [31]. Velazquez and Alter reviewed the reported associations between anabolic androgenic steroids and liver tumours in patients with Fanconi's anemia. They concluded that all patients on anabolic androgenic steroids are at risk of liver tumours, regardless of the underlying diagnosis [17].

Androgen receptors (AR) are present in the normal liver tissue from both male and female mammalians, but their expression and activation is reported to be increased in the tumour tissue and in the surrounding liver tissue of individuals with HCC [33-37]. Nagasue et al. showed that individuals with AR negative tumours had a survival rate of 55% five years after surgery, while those with AR positive tumours had a survival rate of 0% [37]. The influence of high serum testosterone levels on the risk of tumour recurrence and long-term prognosis in male patients undergoing hepatectomy for early stage HCC has been studied by Lin et al. They showed patients with high serum testosterone to have significantly higher 5-year tumour recurrence rates and an inferior long-term prognosis than patients with low testosterone levels [38].

The hepatic effect of androgens is clearly receptor mediated, since their effect on tumour growth is inhibited by the concomitant presence of anti-androgen products that specifically block the AR. Orchidectomy or the use of anti-androgen treatment protects male rodents from tumour development [39-41]. Moreover, ovariectomized female rodents receiving testosterone have a susceptibility to tumour development similar to that of intact males [42].

The results of a follow-up study of 46 males with HCV related cirrhosis by Tanaka et al showed that elevated serum testosterone levels together with decreased estrogens may promote the development of HCC in cirrhosis [43]. The male predominance in HBV-related HCC is much higher than that of HCV-related HCC with a ratio of 5-7: 1 vs. 2-3: 1 [44, 45]. In addition, among male HBV carriers, those with a higher level of

serum androgen and more active AR gene alleles have a significantly increased risk of HCC [46, 47]. This finding prompted investigators to examine whether specific HBV viral factors might also participate in male hepatocarcinogenesis by targeting the AR signalling pathway. Chiu et al showed that HBx, a HBV non-structural gene, increased the anchorage-independent colony forming potency of AR in a mouse hepatocyte cell line [48].

Despite strong evidence of involvement of androgen compounds in the hepatocarcinogenesis, the results of anti-androgenic trials in the treatment of HCC have been quite disappointing, as most of the published studies show a complete lack of effect of this therapeutic approach. Three large studies have been published recently: in the first study, Chao et al. assessed the clinical activity and toxicity of Flutamide, an anti-androgenic compound, in 32 patients with un-resectable HCC [49]. The patients received Flutamide for 8 weeks; at the end of treatment, no complete or partial responses were observed. The authors concluded that HCC may not be an androgen-responsive tumour. In the second study, Grimaldi et al reported a multi centric double blind trial with 244 patients with un-resectable HCC randomized to receive different regimens of anti-androgens or placebo [50]. No significant difference among the groups was reported at the end of the study. In the third study, male patients with advanced HCC were randomized into 2 groups treated with (a) leuprorelin, flutamide and tamoxifen or (b) tamoxifen alone administered until death. 376 male patients were included. At the end of study, no benefit in survival was found with antiandrogenic treatment in male patients with advanced HCC [51].

#### 6.2.1.2. The role of Oestrogens

The normal liver tissue of male and female mammalians has high-affinity, low-capacity, saturable and specific oestrogen receptors [52, 53]. It has been shown that estrogens play an important role in the control of liver cell proliferation [54]. The hepatic

ERs increase and are actively translocated to the nucleus after partial hepatectomy in humans and rats [55]. Anti-oestrogens, like Tamoxifen, reduce the levels of both cytosolic and nuclear ER and inhibit hepatocyte proliferation following partial hepatectomy.

Cirrhotic patients have a unique hormone imbalance with an absolute or relative hyper-oestrogenic state manifested clinically by the occurrence of a 'feminized' phenotypic appearance [56-58]. This feminization is the result of a direct effect on gonads of toxic agents (i.e. alcohol), altered hormone metabolism due to chronic liver disease, and failure of the hypothalamus- pituitary-gonadal axis. The activity of cytosolic ERs is also increased in liver diseases in males, enhancing the responsitivity of male liver to oestrogens [56-59]. Moreover, the serum estradiol to testosterone ratio is higher in individuals with HCC and cirrhosis than in normal individuals or individuals with cirrhosis alone [56]. Castagnetta and colleagues investigated the activity and expression of the aromatase enzyme in non-tumoural, cirrhotic, and malignant human liver tissues and cells. Human hepatocellular carcinoma (HCC) tissues and HepG2 hepatoma cells showed elevated aromatase activity, compared to non-tumoural hepatic tissues where no aromatase activity could be detected. Cirrhotic samples exhibited intermediate enzyme activity. This implies that locally elevated oestrogen formation in malignant human liver tissues and cells may have a role in the development and/or maintenance of human HCC, eventually leading to develop alternative strategies for treatment of HCC patients using anti-aromatase agents [60]. It is not clear if this could be a pathogenic factor in HCC or just an epiphenomenon.

In animal experiments it has been shown that oestrogens may induce also the formation of free radical-mediated DNA and RNA adducts potentially mutagenic [61]. In humans, the chronic use of oestrogens is associated with increased risk of developing liver neoplasms such as benign nodular hyperplasia and hepatic adenoma [62-64]. Oestrogens have also been described as a putative agent of HCC in humans, and the

level of nuclear ERs in the neoplastic liver is higher than in normal tissue [64]. It has also been shown that intrahepatic transplantation of ovarian fragments in ovariectomized rats results in morphological abnormalities. Dombrowski and colleagues investigated the long-term development of these oestrogen-induced foci of altered hepatocytes in a large group of rats. They divided 451 Lewis rats into one test group and 11 control groups and observed them for up to 30 months. Test group animals were ovariectomized and received ovarian transplants into the right lobe of their liver. Different combinations of castration, transplantation of ovarian or testicular fragments, and administration of anti-oestrogenic Toremifene were used in controls. Hepatocellular carcinoma (HCC) appeared only in the test group. At 24 and 30 months, 78% of test group showed at least one carcinoma. Administration of Toremifene in ovariectomized and transplanted animals completely prevented carcinogenesis. They concluded that initially adaptive but preneoplastic alterations in hepatocytes downstream of intrahepatically transplanted ovarian fragments may transform into HCC, indicating a strong hepatocarcinogenic potential of high local levels of endogenous estrogens in the rat liver [65].

In contrast to literature supporting carcinogenic effects of oestrogens, there is some evidence for oestrogen and oestrogen-like compounds to have a beneficial effect on the pathogenesis of hepatocellular carcinoma. The findings of Huang et al suggest that oestrogen and oestrogen-like compounds may induce anti-proliferative and apoptotic effects in Hep3B cells, and the E2 and the E2-like compounds' mediated apoptotic effect was oestrogen receptor dependent [66].

HCC represents a classic case of inflammation-linked cancer [67], and chemically or genetically induced HCC depends on inflammatory signaling [68, 69]. These inflammatory signals have some interactions with sex hormones during HCC carcinogenesis. Recently, Naugler et al in an animal study [70] used the chemical carcinogen diethylnitrosamine (DEN) to induce HCC in mice, which causes HCC in

100% of male mice but only in 10 to 30% of female littermates [71, 72]. The DEN administration caused greater increases in serum interleukin-6 (IL-6) concentration in males than it did in females. DEN exposure promoted production of IL-6 in Kupffer cells in a manner dependent on the Toll-like receptor adaptor protein MyD88, ablation of which also protected male mice from DEN-induced hepatocarcinogenesis. The study that oestrogen inhibited secretion of IL-6 from Kupffer cells exposed to necrotic hepatocytes and reduced circulating concentrations of IL-6 in DEN-treated male mice [70]. As its authors believe, the pathogenesis of HCC in this mouse model differs from that in humans and thus the protective effect of oestrogen may not be directly comparable to human HCC.

Several studies have used Tamoxifen for the treatment of HCC and the results appeared to be initially encouraging. Tamoxifen (TMX) is an anti-oestrogen drug used for the treatment of breast cancer. TMX has several other biologic activities that may have relevance in cancer treatment: inhibition of PKC, calmodulin, TGF-α and TGF-b1 induction; antagonism of oestrogen binding to the erbB-2 oncogene; and activation of NK mediated cytotoxicity. Some of these may be responsible for the reported effects of TMX on various cancers. Most of the studies published in the early 90s reported a reduced tumour growth with prolonged survival in individuals treated with Tamoxifen as compared with untreated controls. The results were consistent with a meta-analysis on the palliative treatment for HCC which indicated Tamoxifen as one of the few therapeutic approaches with a clear and significant beneficial effect [73]. Di Bisceglie et al induced liver tumours by injecting ethyl-nitrosourea in a type of male mice. Two chemopreventive agents were administered over a period of 60 weeks: Tamoxifen and a retinoid, 13-cis-retinoic acid. Animals were killed at 60 weeks and their livers examined for HCC and premalignant lesions. All liver lesions occurred significantly less frequently in the Tamoxifen-treated group than the group given only ethylnitrosourea (HCC developed in 4% vs. 25%). Tamoxifen significantly decreased

the incidence of chemical carcinogenesis in this model, suggesting an important role for estrogens in the pathogenesis of HCC [74].

The positive conclusions about Tamoxifen were later contradicted by the results of two large trials utilizing Tamoxifen. In a multi centric trial, 496 patients with HCC at any stage were randomized into two matched group to receive Tamoxifen or a placebo [75]. The median survival was 15 and 16 months, respectively, and the conclusion was that Tamoxifen is not effective in HCC treatment. The same results were observed by Liu et al: 119 patients with un-resectable HCC were randomized in two matched groups to receive Tamoxifen or a placebo [76]. The median survival was 44 and 41 days, respectively, and no relation between better survival and presence of ER receptors was found.

#### 6.2.1.3. Conclusion

Hepatocellular cell carcinoma shows a distinctive male predominance in both incidence and mortality rates. Except to exposure with aflatoxine, alcohol, and chemical carcinogens, the majority of HCCs are developed in a background of cirrhosis due to viral hepatitis. While the reported prevalence of viral hepatitis is more noticeable in males than in females in some western European populations, this difference is unlikely to be able to explain profound male predominance of HCC worldwide. Experimental and clinical data have shown that both estrogens and androgens have important effects in controlling the replication rate of hepatic cells. Both estrogens and androgens may also have an effect on inducing or at least promoting the growth of liver tumours, including HCC. However, the disappointing results obtained by anti oestrogen and anti-androgen treatments may suggest that either the suppression of their effect, once the tumour has developed, has probably no clinical relevance on the progression of the disease or that ‘clinically’ HCC is not a sex

hormones responsive tumour. The controversial findings of the protective effects of oestrogen may open a different way for the interpretation of these data.

### 6.2.2. Lung Cancer

Lung cancer has been the most common cancer in the world since 1985 [2], and by 2002, there were 1.35 million new cases, representing 12.4% of all new cancers. It was also the most common cause of death from cancer, with 1.18 million deaths, or 17.6% of the world total. Worldwide, it is the most common cancer of men, with the highest rates observed in North America and Europe (Fig 6.2). Moderately high rates of men are also seen in Australia/New Zealand and East Asia i.e. China and Japan. Globally, the incidence rate is 35.5 per 100,000 men versus 12.1 per 100,000 women. This yields an M/F estimate of 3:1 [1].

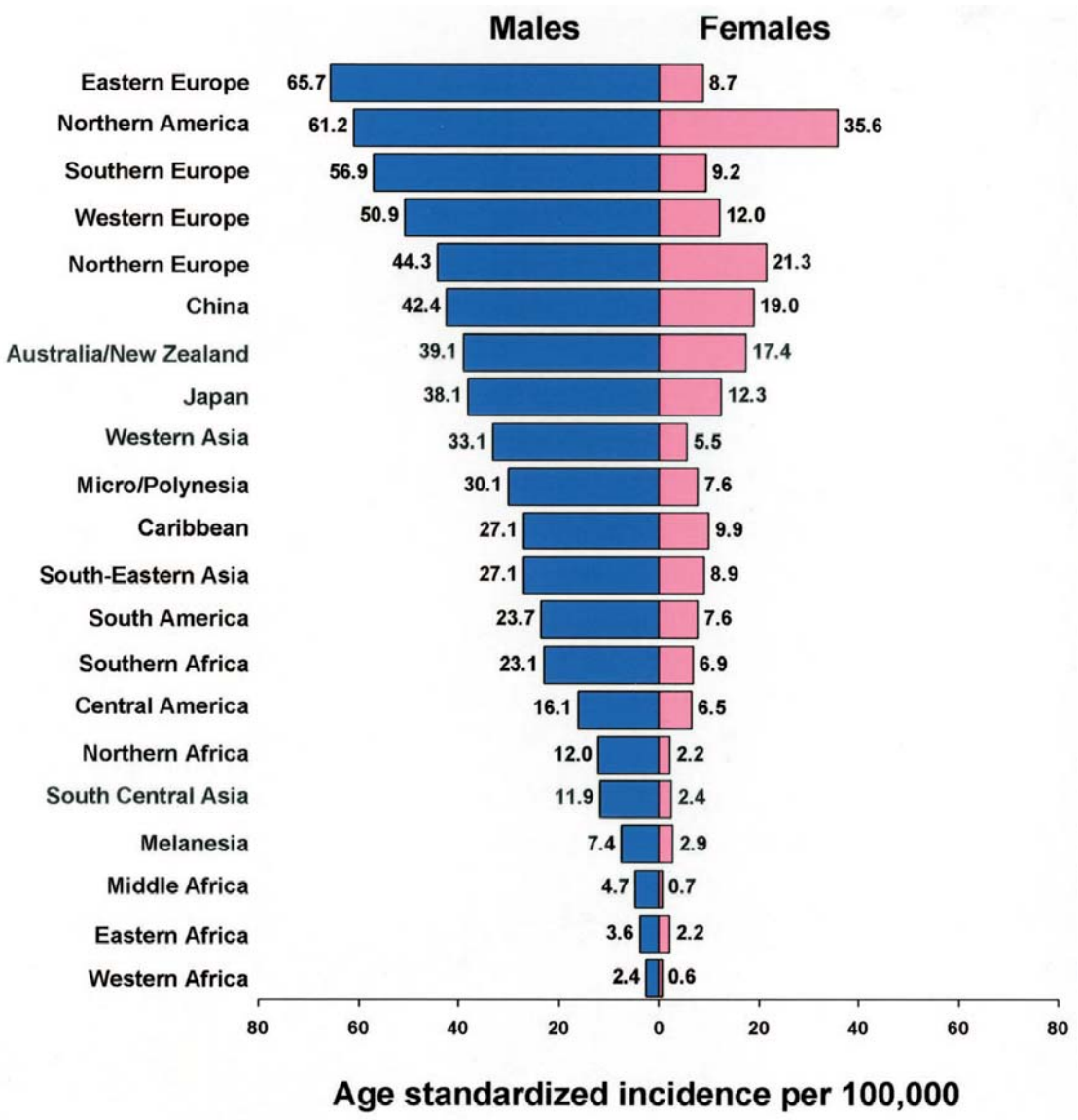



**Fig 6.2:** Age standardized incidence rate of lung cancer by geographical region [(1)]

Lung cancer remains a highly lethal disease. Survival at 5 years measured by the SEER program in the United States is 15%, the best recorded at the population level. The average survival rate in Europe is 10%, not much better than the 8.9% observed in developing countries. The survival rate varies by stage of disease [(1)]. The corresponding 5-year relative survival rates are 51.3% for localized, 17.1% for regional, and 2.1% for metastatic disease [(77)]. Women have a better relative survival

rate compared to men for each stage of the disease, [10] and male sex is a consistently unfavourable prognostic indicator in advanced disease [78-80].

### 6.2.2.1. Male predominance of lung cancer

Lung cancer historically has been more prevalent in men than women; however, the male/female incidence ratio has narrowed dramatically, as the incidence rate in men declines while the rate in women continues to rise slowly [10]. This rising incidence of lung cancer in women is primarily due to an increase in tobacco use which started in the 1940s. The lung cancer death rate in women has subsequently increased rapidly since the 1960s, from about 5 cases per 100,000 women to an estimated 40 per 100,000 in 2000. Today, lung cancer is the most common cause of cancer death in women (27%) in USA, claiming more lives than breast and colorectal cancer combined (15 and 10%, respectively) [81]. The estimated number of lung cancer cases worldwide has increased by 51% since 1985, but the rate of increase is significantly different in male and females, 44% in the former and 76% in the latter. In males, this increase is due solely to population growth and aging; there has actually been a small (3.3%) decrease in the actual age-standardized incidence. However, the ASRs have increased by 22% in females [1].

### 6.2.2.2. Smoking and gender difference of lung cancer

Smoking is the overwhelming cause of lung cancer in both male and females. Although females diagnosed with lung cancer are more likely to be non-smokers than males, [11, 12, 82, 83] overall (both sexes) 85 to 90% of patients with lung cancer are current or former smokers. Smokers are 22 times more likely to die from lung cancer than non smokers [84].The proportion of smoking–related lung cancer can be estimated by comparing observed incidence in different areas with that expected based on rates in non smokers from several large cohort studies [85, 86]. For the year

2000, an estimated 85% of lung cancer in men and 47% of lung cancer in women is the consequence of tobacco smoking. The fractions are lower for women, and several areas, including south-central Asia, have no smoking attributable cases. The highest fractions are in North America (85%), northern Europe (74%), and Australia/New Zealand (72%), where women have been smoking the longest [1].

Primary lung cancer represents different histological types including squamous cell carcinoma, adenocarcinoma, small cell carcinoma, and large cell carcinoma. Non-small cell lung cancers (NSCLC) account for approximately 85% of lung cancer, and include the histological types of squamous cell carcinoma and large cell carcinoma, which arise from epithelial cells, and adenocarcinoma, which develops from glandular tissue in the peripheral regions of the lung. The proportional occurrence of these histological subtypes differs significantly between males and females [10]. Adenocarcinoma is currently the most common histological subtype in both males and females, and females have proportionally less squamous cell carcinoma compared to males (Table 6.2). Cigarette smoking has been linked to all four histological subtypes; however, the proportion of non-smokers is highest in those who develop adenocarcinoma [87].

The incidence rates for the various histological subtypes have changed over time and reflect changes in smoking habits. For example, incidence rates for squamous cell carcinoma decreased in men between 1975 and 1999 while increasing slightly in women. On the other hand, the incidence rate of adenocarcinoma increased both in men and in women during that same time period, with a greater increase observed in women [10, 91]. The increase in adenocarcinoma has been associated with the introduction of low-tar cigarettes that enhance delivery of smoke to peripheral regions of the lungs [92].

**Table 6.2:** Frequency of patients diagnosed with different histological types of lung cancer by gender

| Squamous Cell Carcinoma | | Adenocarcinoma | | Other histologies | | Refs |
|---|---|---|---|---|---|---|
| M (%) | F (%) | M (%) | F (%) | M (%) | F (%) | |
| 36 | 21 | 33 | 45 | 31 | 34 | 10 |
| 32 | 22 | 48 | 60 | 20 | 18 | 11 |
| 38 | 24 | 38 | 47 | 24 | 29 | 12 |
| 31 | 20 | 42 | 50 | 27 | 30 | 88 |
| 41 | 17 | 42 | 74 | 17 | 9 | 89 |
| 30 | 21 | 32 | 40 | 38 | 39 | 90 |

Approximately 10–15% of patients with lung cancer are non-smokers. The ratio of women to men in patients with lung cancer who have never smoked is approximately 3:1 [11, 93]. The risk for developing lung cancer in both smokers and non-smokers is modulated by determinants that may vary between individuals. The development of lung cancer despite the low level of exposure to tobacco suggests that a subset of the general population may be more susceptible to the carcinogenic effects of tobacco smoke. Susceptibility is likely to be determined by each individual's capacity to activate and detoxify carcinogens in tobacco smoke. A positive family history also has been defined as a risk factor in non-smokers; particularly in the development of adenocarcinoma, in females, and in cases with an earlier age at onset [94-97].

### 6.2.2.3. Sex differences in lung tumour biology

The current epidemiological evidence clearly shows sex-specific differences in lung cancer susceptibility and prognosis. Female patients with lung cancer appear to have a better survival rate. In a large population-based study, elderly women with early lung cancer had better risk adjusted survival regardless of the type of treatment compared

to men [98]. On the other hand, women appear to have an increased susceptibility to tobacco carcinogens. Smoking-related lung cancer is induced by the formation of DNA adducts in lung epithelial cells [99]. The formation of DNA adducts is dependent on the enzymatic activation of several tobacco-related pro-carcinogens, including polycyclic aromatic hydrocarbon (PAH), [100]. PAH activation is catalyzed by the cytochrome P450 enzymes CYP1A1 and CYP1B1, and inactivated by glutathione *S*-transferases (GSTs). Levels of lung DNA adducts correlate with the level of CYP1A1 expression. Among smokers, female patients had a 3.9-fold higher median level of CYP1A1 compared to males. Independent of smoking history, the combined variant genotype of CYP1A1 and GSTM1 contributes to an increased risk of lung cancer in women compared to men with the odds ratio 6.54 versus 2.36, respectively [101]. Moreover, the carcinogens of tobacco smoke are associated with specific mutations of a relatively small number of codons of tumour suppressor p53 [102]. Analysis by gender shows that the tobacco-related p53 mutations are more common in females than males, evidence that females may be more susceptible to the carcinogenic effects of tobacco smoke [103-105].

### 6.2.2.4. Sex hormones and Lung Cancer

Gender differences in the histological distribution of lung cancer and a possibly greater susceptibility of females than males to smoking–related carcinogenesis suggest a possible effect of sex specific hormones. Oestrogen plays a role in both normal pulmonary physiology and in the biology of non-small cell lung carcinoma (NSCLC) [106-108]. In vitro studies confirm that NSCLC cells respond to oestrogens and anti-oestrogens by altering endogenous gene expression [109]. ER-beta, and to a lesser extent ER-alpha, are expressed in lung tumours from both men and women. Expression of ER-beta is associated with improved survival, while expression of ER-alpha is a poor prognostic factor [108, 110-112]. In a case control study, a reduction in lung

cancer risk was observed with the use of oral contraceptives, and history of hormonal replacement therapy was also associated with lower risk of lung cancer, particularly after long term usage [113].

The chemopreventive role of phytoestrogens in lung cancer as an exogenous source of sex hormones has been evaluated in few studies. In a large scale population-based case-control study, the highest quartiles of total phytoesterols, isoflavones, lignans, and phytoestrogens were associated with reductions of the risk of lung cancer ranging from 21% to 46%. While there are limitations regarding case-control studies of diet and cancer, this study provided a support for the growing evidence that phytoestrogens are associated with a decrease in the risk of lung cancer [114].

The greater survival rate of female patients with lung cancer has partly been linked to increased expression of the parathyroid hormone-related protein (PTHrP). This protein is commonly expressed in non-small cell lung cancer and could have implications for progression of the disease because it regulates cancer cell growth, apoptosis, and angiogenesis [115]. A recent study showed that lung carcinoma in female mice expressed more PTHrP than in males possibly because of negative regulation by androgens in males [116]. This finding is unique evidence implying a promotional effect of androgens on lung cancer.

#### 6.2.2.5. Conclusion

Male predominance in lung cancer is a universal phenomenon throughout the world. Smoking is the most important risk factor for lung cancer and it explains a major part of gender difference. While the number of cases and incidence rate of lung cancer is more in men than in women, the latter shows more susceptibility to the effects of smoking which in turn modifies large expected M/F ratios. Most histologic types of lung cancer except adenocarcinoma demonstrate significant male predominance and

also a greater relationship with smoking. Sex hormones and genetic susceptibility are among the risk factors associated with the development of lung cancer in either smokers or non-smokers, but their role in male predominance is not clear

### 6.2.3. Bladder Cancer

Bladder cancer with an estimated 357,000 cases in 2002 is the ninth most common cause of cancer for both sexes combined worldwide. It is relatively common in developed countries, where 63% of all incident cases occur (Figure 6.3). Rates are high in many southern and eastern European countries where smoking has been prevalent [(1)], and in parts of Africa and the Middle East where bladder cancer, particularly of the squamous cell type, is linked to chronic infection with *Schistosoma hematobium* [(117)]. Some occupational exposures contribute to the high risk of developed countries. The highest recorded incidence rate is that found in Egypt, where the estimated world-standardized rate in men is 37 per 100,000. In the United States, the incidence in Whites is higher than in Blacks; about double among men and 50% greater among women. It is unlikely that this is due to differences in exposure to environmental carcinogens, and explanations based on differential susceptibility have been proposed, including, for example, genetic polymorphisms of metabolic enzymes such as *N-Acetyltransferase* (NAT) and *Glutathione S-transferase 1* (GSTM1) [(118, 119)].

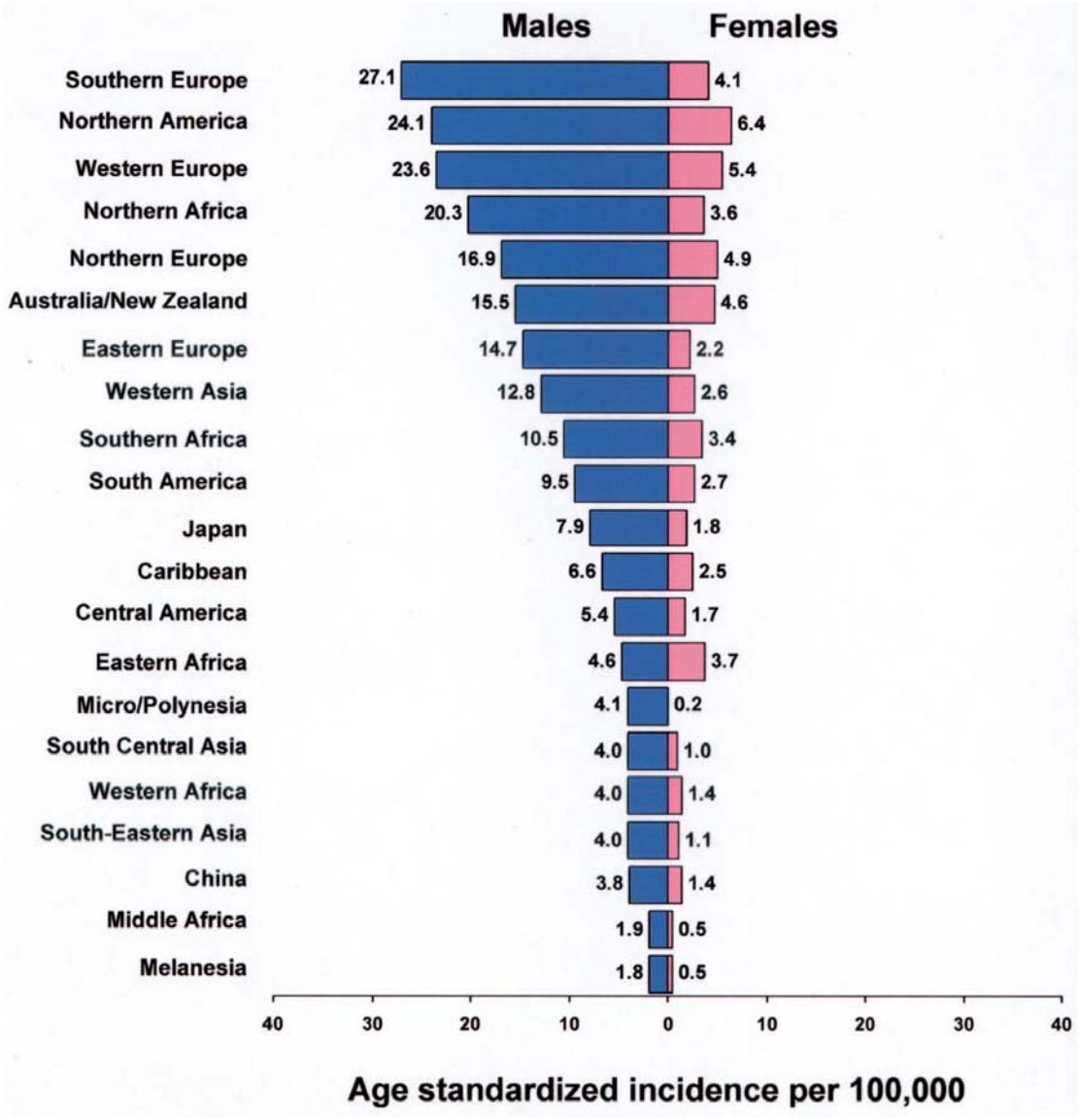



Fig 6.3: Age standardized incidence rate of bladder cancer by geographical region [1]

### 6.2.3.1. Male predominance of bladder cancer

The majority (77%) of bladder tumours occur in men [1]. The M/F ratio varies from 8.2: 1 to 2.5: 1. The maximum M/F ratio has been reported from high incidence areas, i.e. Southern Europe. In most western countries, M/F ratio of bladder cancer is 2.5 to 5 [1, 120-127]. Between 1985 and 2000 the number of bladder cancers diagnosed annually in the United States increased by 33%, at roughly the same rate in both genders [128, 129]. Bladder cancer is virtually never recognized incidentally at autopsy

[130, 131]. This coupled with the consistent method by which bladder cancer has been diagnosed (cystoscopy and biopsy) over the last six decades, indicates that the increase in bladder cancer cannot be attributed to technological innovations or major changes in the delivery of medical care. As a result, the reported differences in incidence rates between the genders cannot be simply explained by a failure to diagnose the disease in particular groups [132].

Cigarette smoking is a well established risk factor of urinary bladder cancer [127, 133-137]. It has been estimated that 65% of bladder cancer in men and 20% to 30% in women is attributable to cigarette smoking [127]. Bladder cancer risk tends to increase with both increasing duration and increasing intensity of smoking [135, 138-145]. As mentioned earlier, there is a huge male predominance in incidence of bladder cancer. Some have speculated that the high sex ratios observed in some countries may be due to the large differences in smoking prevalence between men and women [146]. In support of this hypothesis, Samanic and colleagues removed the number of incident cases due to smoking in men and women from the incidence rates in their study. The M/F ratio fell from 8.2 to 1.7, suggesting that the high M/F ratio may be largely due to smoking, although the contribution of additional factors cannot be ruled out. They proposed two factors to explain the higher population attributable risk (PAR) for smoking in men than in women. These are first, the higher prevalence of smoking among men (37%) compared with women (8.7%), and second, the higher point estimates for smoking in men (OR, 7.4 current; OR, 3.8 former) than women (OR, 5.1 current; OR, 1.8 former). The findings of this study overall suggest that smoking explains almost the entire male excess of bladder cancer in Spain [127]. Only one study contrasts with that of Hartge et al [147], who found that cigarette smoking and occupational exposures explained only a part of the male excess of bladder cancer in the United States.

# Section 3

## Male predominance in incidence of gastric and oesophageal adenocarcinoma

### 6.3.1. INTRODUCTION

A remarkable and unexplained characteristic of upper gastrointestinal adenocarcinoma is its male predominance. This male predominance of gastric cancer is related to the histological subtype of the tumour. Gastric adenocarcinoma may be of the intestinal or diffuse histological subtype as described by Lauren.[148] The intestinal subtype is strongly linked to chronic H. pylori superficial gastritis. According to Correa, the latter may induce intestinal metaplasia of the gastric mucosa from which the intestinal subtype cancer is thought to develop.[149] The diffuse histological subtype of gastric cancer is less strongly associated with H.pylori infection and genetic predisposition is thought to be more important.[150-152] The gender phenomenon is more marked in gastric cancer of the intestinal versus diffuse histological subtype and this has been described well by Sipponen and colleagues.[16] However, few, if any, cancer registries have reliable records of the histological subtype of gastric and oesophageal cancer and therefore true population-based incidence studies of the influence of gender on intestinal versus diffuse gastric cancer are lacking.

Interest in the role of gender in upper gastrointestinal cancer has been rekindled by the rapidly rising incidence of adenocarcinoma of the oesophagus in the western

world.[153] These cancers also demonstrate a marked male predominance and tend to present at a younger age in males.[154] Adenocarcinoma of the oesophagus is considered to be a consequence of chronic damage to the squamous mucosa of the distal oesophagus by acid, pepsin and probably bile refluxing from the stomach and small intestine.[155, 156] In response to this chronic damage, the oesophageal squamous epithelium undergoes metaplasia to become columnar in type and eventually resembling that of the small or large intestine.[157, 158] Oesophageal adenocarcinoma is thought to arise from this intestinal metaplasia and histologically resembles the intestinal subtype of gastric adenocarcinoma.

Global data from cancer registries suggests that the male predominance of upper gastrointestinal cancer is related to the anatomical location, being higher for adenocarcinoma of the oesophagus and lower for adenocarcinoma of the distal stomach.[159] The male to female ratio of age-standardised incidence rates for oesophageal adenocarcinoma in Scotland is of the order of 4.5:1, for adenocarcinoma of the proximal cardia region of the stomach or gastro-oesophageal junction it is 3.5:1 and for more distal gastric cancer it is 2.0:1. [160] However, the proportion of the intestinal histological subtype differs according to anatomical site and it is unclear whether it is the anatomical site or the histological subtype which is associated with the gender phenomenon.

Understanding the point at which the gender phenomenon is acting will facilitate unravelling its mechanism. We have therefore conducted a population-based study to determine whether the gender phenomenon is primarily related to the anatomical site or to the histological subtype of adenocarcinoma of the upper gastrointestinal tract. This has been conducted in the West of Scotland, a region with a moderately high incidence of gastric cancer and with the highest recorded incidence of oesophageal adenocarcinoma in the world [160]. Our findings indicate that the intestinal subtype has the greatest impact on the gender ratio and this is unrelated to whether the carcinoma

has developed in the oesophagus or distal stomach. Our study also indicates that the gender phenomenon is due to the development of the intestinal subtype of cancer being delayed by 17.3 years in females.

## 6.3.2. METHODS & MATERIALS

### 6.3.2.1. Setting

The study was based on patients with a diagnosis of gastric or oesophageal cancer recorded in the West of Scotland Cancer Registry between 1998 and 2002. The Cancer Registry was covering more than a half of the Scottish population at this time. The registry constantly monitors data quality to evaluate reliability of recorded diagnosis. According to a recent reliability report in 1997, there was a 97% agreement in coding the major tumour site category based on ICD-10 and only 2% discrepancy in microscopic verifications of tumours. Registration of cancers based on death certificate only (DCO) criteria for all malignant neoplasms, excluding non-melanoma skin cancer was only 0.4% in 1997 [161]. For the time period included in our study, the estimated completeness of cancer registration was >96% [162].

### 6.3.2.2. Selection Process

We collected the tumour identification number of all cases of gastric and oesophageal adenocarcinoma recorded in the West of Scotland during the five year period 1998-2002. The study was conducted on histology slides and records of 812 randomly selected patients from a total of 3270 cases of gastric and oesophageal cancers recorded in the database. The number of samples was stratified by tumour site to ensure that the sites are present in the sample in the same proportion as in the population. A random sample of pre-defined size was selected from each group of

cancers (approximately 25% for each site). Randomisation was performed with a computerised random number generator (SPSS Inc, Chicago, IL, USA). To be included in the study, all cases were required to have histologic samples available for microscopic verification.

#### 6.3.2.3. Histologic Study

All pathology records of sampled subjects were reviewed for microscopic diagnosis and anatomical site of tumour using ICD-10 and ICD O-2. The histologic subtype of adenocarcinoma was determined by the Lauren classification [148]. By definition, intestinal subtype tumours have a glandular pattern usually accompanied by papillary formations or solid components. The glandular epithelium consists of large pleomorphic cells with large hyperchromatic nuclei often with numerous mitoses. They are usually fairly well polarized columnar cells, sometimes with a prominent brush border and goblet cells. Diffuse subtype was defined as tumour predominantly composed of poorly cohesive or completely un-cohesive infiltrating small tumour cells. Gland formation is inconspicuous, except sometimes in the superficial part of the tumour. Signet ring cells are common and there may be extracellular mucin in stroma.

When the information on the pathology reports was inadequate, the original microscopy slides were re-evaluated by the study pathologists using the Lauren classification. In order to ensure compatibility of reported classifications with our study definitions, at least 10% of all specimens with complete histologic records were selected randomly for re-examination using the same protocol.

#### 6.3.2.4. Statistical Analysis

Binary logistic regression models were used to estimate the relationship between the odds of male gender (dependent variable) and histological subtype, tumour location

and age (independent variables). The histological subtype included intestinal and diffuse subtypes but not mixed subgroup due to a very small percentage of this type in the population sample. All gastric and oesophageal tumours of histology other than adenocarcinoma were excluded from analysis. Tumour location was defined as oesophageal, gastric cardia and gastric non-cardia as defined in the cancer registry database. Patients were categorised into 5 age-groups: <50, 50-59, 60-69, 70-79 and ≥80. The 10 year groupings were arbitrarily chosen on common-sense grounds; the top and bottom groups extend beyond a decade to ensure all groups have an adequate number of patients. Grouping age in this way for the logistic regression means that no assumptions have to be made about the form of the relationship (e.g, linear) between age and the odds of male gender. The logistic regression models were used to estimate the odds of male gender for the categories of the independent variables, the associated 95% confidence intervals and the associated p-values. Logistic regression models were fitted initially for each independent variable separately. A multiple logistic regression model was finally fitted including all the independent variables. All 2-way interactions between the independent variables were initially considered in this multivariable model, but as none were statistically significant at 10% they were omitted from the final analysis.

### 6.3.2.5. Supplementary studies

As the above analysis indicated that male predominance was associated with the intestinal histological subtype and not tumour location, we proceeded to investigate characteristics of the male predominance affecting the intestinal versus diffuse subtype of tumours. This included modelling the age specific incidence in males versus females in the intestinal and diffuse tumours and also of other tumours in our cancer registry.

#### 6.3.2.5.1. Curve fitting age-specific cancer incidence data

A curve fitting approach was taken to quantitatively describe the age-specific incidence of cancer using non-linear regression analysis. Equation 1 was fitted to the age-specific incidence data using the SOLVER function of Excel [163]. The difference between the data and the model (sum of the square differences (SS)) was computed and the target function which was minimised by non-linear regression analysis using Generalized Reduced Gradient (GRG2) non-linear optimization was the root mean square of SS. Curve fits were obtained using similar starting estimates for all age-specific incidence data.

*Equation 1* $$I_{(t)} = a \times (t - d)^b$$

where,

$I_t$ is the age-specific incidence of cancer (per 100,000 person-year) at age t

$t$ is the mean age of the group

$a$, $b$ and $d$ are regression constants where $a$ is a scaling factor, $b$ is a power term that reflects the rate of incidence with age and $d$ is a delay term for the time between birth and age of increased incidence above zero. A logic IF function was used in Excel such that when t < d (t – d <0), $I_{(t)} = 0$. Thus only when d > t was $I_{(t)} > 0$.

#### 6.3.2.5.2. Comparison of gender related, age-specific incidence with other cancers

The 1998-2002 average age-group-specific incidence (per 100,000 person-years) were extracted from the ISD Scottish Cancer Registry for: cancer of the oesophagus, adenocarcinoma (ICD-10: C15, ICD-O-2 various); cancer of the oesophagus, squamous cell (ICD-10: C15, ICD-O-2: 8050-8076); cancer of the lung, squamous cell carcinoma (ICD-10: C33-34; ICD-O-2: 8050-8076), cancer of the lung,

adenocarcinoma (ICD-10: C33-34; ICD-O-2: various); cancer of the lung, small cell carcinoma (ICD-10: C33-34; ICD-O-2: 8040-8045); cancer of the bladder, squamous cell carcinoma (ICD-10: C67; ICD-O-2: 8051-8076); cancer of the bladder, transitional cell carcinoma (ICD-10: C67; ICD-O-2: 8050, 8120-8122, 8130); cancer of the colon (ICD-10: C18; ICD-O-2: various); and cancer of the pancreas (ICD-10: C25; ICD-O-2: various). These cancers were recorded for West of Scotland Cancer Registry matching the population for gastric and oesophageal cancer. The age-specific incidence of these cancers were also analysed by curve fitting, as described previously, to examine gender differences in the incidence rate and the age at which incidence increased above zero.

#### 6.3.2.6. Ethical Considerations

The study protocol was reviewed and approved firstly by the Multi Centre Research Ethics Committee (MREC) which is acting nationally and subsequently by local NHS Ethics Committees.

### 6.3.3. RESULTS

In total 812 incident cancers with histologic diagnosis of oesophageal adenocarcinoma (C15), gastric cardia cancer (C16.0) and gastric non-cardia cancer (C16.1-16.9) were reviewed. Of these, 25 records (3.1 %) were excluded because both original reports and materials were missing (n=9) or they were recorded in duplicate (n=16). After the first round of document review, 3241 slides from 463 cancer cases were reviewed because their original records had inadequate information regarding the Lauren histological subtypes. Among 349 reports with adequate information, 42 reports were selected randomly and related slides were re-evaluated. Classification of only 2 cases (<5%) required to be changed (from diffuse subtype to mixed subtype). The distribution of cancers by sex and anatomical site in the sample studied showed no statistical difference from the correspondent entire cancer registry data.

Regardless of anatomical subsite, the upper GI cancers were more common in males (502, 63.8%) than females (285, 36.2%). Four hundred and five (51.5%) of the cancers originated from the non-cardia region of the stomach, 173 (22.0%) from the gastric cardia and 209 (26.6%) from the oesophagus.

Histologically, 63.8 % of all tumours were of intestinal and 21.3% of diffuse subtype (Table 6.3). Of the remaining 117, 25.6% were mixed type of Lauren classification, 30.8% undifferentiated carcinoma, and 43.6% of other histological diagnosis. The last group included adenosquamous carcinoma (n=1), large cell carcinoma (n=1), leiomyosarcoma (n=1), lymphoma (n=1), carcinoid tumour (n=7), carcinoma in situ (n=7), squamous cell carcinoma (n=10) and metastatic tumours of unknown origin

(n=23). For the purpose of this study, we only analysed the data of patients with either intestinal or diffuse type carcinoma which included more than 85 % of incident cancers.

The proportion of histological subtypes varied with tumour location. Intestinal /diffuse subtype ratio was 163/17 (9.6: 1) for oesophageal adenocarcinomas, 102/32 (3.2: 1) for cardia and 227/119 (1.9: 1) for non-cardia adenocarcinomas (Fig 6.4).

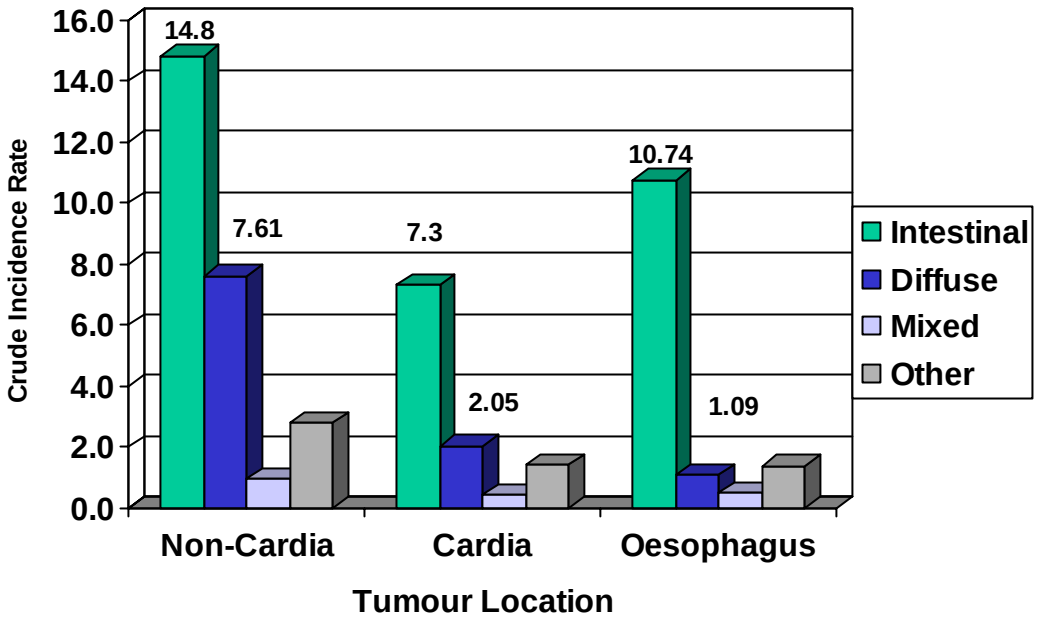



**Fig 6.4:** Crude incidence rates of intestinal versus diffuse subtypes of adenocarcinoma in different tumour location

**Table 6.3:** Crude incidence rates of upper GI cancer of the random sample of West of Scotland by histology and tumour location

| **Tumour Location** | **Gastric Non-Cardia** | | | | **Gastric Cardia** | | | | **Oesophageal adenocarcinoma** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Histology | Number | | Incidence Rate* | | Number | | Incidence Rate* | | Number | | Incidence Rate* | |
| | M | F | M | F | M | F | M | F | M | F | M | F |
| **Intestinal** | 151 | 76 | 10.14 | 4.66 | 76 | 36 | 5.10 | 2.20 | 128 | 35 | 8.60 | 2.14 |
| **Diffuse** | 57 | 62 | 3.82 | 3.79 | 17 | 15 | 1.14 | 0.91 | 9 | 8 | 0.60 | 0.49 |
| **Mixed** | 9 | 6 | 0.60 | 0.37 | 4 | 3 | 0.27 | 0.18 | 6 | 2 | 0.40 | 0.12 |
| **Undifferentiated** | 7 | 8 | 0.47 | 0.49 | 6 | 4 | 0.40 | 0.24 | 9 | 2 | 0.60 | 0.12 |
| **Other** | 10 | 19 | 0.67 | 1.16 | 5 | 7 | 0.34 | 0.43 | 8 | 2 | 0.54 | 0.12 |
| **All types** | 234 | 171 | 15.73 | 10.48 | 108 | 65 | 7.26 | 3.98 | 160 | 49 | 10.75 | 3.00 |

* Crude annual incidence rate, per 100,000 person-years

### 6.3.3.1. Association of male predominance with tumour location versus histological subtype

**6.3.3.1.1. Gender and histological subtypes:** Regardless of anatomical site, the crude incidence rate of intestinal subtype upper GI adenocarcinoma was higher in males at 23.86 per 100,000 person-years versus females at 9.00 per 100,000 person-years, resulting in a M/F ratio of 2.65. In contrast, the crude incidence rate of diffuse subtype adenocarcinoma was similar in males and females (5.58 vs 5.20 per 100,000 person-years) yielding M/F ratio of 1.07 (Table 6.4, and Fig 6.5). Gender effect expressed as M/F incidence ratio varied with age and histological subtype. As shown in Fig 6.6, M/F ratio of intestinal subtype cancer was 3.41 at age less than 50, reached a peak of 7.86 at age 50-59, and then showed a progressive decrease with a minimum of 2.29 at age group 80 years and over. In contrast, M/F ratio of diffuse subtype cancer was 0.89 at age less than 50 and did not show any significant changes with increasing age.

**6.3.3.1.2. Gender and tumour location:** Regardless of histological subtype, the male predominance of adenocarcinoma incidence varied with anatomical location (Table 6.5). Male predominance was greatest in the oesophagus with crude incidence rates of 9.21 and 2.63 in males versus females, respectively (M/F = 3.50). For cardia cancer the crude incidence rates were 6.25 and 3.12 for males and females (M/F= 2.00) and for non-cardia cancer 13.98 and 8.46 (M/F= 1.65). (Fig 6.7)

**Table 6.4:** Distribution of upper gastrointestinal adenocarcinoma in different age groups by gender and histological subtypes

| Histology | | Age groups | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | <50 | | 50-59 | | 60-69 | | 70-79 | | ≥ 80 | | All ages | |
| | | n | Rate* | n | Rate* | n | Rate* | n | Rate* | n | Rate* | n | Rate** |
| Intestinal subtype | Male | 20 | 2.51 | 60 | 32.56 | 94 | 65.38 | 124 | 134.74 | 57 | 177.11 | 355 | 23.86 |
| | Female | 6 | 0.57 | 8 | 4.14 | 21 | 12.50 | 52 | 38.94 | 60 | 77.44 | 147 | 9.00 |
| | Total | 26 | | 68 | | 115 | | 176 | | 117 | | 502 | |
| | M/F | | 3.41 | | 7.86 | | 5.23 | | 3.46 | | 2.29 | | 2.65 |
| Diffuse subtype | Male | 7 | 0.68 | 14 | 7.56 | 25 | 17.38 | 27 | 29.34 | 10 | 31.07 | 83 | 5.58 |
| | Female | 8 | 0.75 | 11 | 5.69 | 17 | 10.11 | 24 | 17.98 | 25 | 32.26 | 85 | 5.20 |
| | Total | 15 | | 25 | | 42 | | 51 | | 35 | | 168 | |
| | M/F | | 0.89 | | 1.33 | | 1.72 | | 1.63 | | 0.96 | | 1.07 |
| Both subtypes | Male | 27 | 2.60 | 74 | 40.16 | 119 | 82.77 | 151 | 164.07 | 67 | 208.18 | 438 | 29.44 |
| | Female | 14 | 1.32 | 19 | 9.83 | 38 | 22.62 | 76 | 56.93 | 85 | 109.71 | 232 | 14.21 |
| | Total | 41 | | 93 | | 157 | | 227 | | 152 | | 670 | |
| | M/F | | 1.97 | | 4.09 | | 3.66 | | 2.90 | | 1.90 | | 2.07 |

**Rate*:** Age-specific incidence rate per 100,000 person-years
**Rate**:** Crude incidence rate per 100,000 person-years

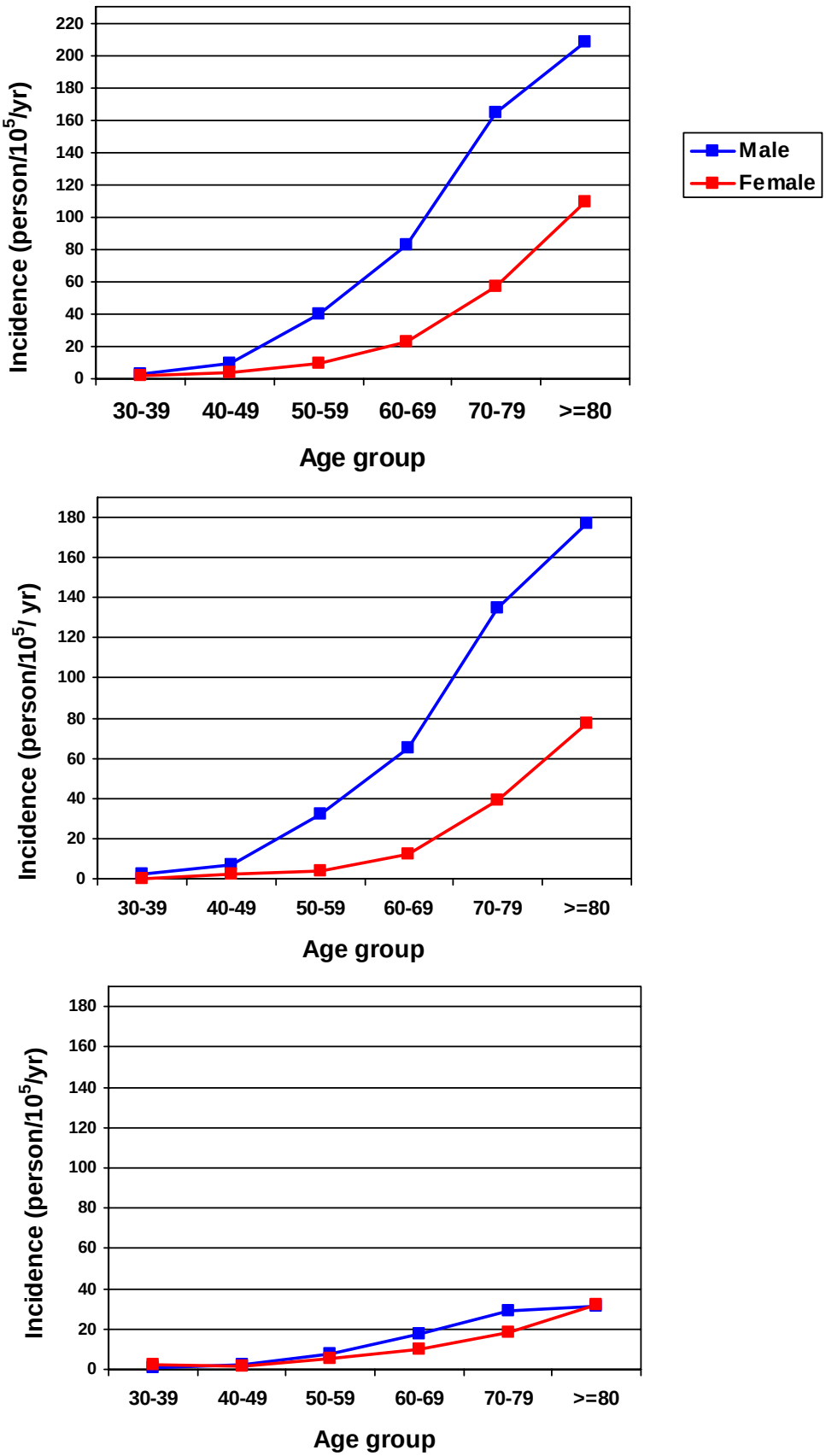



**Figure 6.5:** Age specific incidence rate of upper GI adenocarcinoma by gender, (top) combined intestinal and diffuse subtype, (middle) intestinal subtype and (bottom) diffuse subtype

**Table 6.5:** Distribution of upper gastrointestinal adenocarcinoma in different age groups by gender and tumour location

| Tumour location | | Age groups | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | <50 | | 50-59 | | 60-69 | | 70-79 | | >= 80 | | All ages | |
| | | n | Rate* | n | Rate* | n | Rate* | n | Rate* | n | Rate* | n | Rate** |
| Gastric non-cardia | Male | 9 | 0.86 | 28 | 15.20 | 54 | 37.56 | 69 | 74.97 | 48 | 149.15 | 208 | 13.98 |
| | Female | 11 | 1.04 | 10 | 5.17 | 25 | 14.88 | 41 | 30.71 | 51 | 65.73 | 138 | 8.46 |
| | Total | 20 | | 38 | | 79 | | 110 | | 99 | | 346 | |
| | M/F | | 0.83 | | 2.94 | | 2.52 | | 2.44 | | 2.27 | | 1.65 |
| Gastric cardia | Male | 9 | 0.86 | 15 | 8.14 | 24 | 16.69 | 36 | 39.12 | 9 | 27.97 | 93 | 6.25 |
| | Female | 2 | 0.18 | 5 | 2.59 | 5 | 2.98 | 18 | 13.48 | 21 | 27.11 | 51 | 3.12 |
| | Total | 11 | | 20 | | 29 | | 54 | | 30 | | 144 | |
| | M/F | | 4.78 | | 3.14 | | 5.69 | | 2.90 | | 1.03 | | 2.00 |
| Distal oesophagus | Male | 9 | 0.86 | 31 | 16.82 | 41 | 28.52 | 46 | 49.98 | 10 | 31.07 | 137 | 9.21 |
| | Female | 1 | 0.09 | 4 | 2.07 | 8 | 4.76 | 17 | 12.73 | 13 | 16.78 | 43 | 2.63 |
| | Total | 10 | | 35 | | 49 | | 63 | | 23 | | 180 | |
| | M/F | | 9.56 | | 8.13 | | 5.99 | | 3.93 | | 1.85 | | 3.50 |
| All sites | Male | 27 | 2.60 | 74 | 40.16 | 119 | 82.77 | 151 | 164.07 | 67 | 208.18 | 438 | 29.44 |
| | Female | 14 | 1.32 | 19 | 9.83 | 38 | 22.62 | 76 | 56.93 | 85 | 109.71 | 232 | 14.21 |
| | Total | 41 | | 93 | | 157 | | 227 | | 152 | | 670 | |
| | M/F | | 1.97 | | 4.09 | | 3.66 | | 2.90 | | 1.90 | | 2.07 |

**Rate*:** Age-specific incidence rate per 100,000 person-years
**Rate**:** Crude incidence rate per 100,000 person-years

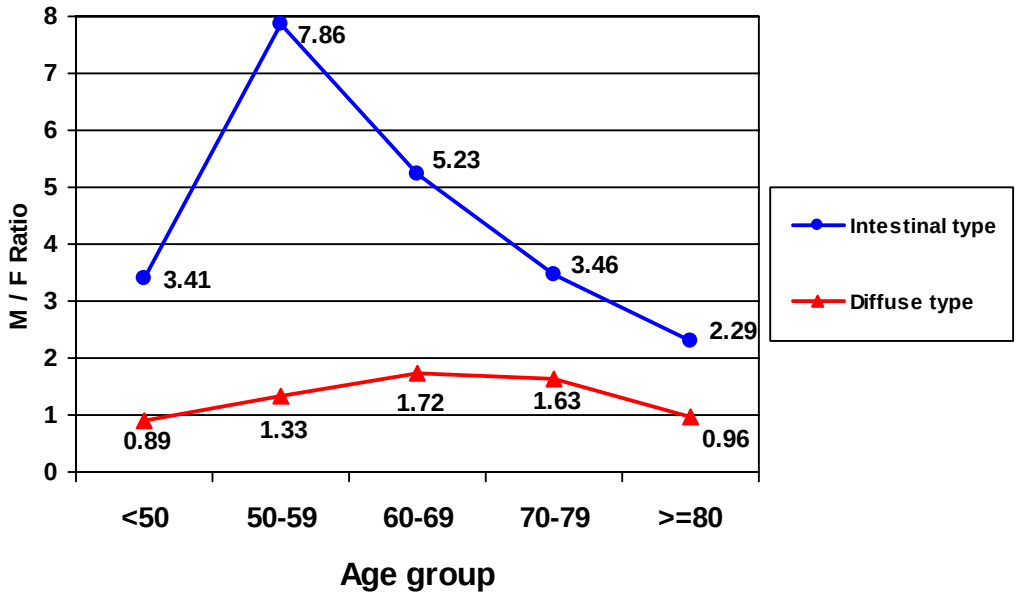



**Figure 6.6:** Male to female ratios of age-specific incidence rate of upper GI adeno-carcinoma by histological subtype. Note that the ratio of the intestinal subtype increases to a maximum at age group 50-59 followed by a progressive decrease.

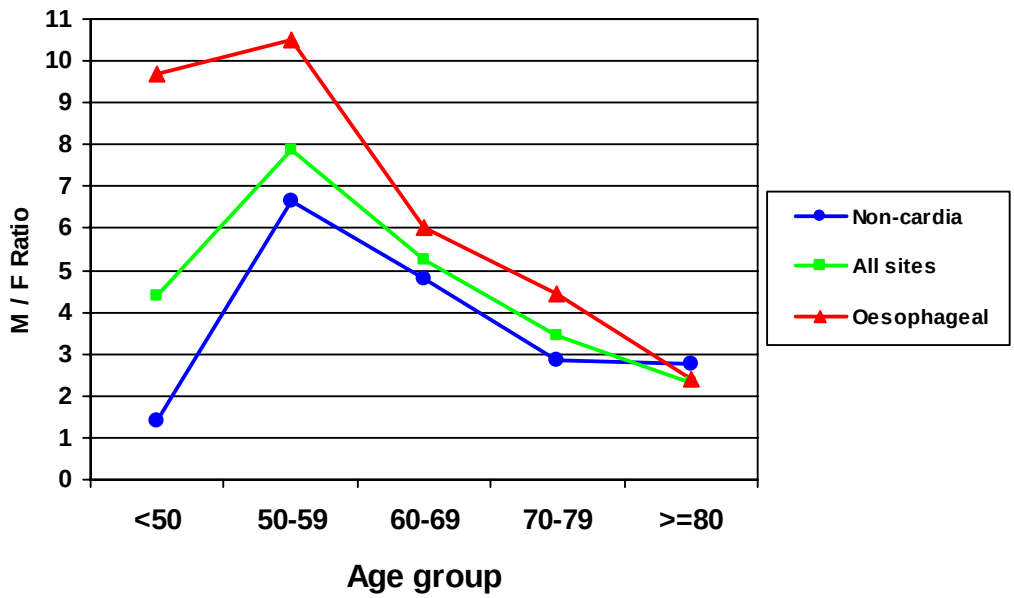



**Figure 6.7:** Male to female ratios of age-specific incidence rate of intestinal subtype upper GI adenocarcinoma by tumour location. For each cancer site the M/F ratio peaks at age group 50-59 years and then shows a progressive marked decrease.

#### 6.3.3.1.3. Multivariable analysis of male predominance risk factors:

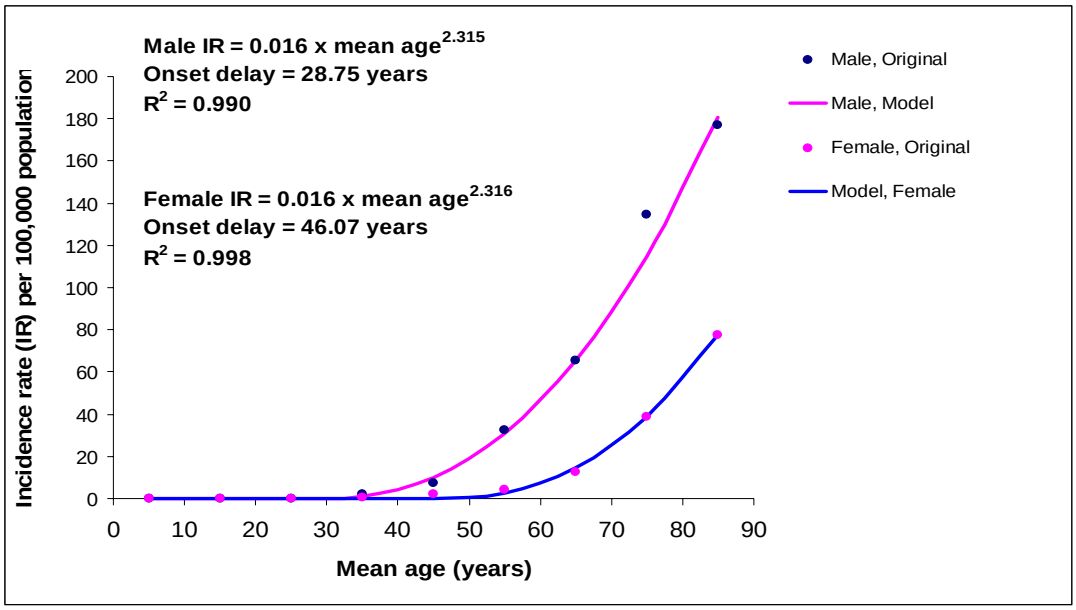
Multivariable logistic regression including histological subtype, tumour location and age indicated that the odds of male gender was mainly related to the histological subtype and age rather than anatomical location. Although the odds of male gender was higher for oesophageal versus non-cardia adenocarcinoma when anatomical location was considered alone in a logistic regression model [Odds ratio (OR)= 2.11; 95% confidence interval (CI): 1.41 - 3.17], this relationship with anatomical location lost statistical significance in the multivariable analysis when histological subtype and age were added [OR=1.37; 95% CI: 0.88 - 2.12]. In addition the overall significance level in the logistic regression model for anatomical location diminished from p<.001 when it was considered alone to p=.333 when considered together with age and histological subtype. This suggests that male predominance in upper GI adenocarcinomas was not primarily a function of tumour location but rather related to histological subtype and age (Table 6.6). Intestinal subtype adenocarcinoma was associated with increased odds of male gender irrespective of anatomical location or age [OR=2.64; 95% CI: 1.78 – 3.90). Increasing age showed an overall inverse relationship with the odds of male gender excluding those aged < 50 years; again this relationship persisted when anatomical location and histological subtype were considered in the same logistic regression model.

**Table 6.6:** Logistic regression analysis of association between gender (in favour of male) and histological subtype, tumour location and age

| | Independent Variable Considered Individually | | | | Multivariable Model | | | |
|---|---|---|---|---|---|---|---|---|
| | P value | Odds Ratio (OR) | 95 % C.I. for OR | | P value | Odds Ratio (OR) | 95 % C.I. for OR | |
| | | | Lower | Upper | | | Lower | Upper |
| Histological subtype | | | | | | | | |
| Diffuse (Referent) | | 1.000 | | | | 1.000 | | |
| Intestinal | 0.000 | 2.473 | 1.728 | 3.539 | 0.000 | 2.637 | 1.784 | 3.896 |
| Tumour Site | | | | | | | | |
| Gastric non-cardia (Referent) | | 1.000 | | | | 1.000 | | |
| Gastric Cardia | 0.355 | 1.210 | 0.808 | 1.811 | 0.983 | 0.995 | 0.648 | 1.529 |
| Distal Oesophagus | 0.000 | 2.114 | 1.410 | 3.168 | 0.161 | 1.368 | 0.883 | 2.121 |
| P value for overall effect | 0.000 | | | | 0.333 | | | |
| Age band | | | | | | | | |
| Age < 50 (Referent) | | 1.000 | | | | 1.000 | | |
| Age 50-59 | 0.093 | 2.019 | 0.890 | 4.581 | 0.165 | 1.821 | 0.782 | 4.240 |
| Age 60-69 | 0.200 | 1.624 | 0.773 | 3.409 | 0.328 | 1.466 | 0.681 | 3.155 |
| Age 70-79 | 0.934 | 1.030 | 0.511 | 2.079 | 0.721 | 0.876 | 0.423 | 1.813 |
| Age >=80 | 0.015 | 0.409 | 0.199 | 0.840 | 0.006 | 0.347 | 0.164 | 0.736 |
| P value for overall effect | | 0.000 | | | | 0.000 | | |

### 6.3.3.2. Characteristics of male predominance of upper GI adenocarcinoma

The rise in incidence with increasing age was much more marked in the intestinal versus diffuse subtype (Fig 6.8). For the diffuse histological subtype the crude and age-specific incidence rates were similar for males and females. Curve fitting of the age-specific incidence data for diffuse subtypes resulted in similar equations, $y = 0.016 \times \text{mean age}^{2.007}$, $R^2 = 0.999$ and $y = 0.016 \times \text{mean age}^{1.954}$, $R^2 = 0.989$, for male and females respectively. The age at which the age-specific incidence curve rose above zero was similar in males (33.0 years) and females (35.8 years). For the intestinal histological subtype, the age-specific incidence data were different for males and females. Curve fitting indicated a similar incidence rate in males ($y = 0.016 \times \text{mean age}^{2.315}$, $R^2 = 0.990$) and in females ($y = 0.016 \times \text{mean age}^{2.316}$, $R^2 = 0.998$). However, the age-specific incidence curve for females did not appear to deviate from zero until an older age compared with male intestinal subtype. The age at which the age-specific incidence curve rose above zero was 28.8 years in males versus 46.1 years in females indicates a delay of 17.3 years in the appearance of intestinal subtype cancer in females.



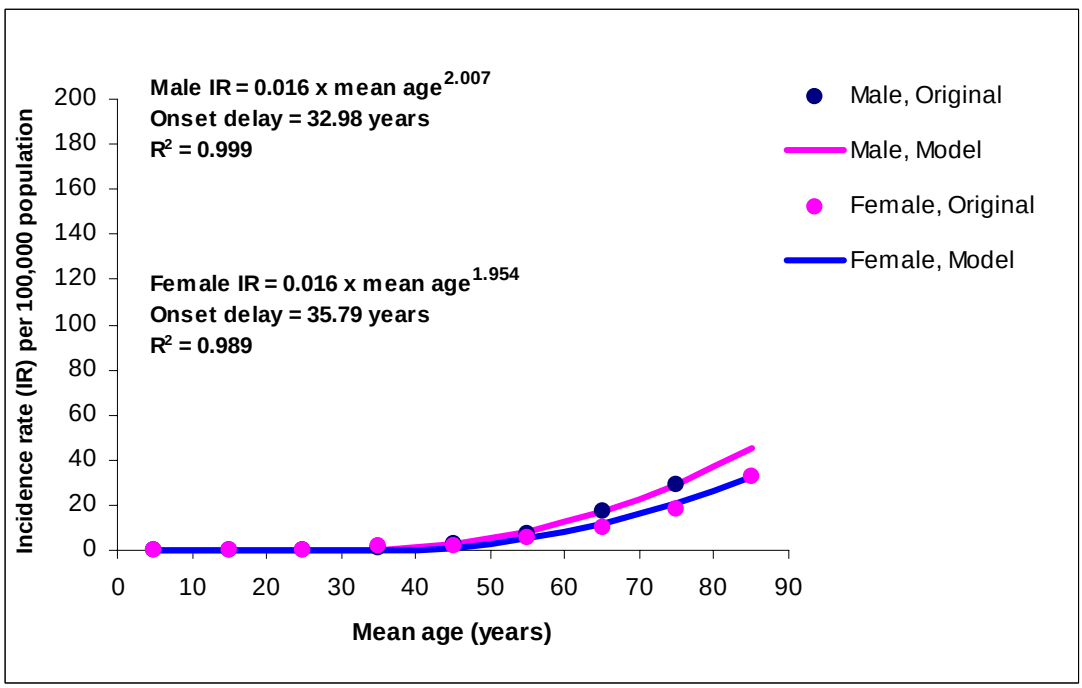




**Figure 6.8:** Modelling of age-specific incidence rate curve of intestinal (top) and diffuse subtypes (bottom) upper GI adenocarcinoma by gender. This shows similar slope of curves but delayed rise in curve in female.

#### 6.3.3.3. Characteristics of male predominance in other cancers:

Analysis of all recorded cases of oesophageal adenocarcinoma in the Scottish Cancer Registry between 1998 and 2002 produced similar age-specific incidence curves to that observed in our random sample of oesophageal adenorcarcinoma with an age delay in the appearance of intestinal subtype cancer in females of 15.6 years (Table 6.7). Analysis of age-specific incidence curves of squamous cell carcinoma of oesophagus, lung cancer (three common histologic types), bladder cancer (two common histologic types), colon cancer (all histologies) and pancreatic cancer (all histologies) showed no evidence of a gender related delay in the incidence of these cancers. This analysis included squamous cell carcinoma of lung and transitional cell carcinoma of bladder, which have a M/F ratio of 2.1 and 2.6 respectively. In the latter cancers, the higher male incidence was due to a higher rate of increase rate rather than any gender specific delay in the rise of incidence (Table 6.7).

**Table 6.7:** Parameters from fit of equation 1 to age-specific incidence rates of upper GI adenocarcinomas compared with other cancers from West of Scotland 1998-2002.

| | M / F ratio | Male | | | Female | | | Gender Bias | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (of crude incidence) | a | b | d | a | b | d | Δa | Δb | Δd |
| Upper GI adenocarcinoma, Intestinal subtype | 2.65 | 0.02 | 2.32 | 28.8 | 0.02 | 2.32 | 46.1 | 0.0 | 0.0 | 17.3 |
| Upper GI adenocarcinoma, Diffuse subtype | 1.07 | 0.02 | 2.01 | 33.0 | 0.01 | 1.95 | 35.8 | 0.0 | -0.1 | 2.8 |
| Oesophageal adenocarcinoma | 2.96 | 0.02 | 2.21 | 33.1 | 0.01 | 2.09 | 48.7 | 0.0 | -0.1 | 15.6 |
| Oesophageal squamous cell carcinoma | 1.07 | 0.02 | 2.16 | 34.7 | 0.01 | 2.15 | 37.0 | -0.1 | -0.1 | 2.3 |
| Squamous cell carcinoma of lung | 2.10 | 0.02 | 2.64 | 38.2 | 0.02 | 2.47 | 38.7 | 0.0 | -0.2 | 0.5 |
| Adenocarcinoma of lung | 1.32 | 0.03 | 2.24 | 34.5 | 0.02 | 2.18 | 32.5 | 0.0 | -0.1 | -2.0 |
| Small cell carcinoma of lung | 1.14 | 0.02 | 2.38 | 34.4 | 0.04 | 2.16 | 37.3 | 0.0 | -0.2 | 2.9 |
| Squamous cell carcinoma of bladder | 0.88 | 0.001 | 2.22 | 38.2 | 0.001 | 2.31 | 38.8 | 0.0 | 0.1 | 0.6 |
| Transitional cell carcinoma of bladder | 2.57 | 0.01 | 2.63 | 40.0 | 0.01 | 2.20 | 36.0 | 0.0 | -0.4 | -4.0 |
| Cancer of the colon | 1.06 | 0.02 | 2.49 | 34.8 | 0.06 | 2.21 | 36.7 | 0.0 | -0.1 | 1.9 |
| Cancer of the pancreas | 1.07 | 0.09 | 1.84 | 39.4 | 0.04 | 1.90 | 36.2 | -0.1 | 0.1 | -3.2 |

**Note:** Equation1: $I_{(t)} = a \times (t - d)^b$ where, *I* is the incidence rate of cancer at age *t*, which is the mean age of the age group, *a* is a scaling factor, *b* is a power term that reflect the rate of increasing incidence with age and *d* is a delay term for the time between birth and age of rise of age-specific incidence curve above zero.

# Section 4

## Evaluation of gender difference in prevalence of gastric precancerous lesions

### 6.4.1. INTRODUCTION

As we showed in the previous section, the incidence of Intestinal subtype gastric adenocarcinoma in males is higher than in females and almost all male predominance of upper gastroesophageal cancer can be explained by a remarkable 17 years' delay in the development of the intestinal subtype in females compared to males. The intestinal subtype of gastric adenocarcinoma arises due to progression of a H.pylori-induced chronic inflammation to atrophic gastritis to intestinal metaplasia and finally to adenocarcinoma. The histological stage in this pathway in which gender acts is unclear.

As mentioned earlier, the Ardabil province in Northwest of Iran is an area with high incidence of gastric cancers, particularly those located at gastric cardia. Due to excellent collaboration of local health and academic authorities with national and international research teams, this area has been considered for many population-based projects on upper gastrointestinal tract cancers. The current study on gender and gastric precancerous lesions is part of a large cross-sectional study on gastric and oesophageal cancer in this region, and we have investigated the influence of

gender on the frequency of different histological inflammatory and precancerous lesions.

## 6.4.2. METHOD & MATERIALS

### 6.4.2.1. Subjects

Participants in the study were selected in collaboration with Ardabil University of Medical Sciences. A list of all the families resident in the Meshkinshahr district was prepared and the rural/urban distribution of the population was computed. The sampling framework included all the families with at least one member aged ≥40 based on the 2001 census. By using stratified random sampling, 650 families were selected from rural and urban areas in proportion to the population size of each area. In families with more than one eligible member, one only was selected randomly and invited by trained native interviewers to participate in the study. Five-hundred and thirty-seven individuals (82.7%) agreed to participate. Before endoscopy, all participants were interviewed and a brief physical examination was performed. Twenty-five individuals were excluded from the study for three reasons: presence of cardiac, respiratory, or other problems which were expected to increase the risk of complications of endoscopy and biopsy for the patients (eighteen cases), pregnancy (one case), and recently taking medications for H.pylori eradication (six cases). Four cases were excluded because of poor cooperation during endoscopy. Finally, 508 participants were recruited to the study after signing an informed consent agreement.

### 6.4.2.2. Endoscopic evaluation and mucosal biopsy

The procedure for endoscopy and tissue sampling was designed by the Digestive Diseases Research Centre (DDRC) and approved by the medical ethics committee of Tehran University of Medical Sciences. All the endoscopy procedures were performed by four experienced gastroenterologists after local anaesthesia by oral administration of 10% Lidocaine spray and intravenous infusion of 5–7.5 mg Midazolam. Before the study the gastroenterologists were informed of the objectives and details of the procedures. The nomenclature and visual diagnostic criteria of the lesions of the upper GI tract were reviewed by the members of the team to minimize inter-observer diagnostic variations. Pentax EG2940 video endoscopes were used for the procedure. All notable lesions were recorded and reported in minimal standard terminology [164]. Biopsy specimens were obtained using conventional disposable biopsy forceps. The first biopsy from the incisura angularis was used for a rapid urease test (RUT). Biopsy sites for histological examination were chosen in accordance with a modified format of the updated Sidney classification recommendations [165]. In this format six different biopsies were performed and oriented on filter paper before fixation. The biopsies included three samples from different parts of the antrum, two from the corpus, and one from the cardia. Samples were placed in six pre-labeled containers with 10% neutral buffered formalin as fixative. Additional separate biopsy samples were taken from any lesions observed endoscopically and treated in the same way as the other samples.

### 6.4.2.3. Histopathological examination of samples

All the specimens were processed, sectioned, and stained with the hematoxyline–eosin method in routine procedures and studied by a team of three pathologists well experienced in upper GI biopsies. All samples with abnormal findings were studied by

the second pathologist of the team. In the event of diagnostic discrepancy the controversial case was agreed to be reviewed by both pathologists in joint sessions to reach a diagnostic consensus.

One out of ten slides was also randomly and blindly re-examined by the second pathologist for diagnostic quality assurance. The histological criteria used for evaluation of gastritis were based on the updated Sydney system for classification of gastritis [165]. PMN and MN cell infiltrations of the mucosa were scored from zero to three on the basis of morphological scales but we used the results as dichotomous data in most of the calculations. The sum of PMN and MN infiltrations was expressed as combined inflammatory score (CIS) for each biopsy site. For evaluation of H.pylori infection the presence of the bacteria in at least one biopsy site or a positive RUT was regarded as indicative of global infection of the stomach.

#### 6.4.2.4. Statistical methods

Relationships between the two components of gastritis (PMN and MN infiltrations) and male gender were indicated by the odds ratio (OR) and the 95% confidence interval (CI) for each subsite.

## 6.4.3. RESULTS

### 6.4.3.1. Chronic Gastritis and Gender

In the general population, the frequency of active inflammation in all biopsy sites between both genders was similar (Table 6.8). Our data also did not show any difference in chronic inflammation between males and females (Table 6.9). The rate of H.pylori infection, based on histology and rapid urease test was similar among both genders (82.1 % and 82.5 % in males and females, respectively), [OR=1.0 (0.6 – 1.7).

**Table 6.8**: Relationship between gender and Active inflammation (PMN infiltration) In different locations of the gastric mucosa

| Biopsy site | | Male | | Female | | Male Vs. Female |
|---|---|---|---|---|---|---|
| | | N | % | N | % | OR (95% CI) |
| **Cardia** | Cardia | 122 | **54.2** | 136 | **50.9** | **1.1 (0.8 – 1.6)** |
| **Corpus** | Greater Curvature | 94 | **40.7** | 124 | **45.8** | **0.8 (0.6 – 1.2)** |
| | Lesser Curvature | 123 | **53.7** | 141 | **52.6** | **1.0 (0.7 – 1.5)** |
| **Antrum** | Prepyloric | 149 | **64.2** | 177 | **66.0** | **0.9 (0.6 – 1.3)** |
| | Incisura Angularis | 148 | **63.8** | 190 | **70.1** | **0.8 (0.5 – 1.1)** |
| | Greater Curvature | 127 | **55.7** | 161 | **59.4** | **0.9 (0.6 – 1.2)** |

**Table 6.9**: Relationship between gender and chronic inflammation (MN infiltration) in different locations of the gastric mucosa

| Biopsy Site | | Male | | Female | | Male Vs. Female |
|---|---|---|---|---|---|---|
| | | N | % | N | % | OR (95% CI) |
| **Cardia** | Cardia | 158 | **70.2** | 185 | **69.3** | **1.0 (0.7 – 1.5)** |
| **Corpus** | Greater Curvature | 159 | **68.8** | 193 | **71.2** | **0.9 (0.6 – 1.3)** |
| | Lesser Curvature | 186 | **81.2** | 208 | **77.6** | **1.2 (0.8 – 1.9)** |
| **Antrum** | Prepyloric | 198 | **85.3** | 219 | **81.7** | **1.3 (0.8 – 2.1)** |
| | Incisura Angularis | 211 | **90.9** | 238 | **87.8** | **1.4 (0.8 – 2.5)** |
| | Greater Curvature | 185 | **81.1** | 214 | **79.0** | **1.1 (0.7 – 1.8)** |

### 6.4.3.2. Precancerous changes and Gender

The frequency of non-metaplastic atrophy at the all biopsy sites was similar in both genders (Table 6.10). Intestinal metaplasia of antrum was more prevalent in males than in females (11.6 % vs. 5.9 %) and showed a significant relationship with gender [OR= 2.1 (1.1 – 4.0)]. A similar relationship was evident in cardia and corpus subsites [OR= 3.1 (1.2 – 8.1) and OR= 7.4 (1.6 – 33.3), respectively]. Considering the presence of intestinal metaplasia in at least one subsite of gastric mucosa, males had more metaplastic changes than females (24.4 % vs. 16.8%) and the subsequent relationship with gender had narrower confidence interval [OR=1.6 (1.1 – 2.5)]. (Table 6.11)

**Table 6.10**: Relationship between gender and mucosal atrophy in different locations of the stomach

| Biopsy Site | | Male | | Female | | Male Vs. Female |
|---|---|---|---|---|---|---|
| | | N | % | N | % | OR (95% CI) |
| **Cardia** | Cardia | 63 | **28.0** | 79 | **29.6** | **0.9 (0.6 – 1.4)** |
| **Corpus** | Greater Curvature | 70 | **30.3** | 104 | **38.4** | **0.7 (0.5 – 1.0)** |
| | Lesser Curvature | 86 | **37.6** | 105 | **39.2** | **0.9 (0.7 – 1.4)** |
| **Antrum** | Prepyloric | 83 | **35.8** | 104 | **38.8** | **0.9 (0.6 – 1.3)** |
| | Incisura Angularis | 89 | **38.4** | 121 | **44.6** | **0.8 (0.5 – 1.1)** |
| | Greater Curvature | 91 | **39.9** | 110 | **40.1** | **1.0 (0.7 – 1.4)** |

**Table 6.11**: Relationship between gender and intestinal metaplasia in different locations of the gastric mucosa

| Biopsy Site | | Male | | Female | | Male Vs. Female |
|---|---|---|---|---|---|---|
| | | N | % | N | % | OR (95% CI) |
| **Cardia** | Cardia | 15 | **6.7** | 6 | **2.2** | **3.1 (1.2 – 8.1)** |
| **Corpus** | Greater Curvature | 12 | **5.2** | 2 | **0.7** | **7.4 (1.6 – 33.3)** |
| | Lesser Curvature | 18 | **7.9** | 15 | **5.6** | **1.5 (0.7 – 3.0)** |
| **Antrum** | Prepyloric | 19 | **8.2** | 19 | **7.1** | **1.2 (0.6 – 2.3)** |
| | Incisura Angularis | 27 | **11.6** | 16 | **5.9** | **2.1 (1.1 – 4.0)** |
| | Greater Curvature | 12 | **5.3** | 21 | **7.7** | **0.7 (0.3 – 1.4)** |
| **All Sites (IM in at least one site)** | | **57** | **24.4** | **46** | **16.8** | **1.6 (1.1 – 2.5)** |

## 6.5. DISCUSSION

This study confirms the long-recognised male predominance of adenocarcinoma of the upper gastrointestinal tract, the crude incidence rates being 29.44 in males and 14.21 in females. It also confirms that the degree of male predominance varies by anatomical site of the adenocarcinoma, being greatest in the oesophagus (M/F ratio = 3.50), less at the cardia (M/F ratio =2.00), and least in the more distal non-cardia region of the stomach (M/F ratio = 1.65). This relationship between anatomical site and male predominance has been observed in several previous studies from different regions of the world.[154, 166]

The proportion of the intestinal to diffuse histological subtypes varied with anatomical locations, being 9.6: 1 in the oesophagus, 3.2: 1 at the cardia and 1.9: 1 in the distal stomach (Table 6.3). A high ratio of the intestinal /diffuse histological subtypes has been reported in the cardia and non-cardia region of the stomach in previous studies [151, 167-170].

A strong association was observed between male predominance and histological subtype. Regardless of anatomical subtype, the crude incidence rate of intestinal type upper gastrointestinal tract adenocarcinoma was higher in males with a M/F ratio of 2.65: 1. In contrast, the crude incidence rates of the diffuse subtype were similar in male and females with a M/F ratio of 1.07: 1.

Applying multivariable analysis to our population-based data allowed us to investigate for the first time whether the gender phenomenon was related to the anatomical site of upper gastrointestinal cancer or to the histological subtype. This

indicated that it was the intestinal subtype that was associated with male predominance rather than anatomical location. The higher male predominance in oesophageal versus gastric adenocarcinoma is explained by the higher incidence of intestinal subtype in the former.

The Lauren histological classification was originally devised to classify gastric adenocarcinoma and has proved to be of aetiopathogenic value [148]. The intestinal histological subtype of gastric cancer develops against a background of chronic H. pylori induced gastritis.[149, 171] The chronic inflammation causes atrophy of specialized gastric glands that are replaced by intestinal metaplasia from which the intestinal type of gastric adenocarcinoma is believed to originate. Oesophageal adenocarcinoma is nearly always intestinal in subtype and histologically indistinguishable from the intestinal subtype of adenocarcinoma of the stomach. Oesophageal adenocarcinoma also resembles intestinal subtype gastric cancer in its pathogenesis in that it develops against a background of chronic mucosal damage. Exposure of the squamous epithelium of the distal oesophagus to refluxing gastric juice causes it to undergo metaplasia to columnar type epithelium resembling the stomach and then to the intestinal type of epithelium [172, 173] from which the oesophageal adenocarcinoma of intestinal phenotype develops. The finding in our current study, that the intestinal type of adenocarcinoma of the oesophagus and stomach show the same male predominance, provides further evidence of similarity of pathogenesis and supports applying the Lauren classification to oesophageal cancers.

We further investigated the male predominance of intestinal type upper gastrointestinal adenocarcinoma by comparing the age-specific incidence rates of the two sexes. Curve fitting indicated that the male and female were described by similar power terms in the functions describing the curves. The only difference between the curves was that the rise in the incidence of female cancer lagged behind that of the

male by 17.3 years. The male predominance of this cancer is due to the rise of cancer incidence with age in males commencing at 28.8 years of age compared to 46.1 years of age for females. Sipponen and Correa have previously reported a delay in the development of the intestinal subtype of gastric cancer in females in the Finnish population.[174] A delay in development of oesophageal carcinoma in females has not been reported previously but there are reports of a delay in development of Barrett's oesophagus in females versus males. [175, 176].

The fact that the rise in age-specific incidence of intestinal subtype is occurring 17.3 years later in females than males, but has the same slope, indicates that there is temporary delay in development of the cancer in females which then disappears around age 46. If the protection against of cancer development persisted throughout life the power term in the function describing the incidence rate would be expected to be different in females compared with males. The maximum difference in the gender incidence ratio will occur at whatever age the process differentially influencing the carcinogenic process in males versus females disappears. The difference in M/F ratio increased to a maximum at 50-59 years of age (7.9: 1) and then showed a marked progressive decrease (Figure 6.6). This indicates the difference in the age-specific incidence of cancer between males and females is limited to <55 years of age.

In contrast to intestinal type adenocarcinoma, the diffuse subtype showed no difference in age-specific incidence between males and females. In addition, the power term in the function describing the incidence rate for diffuse subtype was lower than that for the intestinal subtype. This is consistent with a stronger genetic predisposition being involved in the development of the diffuse subtype of cancer and thus fewer mutations are required to complete the carcinogenic process [177].

Applying similar curve-fitting analysis to a range of other types of cancers in the same population over the same time period revealed no evidence of a gender based delay phenomenon. In particular, cancers such as squamous carcinoma of lung and

transitional cell carcinoma of bladder which have a strong male predominance related to smoking [178, 179] showed differences in power term in the function describing the incidence rate between the genders but no evidence of a delay in onset.

In summary, our study indicates (I) that the male predominance of gastrointestinal adenocarcinoma is related to the intestinal subtype and is independent of whether the cancer arises in the oesophagus or proximal or distal stomach; (II) that the male predominance of the intestinal subtype is due to a delay of 17.3 years in its rise in incidence in females; (III) that this delay is due to differences between males and females of less than 55 years of age.

The reason for the difference in the development of the intestinal subtype of upper gastrointestinal cancer in females versus males is unclear and deserves further consideration and investigation. The fact that the delay is occurring at age less than 55 years makes it likely to be related to an endogenous protective effect associated with the reproductive years in the female. Fox et al reported gender specific *H.pylori*–related carcinogenesis in INS-GAS mice which was explained by a protective effect of 17β-estradiol [180-182]. In humans, delayed menopause and hormone replacement therapy may protect against gastric cancer [183-185]. The intestinal subtype of cancer arises against a background of chronic inflammation and tissue damage. The female sex hormone, oestrogen, is known to suppress the inflammatory response and cytokine production in certain tissues and might be exerting similar effects in the upper GI tract [186, 187]. In addition, females have lower body iron stores during their reproductive years and this might modify the degree of DNA damage arising from chronic inflammation [188 -191].

Several observations indicate that the delayed development in females is unlikely to be explained by different lifestyle factors such as smoking. Firstly, in cancers with male predominance due to exogenous lifestyle factors (i.e. lung, bladder), the age specific incidence data demonstrate differences in the power terms for the function

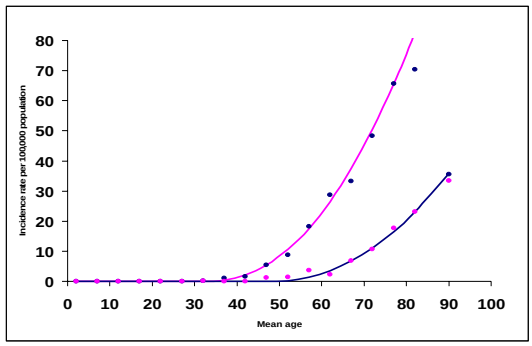
describing the data rather than a delay in appearance of the cancer. Secondly, recent studies have reported male predominance of gastric cancer in never-smokers [192, 193]. Thirdly, male predominance is observed in animal models of gastric cancer raised in an identical environment [194]. Fourthly, smoking rates in the U.K. available from 1978-1998 are similar for males and females under 50 years of age.[195]

The intestinal subtype of gastric adenocarcinoma arises due to progression of chronic superficial gastritis to atrophic gastritis to intestinal metaplasia to dysplasia and finally cancer [149]. Where the gender acts in the carcinogenesis cascade was one of the questions we tried to investigate. We were able to analyse data of our population-based cross sectional study of gastric precancerous lesion in Ardabil, Northwest of Iran [196]. The results of histologic examination of gastric biopsies from different pre-determined sites revealed similar rates of chronic gastritis (including both PMN and MN infiltrations) among men and women. The frequency of atrophic gastritis in men and women also showed no difference. These results were consistent with the results of Watabe *et al* who showed a similar rate of serologic atrophic gastritis among Japanese men and women [197].

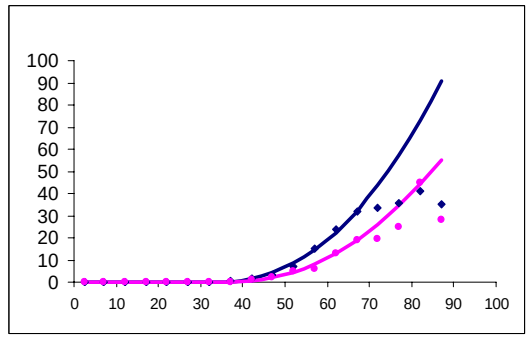
In contrast to inflammatory and atrophic lesions, intestinal metaplasia of the gastric mucosa was more prevalent in men. This was evident in all three subsites. This finding is consistent with the results of You *et al* who showed a higher prevalence of intestinal metaplasia and glandular dysplasia in men in a Chinese population [198]. This suggests that the gender phenomenon is acting at or after the metaplastic stage. With respect to the oesophagus, columnar epithelial metaplasia is more common in males than females (M/F = 1.7:1) and specialised intestinal epithelial more markedly so (M/F = 2.1:1). This again indicates the gender phenomenon is evident at and after the metaplastic stage.[199]

In conclusion, this study indicates that the marked male predominance of upper gastrointestinal adenocarcinoma is due to a more than 17 years delay in the

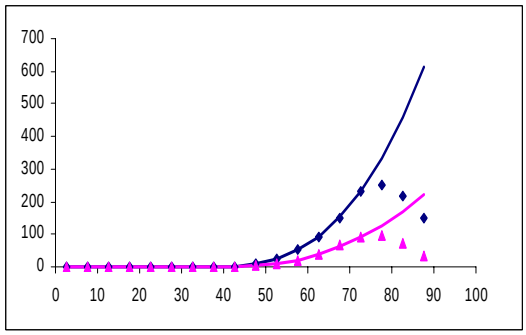
development of the intestinal subtype of the cancer. The basis of this phenomenon requires investigation as it accounts for a substantial proportion of upper gastrointestinal cancers and of such cancers occurring at a younger age when the personal, social and economical implications are greatest. It is likely also to give valuable new insights into the control of the carcinogenic process.



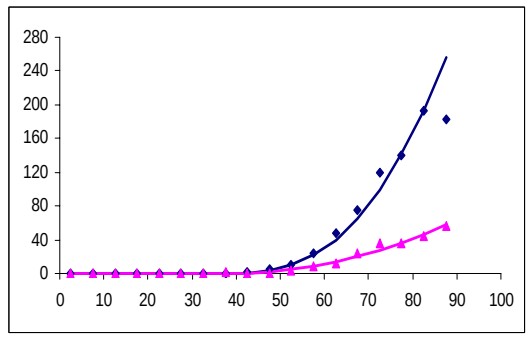

Oesophageal Adenocarcinoma
Delay = 48.3 – 33.1= 15.2 years



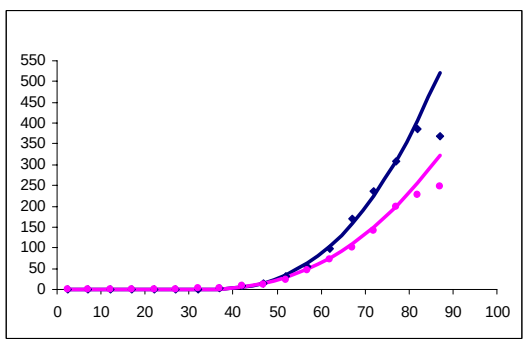
Oesophageal Squamous Cell Carcinoma
Delay = 36.9 – 34.3= 2.6 years



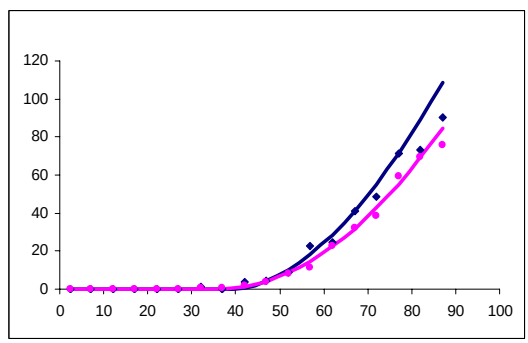
Squamous Cell Carcinoma of Lung
Delay = 38.7 – 38.2= 0.5 years



Transitional Cell Carcinoma of Bladder
Delay = 35.9 – 38.2= -2.3 years



Cancer of the Colon
Delay = 32.6 – 33.9= -1.3 years



Cancer of the Pancreas
Delay = 36.4 – 39.2= -2.8 years

**Fig 6.9:** Modelling of age-specific incidence curves of oesophageal adenocarcinoma compared to other individual cancers with marked male predominance. Note that the delayed development of oesophageal adenocarcinoma are not seen in other tumours. All the row data are obtained from Scottish Cancer Registry, West of Scotland,1998-2002.

# Chapter 7 Conclusion

Gastric and oesophageal cancers were responsible for more than one million deaths in 2002 [1, 2]. Although the global incidence of gastric cancer is decreasing, this malignancy is still the fourth most common cause of cancer worldwide [3]. The incidence of oesophageal adenocarcinoma is rising rapidly, three-fold in the last two decades [4-7]. The incidence of adenocarcinoma of gastric cardia is stable.

In the pathogenesis of both gastric and oesophageal adenocarcinomas, the state of the gastric mucosa and its secretory function plays a central role. Non-cardia adenocarcinoma develops in subjects with *H.pylori* associated atrophic gastritis and hypochlorhydria. Little is known about the gastric phenotype in patients with adenocarcinoma of the cardia and gastroesophageal junction.

In the first study we aimed to investigate the association between the pattern of *H.pylori* gastritis and gastric secretory function in 255 *H.pylori*-infected patients with dyspepsia who had a normal endoscopy. Our findings demonstrate the strong correlation between *H.pylori* body gastritis and reduced gastric acid secretion. Both inflammation and atrophy of the body mucosa were strongly associated with reduced gastric acid secretion. The effect of the atrophy is clearly explained by the loss of acid secreting parietal cells. The inflammation of the body mucosa is thought to produce functional inhibition of acid secretion. We, and others, have shown that eradicating

*H.pylori* in patients with low acid secretion produces recovery of secretion, which is associated with resolution of the body gastritis without any change in atrophy [8, 9]. The mechanism by which *H.pylori*-induced inflammation impairs the function of the oxyntic mucosa is not clear. However, the infection is known to stimulate production of interleukin-1 beta, which is a powerful inhibitor of acid secretion, blocking the release of histamine from the ECL cells and also inhibiting the function of the parietal cells [10, 11]. The current study clearly showed that atrophy and inflammation are both independently associated with low acid secretion.

In the next study, we compared cancers at the cardia and non-cardia subsites with respect to pre-morbid gastric mucosal atrophy and acid secretion. This study demonstrates the association between the pre-morbid state of the gastric mucosa and the location and histological subtypes of gastric adenocarcinoma presenting over subsequent years. Cancers of the mid and distal stomach of all histological subtypes were positively associated with *H.pylori* infection, atrophy and hypochlorhydria. Cardia cancer was more complex; it was negatively associated with *H.pylori* infection and the predominant intestinal subtype of cardia cancer was not associated with gastric atrophy. However, in subjects with *H.pylori* infection cardia cancer was positively associated with atrophy and hypochlorhydria. These findings can be explained by cardia cancer being of two distinct aetiologies, some cases being similar to non-cardia cancer and others having a different aetiology.

The multistage process of non-cardia cancer development has been traditionally more strongly linked to the development of the intestinal histological subtype of cancer as the diffuse type may develop in subjects with normal gastric mucosa [12]. In the latter cases, there is often a strong hereditary predisposition with inherited germ-line mutations [13]. Our study, however, along with that of Uemura *et al* indicates that atrophy and hypochlorhydria are associated with increased risk of the both diffuse and intestinal subtypes of gastric cancer [14].

The state and function of the gastric mucosa associated with cardia cancer is more complex than that associated with non-cardia cancer. In contrast to non-cardia cancer, there was a negative association between *H.pylori* infection and cancer of the cardia. As *H.pylori* infection causes atrophy and hypochlorhydria, we expected to find a lower prevalence of atrophy and hypochlorhydria in the cardia cancers than controls due to the lower prevalence of *H.pylori* in the former, as shown by Kamangar et al [(15)]. Such a finding would be consistent with *H.pylori* protecting from cardia cancer by the same mechanism by which it predisposes to non-cardia cancer, i.e. by reducing gastric acidity. However, despite the significantly lower prevalence of *H.pylori* infection in the cardia cancer patients (43% vs. control 71%) the prevalence of atrophy was at least as high in the cases (18%) as in the controls (13%). The reason for this unexpected finding was that the prevalence of atrophy within the *H.pylori* positive cardia cancer patients was significantly higher than the *H.pylori* positive controls. Cardia cancer patients were thus characterised by having a significantly lower prevalence of *H.pylori* infection but higher prevalence of atrophy in those with the infection as compared to the controls.

The lower prevalence of *H.pylori* infection is consistent with *H.pylori* having some protective effect. However, the high prevalence of atrophic gastritis within the *H.pylori* infected subjects suggests that atrophic gastritis due to *H.pylori* predisposes to cardia cancer. The most plausible explanation for our findings is that cancer of the cardia region is of heterogeneous aetiology and arising by two different pathways with *H.pylori* exerting an opposite influence on the two pathways.

The positive association with atrophic gastritis in the *H.pylori* infected cardia cancer patients is consistent with a subgroup of cardia cancers having a similar aetiology to non-cardia cancer. The serological markers of atrophy detect that mostly involving the body mucosa [(16-18)]. Body atrophy induced by *H.pylori* gastritis causes distal regression of the apparent cardia-oxyntic junction due to loss of specialised cells [(13)].

Our finding is consistent with a proportion of the cardia cancers having arisen from this process and thus being of similar aetiology to non-cardia cancer. Ye *et al* recently reported that cardia cancer was not associated with *H.pylori* infection but was associated with gastric atrophy and their observation is thus also consistent with atrophy being involved in a subgroup of cardia cancers [19].

On the other hand, several studies have reported a negative association between *H.pylori* infection and oesophageal adenocarcinoma [19, 20]. A subgroup of the cardia cancers may have similar aetiology to oesophageal adenocarcinoma and be subject to an *H.pylori* protective influence. Proximal expansion of the cardia mucosa can arise by metaplasia of oesophageal mucosa which the same process is thought to lead to oesophageal adenocarcinoma and to be induced by reflux of gastric acid [21-23]. The mechanism by which *H.pylori* infection may protect from this process is unclear but may be due to the fact that it causes a fall in acid output with advancing years due to development of atrophy [24, 25]. Other possible mechanisms such as mucosal changes of the cardia and highly alkaline ammonia produced by locally-colonising *H.pylori* might protect the cardia and adjacent distal oesophageal mucosa against cancer [26].

The analysis of the histological subtypes provided further evidence of two distinct aetiologies of cardia cancer. Atrophy increased the risk of the diffuse subtype of cardia cancer to a similar extent to which it increased the diffuse subtype of non-cardia cancer. However, atrophy did not increase the risk of intestinal-type cardia cancer, which was in contrast to the increased risk of the intestinal-type in the non-cardia region. The contrast in associations between atrophy and the intestinal-type in the cardia versus non-cardia regions was statistically highly significant using either low PG I/II or high gastrin as risk indicators. The diffuse type cancers at the cardia thus appear aetiologically similar to diffuse non-cardia cancers, whereas the intestinal-type cancers at the cardia (or at least the majority of them) are aetiologically distinct from intestinal-type cancers in the non-cardia region.

These findings indicate two aetiologies of cardia cancer, one associated with *H.pylori*-related atrophic gastritis, resembling non-cardia cancer, and the other associated with non-atrophic gastric mucosa, resembling oesophageal adenocarcinoma. Serological markers of gastric atrophy may provide the key to determining gastric versus oesophageal origin of cardia cancer.

In the next study, we extended our investigation of the aetiology of cardia cancer by examining the association of both serological evidences of gastric atrophy and gastroesophageal reflux disease (GORD) symptoms with adenocarcinoma of the oesophagus, cardia and non-cardia regions of the stomach. This has been performed for the different histological subtypes of the cancer. We have also included *H.pylori* status and smoking history which are other well-established risk factors for upper GI cancer. This has been undertaken in a population in Northwest Iran with a high incidence of upper gastrointestinal cancer. Serum pepsinogen I/II was used as a marker of atrophic gastritis and categorised to five quintiles. History of GORD symptoms, smoking and *H.pylori* infection was incorporated in logistic regression analysis. Lauren classification was used to subtype gastric and oesophageal adenocarcinoma.

Atrophic gastritis was associated with increased risk of both the intestinal and diffuse histological subtypes of non-cardia cancer but the association was stronger for the former as previously reported [27-29]. Whereas, the intestinal subtype is nearly always a consequence of atrophic gastritis and intestinal metaplasia, the diffuse histological subtype sometimes develops in a non-atrophic stomach with a strong genetic predisposition being an important factor in some of these cases [30, 31].

An association between *H.pylori* infection and non-cardia cancer was present in the univariate analysis consistent with previous reports [14]. However, this association was lost in multivariate analysis when atrophy and lifestyle factors were included. This is consistent with *H.pylori*-induced atrophic gastritis being the pre-cancerous

lesion rather than *H.pylori* infection itself. High prevalence of *H.pylori* infection in the background population shown in the current and previous studies may explain its weak relationship with gastric cancer risk [32].

In contrast to non-cardia cancer, oesophageal adenocarcinoma was positively associated with reflux symptoms. This is consistent with previous reports and the currently accepted hypothesis that gastro-oesophageal reflux causes columnar and intestinal metaplasia which then progresses to intestinal subtype adenocarcinoma [33, 34]. Consistent with this, the great majority of oesophageal adenocarcinomas in our study were of the intestinal histological subtype. There was also a positive association with smoking as previously reported [35, 36]. There was no association with gastric atrophy.

As shown in the previous part of the study which was conducted on a Norwegian population, cardia cancer in an Iranian population also showed a complex relationship with gastric atrophy. Severe gastric atrophy indicated by the lowest pepsinogen I/II quintile of <2.37 was associated with an increased risk of cardia cancer. However, unlike non-cardia cancer, there was no evidence of a progressive rise in cancer incidence with falling pepsinogen I/II ratio. Rather, the relationship between pepsinogen I/II ratio and cancer risk showed a quadratic pattern with the risk of cardia cancer being highest for the lowest and highest pepsinogen I/II ratios and lowest for the intermediate ratios. A plausible explanation for this complex association between cardia cancer and atrophic gastritis is that there are two distinct aetiologies of cardia cancer.

Reflux symptoms were also found to be a risk factor for cardia cancer with GORD symptoms of >2 time per week increasing the risk of cardia cancer with OR (95% CI):10.08 (2.29–44.36). Reflux symptoms have been reported previously to be a risk factor for cardia cancer but not as strong a risk factor as for oesophageal adenocarcinoma [37]. In our study, we were able to investigate the interaction of reflux

symptoms and atrophy in the aetiology of cardia cancer. This showed that reflux symptoms were associated with cardia cancer only in non-atrophic subjects, with a powerful OR (95% CI): 8.02 (2.25 – 28.58). This is again consistent with two distinct aetiologies of cardia cancer, one being associated with atrophic gastritis and resembling non-cardia cancer and one associated with reflux and resembling oesophageal adenocarcinoma.

Further evidence of two distinct aetiologies of cardia cancer was apparent on examining the atrophy-cancer and GORD-cancer associations separately in the two histological subtypes. The association between atrophy and intestinal subtype adenocarcinoma was weaker in the cardia than in the non-cardia region of the stomach. This is consistent with the intestinal subtype cardia cancer being a mixture of tumours positively associated with atrophy and tumours un-associated or negatively associated with atrophy (similar to oesophageal intestinal subtype adenocarcinoma).

The association of atrophy with diffuse cancer was stronger in the cardia than in the non-cardia region of the stomach. This difference may be related to the different topographic distribution and extent of atrophy required to produce cancer at those two sites and the ability of PGI/II to detect the atrophy associated with cancer at these two sites. Atrophy tends to start in the distal stomach at the junction between the antrum and body mucosa and progress proximally [38, 39]. Cancers tend to develop within atrophic mucosa and thus cancers of the distal stomach may develop in subjects with less extensive atrophy than would be required to produce cancer up at the cardia region. Furthermore, PGI/II is a reliable marker for detecting extensive atrophy involving the body mucosa but a poor marker for detecting early atrophy or that confined to the antral mucosa [40, 41].

The association between GORD symptoms and cardia adenocarcinoma was also related to the histological subtype. GORD symptoms were strongly associated with the intestinal subtype cancers at the cardia and this relationship was similar to that for

oesophageal adenocarcinoma. This association with GORD symptoms and intestinal subtype adenocarcinoma at the cardia is consistent with some of these cancers occurring by the same mechanism as oesophageal adenocarcinoma which is also of the intestinal subtype; the reflux of gastric juice leading to columnar intestinal metaplasia, dysplasia and adenocarcinoma. In contrast, there was no relationship between GORD symptoms and diffuse subtype adenocarcinomas at the cardia.

The above observations imply that there are not only two distinct aetiologies of cardia cancers but that the structural and functional state of the stomach associated with them is profoundly different. One type is associated with a non-atrophic healthy gastric mucosa producing sufficient acid and pepsin to damage the mucosa of the gastro-oesophageal junction and lead to columnar intestinal metaplasia and intestinal subtype cancer. The other is associated with atrophic gastritis of sufficient severity and extent to involve the proximal stomach leading to the development of intestinal or diffuse subtype cancer from the atrophic gastric mucosa.

Another important but poorly understood risk factor for upper GI adenocarcinoma is male gender. In our last study, we investigated the relationship between gender and upper gastrointestinal adenocarcinoma. Male gender is a well-established risk factor for oesophageal adenocarcinoma [42, 43]. Male predominance of gastric cancer is related to the histological subtype of the tumour being more marked in the intestinal versus diffuse histological subtype. In addition, global data suggests that the male predominance of upper gastrointestinal cancer is related to the anatomical location, being higher for proximal and lower for distal tumours [44]. However, the proportion of the intestinal histological subtype differs according to anatomical site and it was unclear whether it is the anatomical site or the histological subtype which is associated with the gender phenomenon. We conducted a population-based study to investigate this.

The study was based upon 3270 gastric and oesophageal cancers recorded in West of Scotland Cancer Registry between 1998 and 2002. The Lauren subtype of adenocarcinoma was determined by reviewing 1204 reports and 3241 slides in a sample of 812 cases. Logistic regression models were used to estimate relationship between male predominance and histological subtype, tumour location and age.

We found that the crude incidence rate of intestinal subtype was higher in males versus females, giving M/F of 2.65. M/F ratio of intestinal subtype cancer was 3.41 at age <50, reached a peak of 7.86 at age 50-59, and then showed a progressive decrease throughout the life. In contrast, the incidence rate of diffuse subtype adenocarcinoma was similar in both sexes, yielding M/F of 1.07. Multivariable analyses including histological subtype, tumour location and age indicated that the male predominance was related to the histological type rather than anatomical location. Intestinal subtype tumour showed similar male predominance of incidence irrespective of its anatomical location (OR, 95% CI: 2.64, 1.78 – 3.90). Further analysis of the age-specific incidence curves indicated that the male predominance of intestinal subtype was due to a 17.3-year delay of development of this cancer in females.

The reason for the difference in the development of the intestinal subtype of upper gastrointestinal cancer in females versus males is unclear and deserves further consideration and investigation. The fact that the delay is occurring at an age of less than 55 years old makes it likely to be related to an endogenous protective effect associated with the reproductive years in the female. Fox et al reported gender specific *H.pylori*–related carcinogenesis in INS-GAS mice which was explained by a protective effect of 17β-estradiol [45-47]. In humans, delayed menopause and hormone replacement therapy may protect against gastric cancer [48-50]. The intestinal subtype of cancer arises against a background of chronic inflammation and tissue damage. The female sex hormone, oestrogen, is known to suppress the inflammatory response

and cytokine production in certain tissues and might be exerting similar effects in the upper GI tract [51, 52]. In addition, females have lower body iron stores during their reproductive years and this might modify the degree of DNA damage arising from chronic inflammation [53-56].

Several observations indicate that the delayed development in females is unlikely to be explained by different lifestyle factors such as smoking. Firstly, in cancers with male predominance due to exogenous lifestyle factors (i.e. lung and bladder), the age specific incidence data demonstrate differences in the power terms for the function describing the data rather than a delay in appearance of the cancer. Secondly, recent studies have reported male predominance of gastric cancer in never-smokers [57, 58]. Thirdly, male predominance is observed in animal models of gastric cancer raised in an identical environment [59]. Fourthly, smoking rates in the U.K. available from 1978-1998 are similar for males and females under 50 years of age [60].

Where gender acts in the gastric carcinogenesis cascade was one of the questions we tried to investigate. We were able to analyse data of our population-based cross sectional study of gastric precancerous lesion in Ardabil, Northwest of Iran [61]. The results of histologic examination of gastric biopsies from different pre-determined sites revealed similar rates of chronic gastritis (including both PMN and MN infiltrations) among males and females. The frequency of atrophic gastritis in males and female also showed no difference. These results were consistent with the results of Watabe *et al* who showed similar rate of serologic atrophic gastritis among Japanese males and females [62].

In contrast to inflammatory and atrophic lesions, intestinal metaplasia of gastric mucosa was more prevalent in males, which was evident in all three subsites. This finding is consistent with results of You *et al* who showed a higher prevalence of intestinal metaplasia and glandular dysplasia in a Chinese population [63]. This suggests that the gender phenomenon is acting at or after the metaplastic stage.

With respect to the oesophagus, columnar epithelial metaplasia is more common in males than females (M/F = 1.7:1) and specialised intestinal epithelial more markedly so (M/F = 2.1:1). This again indicates that the gender phenomenon is evident at and after the metaplastic stage [64].

We believe our consecutive studies presented in this thesis have provided new insights to major risk factors of upper gastrointestinal adenocarcinomas. The critical significance of the gastric mucosal state (including its pathological pathways) leading to altered acid secretion has been emphasized. In particular, application of gastric mucosal and secretory state data to distinguish two types of cardia cancer is one of the advances that should be replicated in other studies using different populations.

Introduction of gender as a risk factor for upper gastrointestinal cancers was one of the main targets of our study. This is a relatively dark side of the upper GI cancer research and we tried our best to provide a simple, but robust evidence of male predominance of gastric and oesophageal adenocarcinoma. The marked delay phenomenon in development of intestinal subtype adenocarcinoma in females and lack of similar delay pattern in other cancers with prominent male predominance make this group of cancers very special to cancer research. Many endogenous factors including long- term exposure to feminine hormones, a different pattern of cell-mediated immunologic response to long-term *H.pylori* infection, differences in aging and immunosenescence phenomena, and different systemic and mucosal iron kinetics are attractive factors to be considered for future investigations. Environmental factors are less likely to be justified for investigations on gender effect.

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