

**GASTROINTESTINAL MOTOR AND
SENSORY FUNCTION IN COMPLICATED
AND UNCOMPLICATED PEPTIC ULCER
DISEASE**

A thesis submitted by

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THESIS SUMMARY

Peptic ulcer disease is common. The exact pathophysiology of peptic ulcer disease is still unclear. However, major causes of peptic ulcer disease are *Helicobacter pylori* infection and Non-steroidal anti-inflammatory drugs (NSAIDs) used. Peptic ulcer disease usually manifests as either dyspepsia or, less commonly, with life threatening complications such as bleeding and perforation (Linder and Wilcox 2001). Over the past two decades the incidence of uncomplicated peptic ulcer disease has dropped substantially, whilst the incidence of peptic ulcer bleeding seems to have remained unchanged (Lassen, Hallas et al. 2006; Post, Kuipers et al. 2006). Approximately 30% to 50% of patients with bleeding peptic ulcer are asymptomatic until bleeding occurs (Croker 1991) even though the endoscopic assessment may reveal multiple ulcer scars suggestive of previous ulceration. Moreover, the majority of patients dying from peptic ulceration have no symptoms of ulcer disease until the presentation of their final, fatal illness (Pounder 1989).

The mechanism of ulcer pain is still unclear. However, several factors have been associated with silent peptic ulceration. Older age and NSAIDs have been shown to be associated with asymptomatic peptic ulcer (Clinch, Banerjee et al. 1984; Mellem, Stave et al. 1985; Dew 1987), although this notion has been challenged

(Wilcox and Clark 1997; Lu, Chang et al. 2004). While, no study has reported in relate to visceral sensory function in patients with peptic ulcer disease. Altered gastric motor function has been proposed to be associated with the pathogenesis of peptic ulcer disease, though this idea is not well acknowledged. Gastric motor function and visceral sensory function may be the key responsible for the difference between clinical manifestations of complicated and uncomplicated peptic ulcer.

The incidence and proportion of patients between symptomatic and asymptomatic peptic ulcer is unknown. Few studies have shown the factors that could be associated with asymptomatic peptic ulcer such as age, NSAIDs and ulcer size.

This research aims of this thesis were, therefore, to examine: (i) the effect of age on gastric sensory function using the nutrient challenge test; (ii) assess symptom profiles and compare visceral sensory thresholds in patients with bleeding peptic ulcer, uncomplicated peptic ulcer disease and healthy controls; (iii) assess gastric emptying in patients with bleeding peptic ulcer, uncomplicated peptic ulcer and healthy controls, and the relationship between symptoms and gastric emptying; (iv) study the link between immune activation and clinical manifestation of patients with peptic ulcer disease and explore the link between anxiety or depression and the release of inflammatory cytokine; (v) determine the incidence and risk factors of uncomplicated peptic ulcer disease, bleeding peptic ulcer and asymptomatic peptic ulcer, and changes of epidemiology of peptic ulcer disease

over a 10-year period, between 1997 to 2007, at the Royal Adelaide Hospital; (vi) compare the symptoms reported in patients with uncomplicated peptic ulcer compared with bleeding peptic ulcer after 1 year of ulcer healing; (vi) assess the distribution of GNB3 C825T polymorphisms and the association between GNB 3 825 polymorphisms and symptoms during a nutrient challenge in healthy subjects.

The current study indicates that elderly people have decreased gastric visceral sensation compared with younger people. This study also shows that patients with uncomplicated peptic ulcer have an augmented symptom response and significantly delayed gastric emptying whilst patients with bleeding peptic ulcer have a symptom response to a test meal and gastric emptying time that is not different from that in healthy control, suggesting fundamental difference in visceral sensitivity and abnormal gastric motor function suggesting a between patients with bleeding or asymptomatic ulcers and those with symptomatic or uncomplicated ulcers. Our findings also showed that there were increased levels of systemic proinflammatory cytokines in patients with uncomplicated peptic ulcer, despite the ulcer had healed, compared with patients with bleeding peptic ulcer and healthy control. The findings further support the notion that patients with uncomplicated peptic ulcer share similarities with patients with functional dyspepsia.

The study in this thesis also demonstrates the epidemiology of symptomatic and asymptomatic peptic ulcer disease over the past 10 years at the Royal Adelaide

Hospital, including the risk of being asymptomatic peptic ulcer. The study shows that over the last 10 years, the incidence of uncomplicated peptic ulcer has decreased whereas bleeding peptic ulcer has remained stable.

The work described in Chapter 15 demonstrates that most patients with dyspeptic symptoms prior to the diagnosis of peptic ulcer disease continue to have dyspeptic symptoms 12 months after ulcer healing and *Helicobacter pylori* eradication. Patients with persistent dyspeptic symptoms have higher level of anxiety and depression score than patients without symptoms. The data suggest that most patients with symptomatic peptic ulcer disease have concomitant functional dyspepsia, which may have led to the diagnostic endoscopy being performed that probably prevented the development of a life threatening ulcer bleed.

Based upon our data it might be speculated that mechanisms that are involved into the manifestation of symptoms in patients with functional dyspepsia may actually prevent the manifestation of ulcer complications since ulcers manifest with symptoms that trigger health care seeking and treatment before complications occur.

Chapter 16 of this thesis might potentially explain one of the mechanisms of abdominal pain. The work described in this study shows that GNB3 825T-CC plays a role in the processing of visceral sensory information or the gastrointestinal motor responses to a nutrient challenge.

By doing so, the research studies which were conducted as part of this thesis have significantly improved and added new knowledge in the fields of gastric motor and sensory function in peptic ulcer disease.

In conclusion, the thesis has highlighted the differences in gastric motor function, gastrointestinal sensory function and immune activation between patients with uncomplicated peptic ulcer disease and bleeding peptic ulcer. This thesis also showed the epidemiologic data of patients with symptomatic and asymptomatic peptic ulcer disease, including the risk factors of being asymptomatic peptic ulcer. The results have important therapeutic implications, and suggest aggressive management of patients with peptic ulcer disease. In addition, the research study also suggests further studies in the areas of the mechanism and pathogenesis of peptic ulcer pain, and abdominal pain, which are likely to result in better strategies to manage and prevent this important clinical condition.

Declaration

For a thesis that does not contain work already in the public domain

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Montri Gururatsakul

September 2013

DEDICATION

I dedicate this thesis to my dearest parents, Pramote and Harmitpal Kaur Gururatsakul.

To my dearest wife, Navarat Sachayansrisakul, without whom this would not have been undertaken. I am forever grateful for your unconditional love and support.

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PUBLICATIONS ARISING FROM THE THESIS

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SECTION 1:

LITERATURE REVIEW

CHAPTER 1: ANATOMY OF THE STOMACH, PYLORUS AND DUODENUM

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1.1 INTRODUCTION

The motility and sensation of the upper gastrointestinal tract, including the oesophagus, stomach and duodenum, is complex. To understand the motor and sensory functions and mechanism of these regions, it is important to know their basic anatomy, embryological, histology and innervation. This chapter will review the gross anatomy, embryological development, muscular anatomy, microscopic anatomy, and neuroanatomy of the stomach and duodenum.

1.2 EMBRYOLOGICAL DEVELOPMENT OF THE STOMACH AND DUODENUM

The stomach, duodenal bulb and second part of the duodenum develop from foregut, while the third and fourth part of the duodenum develops from mid gut. Around the middle of the fourth week of gestation, the stomach develops as a fusiform enlargement. The dorsal part grows faster than the ventral part making the dorsal part become greater curvature and the ventral part the lesser curvature. The stomach subsequently rotates 90 degrees clockwise on its longitudinal axis. As in the oesophagus, the epithelium and glands in the stomach derive from the embryonic endoderm while the muscle and serosal layers derive from the

mesoderm. At the same time, the duodenum grows and forming a C-shaped and rotates to the right side.

The organs which derive from the foregut (oesophagus, stomach, duodenal bulb and second part of duodenum) receive their arterial blood supply from the celiac trunk, whereas the organs which derive from the midgut (third and fourth of the duodenum) are supplied by the superior mesenteric artery.

1.3 STOMACH AND DUODENUM

1.3.1 Gross anatomy

The stomach is a J-shaped canal which is connected proximally to the oesophagus at the gastro-oesophageal junction and distally to the duodenum, making the location of the stomach variable.

The stomach is divided anatomically into 5 sections: 1) the cardia, adjacent to the gastro-oesophageal junction which is below the diaphragmatic hiatus, and located slightly left of the midline and the most fixed portion of the stomach; 2) the fundus, adjacent to cardia and superior to the horizontal plane of the gastro-oesophageal junction; 3) the corpus or body, the largest portion of the stomach

and between the fundus and incisura angularis; 4) the antrum, which extends from the body to the pylorus, basically between the incisura angularis and the pylorus; 5) the pylorus, tubular shape, that separates the antrum from the duodenum.

When the stomach is empty, the inner surface of the stomach is raised into a series of longitudinal folds called rugae. Rugae are composed of mucosa and submucosa, they are more prominent in the narrowing regions such as body, antrum and pylorus, but not in fundus. The rugae assist the stomach to distend, and when the stomach fully distends, the rugae disappear.

The duodenum is a tubular C-shaped structure, 25-30 cm in length, with the head of pancreas located in its curve. The duodenum is divided into 4 parts: the duodenal bulb, which extends from the pylorus; the second part, which contains the papilla of Vater that drains bile from liver and gall bladder and pancreatic juice from pancreas; the third part, and the fourth part that is connected to the jejunum at the duodenojejunal flexure, formed by the ligament of Treitz.

The stomach receives its arterial blood supply from branches of the coeliac trunk, from which arise the common hepatic artery, left gastric artery and splenic artery. The branches of splenic artery are the short gastric branches and the left gastroepiploic artery, which supply the fundus and body along the greater curvature respectively. The branches of common hepatic artery are hepatic artery proper, right gastric artery and gastroduodenal artery which gives branches to the

right gastroepiploic artery and superior pancreatico duodenal artery which supply duodenum and head of pancreas. The right gastroepiploic artery supplies the antrum part of greater curvature and anastomoses to left gastroepiploic artery. The lesser curvature is supplied by the left gastric artery at the fundus and right gastric artery at the body and antrum.

The venous drainage of the stomach usually accompanies the main arteries and eventually drains into portal vein. The left and right gastric veins which drain the lesser curvature of the stomach, and superior pancreatico duodenal vein drain directly to portal vein. The right epiploic vein drains the lower part of greater curvature to the superior mesenteric vein and later on to portal vein. The left epiploic vein drains the above portion of greater curvature becomes splenic vein which later on combines with short gastric veins which drain fundus part of the greater curvature and eventually drain to portal vein.

Most of the lymphatic drainage from lymph nodes around the stomach (splenic, gastro-omental, gastric, superior mesenteric and pyloric lymph nodes) is all connected and drains to the coeliac nodes. The gastro-omental nodes drain to gastric node and later to celiac nodes and ultimately to thoracic duct.

1.3.2 Muscular anatomy of the gastric wall

The muscular layer of the gastric wall consists of 3 layers including the outer longitudinal muscle, inner circular layer and innermost oblique fibres. The longitudinal muscle is connected proximally to the oesophageal longitudinal muscle, runs distally to the pylorus, and is connected distally to the duodenum. It is thicker along lesser and greater curvatures of the stomach. The middle circular muscle is present throughout the stomach wall and is connected to circular muscle of duodenum. The circular muscle at the pylorus, particularly the pyloric sphincter, is thicker than other parts of the stomach. The inner oblique muscle starts from cardia and medial aspect of the fundus and ends anteriorly and posteriorly at the greater curvature. The muscle of the duodenum is similar to oesophagus and other part of gastrointestinal tracts which consists of outer longitudinal and inner circular muscle.

1.3.3 Microscopic anatomy and their physiology of the wall of the stomach, pylorus, and duodenum

The stomach is histologically divided into 3 parts – cardiac region, pyloric region and fundic region and consists of four layers - mucosa, submucosa, muscularis externa and serosa. The inner surface of the stomach is lined with simple

columnar epithelium which produces the mucous to protect the epithelium from the gastric juice.

The fundic or gastric glands produce gastric juice which contains hydrochloric acid (HCl) (produced by parietal cells), pepsin (produced by chief cells), mucus, and intrinsic factor. The glands are composed of 3 regions which are isthmus, neck and base. Gastric glands consist of 4 different cell types which are mucous neck cells, chief cells, parietal cells, and enteroendocrine cells. Mucous neck cells are located at the neck region of gastric glands, they secrete soluble mucus and their main role is to function as a stem cell precursor for surface mucous, parietal, chief and endocrine cells (Redel 2002). Chief cells are located in the deep part of the gastric gland (base), they secrete pepsinogen and leptin (Bado, Levasseur et al. 1998). Parietal cells are also found in the neck region of the gastric glands among mucous neck cells, they secrete HCl and intrinsic factor. It is estimated that human stomach contains around 1×10^9 parietal cells (Naik, Bajaj et al. 1971), which comprises 80% of the organ.

Three types of receptors are located at the membrane of parietal cells: gastrin, histamine and acetylcholine M_3 receptors. Activation of these receptors stimulates the parietal cells to produce HCl and intrinsic factor. Gastrin receptors are the major stimulant of acid secretion, and are activated by gastrin which is secreted by G cells (Royston, Polak et al. 1978). Several types of neuroendocrine cells are located at the neck and base of the gastric glands, including enterochromaffin

cells which contain serotonin, enterochromaffin-like cells containing histamine, D cells which contain somatostatin and amylin, and A-like or Gr cells which contain ghrelin and obestatin (Schubert and Peura 2008).

Generally, the function of gastrin is to stimulate gastric acid secretion via parietal cells. Ghrelin stimulates gastric hormone secretion and appetite and perception of hunger but inhibits lipid metabolism and fat utilisation in adipose tissue. Histamine stimulates gastric acid secretion, while somatostatin inhibits gastrin release, gastric acid secretion and release of other gastrointestinal hormones (Ross and Pawlina 2003).

Cardiac glands occupy only small region of the stomach (cardia) that surrounds the oesophageal orifice. Cardiac glands are composed of mucus-secreting cells which contribute to protecting the oesophageal epithelium from acid reflux.

Pyloric glands, similar to parietal glands, contain surface mucous cells, mucous neck cells, D cells, and enterochromaffin cells. In addition, pyloric glands also contain G cells which are located at the base. They are activated by gastric distension, vagal stimulation, dietary amino acids, and peptides and play an important role in stomach physiology.

The lamina propria of the stomach is composed of reticular fibres, smooth muscle and immune cells such as lymphocytes, macrophages and plasma cells. The

muscularis mucosae is composed of thin two muscular layers, inner circular and outer longitudinal muscles.

The gastric submucosa and muscularis externa are similar to those of the oesophagus. The submucosa is composed of a dense connective tissue containing blood vessels, nerve fibres and the submucosal plexus (Meissner's) plexus. The muscularis externa consists of three muscular layers as described (outer longitudinal, middle circular and inner oblique layer). The myenteric (Auerbach's) plexus lies between these muscle layers. The important role of these muscles is to mix the content of the stomach, helping with digestion and forcing the content into duodenum. The gastric serosa is connected to the parietal peritoneum.

The duodenum also contains of four layers, similar to those of the oesophagus and the stomach. However there are plicae circulares along the surface of small intestine. Five types of cells are found in the mucosa, including enterocytes, goblet cells, paneth cells, enteroendocrine cells, and M cells. Enteroendocrine cells secrete cholecystokinin (CCK), secretin, gastric inhibitory peptide (GIP) and motilin. Generally, CCK stimulates gallbladder contraction, pancreatic enzyme secretion, pancreatic bicarbonate secretion and pancreatic growth, and inhibits gastric emptying, while secretin stimulates pancreatic enzyme secretion, pancreatic bicarbonate ion secretion and pancreatic growth, and inhibits gastric

acid secretion. GIP stimulates insulin release and inhibits gastric acid secretion, while motilin stimulates gastric and intestinal motility.

Submucosal glands or Brunner's glands are located in submucosa, and their main role is to secrete neutral and alkaline glycoproteins and bicarbonate ions in order to neutralise the acid containing chyme delivered from the stomach. Two types of muscular layers (circular and longitudinal) in the muscularis externa are responsible for 2 types of contractions, which are segmentation and peristalsis. Segmentation is mainly engaged by inner circular layer, while peristalsis is engaged by both types of muscles.

1.4 NEUROANATOMY OF STOMACH AND DUODENUM

The oesophagus receives both parasympathetic (vagus) and sympathetic (spinal) innervation (Goyal and Sivarao 1999). The vagus nerve is composed of motor (efferent) and sensory (afferent) fibres, although the afferent neurons constitute 80% of the fibres in the vagus nerve (Berthoud and Neuhuber 2000). The right vagus nerve gives a branch to the right recurrent laryngeal nerve which supplies the cervical oesophagus, whereas the left vagus nerve gives branches to the left recurrent laryngeal nerve. The vagus nerve then descends to the superior mediastinum and gives branches to the oesophagus and tracheobronchial tree at

the tracheal bifurcation. The branches along the oesophagus form anterior and posterior plexus. The anterior branches pass along the oesophagus, and innervate stomach (fundus, body and lesser curvature) and liver (hepatic plexus) through the diaphragmatic hiatus. The posterior branches pass along the oesophagus and innervate the stomach and give branches to the coeliac plexus (Mercer and Lucius 1988). The spinal afferents have their cell bodies in the dorsal root ganglia and project to the spinal cord at T1-L2 (Goyal and Sivarao 1999). Vagal and spinal afferents terminate within all three layers of the gastrointestinal wall. There are also interstitial cells of Cajal located in between nerves and smooth muscle cells; they act as modulators of the nerve-smooth muscle interaction and muscle-muscle conduction pathways (Sanders 1996; Clouse and Diamant 2002). Nerve terminals in the serosa and muscle usually convey mechanosensory information (distension and contraction), whereas nerve terminals in the mucosa convey chemosensory information (Grundy 2002). The mechanism of abdominal pain and visceral nociception will be described in Chapter 3 and 4.

CHAPTER 2: HUMAN UPPER GASTROINTESTINAL MOTOR FUNCTION

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2.1 INTRODUCTION

The purpose of this chapter is to review gastric motor function including gastric motility, gastric accommodation, and gastric emptying. In addition, the assessment of gastric motor function will be reviewed.

2.2 GASTRIC MOTOR FUNCTION

The main functions of the stomach are to receive the food from the oesophagus, store it, mix it with gastric secretion, reduce the food to small particles with digestive enzyme, and then deliver it to the duodenum. The function of the duodenum is to mix the food from stomach with bile and digestive enzyme, neutralise the acid and deliver it to small intestine for absorption.

The stomach can be divided functionally into 3 parts, proximal stomach (cardia, fundus and proximal corpus), distal stomach (distal corpus and antrum) and pylorus. Its motor function is regulated by feedback from small intestine. Smooth muscle cells, interstitial cells of Cajal, enteric nerves and the vagus nerve are responsible for gastric motility. As described previously, stomach contains of three muscle layers; outer longitudinal muscle, intermediate oblique muscle layer and inner circular muscle layer, while the myenteric plexus is located in between the circular and longitudinal muscle layers. Interstitial cells of Cajal normally

generate electrical slow wave-activity in the gastrointestinal tract. In the stomach, these cells are mainly located between the circular and longitudinal muscle layers (Song, David et al. 2005; Sanders, Koh et al. 2006). Slow waves are generated at the frequency of 3 cycles per minute and characterised by a phasic contractile activity (distal stomach), while the proximal stomach generates tonic contractile activity. Slow waves determine the maximal frequency of phasic contraction. The vagus nerve in the stomach mainly functions as a sensory nerve, as 80% of the fibres are afferent fibres. However, when the 20% motor fibres are activated, they regulate gastric motility from vagovagal reflex. The stomach is also innervated by splanchnic nerves (sympathetic), which mainly inhibit gastric motility by inhibiting acetylcholine release from the myenteric plexus (Tack 2007).

In the fasting (interdigestive) state, the proximal stomach takes part in the migrating motor complex (MMC) that helps to evacuate indigestible particles from the stomach (Vantrappen, Janssens et al. 1977). The interdigestive MMC consists of three phases, phase I (quiescence): absence of contractile activity, phase II (intermittent pressure activity): produces irregular contractile activity, and phase III (activity front): may display up to its maximum contractility (3 per minute in stomach and 12 per minute in the duodenum) (Camilleri 2000), then followed by phase I again as a cycle. This cycle takes approximately 90 to 120 minutes. The MMC is largely controlled by both extrinsic nerves (especially vagus nerve) and peptide hormones (especially motilin) (Quigley 2006). Phases I and II are controlled mainly by vagus nerve, while phase III may be induced by

motilin (Vantrappen, Janssens et al. 1979). The fundic contractions are controlled by balance between cholinergic (excitatory) and nitrenergic (inhibitory) input, thus fundus is controlled mainly by cholinergic input in fasting state.

When the food is ingested and enters the stomach, gastric motility changes from the interdigestive state to the fed or postprandial state. During the postprandial pattern, the MMC is suppressed by the vagus nerve; tonic and phasic contractions in the proximal stomach are inhibited, thus the proximal stomach is relaxed to increase the volume without changing of pressure to store the food and pass the digested food to the duodenum at the optimum rate (gastric reservoir function). Two phases occur at this stage; first the lower oesophageal sphincter and proximal stomach rapidly relax to store the food bolus, a process known as receptive relaxation, and which is mediated by the vagus nerve. Second, a long-lasting relaxation of the proximal stomach occurs, the purpose of which is to store food, a process known as adaptive relaxation or gastric accommodation. Gastric accommodation can enlarge the stomach up to 2-3 times its standard volume (Tack, Piessevaux et al. 1998), and is normally induced by stimulation of mechanoreceptors. Gastric relaxation is usually mediated by vagovagal reflex, the afferent vagal nerve relay signals to nucleus solitarius, then the nucleus solitarius sends axons to dorsal motor nucleus to mediate vagal efferent to the fundus by either inhibition of excitatory or exhibition of inhibitory (Rogers, Hermann et al. 1999). The main inhibitory neurotransmitters are nitric oxide (NO) and vasoactive inhibitory peptide (VIP).

After gastric accommodation, the food is gradually propelled to the antrum where it is ground, mixed, digested, and eventually evacuated to the duodenum (gastric emptying).

2.2.1 Pattern of gastric emptying

The stomach empties its content in pulsatile fashion (Heddle, Dent et al. 1988; Anvari, Yu et al. 1995), which is usually controlled by intra-gastric volume and small intestine feedback mechanisms (Azpiroz and Malagelada 1985). As described previously, patterns of gastric emptying are different among non-nutrient liquid, nutrient liquids, solids, and fats.

2.2.1.1 Non-nutrient liquids

Gastric emptying of non-nutrient liquids follows a simple exponential pattern (Hunt and Spurrell 1951; Moore, Christian et al. 1981) and are associated with rapid gastric emptying. When liquids are ingested, they disperse throughout the stomach and begin emptying without any lag period (Quigley 2006). Surgical resection of the canine fundus (Wilbur, Kelly et al. 1974) and proximal vagal denervation (Wilbur and Kelly 1973) is associated with more rapid liquid gastric

emptying. In addition, transection of extrinsic antral nerves (Mroz and Kelly 1977) and distal antrectomy (Dozois, Kelly et al. 1971) have no impact on liquid gastric emptying. Therefore, proximal stomach is the main component that controls gastric emptying of liquid (Dozois, Kelly et al. 1971; Kelly 1980), although it has been shown that distal stomach may also play an important role in gastric emptying of liquid meal (Collins, Houghton et al. 1991). Gastric emptying, is influenced by posture (Hancock, Bowen-Jones et al. 1974; Moore, Datz et al. 1988; Horowitz, Jones et al. 1993). A study from Anvari et al suggested that gravity has an impact on liquid gastric emptying rate as emptying is more rapid in the sitting position compared with the left lateral position (Anvari, Horowitz et al. 1995). Burn-Murdoch et al showed that people who are lying on their right side had more rapid gastric emptying rate than left side (Burn-Murdoch, Fisher et al. 1980).

2.2.1.2 Nutrient liquids

Gastric emptying of nutrient liquids rate is influenced by both the volume and nutrient density. The greater the intragastric volume and the higher the nutrient density the slower is the gastric emptying rate (Hunt, Smith et al. 1985). Increasing the caloric content of the nutrient liquids slows gastric emptying (Brener, Hendrix et al. 1983; Collins, Horowitz et al. 1983). The gastric emptying rate of nutrient liquids is approximately linear (Brener, Hendrix et al. 1983). It is

also determined by an interaction between gastric volume and nutrient-induced duodenal feedback, as intraduodenal glucose infusion slow the gastric emptying rate (Brener, Hendrix et al. 1983; Moran, Wirth et al. 1999). Similar to non-nutrient liquids, the proximal stomach is the main part that controls gastric emptying of non-nutrient liquids.

2.2.1.3 Solids

The emptying of digestible solids consists of two phases, a lag phase and a linear emptying phase. It is primarily a function of the distal stomach (Kelly 1980). Once solids are ingested, they are stored in the proximal stomach and then gradually distributed to the antrum (Collins, Houghton et al. 1991) for trituration into small particles (<1 mm) (Kelly 1980). This process is known as the lag phase. Emptying occurs after the solids are ground into small particles. Solids are emptied in a linear fashion by antral contractions. The proximal antrum contracts and propels the food through antrum toward the pylorus. The pyloric sphincter allows only food particles size less than 1 mm in diameter pass to the duodenum (Urbain, Siegel et al. 1989). Larger particles will be propelled back from the distal antrum to the proximal antrum and ground until they are small enough. Solid emptying is inversely related to meal volume, caloric density and content (greater volume or higher caloric density of the solid meals slows the gastric emptying) (Moore, Christian et al. 1984). Data have also shown that transection of extrinsic

antral nerves (Mroz and Kelly 1977) and distal antrectomy (Dozois, Kelly et al. 1971) accelerate solid gastric emptying.

2.2.1.4 Mixed meal

When liquids and solids are ingested simultaneously, the gastric emptying of both liquids and solids is delayed (Horowitz, Maddox et al. 1989) compared with liquids or solids emptying alone. Liquids are emptied initially while the solids are retained in the proximal stomach. Once about 80% of the liquid has emptied, the process of solid emptying starts (Houghton, Read et al. 1988).

2.2.1.5 Fats

Fats become liquid at body temperature (in the stomach), and their low density and poor water-solubility lead them to float on the liquid content in the stomach but not to mix. The characteristic of gastric emptying of fats is similar to solids as a linear fashion (Meyer, Mayer et al. 1986).

2.2.2 Small intestinal feedback regulation of gastric motor function

Mechanical or chemical stimulation in the small intestine leads to relaxation of the proximal stomach and suppression of gastric motility. The purpose of this feedback regulation is to slow down the flow rate of chyme from the stomach to the duodenum (Heddle, Miedema et al. 1993). Cholecystokinin (CCK) (Liddle, Morita et al. 1986; Kleibeuker, Beekhuis et al. 1988), Glucagon-like peptide-1 (GLP-1) (Mojsov, Heinrich et al. 1986) and peptide YY (Wen, Phillips et al. 1995) are perhaps important gastrointestinal peptide hormones, which are produced in intestinal mucosa. They are stimulated by nutrients in the gut and have a major inhibitory effect on gastric emptying.

2.3 ASSESSMENT OF GASTRIC MOTOR FUNCTION

A variety of methods has been developed to assess gastric motor function. The ideal test should be able to measure contractile activity, intraluminal pressure and wall motion accurately, and should be safe, non-invasive, and cost effective. However, this ideal test does not exist. Gastric motor function can be measured by scintigraphy, stable isotope breath tests, electrogastrography, ultrasonography, antroduodenal manometry, magnetic resonance imaging, water and nutrient drink tests, etc... Each test has its own merits and disadvantages, which will be reviewed in this session.

2.3.1 Barostat measurements

The barostat is the first method and remains the gold standard for measurement of proximal gastric motility. The gastric barostat involves transoesophageal introduction of a polyethylene balloon. The balloon bag is positioned in the gastric fundus. The barostat measures gastric tone by recording the volume changes in the balloon. It is also able to measure gastric sensitivity by slowly increasing the pressure of the balloon bag and ask subjects to report their perception of upper abdominal sensations including discomfort and pain. Additionally, it is able to measure gastric accommodation when the subjects ingest the meal (postprandial) by recording the balloon volume (Tack, Piessevaux et al. 1998). Reproducibility for measurement of gastric sensitivity and accommodation has been reported to be good (Sarnelli, Vos et al. 2001). The disadvantages of the barostat are that it is invasive, uncomfortable, and requires special expertise. For measurement of gastric sensitivity, it requires subjects to report their sensation which is subjective, thus results may be altered by subjects who are having uncomfortable experience or stressed (De Schepper, Cremonini et al. 2004). The balloon may also alter intragastric distribution of the meal and induce exaggerated proximal gastric relaxation (Mundt, Hausken et al. 2002) and interfere gastric physiology (de Zwart, Haans et al. 2007).

2.3.2 Scintigraphy

Scintigraphy is considered the gold standard for evaluating gastric emptying (Mariani, Boni et al. 2004), and is also the most widely studied and available method (Bratten and Jones 2006). It is also often used to confirm gastric dysmotility (Camilleri, Hasler et al. 1998). The subject ingests a test meal, which contains a radionuclide label, and sits in front of a gamma camera for at least 90 minutes. The meals can be liquid, solid or mixed. The most common radionuclide imaging agent is ^{99m}Tc -sulfur colloid. In case of mixed meal ingestion, ^{111}In -DTPA is commonly used for liquid meal, thus the study can be analysed in both liquid and solid emptying phase. For analysis, regional of interests (ROIs) are drawn around the proximal stomach, distal stomach and small intestine, and the percentage of gastric retention at specific time point (usually T_{50} , T_{lag} , and maybe minute 90 depending on centre's method) is calculated. Disadvantages of scintigraphy are that it involves radiation exposure, is time consuming, and the results can vary from centre to centre. Thus each centre needs to establish its own normal values.

2.3.3 Ultrasonography

The principle of conventional ultrasound is to calculate the proximal gastric area and the proximal gastric diameter (De Schepper, Cremonini et al. 2004). Changes in these measurements can be used to determine gastric emptying. Ultrasound is able to measure gastric emptying (Bolondi, Bortolotti et al. 1985), antral motility and transpyloric flow (Hausken, Odegaard et al. 1992). The advantages of this test are that it is non-invasive, relatively inexpensive, and widely available. Additionally, three dimensional ultrasound has been shown to have more accuracy in calculating gastric emptying rate than conventional 2D ultrasound (Gilja, Detmer et al. 1997). However, this test is highly operator dependent, and factors such as the costal margin and bowel gas may limit the study.

2.3.4 Magnetic resonance imaging (MRI)

MRI is a safe and very accurate test that can assess gastric anatomy, emptying (Schwizer, Maecke et al. 1992; Kunz, Crelier et al. 1998; Feinle, Kunz et al. 1999), motility (Marciani, Young et al. 2001; de Zwart, Mearadji et al. 2002), accommodation, secretion, and intragastric meal distribution. A test meal is usually labelled with gadolinium as an MRI marker to measure gastric emptying. Disadvantages of MRI are only that it is a very expensive test and usually able to

test in the supine position which may alter the result of gastric emptying, especially liquid, as a result of gravity.

2.3.5 Single photon emission computed tomography (SPECT)

SPECT is able to noninvasively measure gastric accommodation (Kuiken, Samsom et al. 1999). Intravenous ^{99m}Tc -pertechnetate is used to label the gastric wall as it is taken up and excreted by gastric mucosa (parietal and mucin secreting cells) throughout the stomach. The label can be visualised topographically by SPECT (using a dual head gamma camera system) in supine position (Kuiken, Samsom et al. 1999). While SPECT is a high-quality test to measure gastric accommodation, it involves radioactivity and is not widely available. In addition, it cannot assess gastric tone and sensation compared with the gold standard barostat technique.

2.3.6 Stable isotope breath tests

This is a reliable non-invasive technique for gastric emptying that does not involve radioactive agents. A nonradioactive isotope, ^{13}C , is bound to a medium chain triglyceride (octanoic acid) or proteinaceous algae (Spirulina) and mixed with the test meal. After ingestion, the ^{13}C -labeled substrate is absorbed in the

duodenum, metabolised in the liver and excreted through the lungs as breath $^{13}\text{CO}_2$. The $^{13}\text{CO}_2$ is measured by mass spectrometry or laser infrared spectroscopy at regular intervals. The gastric emptying rate can be calculated from the rate of excretion of breath ^{13}C . The technique has been validated against scintigraphy and shows good correlation with liquid and solid gastric emptying (Mossi, Meyer-Wyss et al. 1994; Braden, Adams et al. 1995; Lee, Camilleri et al. 2000). The only limitation of this test is that the excretion of ^{13}C is altered in patients with severe liver or lung disease, and the calculation may not be valid.

2.3.7 Nutrient drink test

The nutrient challenge test has been used in various studies of patients with functional dyspepsia, irritable bowel syndrome and healthy subjects (Holtmann, Talley et al. 1996; Boeckxstaens, Hirsch et al. 1999; Kim, Myung et al. 2000; Mulak 2003; Tack, Caenepeel et al. 2003; Delgado-Aros, Camilleri et al. 2004; Haag, Talley et al. 2004; Choung, Talley et al. 2007) and correlates well with mechanosensory thresholds as measured by the barostat (Holtmann, Talley et al. 1996). This test is believed to be able to assess gastric accommodation and visceral sensitivity noninvasively, and the cost is low. Subjects ingest water (Jones, Hoffman et al. 2003) or nutrient liquid meal (Nutridrink ®, Ensure ®, or Nutricia ®) at a certain filling rate which can be 15-30 ml/minute or 200 ml/5 minute. At 5 minute intervals, subjects are asked to score their sensations of level

(fullness, pain, nausea, heartburn, regurgitation) on a visual analogue scale. Then the total volume of liquid meal and score on visual analogue scale is record and analysed. The test can be combined with scintigraphy to access gastric accommodation, emptying, and sensitivity simultaneously. However, the test depends on subject's report which is subjective, thus the test may be influenced by sensory and psychological factors.

2.3.8 Conclusion

A variety of tests is available to assess gastric motor function. However, a test which can assess all gastric motor function simultaneously, is inexpensive, minimal invasive and does not involve radioactivity does not exist. Therefore, selecting the test is very important, and depends on what measurement the investigator would like to observe (gastric accommodation, contraction, or emptying). The investigator also has to be aware of the subjects' conditions; whether they can handle the invasive tests, or tests that involve radioactivity.

2.4 SUMMARY

Gastric motor function is complex and involves wide range of gastric motor patterns. The stomach approach the meal differently depends on the food component. The liquid meal is emptied mainly by the proximal stomach, while distal stomach is the main role to empty solids. Stimulation of the small intestine by food also potentially leads to relaxation of the proximal stomach and suppression of gastric motility.

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Figure 2.1

A normal gastric accommodation response recorded by a barostat balloon after ingest meal. The balloon is placed at the proximal stomach, it is clearly seen that intragastric volume dramatically increases after the meal is ingested (Tack, Piessevaux et al. 1998).

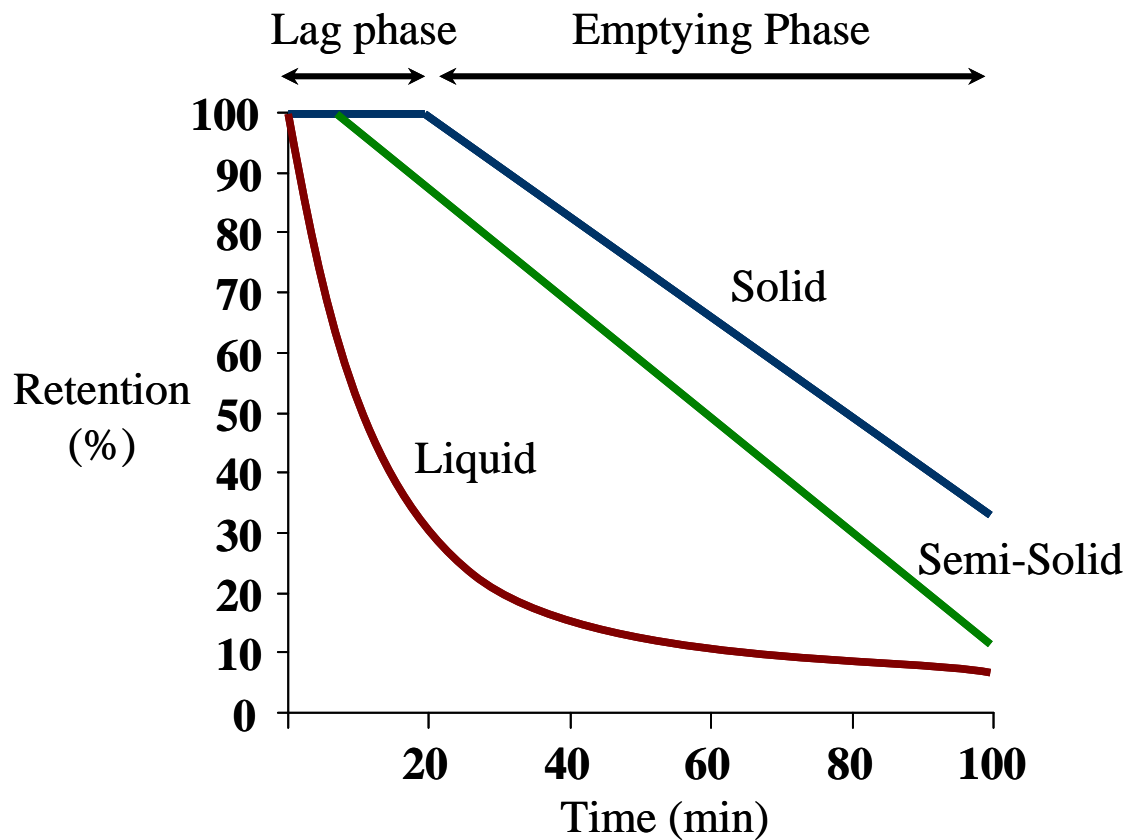


Figure 2.2

Gastric emptying curves for solid, semi-solid and liquid. The graph shows percent retention in the total stomach over time. Solid (pancake) and semi-solid (porridge) is emptied in a linear fashion, while liquid (10% dextrose) is emptied in a monoexponential curve.

CHAPTER 3: HUMAN UPPER GASTROINTESTINAL SENSORY FUNCTION

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3.1 INTRODUCTION

The purpose of this chapter is to review gastric sensory function, including sensory innervations, mediators of sensation in the upper gastrointestinal tract and the gut sensing mechanism. The neuroanatomy of the upper gastrointestinal tract has already been described in Chapter 1. This chapter will provide the background for the studies reported in this thesis in which alterations in gastric perceptions associated with peptic ulcer disease. In addition, assessment of gastric sensory function will also be reviewed.

3.2 PURPOSE OF GASTRO-OESOPHAGEAL SENSORY FUNCTION

There are two main purposes of gastro-oesophageal sensory function. First, to perceive content in the gut. Second, sensory function serves to warn the body to response to noxious stimuli, this kind of sensory function usually involves conscious perception and leads to gut symptoms.

The perception of content is usually coordinated with the motor function of the gut (in response to its content). This kind of sensory function happens without awareness, or does not give rise to conscious perception, and is the major type of

sensory function in the gut. For example, it has been shown that oesophageal acid perfusion which did not induce symptoms of heartburn or chest pain (i.e. did not reach the conscious perception level) in healthy volunteers, nevertheless induced cerebral cortical activity as measured by functional MRI (Kern, Birn et al. 1998). Similarly, Kern et al showed that the afferent signals induced by rectal balloon distension which did not reach the perception level (subliminal) were nevertheless registered in the cerebral cortex as detected by functional MRI (Kern and Shaker 2002).

3.3 SENSORY INNERVATION IN THE UPPER GUT

The gut normally responds to a number of different stimuli such as distension, mechanical, distortion of the mucosa by compression or stroking, and chemical stimuli from the lumen. The nociceptors in the gut are normally activated by distension of hollow organs, traction on the mesentery, ischemia and inflammation (Blackshaw and Gebhart 2002).

The gastrointestinal tract is innervated by two primary sensory pathways. First, are the intrinsic primary afferent neurons (IPANs), which are located within the submucosal and myenteric plexus, and whose main role is to activate enteric reflexes that regulate motility, secretion and blood flow (Bielefeldt and Gebhart 2008). Second, the upper gut is innervated by extrinsic primary afferent neurons

(EPANs) in the vagus and spinal afferents. The vagal afferent fibres have their cell bodies in the nodose ganglion and project to the nucleus solitarius which is located in the brain stem. The spinal afferent fibres have their cell bodies in the dorsal root ganglia and project to the spinal cord at T1-L2 (Goyal and Sivarao 1999). The spinal afferent fibres which innervate the upper gut are splanchnic afferent fibres, and most of spinal afferent fibres are unmyelinated C-fibres.

It is believed that vagal afferent fibres are present as mechanoreceptors, chemoreceptors and thermoreceptors. They are mainly located superficially in the gut in the mucosal and sub-mucosal layers. Their location thus facilitates their being activated by low intensity stimuli such as stroking or nutrient contact (within physiological levels of distension). Thus, vagal afferent fibres mainly mediate non-noxious sensation, including local reflexes, such as gastric accommodation and gastrocolic reflex (Mertz 2002). However, recent studies have shown that they may also mediate nociceptive information (Sengupta, Hummel et al. 1993; Traub, Sengupta et al. 1996; Bielefeldt and Gebhart 2008).

Spinal afferent fibres are located mainly in muscularis and serosae layers and are thought to mediate noxious sensation such as pain. Thus they are activated by high intensity stimuli such as distension and stretch. However, recent studies have shown that both vagal and spinal afferents may mediate physiological and noxious sensation (Grundy 2002).

3.3.1 Sensory innervations in the stomach

The stomach is innervated by vagal and spinal (splanchnic) afferent fibres. Three types of receptors have been described in the vagal afferent fibres, based on the layer of the gut. First, are the mucosal receptors, which are silent at rest but are stimulated after acute inflammation or damage. They are located in the gastric mucosa and respond to light stroking and chemical stimuli (Clarke and Davison 1978), are relatively rapidly adapting to continuous stimulation, but do not respond to distension. Thus they are polymodal in character and responsible for the sensations of satiety, nausea and vomiting. They also respond directly to local mediators such as cholecystinin (CCK), 5-hydroxytryptamine (5-HT), noradrenaline, opioids, bradykinin, purine, and prostaglandins (Blackshaw and Gebhart 2002). Second, are the tension receptors. These are slowly adapting, mechanosensitive to contractions and distension, and are important for signalling food intake and responsible for sensation of satiety and fullness. They are located in the smooth muscle (Blackshaw and Grundy 1990). Third, are the tension and mucosal receptors, which are located only in the oesophageal striated muscle, and have characteristics of both tension and mucosal receptors.

Several types of receptors have been described in the spinal afferent fibres. First, are tonic mechanoreceptors, responding to contractions and distension (Sengupta, Saha et al. 1990), give rise to sensations of fullness. They can also signal the

sensations of discomfort and pain when inflammation occurs. Second, are high threshold mechanoreceptors, considered to be mechanonociceptors as they respond only to noxious stimuli from distension. Third, are the silent nociceptors, which are activated by inflammatory mediators during and after inflammation. Fourth, are mucosal receptors that have characteristics similar to those of mucosal receptors in vagal afferents (Sengupta, Saha et al. 1990).

3.3.2 Mechanisms of sensory stimulation

Stimulation of vagal and spinal afferents fibres usually involves 2 mechanisms, including mechanical and chemical mechanisms. Thus the upper gut responds to mechanical (distension) and chemical (nutrient, acid) stimuli.

3.3.2.1 Mechanical mechanisms

As discussed previously, both vagal and spinal afferents have characteristics of mechanoreceptors (respond to tension and stretch). Vagal afferents are mainly activated by low-threshold stimuli, while spinal afferents are mainly activated by high-threshold stimuli or pain. However, high-threshold mechanoreceptors have been described in vagal afferent fibres in the rat (Sengupta, Hummel et al. 1993).

3.3.2.2 Chemical mechanisms

The oesophageal mucosa usually responds to chemical stimuli, including acid and pH. In the stomach, vagal afferent fibres convey chemonociceptive information. Vagal afferent fibres in the gut are also sensitised by CCK (Blackshaw and Grundy 1990), 5-HT (Hillsley and Grundy 1998), somatostatin, IL-1 β and GLP-1 (Berthoud and Neuhuber 2000). Therefore, increased production of these mediators is associated with increased gastrointestinal perception.

Capsaicin is the main ingredient in red peppers, and excites the visceral sensory afferents through transient receptor potential V1 receptor (TRPV1). It has been reported recently that infusing of red pepper sauce to the stomach was associated with hypersensitivity (Lee, Vos et al. 2004). In addition, TRPV1 up regulation has been reported in patients with irritable bowel syndrome (Akbar, Yiangou et al. 2008).

3.3.2.3 Mediators of sensation

In some cases, mediators directly stimulate visceral sensory nerve endings. Thus the afferent neuron does not respond directly to the stimulus, but they are activated by mediators which produced by enterochromaffin cells (release 5-HT)

and enteroendocrine cells (release CCK). This kind activation usually occurs with vagal mucosal afferents. In spinal afferents however the signalling pathways are different. They are stimulated not as selectively as vagal afferents, and are usually activated by substances released under conditions of inflammation, injury or ischemia from platelets, leukocytes, lymphocytes, macrophage, mast cells, glia, fibroblasts, blood vessels, muscle, and neurons (Grundy 2004). Bradykinin is one of the mediators which formed during tissue damage and activate both high and low thresholds mechanical afferent fibres. Cholecystokinin is released by the small intestinal mucosa, when fat is digested. CCK is associated with the sensation of satiety and reflex inhibition of gastric motility and activates vagal afferent fibres (Blackshaw and Grundy 1990). 5-HT is released from GI mucosa after meal ingestion, mechanical and chemical stimuli. Seven types of 5-HT have been described in the GI tract. However, only 5-HT₃ so far has been confirmed to be associated with visceral sensitivity (nausea, vomiting, pain and discomfort). Studies have shown that 5-HT₃ receptor antagonist medication was helpful in patients with functional dyspepsia and irritable bowel syndrome (Maxton, Morris et al. 1996; Camilleri, Northcutt et al. 2000).

3.4 VISCERAL PERCEPTION

As described previously most of the sensory information from the GI tract that is activated by mechanical or chemical stimuli does not reach conscious perception.

Thus gastrointestinal symptoms represent the final consequence of activation mostly from mechanonociceptors and chemonociceptors and relayed through vagal or spinal afferent fibres. The sensations of satiety, nausea and hunger are associated with vagal afferent fibres while spinal afferents are associated with the sensations of fullness, bloating, discomfort and pain (Blackshaw and Gebhart 2002).

Most of the sensory information from external and internal primary afferent neurons does not reach level of consciousness, thus human beings do not perceive any sensation at normal stage. When noxious stimuli occur is perceived, it is often difficult to determine the location and type of stimulus because of the low density of visceral innervation and polymodal character of visceral afferents. Thus the characteristic of usual pain tends to be dull, burning, poorly localized, more gradual in onset and longer in duration

3.4.1 Visceral perception in functional gastrointestinal disorders

Functional GI disorders consist of a heterogeneous group which is characterised by a number of pathophysiologic mechanisms. The pathophysiology of functional gastrointestinal disorders especially functional dyspepsia and functional heartburn will be explained in more detail in Chapter 6. One of the mechanisms which potentially explain the symptoms of functional GI disorders is visceral

hypersensitivity. It has been shown that patients with functional heartburn have a lower threshold for oesophageal sensation and pain to oesophageal balloon distension and acid perfusion (Rodriguez-Stanley, Robinson et al. 1999), while patients with functional dyspepsia have gastric hypersensitivity in response to gastric balloon distension (Lemann, Dederding et al. 1991). Also patients with irritable bowel syndrome exhibit increased perception of rectal and colonic distension compared with healthy subjects (Ritchie 1973; Whitehead, Holtkotter et al. 1990). These data suggest that in the normal physiologic condition healthy subjects are expected to have no perception, but patients with functional gastrointestinal disorder may have a sensory dysfunction, which could perceive the physiological stimuli, thereby experience gastrointestinal symptoms (hyperalgesia) (Mayer and Raybould 1990).

3.5 ASSESSMENT OF GASTRIC SENSORY FUNCTION IN HUMANS

A variety of methods has been developed to assess gastric sensory function. The ideal test should be reproducible, safe, non-invasive, cost effective, and able to assess subconscious and conscious sensation. However, such an ideal test does not exist. As the sensory pathways of the stomach are vagal and splanchnic (spinal) pathways, which can be activated by tension, chemical, electrical and thermal stimuli, thus gastric sensory function can be measured by gastric balloon

distension (barostat), water and nutrient drink tests, etc... Each test has its own merits and disadvantages, which will be reviewed in this section.

3.5.1 Barostat

The barostat technique has been described in Chapter 2. The barostat is generally regarded as the gold standard for the assessment of gastric sensitivity, and is also able to measure gastric tone, and accommodation when the subject ingests a meal (Schmidt, Abrahamsson et al. 2008). Gastric sensitivity is measured by slowly increasing the pressure of the balloon bag and asking subjects to report their perception of upper abdominal sensations including discomfort and pain on a visual analogue scale. Assessment of gastric sensory function does not appear to be influenced by the mode of distension (Holtmann, Gschossmann et al. 1995). Disadvantages of the barostat are that it is invasive, uncomfortable, and requires special expertise. For measurement of gastric sensitivity, it requires subjects to report their sensation which is subjective, thus results may be altered by subjects who are having uncomfortable experience or stressed (De Schepper, Cremonini et al. 2004).

3.5.2 Water load test and nutrient drink test

Drinking tests, with water or nutrients (Jones, Hoffman et al. 2003; Haag, Talley et al. 2004), have been developed as a potential non-invasive approach to assess gastric sensory function. A standardised nutrient challenge is believed to be an effective visceral sensory stimulus in healthy subjects (Choung, Talley et al. 2007) and in patients with functional dyspepsia (Haag, Talley et al. 2004). The nutrient challenge test has been used in various studies (Holtmann, Talley et al. 1996; Boeckxstaens, Hirsch et al. 1999; Kim, Myung et al. 2000; Mulak 2003; Delgado-Aros, Camilleri et al. 2004; Haag, Talley et al. 2004; Choung, Talley et al. 2007) in patients with functional dyspepsia, irritable bowel syndrome and healthy subjects although the volumes and rates of ingestion have varied among the studies. A recent study has shown that slowly drinking nutrient was associated smaller volume of consumption compared with rapid drinking (Abid, Anis et al. 2009). A study from Haag et al chose to use a 200 ml of nutrient challenge every 5 minutes because it reflects real life and should reflect mechanosensory function (Haag, Talley et al. 2004). The results correlated well with mechanosensory thresholds as measured by the barostat (Holtmann, Talley et al. 1996). However, Boeckxstaens et al did not find a correlation between a rapid caloric or water drinking test and sensitivity to gastric distension (Boeckxstaens, Hirsch et al. 2001). A water load test has been used to assess gastric sensation (the sensation of fullness after meal ingestion) (Koch, Hong et al. 2000), whilst the nutrient drink

test has been used to assess the symptom of early satiety and predicts gastric accommodation (Tack, Caenepeel et al. 2003). A nutrient drink may also assess global gastric function. However, the test depends on subject's report which is subjective, thus the test may be influenced by sensory and psychological factors.

3.5.3 Measurement of conscious perception

Pain and other sensations are usually reported on questionnaires or a visual analogue scale. Subjects are asked to place a mark on visual analogue scale, which should be only a straight line without any division but clearly defined both end points from nothing to the worst of each feeling (i.e., nothing to unbearable pain). The visual analogue scales have been reported as valid and reliable tool to measure the intensity of pain (Price, McGrath et al. 1983). Another method is to report on descriptor scales which contain 5 to 7 steps (Mulak 2003). Questionnaires are also useful for reporting the location and type of sensation in addition to the intensity of sensation.

3.5.4 Measurement of central responses: brain imaging techniques

New brain imaging techniques, such as positron emission tomography (PET), single-photon emission computer tomography (SPECT) and functional magnetic

resonance imaging (fMRI) are able to evaluate brain regions activated by visceral stimulation. They therefore help to assess sensorimotor pathways between the brain and the periphery, including the brain-gut axis, and to differentiate whether the responses are recorded through perception or through reflex pathways (Azpiroz 2002).

3.5.5 Conclusion

A variety of tests is available to assess gastric sensory function. However, a test which can assess all gastric functions simultaneously, and is inexpensive, minimally invasive, subject friendly, and does not involve radioactivity does not exist. Therefore, selecting the test is very important, and depends on what measurement the investigator would like to observe. The investigator also has to be aware of subjects' conditions, whether they can handle the invasive tests, which may alter the results caused by subjects' uncomfortable experience or stressed.

3.6 SUMMARY

In summary, visceral sensation is a complex process. It is mediated by two types of afferent fibres, vagal and spinal. Most of the afferent fibres are polymodal, and

can be activated by chemical, thermal, electrical, or mechanical stimuli. The majority of vagal afferent fibres are responsible for physiologic stimuli, whilst spinal afferent fibres are responsible for noxious stimuli. However, vagal afferent fibres also are able to convey noxious information at a certain level, while spinal afferent also are able to convey physiologic information at some point. Their roles depend on their location in the gut, (oesophagus, stomach, intestine, or colon), and depend on species of the animals studied.

CHAPTER 4: EFFECTS OF AGEING ON GASTRIC MOTOR AND SENSATION

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4.1 INTRODUCTION

Alterations in gastrointestinal motor function and sensation occur with advancing age. Complications of gastro-oesophageal diseases also appear to occur predominantly in elderly patients, such as complication of gastro-oesophageal reflux disease (Barrett's oesophagus) and peptic ulcer disease (bleeding peptic ulcer and perforated peptic ulcer). In addition, elderly patients are less likely to experience gastrointestinal symptoms before the complications occur. Therefore, lack of gastrointestinal symptoms in elderly patients, which might be caused by alteration of gastrointestinal motor and sensory functions, may result in them presenting late with complications rather than earlier with their primary diseases. The effects of ageing on gastric motor and sensory function will be reviewed in this chapter.

4.2 EFFECTS OF AGEING ON GASTRIC MOTOR FUNCTION

Elderly healthy subjects have been reported to have slower gastric emptying for both liquids and solids compared with younger subjects (Horowitz, Maddern et al. 1984; Clarkston, Pantano et al. 1997). Also a study from Rayner et al, showed that gastric accommodation response to gastric balloon distension appeared to be delayed in older subjects, and with lower gastric tone (Rayner, MacIntosh et al. 2000). However, these notions have been challenged (Madsen and Graff 2004), as

the differences in gastric emptying were fairly small and within the range observed in the younger subjects. A study from Huang et al showed that ageing is associated more frequent peristaltic contractions (Huang, Chen et al. 1995). Nevertheless, there is no clear evidence suggests that slowed gastric emptying or more frequent peristaltic contractions in elderly are related to abnormal gastrointestinal symptoms such as abdominal pain, discomfort or bloating.

Disordered gastric motor function may be more common in the elderly as a result of elderly people having more co-morbidity. For example, patients with long-standing type II diabetes is associated with abnormally delayed gastric emptying (Horowitz, Harding et al. 1989; Horowitz, Su et al. 2001).

4.3 EFFECTS OF AGEING ON GASTRIC SENSORY FUNCTION

Ageing has an impact on sensory function in most of the organs including the gastrointestinal system. Ageing is associated with diminished perception of pharyngeal (Aviv, Martin et al. 1994), oesophageal (Lasch, Castell et al. 1997), and rectal sensations (Lagier, Delvaux et al. 1999). Ageing has been shown to be associated with increased visceral pain thresholds in other part of gastrointestinal tract as well as somatic tissues. Lasch et al (Lasch, Castell et al. 1997) reported an age-related decrease in oesophageal pain sensation response to intraoesophageal balloon distension, and Lagier et al (Lagier, Delvaux et al. 1999) showed that

healthy aged subjects had significantly higher rectal sensory thresholds than did young subjects. In addition, healthy aged subjects have been reported to have higher somatosensory thresholds than did young subjects (Lautenbacher, Kunz et al. 2005). Decreased visceral sensation with age may explain the decreasing prevalence of abdominal pain (Kay, Jorgensen et al. 1994) and functional dyspepsia with increasing age (Jones, Lydeard et al. 1990).

Older patients with functional dyspepsia have higher pain, discomfort and fullness thresholds than do younger patients in response to gastric balloon distension (Mertz, Fullerton et al. 1998). In addition, peritonitis and abdominal complaints are often more occult in older persons (Wroblewski and Mikulowski 1991). Painless peptic perforation also occurs more common in elderly patients (Coleman and Denham 1980). A study from Japan showed that only 46.5% of older patients (60-69 years old) with gastric cancer who initially presented with symptoms had epigastric pain compared with 71.6% of young patients (less than 40 years old) (Eguchi, Takahashi et al. 1999). Complicated ulcers (e.g. ulcers with life threatening bleeding) appear to occur predominantly in elderly patients (Hernandez-Diaz and Rodriguez 2002; Targownik and Nabalamba 2006). An age-related reduction in visceral sensory function may result in elderly patients being less symptomatic such that they do not present with their ulcer disease until life threatening bleeding occurs. Hilton and colleagues (Hilton, Iman et al. 2001) showed that 30% of patients over 60 years old with peptic ulceration were asymptomatic compared with only 7% of younger patients.

At present, the mechanism of ageing on visceral sensation is unclear. Aged Long-Evans rats who were injected with formalin into their hind paws had less response to the pain than did middle-aged rats (Gagliese and Melzack 1999). Possibly the function of gastric mechanoreceptors, afferent pathways or central processing of visceral signals might be impaired in elderly subjects. Previous studies have shown a relationship between age and a reduction in the number of myenteric oesophageal neurons in humans (Meciano Filho, Carvalho et al. 1995) and peripheral afferent fibre function in human (Chakour, Gibson et al. 1996). However, data on the effect of age on visceral sensory function of the stomach are lacking.

4.4 EFFECTS OF AGEING ON ABSORPTIVE FUNCTION

Healthy elderly people usually have normal gastric absorptive function. However, the prevalence of atrophic gastritis increases with advancing age (Krasinski, Russell et al. 1986) which may cause decreased secretion of acid and intrinsic factor (Suter, Golner et al. 1991). Moreover, atrophic gastritis may affect calcium bioavailability by limiting its ability to dissociate from food complexes (Russell 1992). Thus, age itself does not affect absorptive function of the stomach, but the increasing prevalence of atrophic gastritis with age is associated with decreased absorptive function.

4.5 SOCIAL AND PSYCHOLOGICAL ASPECTS

Social and psychological issues are related to ageing potential confounders. Depression and anxiety are believed to be associated with disorders of sensory function in the gastrointestinal tract (Lagier, Delvaux et al. 1999). Previous studies also have suggested that anxiety and depression influence gastric sensorimotor function. Studies of Bland et al (Bland, Newman et al. 1988), Regier et al (Regier, Farmer et al. 1993), and from the Australian Bureau of Statistics (1998) (McLennan) showed that the prevalence of anxiety disorders is decreased in people over 65 years of age, and those of Henderson et al, (Henderson, Jorm et al. 1998) who reported that the mean scores for anxiety in the general population decline with age. There are several possible reasons to explain this prevalence. For example; elderly people are less exposed to problems with relationships and work, or less likely to be seriously short of money (Henderson, Jorm et al. 1998). Age may also be correlated with decreased emotional responsiveness, and increased emotional control (Jorm 2000). The association between social and psychological aspects and gastric sensory and motor function will be discussed in detail in Chapters 11 and 12.

4.6 SUMMARY

Ageing itself is not associated with alteration of gastric motor function and absorptive function. However, increasing age is associated with more co-morbidities such as diabetes mellitus and atrophic gastritis thereby making the elderly more likely to manifest delayed gastric emptying and malabsorption. On the other hand, ageing has a significant impact on the gut sensation including pharynx, oesophagus and rectum. Ageing is associated with decreased visceral sensitivity. However, little attention has been paid to the effects of ageing on gastric sensitivity. The visceral sensory function of the stomach has not been studied in relation to ageing, and is potentially important given its role to explain the reason why peritonitis and abdominal complaints are more occult in older persons, or asymptomatic peptic ulcers commonly occur in elderly. This issue is addressed in the study reported in Chapter 10 of this thesis.

CHAPTER 5: IMMUNE ACTIVATION IN GASTROINTESTINAL DISEASES

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5.1 INTRODUCTION

The main function of the gut is to absorb nutrient. At the same time, however, the surface of the gut has a role in protection against bacteria, viruses, toxin, and different antigens from entering the blood and lymph that could be harmful to the organism. Therefore, nutrients are the main influence on the immune system in the gastrointestinal tract. Enzyme in the saliva is the first step helping with immune function. The low pH of gastric juice effectively sterilises food, and also can be fatal for many micro-organisms. Lymphoid tissue along the gut, such as tonsils, adenoids, Peyer's patches, and lymphoid tissue in the oesophagus, stomach, appendix and large intestine, also has a major role in pathogen prevention. Immune responses from the gut largely occur in small intestine as a result of Peyer's patch situated in the terminal ileum. Nutrients are essentially divided to two categories: non-pathogenic and pathogenic, such as infection, which stimulates gastrointestinal immune system. Although the gastrointestinal immune system works independently from the body immune system, local inflammation in the gut essentially influences the systemic immune response.

Cytokines are small cell-signalling protein molecules, which are produced by mononuclear cells such as lymphocyte and macrophages. They serve as local mediators and are involved with variety of biological processes, including cell activation, growth and differentiation, and are particularly involved in the

development of inflammation and immunity (Papadakis and Targan 2000). Cytokines have autocrine, paracrine and endocrine activities, which can mediate the local and systemic presentations of intestinal inflammation (Sartor 1994). Cytokines directly or indirectly mediate and amplify the immune response, induce tissue injury, and mediate complications of the inflammatory response in the gut such as diarrhoea and fibrosis. On the other hand, cytokines also have a role in suppressing inflammation and mediating repair and healing (Sartor 1994).

Two general categories of cytokines can be distinguished; pro-inflammatory, such as tumour necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, IL-8, and IL-12, and anti-inflammatory cytokines such as IL-10. The amount of peripheral cytokine production usually depends on the state of immune activation. In pathological conditions such as acute or chronic inflammation, the immune response and macrophages are activated which leads to increased production of cytokines especially TNF- α , IL-1, and IL-6.

Mononuclear cells respond quickly to infectious agents by secreting pro-inflammatory cytokines. It is believed that in inflammatory conditions, cytokines are produced not only at the site of local inflammation but are also released as part of a systemic immune response. Cytokine production, such as TNF- α , IL-1 β and IL-6, are believed to be associated with severity of inflammation and disease activity in patients with inflammatory bowel disease (Nakamura, Saito et al. 1992; Holtkamp, Stollberg et al. 1995).

However, in functional dyspepsia and irritable bowel syndrome (IBS), little attention has been paid to the association between immune activation and gastrointestinal symptoms; although these patients are found to have normal endoscopy and colonoscopy. The association between immune activation and abdominal symptoms, psychological disorders, upper gastrointestinal diseases and their function will be discussed in this chapter. This chapter will focus only on TNF- α , IL-1 β , IL-6, and IL-10 as these cytokines are predominantly involved with inflammatory process which is associated with gastrointestinal diseases.

5.2 CYTOKINE FUNCTION

5.2.1 Tumour necrosis factor (TNF) – α

TNF- α is a pro-inflammatory cytokine that is secreted predominantly by macrophages, monocytes and activated T lymphocytes (Papadakis and Targan 2000). TNF- α is released in response to lipopolysaccharides, found in outer membrane of gram-negative bacteria, endotoxins, bacterial agents, and IL-1 in various organs and is usually released concurrently with IL-1 and IL-6. Thus, increases in TNF- α are usually associated with increased IL-1 and IL-6.

The primary role of TNF- α is to activate immune cells, induce apoptotic cell death, induce inflammation, and potentially inhibit tumour genesis, and viral replication. It also induces neutrophil proliferation during inflammation. Human gut mucosa is infiltrated with a large number of mononuclear cells. TNF- α is usually one of the most sensitive cytokines which is released early in an inflammatory reaction. TNF- α plays an important role in the regulation of immune functions and is thus targeted to treat mucosal inflammation in Crohn's disease, and chronic inflammatory diseases such as rheumatoid arthritis, or conditions such as psoriasis and asthma. Several studies have shown increased of TNF- α production in mucosal biopsies in patients with Crohn's disease (Reimund, Wittersheim et al. 1996). Anti-TNF- α medication (such as infliximab) now play a role in treatment of patients with Crohn's disease (Present, Rutgeerts et al. 1999).

5.2.2 Interleukin (IL) 1- β

The IL-1 superfamily is composed of two distinct proteins, IL-1 α and IL-1 β . Similar to TNF-alpha, IL-1 β is also produced primarily by macrophages in response to injury and infection. IL-1 β functions as an important mediator of the inflammatory response, and is involved in cellular activities such as cell proliferation, differentiation, and apoptosis. IL-1 β is also a proinflammatory cytokine involved in immune defence against infection.

In gastrointestinal tract, IL-1 β had been related to gastric ulcer, *Helicobacter pylori*, and gastric cancer. IL-1 β has been shown to have a inhibitory effect on gastric acid secretion (Wallace, Cucala et al. 1991). IL-1 β is enhanced in gastric epithelial cell in *Helicobacter pylori* infected patients (Jung, Kim et al. 1997), and IL-1 polymorphisms have also been found to be associated with an increased risk of gastric cancer (El-Omar, Carrington et al. 2000).

5.2.3 Interleukin (IL) 6

IL-6 is produced by T-lymphocytes and macrophages in order to stimulate the immune response and leads to inflammation. IL-6 is therefore a pro-inflammatory cytokine, which is involved mainly in the immune response, inflammation and haematopoiesis (Hirano 1998). It is also involved in malignant transformation and tumour progression (Hirano 1998). In addition, it has also has shown to be associated with various cancers such as prostate cancer (Hobisch, Rogatsch et al. 2000), and lung cancer (Hoheisel, Izbicki et al. 1998).

IL-6 is one of the most important mediators for fever and the acute response phase. In the gastrointestinal tract, *Helicobacter pylori* infection enhances mucosal IL-6 production (Basso, Scrigner et al. 1996). IL-6 is also associated with colon cancer

(Galizia, Lieto et al. 2002), Barrett's oesophagus (Dvorakova, Payne et al. 2004) and oesophageal squamous cell carcinoma (Wang, Chow et al. 1999).

5.2.4 Interleukin (IL) 10

IL-10 is an anti-inflammatory cytokine which is produced primarily by monocytes. It also has inhibitory effects on cytokine synthesis in certain T cells (Fiorentino, Bond et al. 1989) and inhibitory effects on the production of IL-1 α , IL-1 β , IL-6, and TNF- α (de Waal Malefyt, Abrams et al. 1991; Fiorentino, Zlotnik et al. 1991). However, the role of IL-10 in the gastrointestinal tract is largely unknown. A study from Bodger et al showed that gastric mucosal production of IL-10 increased in chronic gastritis associated with *Helicobacter pylori* infection compared with *H. pylori* negative chronic gastritis and *H. pylori* negative normal mucosa (Bodger, Wyatt et al. 1997). This study also showed that increased TNF- α in chronic gastritis was associated with *H. pylori*, infection, and suggested that secretion of IL-10 may limit tissue damage caused by inflammation whilst at the same time being it may responsible for failure of elimination of *H. pylori* by limiting immune activation (Bodger, Wyatt et al. 1997).

5.3 IMMUNE ACTIVATION AND ABDOMINAL SYMPTOMS

The association between immune activation and abdominal symptoms has received little attention. Most attention has been focussed on gastric inflammation (gastritis, gastric ulcer, *H. pylori* infection or gastric cancer) or inflammatory bowel disease. Nevertheless, a few studies have shown an association between immune activation and irritable bowel syndrome (IBS) (Liebregts, Adam et al. 2007) and functional dyspepsia (Liebregts, Adam et al. 2008).

An association has been shown between the level of immune activation and the frequency and severity of symptoms in patients with functional gastrointestinal disorders. Holtmann et al investigated the association between the immunoglobulin (Ig) G response to *Helicobacter pylori* and the frequency of symptoms in patients with functional dyspepsia. They found that high titres of *H. pylori* antibodies were significantly more common in patients with frequent dyspepsia (Holtmann, Gschossmann et al. 2001), suggesting that symptoms may have been related to the pressure of ongoing immune activation.

Gastroenteritis also could potentially precipitate the onset of symptoms of functional dyspepsia (post-infectious). A study from Mearin et al showed that 1 in 7 of patients with *Salmonella* gastroenteritis develop functional dyspepsia after 12 months follow up (Mearin, Perez-Oliveras et al. 2005). In animal models, acute

inflammation from induced colitis is associated with alteration of visceral sensory function (decreased visceral sensory threshold) (Gschossmann, Adam et al. 2002). In addition, visceral hyperalgesia still occurs even after the lesion has healed (Adam, Liebrechts et al. 2006).

One of the main factors that increase risk of post-inflammatory abdominal symptoms is the severity and duration of the acute inflammation (Adam, Liebrechts et al. 2006). It might be speculated, therefore, that immune activation is associated with abdominal symptoms, and that the level of immune activation may help to predict the pattern and severity of abdominal symptoms.

Evidence for underlying immune activation in functional gastrointestinal disorders has been increasing recently. Spiller et al has shown that intraepithelial lymphocytes from rectal biopsies from post-infectious IBS are significantly greater compared with controls (Spiller, Jenkins et al. 2000). Gwee et al found that post-infectious IBS patients exhibited significantly higher expression of IL-1B mRNA in rectal mucosal biopsies than those in patients who had confirmed acute gastroenteritis but who did not develop IBS at 3 months after infection and healthy controls (Gwee, Collins et al. 2003). Liebrechts, et al showed the link between pro-inflammatory cytokine levels, including TNF- α , IL-1 β , and IL-6, and the clinical presentation of IBS, and suggested that inflammatory cytokines may help to explain symptom manifestation in IBS. However, this study measured pro-inflammatory cytokines from cultured peripheral blood

mononuclear cells (PBMC), which may not truly reflect the cytokine levels in the intestinal mucosa, though PBMC-mediated cytokine production has been reported to reflect the severity of inflammation (Nakamura, Saito et al. 1992) and disease activity (Holtkamp, Stollberg et al. 1995) in inflammatory bowel diseases. Another study from Liebrechts, et al (Liebrechts, Adam et al. 2008) also showed that cytokine production from PBMC was linked to the symptom severity in patients with functional dyspepsia. Therefore, immune activation is believed to play a critical role for manifestation of functional gastrointestinal disorders.

5.4 IMMUNE ACTIVATION AND PSYCHOLOGICAL DISORDERS

Immune activation is often associated with psychological disorders especially depression and anxiety. Chronic inflammatory disease such as rheumatoid arthritis, which is associated with increased immune activation, is often associated with depression (Maes, Bosmans et al. 1991). Maes et al have reported that PBMC from patients with major depression produced IL-1 β , and IL-6 significantly higher than controls (Maes, Bosmans et al. 1991). While Smith et al suggested that IL-1, interferon-gamma (IFN- γ), and TNF- α produced by monocytes/macrophages was the cause of depression (Smith 1991). Administration of pro-inflammatory cytokines can also induce psychological change. Previous studies have shown that human volunteers develop symptoms of schizophrenia after given IL-2 intravenously (Denicoff, Rubinow et al. 1987;

Smith 1991). Furthermore, increased immune activation is associated with development of major depressive disorder in patients treated with interferon for hepatitis C (Wichers, Kenis et al. 2006). In healthy subjects, Lipopolysaccharide induce immune activation is associated with the levels of depression and anxiety (Yirmiya, Pollak et al. 2000).

There are several possible mechanisms by which immune activation may be associated with psychological disorders. Apart from inflammatory conditions, which can stimulate cytokine production, the neuroendocrine system is also believed to have influence on the immune system. Corticosteroids, produced by the adrenal cortex, have been shown to stimulate the immune response and cytokine production (Cupps and Fauci 1982). Cytokines could potentially disturb the negative feedback inhibition of circulating corticosteroids on hypothalamic-pituitary-adrenal (HPA) axis and result in HPA axis hyperactivity which is commonly found in patients with depression (Schiepers, Wichers et al. 2005). The release of pro-inflammatory cytokines may induce HPA axis hyperactivity directly or indirectly (via activation of nociceptive, visceral and somatosensory afferents) which may trigger psychological symptoms (Chrousos 1995). Liebrechts et al reported the association between increased production of TNF- α and anxiety in IBS patients (Liebrechts, Adam et al. 2007).

5.5 IMMUNE ACTIVATION AND UPPER GASTROINTESTINAL DISEASES

5.5.1 Immune activation and peptic ulcer disease

An immune response plays a role in peptic ulcer disease, especially those patients with *Helicobacter pylori* infection. Most of the studies have compared between the association between *H. pylori* infected gastric ulcer and *H. pylori* negative healthy controls. All the *Helicobacter pylori* strains are associated with the immune activation system, including macrophages, B lymphocytes, T lymphocytes, polymorphonuclear cells and plasma cells. Usually, once hosts are infected by *H. pylori*, they try to eliminate *Helicobacter pylori* by produce pro-inflammatory cytokines such as IL-1B, Il-6 and TNF- α . These pro-inflammatory cytokines have been shown to be increased in the antral mucosa in patients with *Helicobacter pylori* associated gastric ulcer (Noach, Bosma et al. 1994), and chronic gastritis (Crabtree, Shallcross et al. 1991) with *Helicobacter pylori* infection. Even asymptomatic *H. pylori* infected patients were found to have higher levels of pro-inflammatory cytokine production than healthy *H. pylori* negative volunteers (Lindholm, Quiding-Jarbrink et al. 1998). Interestingly, a study from Japan has shown that injection of IL-1B could induce recurrence of healed gastric ulcer in rats (Watanabe, Arakawa et al. 1997). Therefore, immune

activation may be involved in gastric ulceration either indirectly via mucosal injury related to *H. pylori* or by directly provoking gastric ulcer.

5.5.2 Immune activation and functional gastrointestinal disorders

As mentioned previously, a study from Liebrechts et al has shown an increased production of pro-inflammatory cytokine TNF- α , IL-1B and IL-6 in post-infectious IBS patients, and suggested that immune activation predicts the abdominal symptom pattern and severity (Liebrechts, Adam et al. 2007). A study from Holtmann, et al examined the association between immunoglobulin (Ig) G response to *H. pylori* and the frequency of symptoms in patients with functional dyspepsia, and found that high titres of *H. pylori* antibodies were significantly more common in patients with frequent dyspepsia (Holtmann, Gschossmann et al. 2001). In addition, Liebrechts, et al showed that immune activation was linked to the symptom severity in patients with functional dyspepsia (Liebrechts, Adam et al. 2008). Therefore, immune activation is believed to play a critical role for manifestation of functional gastrointestinal disorders.

5.5.3 Immune activation and gastro-oesophageal reflux diseases

Immune activation is also associated with gastro-oesophageal reflux disease especially reflux oesophagitis (erosive). In an animal model of chronic and reflux oesophagitis in rats, Hamaguchi et al found that IL-1 β , and TNF- α in oesophageal tissue were significantly increased in reflux oesophagitis compared with normal oesophagus (Hamaguchi, Fujiwara et al. 2003). The mechanisms of increased expression of immune activation are unknown. Gastric acid might be one of the reasons responsible since the same study also showed significantly decreased levels of cytokine production after rabeprazole was administered to the rats (Hamaguchi, Fujiwara et al. 2003). Therefore, acid reflux might directly stimulate the cytokine cascades, or indirectly activate cytokine production via inductions of inflammation and activated macrophages to produce cytokines.

In humans, endoscopic oesophageal mucosal biopsies from patients with oesophagitis have been to produce significantly greater amount of IL-1B and IL-6 compared with those from controls (Rieder, Cheng et al. 2007). In addition, supernatant from the biopsy samples from patients with reflux oesophagitis reduced oesophageal muscle contraction. Thus, it seems that cytokine production from the oesophageal mucosal may also have a role in the oesophageal motor dysfunction associated with reflux disease. Studies also have been done in non-erosive reflux disease (NERD), and have found that the oesophageal mucosa of

patients with NERD produced significantly higher levels of IL-1B and IL-8 than healthy controls (Monkemuller, Wex et al. 2009).

In Barrett's oesophagus, Dvorakova et al reported increased IL-6 production from oesophageal mucosal biopsies from Barrett's mucosa compared those with taken from normal oesophageal mucosa adjacent to the Barrett's mucosa, squamous epithelium 5 cm away from the Barrett's mucosa and duodenum, even though there was no inflammation found in Barrett's biopsies (Dvorakova, Payne et al. 2004). The inflammatory cells in the Barrett's metaplasia also produce significantly higher TNF-a (Harrison, Perry et al. 2000), IL-1B and IL-8 (Fitzgerald, Abdalla et al. 2002) compared with inflammatory cells in controls. IL-6 also has shown to be associated with various cancers such as prostate cancer (Hobisch, Rogatsch et al. 2000), lung cancer (Hoheisel, Izbicki et al. 1998), colon cancer (Galizia, Lieto et al. 2002) and oesophageal squamous cell carcinoma (Wang, Chow et al. 1999).

5.6 SUMMARY

Immune activation plays an important role in gastrointestinal diseases, including gastro-oesophageal reflux diseases, peptic ulcer disease, gastritis, and functional gastrointestinal disorders. Their individual function is still unclear as they have variety of specific biological activities and are able to activate or block each

other's activities. Whilst their role in abdominal symptoms in functional gastrointestinal disorders has been vigorously studied recently, the exact mechanisms are still largely unknown. Nevertheless, it seems that there are subtle inflammatory changes in the gut in patients with functional gastrointestinal disorders, even though the endoscopic findings and other standard investigations are normal. Functional gastrointestinal disorders are a heterogeneous group of disorders which are characterised by a number of pathophysiologic mechanisms. Abnormal immune activation appears to be one of the mechanisms that potentially explain the cause of gastrointestinal symptoms in these patients.

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6.1 INTRODUCTION

Upper gastrointestinal symptoms are common complaints which bring people to seek medical attention. Common upper gastrointestinal symptoms are reflux (or heartburn) and dyspepsia. Patients are frequently referred by general practitioner to gastroenterologist due to these complaints.

The severity of upper gastrointestinal complaints is not able to predict the severity of diseases or lesions. It is well known that the extent and severity of erosions is not directly associated with an increased risk for dyspeptic symptoms or more severe symptoms (Collins, Davies et al. 1986; Holtmann, Gschossmann et al. 2002). Conversely patients with functional gastrointestinal disorders (FGID) do not have less severe symptoms. Patients with FGID usually have as poor a quality of life as patients with peptic ulcer disease who suffer from dyspeptic symptoms. This chapter will pay attention to common upper gastrointestinal symptoms, such as dyspepsia, and focus on common upper gastrointestinal diseases, such as peptic ulcer disease and functional dyspepsia, and their complications, particularly bleeding peptic ulcer.

6.2 COMMON UPPER GASTROINTESTINAL COMPLAINTS

6.2.1 Heartburn

Gastro oesophageal reflux disease (GORD) is one of the most common diseases with which patients present to physicians (Shaheen and Ransohoff 2002). The most common manifestations of GORD are heartburn and acid regurgitation. Heartburn is usually described as a sensation of burning or discomfort behind the sternum. However, the term heartburn is often misunderstood. Thus it is better to describe heartburn as a burning feeling rising from the stomach or lower chest up toward the neck (Carlsson, Dent et al. 1998).

Heartburn is one of the most common gastrointestinal complaint in the Western population (Locke, Talley et al. 1997). Approximately 40% of the adult population experience heartburn at least once a month. Around 20% of adults have reflux symptoms once a week and 7% has them daily (Conio, Lapertosa et al. 2003). Symptoms are even more common in pregnancy, when up to 25% of women experience heartburn daily (Nebel, Fornes et al. 1976). In Australia, the frequency of heartburn increases with age and is more common in men than women (Bolin, Korman et al. 2000). Despite the high prevalence of heartburn, not everyone seeks medical attention as many people assume that having heartburn is

normal. Nevertheless, the severity of heartburn symptom does not correlate with the amount of acid reflux or evidence of pathological gastro oesophageal reflux (Moss, Armstrong et al. 2003). Around 85% of acid reflux episodes are asymptomatic (Baldi, Ferrarini et al. 1989), and around one third of patients with Barrett's oesophagus, which caused by long term reflux, are not sensitive to acid (Johnson, Winters et al. 1987). On the other hand, many patients who suffer from heartburn have only low levels of acid reflux. It is believed that age is one of the factors associated with decreased reflux sensation (Fass, Pulliam et al. 2000). The physiologic mechanism of heartburn is unknown. As described in the previous chapter, chemoreceptors and mechanoreceptors are situated along oesophageal mucosa which could potentially transfer signal when reflux occurs and relay signals through vagal pathway.

6.2.2 Dyspepsia

Dyspepsia is described as pain or discomfort centred in the upper abdomen but also includes bloating, early satiety, postprandial fullness, nausea, anorexia, heartburn, regurgitation and burping (belching) (McQuaid 2002). Thus there is sometimes overlap between reflux disease and functional dyspepsia (McQuaid 2002).

More than 25% of the general population experience frequent dyspepsia (Grainger, Klass et al. 1994; Heading 1999), which has a significant impact on their quality of life and results in a substantial cost of diagnostic work up and treatment. However, only one half of patient seek consultation within 6 months of the first symptoms (Westbrook, McIntosh et al. 2000). Dyspepsia is more common in women than men (Agreus, Svardstudd et al. 1994). Moreover, dyspeptic symptoms appear to be decreased with age (Agreus, Svardstudd et al. 1994), and epidemiologic studies showed that dyspepsia is less common after the age 50 (Tibblin 1985; Tibblin, Bengtsson et al. 1990).

About forty percent of patients with dyspepsia who seek medical attention have peptic ulcer disease and gastro-oesophageal reflux disease. Over one half of dyspeptic patients have normal endoscopy and normal standard diagnostic investigations for dyspepsia, and are judged to have so called functional dyspepsia. Although these patients do not have any lesions, it has been shown that their quality of life is affected by dyspeptic symptoms to the same degree as patients with peptic ulcer disease (Nakao, Konishi et al. 2007). Thus severity of dyspeptic symptoms is a very poor predictor for severity of mucosal damage.

6.3 PEPTIC ULCER DISEASE

6.3.1 Definition of peptic ulcer

Peptic ulcer is defined histologically as a necrotic mucosal defect that extends through mucosa and muscularis mucosa (Feldman, Friedman et al. 2002). Any lesion more superficial than this would be judged as gastritis (erosion). In common practice, peptic ulcer is defined by endoscopy as a breach of mucosa and muscularis mucosa. Most studies define a peptic ulcer as a mucosal break at least 3 mm in diameter with visible depth (Taha, Hudson et al. 1996; Hawkey, Karrasch et al. 1998; Yeomans and Naesdal 2008), though a few studies have defined peptic ulcer as a mucosal break of at least 5 mm (Chan, Hung et al. 2002).

6.3.2 Epidemiology

Approximately 500,000 new cases and 4 million recurrent peptic ulcers occur each year in the United States of America. Also around 10% of Western countries population experience a peptic ulcer problem once in their life time (Feldman, Friedman et al. 2002). The incidence of duodenal ulcer in the Australian population is 3.8 cases per 1,000 population, and that of gastric ulcer is 0.7 cases

per 1,000 population. Approximately 70,000 patients per year require treatment for peptic ulcer disease in Australia (Hugh, Coleman et al. 1984). According to Australian Bureau of Statistics (ABS) National Health Survey, 506,000 people in Australia self-reported having peptic ulcer disease in 2001 (2004). Peptic ulcer disease occurs more commonly in men than women (Post, Kuipers et al. 2006).

The epidemiology and care of peptic ulcer has changed over the last few decades. The incidence of uncomplicated peptic ulcer disease has progressively decreased over time since 1950s (Kurata and Haile 1984; Munnangi and Sonnenberg 1997; Lassen, Hallas et al. 2006; Post, Kuipers et al. 2006). In Denmark, the incidence of uncomplicated duodenal ulcer has dropped from 0.55 cases per 1,000 in 1993 to 0.37 cases per 1,000 in 2002, while the incidence of uncomplicated gastric ulcer has dropped from 0.56 cases per 1,000 to 0.4 cases per 1,000.

The decreasing of incidence of uncomplicated peptic ulcer disease is caused mainly by better living conditions (Roosendaal, Kuipers et al. 1997; Loffeld and van der Putten 2003), widespread use of proton pump inhibitors (PPIs) (Van Soest, Siersema et al. 2006) in patients with dyspepsia and peptic ulcer disease, and eradication of *H. pylori* in patients with peptic ulcer disease which leads to much lower rate of recurrence peptic ulcer. In contrast, the incidence of complicated peptic ulcer, including bleeding peptic ulcer (0.55 cases per 1,000) and perforated peptic ulcer (0.1 cases per 1,000), has remained relatively stable over the same period of time (Lassen, Hallas et al. 2006; Post, Kuipers et al.

2006), which could be attributed in part to the increased use of non-steroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin (Higham, Kang et al. 2002). In addition, the incidence of complicated peptic ulcer has increased among elderly subjects (Hernandez-Diaz and Rodriguez 2002; Higham, Kang et al. 2002; Targownik and Nabalamba 2006) and the mortality rate remains approximately 10% (Morgan and Clamp 1984; Branicki, Coleman et al. 1990; Katschinski, Logan et al. 1994).

Peptic ulcer disease is the most common cause of upper gastrointestinal bleeding (Ramirez, Cifuentes et al. 1993; Longstreth 1995; Arlt and Leyh 2001; Church and Palmer 2003; Huang and Lichtenstein 2003; Leerdam, Vreeburg et al. 2003; Palmer 2004; Fiore, Lecleire et al. 2005; Kasper, Braunwald et al. 2005), especially in the elderly (Antler, Pitchumoni et al. 1981), and accounts for approximately 50 % of patients (Croker 1991; Wilcox, Shalek et al. 1994; Shearman, Finlayson et al. 1997; Arlt and Leyh 2001; Huang and Lichtenstein 2003). The main pathogenetic factors in peptic ulcer disease are *Helicobacter pylori* and NSAIDs (Arlt and Leyh 2001; Shiotani and Graham 2002; Huang and Lichtenstein 2003). Approximately half of the patients with bleeding peptic ulcer have a history of NSAIDs or aspirin use (Croker 1991; Paspatis, Metrella et al. 2000; Leerdam, Vreeburg et al. 2003). In the last few decades, whilst the frequency of *Helicobacter pylori* is decreasing and consumption of selective COX II inhibitors is increasing, the incidence of bleeding peptic ulcer disease has remained relatively constant, perhaps because of increased use of aspirin and

NSAIDs (Shiotani and Graham 2002). In patients with chronic NSAIDs use, the prevalence of gastric ulcer ranges between 9% to 31%, and that of duodenal ulcer is about 0% to 19%. NSAIDs decrease mucosal prostaglandin production which leads to reduced epithelial mucus, bicarbonate secretion, mucosal proliferation, epithelial proliferation, and mucosal resistance to injury (Shiotani and Graham 2002).

6.3.3 Pathophysiology of peptic ulcer disease

The major pathophysiological factors in peptic ulcer disease are epithelial defence mechanisms and abnormalities in gastric acid secretion, acid homeostasis and gastroduodenal motility (Feldman, Friedman et al. 2002).

6.3.3.1 Epithelial defence mechanisms

The gastric and duodenal mucosa are covered by mucus and bicarbonate in order to protect against gastric acid. Mucus and bicarbonate are secreted by gastric epithelium and by Brunner's glands in the duodenum. The epithelium also has a role in acid protection. The apical cell membranes and the tight junctional complexes between the surface cells limit the penetration of hydrogen ion into the mucosa. In addition, mucosal blood flow transports bicarbonate to the surface

epithelial cells as well as the processes involved in rapid mucosal repair. Prostaglandin also has an important role in alkali secretion to protect gastric and duodenal mucosa from the acid.

6.3.3.2 Abnormalities in gastric acid secretion and acid homeostasis

Gastric and duodenal ulcers have different pathogeneses. Duodenal ulcer disease is usually associated with acid hypersecretion. In 1952, Cox reported that duodenal ulcer patients had higher mean numbers of gastric parietal cells than do healthy controls and gastric ulcer patients (Cox 1952), although a few patients with duodenal ulcer had similar numbers to healthy controls. In addition, duodenal ulcer patients had increased basal and maximal acid secretion (Blair, Feldman et al. 1987), postprandial serum gastrin levels, rates of post-prandial acid delivery into the duodenum (Malagelada, Longstreth et al. 1977), and 24-hour acid output (Merki, Fimmel et al. 1988) compared with healthy controls. A study from El-Omar et al showed that patients with *H. pylori* infected duodenal ulcer had increased acid output than healthy controls, and the amount of acid output returned to normal after *H. pylori* eradication (el-Omar, Penman et al. 1995).

The location of ulcer could potentially explain the pathophysiology of peptic ulcer disease. Ulcers located at the antrum of the stomach and duodenum are usually associated with gastric acid hyper-secretion. Ulcers located at the body

and fundus of the stomach are associated with acid hypo-secretion. The antrum of the stomach does not contain much acid-secreting (oxyntic) mucosa which consists of abundant parietal cells. While the most common location of gastric ulcer is at the antrum, it seems that gastric ulcers are more likely to occur at the non-acid secretion epithelium.

Gastric ulcers are usually associated with acid hypo-secretion essentially as a result of chronic gastritis and gastric atrophy which majority caused by *H. pylori* (Grossman, Kirsner et al. 1963), especially patients who have ulcers located at the proximal stomach. In contrast, patients with duodenal ulcer are often associated with increased acid secretion.

6.3.4 *Helicobacter pylori*

Helicobacter pylori was discovered by Professor Barry Marshall and Professor Robin Warren in the early 1980s. *H. pylori* is one of the major causes of peptic ulcer disease. It is well established that *H. pylori* is associated with gastritis, peptic ulcer disease, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma.

6.3.4.1 Epidemiology of *Helicobacter pylori*

More than half of the world's population is infected by *H. pylori* (1993), especially developing countries. In developed countries, 20% of people younger than 40 years old and 50% of people older than 60 years have *H. pylori* infection (Graham, Malaty et al. 1991), while up to 80-90% of adults in developing countries have *H. pylori* infection. The prevalence of *H. pylori* increases with age. It is inversely correlated with socioeconomic status and level of education (Graham, Klein et al. 1988; Graham, Malaty et al. 1991). In developed countries, the incidence of *H. pylori* has been decreasing over the last few decades, probably because of improvements in living conditions and common use of antibiotics. In developing countries, however, the incidence of *H. pylori* still continues to be high (Parsonnet 1995). Transmission of *H. pylori* is most likely by the oral-oral, faecal-oral or person to person route, as it has been shown that people who live together and share facilities have higher risk of *H. pylori* infection (Go 2002).

Most of the people who are infected by *H. pylori* will develop gastritis and may progress over decades to peptic ulcer, atrophic gastritis, or gastric atrophy. *H. pylori* are strongly associated with peptic ulcer disease. In the U.S.A., around 80% of duodenal ulcer patients are infected by *Helicobacter pylori*, and 60% of gastric ulcers (Graham, Klein et al. 1988; Feldman, Friedman et al. 2002).

6.3.4.2 Chronic infection

H. pylori can easily survive in the stomach, which is a highly acidic environment, as a result of several factors. First, *H. Pylori* have flagella which help them to move around the stomach easily. Second, their shape (corkscrew shaped) helps them to attach with gastric mucosa. Lastly, *H. pylori* produce the enzyme urease. Urease breaks down urea, which is a common waste product found in human fluid, to ammonia and carbon dioxide. Ammonia neutralizes acid and creates a less acidic environment in which *H. pylori* can survive.

H. pylori induce gastric inflammation. Studies have shown that *H. pylori* infection stimulates immune activation and increases levels of IL-1, IL-6, IL-8, IL-10, IL-12, CD4⁺ T-cells, CD8⁺ T-cells and CD19⁺ B-cells (Basso, Scrigner et al. 1996; Bodger, Wyatt et al. 1997; Jung, Kim et al. 1997; Goll, Husebekk et al. 2005). As the host tries to eliminate *H. pylori* by stimulating immune activation, however, the immune response fails to clear the infection, leading to persistent inflammation, gastritis and eventually acid hyper-secretion and tissue damage (Beswick, Suarez et al. 2006).

The exact mechanism of *H. pylori*-induced peptic ulcer disease is still unclear. *H. pylori* could impair the gastrointestinal mucus layer and duodenal bicarbonate secretion and eventually lead to mucosal injury which later results in peptic ulcer

disease (Sarosiek, Marshall et al. 1991). Interestingly, despite numerous people having *H. pylori* infection, less than 20% of infected people will develop peptic ulcer disease (Blaser 1997). Also less than 1 % will develop gastric cancer (Go 2002). Gastric cancer may be caused by long term atrophic gastritis and gastric atrophy from *H. pylori* infection (Hansson, Nyren et al. 1996). *H. pylori* also increases the risk of peptic ulcer bleeding (Stack, Atherton et al. 2002). Alternatively, *H. pylori* may cause acid hyper-secretion and lead to pre-pyloric ulcer and duodenal ulcer, which may protect against gastric cancer (Hansson, Nyren et al. 1996). Basically, *H. pylori* have ability to decrease or increase acid secretion depending on the location of infection. Infection or gastritis that occurs predominantly at the antrum, leads to acid hyper-secretion and duodenal ulcer. If gastritis occurs predominantly in the fundus or body of the stomach, it leads to gastric atrophy, acid hypo-secretion and gastric ulcer or even gastric cancer. So far, it is still unknown why only 20% of people who have *H. pylori* infection develop peptic ulcer while the others do not, and why men have peptic ulcer disease more commonly than women when they are equally infected.

6.3.4.3 Helicobacter pylori and abdominal pain

People who are infected by *H. pylori* are usually asymptomatic. It has been shown that *H. pylori* has no influence on gastric motor and sensory function (Mearin, de Ribot et al. 1995; Saslow, Thumshirn et al. 1998). The relationship between *H.*

pylori and dyspepsia, however, is unknown. Whilst some studies have reported that *H. pylori* infection is not associated with dyspeptic symptoms in non-ulcer dyspepsia (Rothenbacher, Peter et al. 1998; Bode, Brenner et al. 2000), others have shown that eradication of *H. pylori* reduces the number of people consulted with dyspepsia (Lane, Murray et al. 2006). Although it is generally recommended to eradicate *H. pylori* infection in patients with non-ulcer dyspepsia who have *H. pylori* infection, whether treatment leads to long term improvement of dyspepsia remains controversial (Laine, Schoenfeld et al. 2001; Chiba, Van Zanten et al. 2002; Veldhuyzen van Zanten, Talley et al. 2002; Delaney, Ford et al. 2005). A study from Treiber et al showed that about one-third of patients with peptic ulcer disease still had dyspeptic symptoms after treatment with *H. pylori* eradication (Treiber, Schwabe et al. 2004).

6.3.4.4 Helicobacter pylori and gastro-oesophageal reflux disease

While the incidence of peptic ulcer disease and *H. pylori* infection has been decreasing over years, at the same time, the incidences of gastro-oesophageal reflux disease (GORD), Barrett's oesophagus, and oesophageal adenocarcinoma have increased dramatically (Blot, Devesa et al. 1991; el-Serag and Sonnenberg 1998). In addition, some patients appear to develop GORD after *H. pylori* eradication therapy (Schutze, Hentschel et al. 1995; Labenz, Blum et al. 1997). These initiated an idea that maybe *H. pylori* does not infect people, but only

colonise in the human's stomach. It has been suggested that acid hypo-secretion as a consequence of *H. pylori* related gastritis may protect against GORD (Blaser 1997; Blaser 1999; Fallone, Barkun et al. 2000; Souza and Lima 2009). However, this notion remains controversial (Moayyedi, Bardhan et al. 2001; Malfertheiner, Dent et al. 2002).

6.3.5 Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs and aspirin are among the most commonly used medications in general population worldwide, especially for pain and inflammation. Up to 70% of people older than 65 years take NSAIDs at least once a week and up to 34% take NSAIDs daily (Talley, Evans et al. 1995). However, non-steroidal anti-inflammatory drugs are associated with common gastrointestinal side effects (Thiefin 1991; Thiefin 1991; Lanas 2001), for example, dyspepsia and peptic ulcer (Thiefin 1991; Langman, Weil et al. 1994; Lanas 2001). Moreover, consumption of non-steroidal anti-inflammatory drugs is linked with serious complications of upper gastrointestinal diseases, such as acute upper gastrointestinal bleeding and peptic perforation (Thiefin 1991; Lasch, Castell et al. 1997; Lanas 2001). Up to 25% of patients on long-term NSAIDs experience adverse gastrointestinal effects (Chan and Graham 2004), and serious side effects and complications of non-steroid anti-inflammatory drug occur in 1-2 % of the patients who are prescribed with NSAIDs for 6-12 months period (Lanas 2001).

In addition, it is well established that up to 10-30% of patients with peptic ulcer disease are caused by NSAIDs (Kremer 2000). Gastric ulcer is far more common than duodenal ulcer as a result of NSAID-induced damage to the gastric mucosal barrier. The risk of adverse gastrointestinal effect from NSAIDs increases with advancing age (over 65 years) (Russell 1999).

Interestingly, while up to 40% of patients taking NSAIDs develop dyspepsia, at least 50 % of patients taking NSAIDs who develop dyspeptic symptoms have a normal endoscopy (Armstrong and Blower 1987). In addition, up to 60% of patients who used NSAIDs have no symptoms of peptic ulcer before admission with complications (Armstrong and Blower 1987).

NSAIDs cause ulceration by disrupting the gastric mucosal barrier of bicarbonate and hydrophobic mucus through various mechanisms. The main principle mechanism is that NSAIDs inhibit cyclo-oxygenase (COX); both COX-1 and COX-2 forms. COX-1 is expressed in most tissues including stomach. Its main role in the stomach is to stimulate prostaglandin synthesis. COX-2 on the other hand is normally expressed only under conditions of pain and inflammation. Prostaglandins have a very important role in gastric mucosal protection, including maintenance of mucosal blood flow, stimulation of bicarbonate and mucus secretion, and regulation of cell division (Hawkins and Hanks 2000). Thus, conventional NSAIDs directly inhibit prostaglandin synthesis and render the gastric mucosa vulnerable to gastric acid. Moreover, recent study has shown that

NSAIDs also directly stimulate parietal cells to secrete acid (Arena, Buchinger et al. 2009).

Among NSAIDs, it has been shown that ibuprofen is associated with the lowest risk of gastro duodenal toxicity, while azapropazone and ketoprofen carry the highest risk (Langman, Weil et al. 1994). Specific COX-2 inhibitors medications have been established recently, which have been shown to be associated with much lower risk of gastro-duodenal toxicity (Simon, Weaver et al. 1999).

NSAIDs also increase the risk of peptic ulcer complications. Several studies have shown that consumption of NSAIDs increases the risk of ulcer bleeding about 4-6 times (Laine and Peterson 1994; Shearman, Finlayson et al. 1997; Arlt and Leyh 2001). Among non-steroid anti-inflammatory drugs, Ketorolac and Piroxicam appear to be associated with the highest risk of upper gastrointestinal bleeding (Henry, Dobson et al. 1993; Lanas, Serrano et al. 2003; Laporte, Ibanez et al. 2004), whilst Ibuprofen poses the lowest risk. Approximately half of the patients with upper gastrointestinal bleeding have a history of NSAIDs use (Crocker 1991). Moreover, 50% of patients who used NSAIDs had no symptoms of peptic ulcer before admission with bleeding suggesting that NSAIDs may lead to painless ulceration (Crocker 1991).

6.3.5.1 Aspirin (Acetylsalicylic acid)

Aspirin is also classified as an NSAID, but aspirin is more commonly used for prevention of cardiovascular events (Chan and Graham 2004). Aspirin is the most widely used medication in the world, as it is recommended that most of the people aged over 50 years old take low dose aspirin daily to reduce the risk of many cardiovascular diseases that are associated with aging such as heart disease and stroke. Up to 60% of people older than 65 years old take aspirin regularly (Talley, Evans et al. 1995).

Aspirin causes gastric mucosal damage (Grahim and Smith 1986), and increases the risk of upper gastrointestinal bleeding around three fold (Lanas 2001). Even low dose aspirin (80 – 100 mg/day) increases the risk of upper gastrointestinal bleeding by 1.5 to 2 times. (Capet, Czernichow et al. 2001; Lanas 2001; Chan and Graham 2004).

The notion that *H. pylori* and NSAIDs are synergistic in their gastric toxic effect is still controversial. Nevertheless, the risk of bleeding increases dramatically by 20 fold (Huang, Sridhar et al. 2002; Papatheodoridis, Sougioultzis et al. 2006) in the presence of *Helicobacter pylori* and NSAID use. There is also evidence that eradication of *Helicobacter pylori* is associated with a decreased risk of upper gastrointestinal bleeding in patients taking NSAIDs. While Laine does not believe that *H. pylori* and NSAIDs act synergistically to increase the risk of peptic ulcer

disease (Laine 2001; Laine 2003), a systematic review and meta-analysis showed that *H. pylori* infection and NSAIDs use independently increase the risk of both uncomplicated peptic ulcer and bleeding peptic ulcer (Ji and Hu 2006).

So far, the two factors which have been recognized as the most important causes of peptic ulcer disease, *H. pylori* and NSAIDs, account for more than 90% of peptic ulcer disease. The other factors such as smoking, stress, alcohol and other medications will be briefly discussed in the next section.

6.3.6 Non-NSAID non-*H. pylori* peptic ulcer disease

While the incidence of *H. pylori* induce peptic ulcer disease has been declining as a result of better living condition and very effective of *H. pylori* eradication, the incidence of non-NSAID non-*H. pylori* peptic ulcer has been increasing over the last few decades (Hung, Ching et al. 2005; Chow and Sung 2009). The proportion of confirmed non-NSAID and non-*H. pylori* peptic ulcer is around 20-40% of in both gastric and duodenal ulcers (Peterson, Ciociola et al. 1996; Jyotheeswaran, Shah et al. 1998). The pathogenesis of non-NSAID non-*H. pylori* ulcer is largely unknown as most studies have focused on NSAIDs and *H. pylori*. McColl et al reported that gastric acid hyper secretion and rapid gastric emptying may be the possible causes of duodenal ulcer (McColl, el-Nujumi et al. 1993).

6.3.7 Other risk factors for peptic ulcer disease

6.3.7.1 Selective Cyclo-oxygenase II inhibitors (COX-2 inhibitors)

Cyclo-oxygenase-2 (COX-2) inhibitors are new drugs that have become commonly used over the last few years. COX-2 inhibitors are associated with a significantly lower risk of upper gastrointestinal bleeding compared to non-selective NSAIDs (Arlt and Leyh 2001; Jones and Hawkey 2001; Leerdam, Vreeburg et al. 2003; Norgard, Pedersen et al. 2004; Watson, Yu et al. 2004). However, a study of Lanas et al showed that taking COX-2 inhibitors concomitantly with aspirin increase risk of bleeding peptic ulcer similar to NSAIDs and aspirin (Lanas, Garcia-Rodriguez et al. 2006), possibly due to their lack of clinical antiplatelet effect and use of aspirin undoes the selective advantages of COX-2 inhibitors.

6.3.7.2 Non-aspirin anti-platelet agents

Non-aspirin anti-platelet agents, such as clopidogrel, are another class of medication used for reducing the risk of cardiovascular diseases and stroke, with similar results to aspirin, but with less adverse gastrointestinal effects. Clopidogrel acts through a different mechanism on platelet aggregation.

Clopidogrel has been shown to be more efficient than aspirin for reducing the risk of cardiovascular diseases and cerebral ischemic stroke (Harker, Boissel et al. 1999), and has a lower incidence of gastrointestinal side effects including dyspepsia, peptic ulcer disease and bleeding peptic ulcer (Harker, Boissel et al. 1999).

6.3.7.3 Corticosteroids

Studies on the association between the use of corticosteroids and upper gastrointestinal bleeding and mucosal injury have shown conflicting results (Conn and Poynard 1994). However, the risk of bleeding is much greater if the patients are prescribed a corticosteroid in combination with a NSAID (Shearman, Finlayson et al. 1997; Hernandez-Diaz and Rodriguez 2001). Thus it is believed that corticosteroid may delay the healing process of peptic ulcer caused by NSAIDs, increase acid secretion and decrease mucus production (Schacke, Docke et al. 2002).

6.3.7.4 Anticoagulation

The consumption of anticoagulants alone does not affect the gastro-duodenal mucosa. However, anticoagulation increases the risk of upper gastrointestinal

bleeding and hospitalisation from bleeding peptic ulcer by 2 folds (Shearman, Finlayson et al. 1997). Once bleeding peptic ulcer occurs, anticoagulant aggravates the bleeding. In addition, taking on anticoagulant concurrently with NSAIDs is associated with a 12-fold increase risk of bleeding peptic ulcer (Shorr, Ray et al. 1993).

6.3.7.5 Calcium channel blockers

Calcium channel blockers are commonly used to treat patients with hypertension. Several studies have shown that the prescription of calcium channel blockers is associated with an increased risk of upper gastrointestinal bleeding (Pahor, Guralnik et al. 1996; Kaplan, Heckbert et al. 2000). However the findings are controversial (Smalley, Ray et al. 1998; Suissa, Bourgault et al. 1998; Lanas, Serrano et al. 2003), and some studies have even suggested that they may decrease the risk of upper gastrointestinal bleeding compared with other hypertensive drugs (Leenen, Nwachuku et al. 2006). This hypothesis requires further investigation and more significant information.

6.3.7.6 Selective serotonin reuptake inhibitors (SSRIs)

Selective serotonin reuptake inhibitors are commonly used anti-depressant drugs. It has been suggested that consumption of selective serotonin reuptake inhibitors increases the risk of upper gastrointestinal bleeding (van Walraven, Mamdani et al. 2001; Dalton, Johansen et al. 2003), especially when used in combination with NSAIDs or aspirin (de Abajo, Rodriguez et al. 1999). Animal models have shown recently that SSRIs alone do not cause gastric mucosal damage, but SSRIs given concomitantly with NSAID aggravate gastric ulcer more than NSAID alone. In addition, mucosal damage was worse when exposed to exogenous 5-HT and better when exposed to a selective 5HT₃ antagonist, suggesting that SSRIs may cause gastric ulcer through the activation of 5HT₃ receptors (Nukui, Kojyo et al. 2009). However, this apparent association has not been confirmed.

6.3.7.7 Psychological factors

The impact of psychological factors in peptic ulcer disease and bleeding peptic ulcer is unclear (Shearman, Finlayson et al. 1997). However, there is strong evidence from epidemiologic studies to suggest that psychological factors are related to peptic ulcer. For example, the incidence of peptic ulcer disease of population in London during World War II, and the incidence of peptic ulcer disease in Hong Kong during a period of economic stress, were higher compared

to the normal situation (Shearman, Finlayson et al. 1997). Stress may increase risk of peptic ulcer by increasing duodenal acid load (Shiotani and Graham 2002).

6.3.7.8 Genetic factors

Evidence from a number of studies indicates that peptic ulcer is related to hereditary factors. Family studies and twin studies have revealed genetic influences in peptic ulcer disease (Shearman, Finlayson et al. 1997). However, there is no genetic marker that can identify the role of genetic factors and peptic ulcer disease.

6.3.8 Mortality of peptic ulcer disease

Despite the effective treatment of peptic ulcer disease with PPIs and *H. pylori* eradication, the mortality rate of bleeding peptic ulcer disease remains approximately 10% (Morgan and Clamp 1984; Branicki, Coleman et al. 1990; Katschinski, Logan et al. 1994). Patients usually die from the complications of ulcers, predominantly bleeding and perforation. Most of the patients who die from peptic ulcer are elderly with significant co-morbidities. A study from England showed that patients who were older than 65 years of age had 20 times higher

mortality rate from gastric ulcer compared with patients age between 35-40 years old (Higham, Kang et al. 2002).

6.3.9 Symptoms of peptic ulcer disease

Patients with peptic ulcer disease usually present with either dyspepsia or a complication of peptic ulcer such as upper gastrointestinal bleeding and perforation. Approximately 25% of patients with peptic ulcer disease experience complications (Shiotani and Graham 2002). Classic symptoms of duodenal ulcer include epigastric pain in 2-3 hours after meals and relieved by the intake of food or antacids (Feldman, Friedman et al. 2002; Shiotani and Graham 2002), while those of gastric ulcer include epigastric pain that occurs quickly after meals. Nevertheless, it is often difficult to determine by history whether a patient with epigastric pain has gastric or duodenal ulcer.

6.3.10 Mechanism of peptic ulcer pain

Although the mechanism of peptic ulcer pain has been extensively studied over the past few decades, it remains unclear. Even the true mechanism of abdominal pain is still unclear. A study from Wolf and Wolff reported that patients developed stomach pain when the area of inflamed gastric mucosa was stimulated

by touch or chemical through a gastrostomy tube, while the patient felt nothing when the same stimuli applied on the normal gastric mucosa (Wolf and Wolff 1943). This observation has been confirmed by a study of Nathan (Nathan 1981). The relevance of gastric acid bathing the ulcer crater is controversial. Ruffin et al showed in 1950s that only 37% of the patients with active peptic ulcer developed ulcer pain when the acid was introduced to the stomach (Ruffin, Baylin et al. 1953). Subsequently, Kang et al showed in 1986 that only 16 out of 40 patients with duodenal ulcer reported ulcer pain when acid was applied to the ulcer crater, while healthy volunteer did not feel anything, and suggested that acid may be one of the pathogenesis of ulcer pain (Kang, Yap et al. 1986). Nevertheless, many studies have reported that patients with peptic ulcer disease have significantly better improvement in dyspeptic symptoms when they were treated with acid suppression medications including PPI and H2-blockers (Salas, Ward et al. 2002), strongly suggesting that acid plays a role in a mechanism of peptic ulcer pain.

H. pylori may also play a role in peptic ulcer pain, however, about one-third of patients with peptic ulcer disease continue to have dyspeptic symptoms after *H. pylori* eradication (Treiber, Schwabe et al. 2004). *H. pylori* might be indirectly associated with peptic ulcer pain by increasing the acid secretion.

Dang et al reported that gastric ulcer and inflammation enhanced the activation of nodose ganglions and dorsal root ganglions in rats, suggesting that both vagal and spinal afferents may be involved in ulcer pain (Dang, Bielfeldt et al. 2005). While

another study showed that having gastric ulcer increased response to acid administration in the rat models, compared with controls via vagal afferents (Lamb, Kang et al. 2003).

It is believed that chemical nociceptors, which are situated along the gut mucosa and submucosa, relay the pain signals when they are directly activated by substance released (substance P, serotonin, bradykinin, CGRP, prostaglandin, etc...) when inflammation occurs (gastric ulcer). However, it is not known clearly why many patients with peptic ulcer disease do not develop any dyspeptic symptoms, and why some patients have typical ulcer symptoms but normal endoscopy.

Disordered gastric motility or spasm at the ulcer site has also been proposed to be a cause of ulcer pain (Ruffin 1959). While a number of patients with peptic ulcer disease still have ulcer symptoms even though the ulcer had healed (Isenberg, Peterson et al. 1983).

Immune activation plays an important role in peptic ulcer disease, especially that related to *H. pylori* infection. As immune activation is associated with severity of abdominal symptoms in irritable bowel syndrome (Liebrechts, Adam et al. 2007) and functional dyspepsia (Liebrechts, Adam et al. 2008), it could be speculated immune activation may also be associated with symptoms in peptic ulcer disease,

particular after the ulcer has healed. However, this has not been studied so far. This issue is addressed in the study reported in Chapter 13 of this thesis.

6.3.11 Complications of peptic ulcer disease

6.3.11.1 Bleeding peptic ulcer

Approximately 150,000 patients per year are hospitalized with bleeding peptic ulcer in United States of America (Feldman, Friedman et al. 2002). Epidemiologic studies of bleeding peptic ulcer show that the number of patients with bleeding peptic ulcer increases in cold seasons such as winter, and decreases in hot seasons such as summer. Bleeding duodenal ulcer is more common than bleeding gastric ulcer. The risk of recurrent bleeding in patients with a history of bleeding peptic ulcer is around 5-12% per patient year (Arlt and Leyh 2001).

Peptic ulcer is the most common cause of upper gastrointestinal bleeding (Ramirez, Cifuentes et al. 1993; Longstreth 1995; Arlt and Leyh 2001; Church and Palmer 2003; Huang and Lichtenstein 2003; Leerdam, Vreeburg et al. 2003; Palmer 2004; Fiore, Lecleire et al. 2005; Kasper, Braunwald et al. 2005), accounts for 50% of cases (Crocker 1991; Wilcox, Shalek et al. 1994; Shearman, Finlayson et al. 1997; Arlt and Leyh 2001; Huang and Lichtenstein 2003). About

half of the patients with bleeding peptic ulcer have a history of NSAID or aspirin use (Crocker 1991; Paspatis, Metrella et al. 2000; Leerdam, Vreeburg et al. 2003). Despite improvements in medical care, over the last few decades, the mortality rate of bleeding peptic ulcer still remains at 10 % (Ruigomez, Rodriguez et al. 2000). The admission rate and number of patients with bleeding peptic ulcer have also remained relative stable over recent decades (Lassen, Hallas et al. 2006; Post, Kuipers et al. 2006).

Most of the patients who are diagnosed with peptic ulcer have suggestive symptoms, for instance, upper abdominal pain or heartburn before they are admitted to hospital. Approximately 20% of patients with bleeding peptic ulcer present with melena, 30% present with hematemesis, and 50% present with both of hematemesis and melena, while around 5% may present with hematochezia (Feldman, Friedman et al. 2002). Duodenal ulcers are more common site of bleeding than gastric ulcers (Shearman, Finlayson et al. 1997; Arlt and Leyh 2001). The main risk factors for bleeding peptic ulcer are *Helicobacter pylori* and non-steroidal anti-inflammatory drugs (NSAIDs) (Arlt and Leyh 2001; Huang and Lichtenstein 2003), similar to peptic ulcer disease which have been discussed previously.

6.3.11.2 Peptic perforation

Peptic perforation is a complication of chronic peptic ulcer disease, but not as common as bleeding peptic ulcer. A study from the Netherlands reported that the incidence of perforated peptic ulcer in 2003 was around 8 per 100,000 which is similar to that in 1980. Thus the admission rate for perforating ulcer has remained relatively stable in both gastric and duodenal ulcer in the Netherlands (Post, Kuipers et al. 2006). However, the incidence has been decreasing in Denmark and Sweden (Lassen, Hallas et al. 2006; Hermansson, Ekedahl et al. 2009), which may be partly caused by the introduction of PPI therapy. Nevertheless, perforated peptic ulcer is associated with high morbidity and mortality rate. Many patients with perforated peptic ulcer are asymptomatic before the perforation occurs.

6.3.11.3 Pyloric stenosis

Pyloric stenosis is usually a rare complication of peptic ulcer disease as a result of refractory peptic ulcer disease. Treatment of choice is endoscopic dilatation.

6.3.12 Asymptomatic peptic ulcer

Asymptomatic peptic ulcer disease is not uncommon. The proportion of asymptomatic peptic ulcer is not known exactly, as it is impossible to know whether a person has peptic ulcer disease unless he or she undergoes endoscopy. As peptic ulcer disease usually presents as either dyspepsia or acute bleeding, and peptic ulcer disease is generally diagnosed by symptoms and confirmed endoscopy, patients with asymptomatic uncomplicated peptic ulcer disease would not be found to have ulcer as they do not seek medical attention and endoscopy is not necessary unless the ulcers are found unintentionally from other indication, not dyspepsia.

Bleeding from peptic ulcer occurs when the ulcer erodes into an artery in the stomach or duodenum (Shearman, Finlayson et al. 1997). Therefore, bleeding generally occurs from a chronic peptic ulcer which extends deeper until it erodes into an artery. Patients with bleeding peptic ulcer commonly have very few symptoms or are asymptomatic until the bleeding occurs. In this case, it is the bleeding that the patients to the hospital. Moreover, the majority of patients dying from peptic ulceration have no symptoms of ulcer disease until the presentation of their final, fatal illness (Pounder 1989). Data on the prevalence of ulcer symptoms prior to ulcer bleeding are few. The proportion of patients without symptoms has been reported to range from 43%-87% (Coggon, Langman et al. 1982; Mellem, Stave et al. 1985; Matthewson, Pugh et al. 1988; Croker 1991; Wilcox and Clark

1997), even though the endoscopic assessment may reveal multiple ulcer scars suggestive of previous ulceration.

Although an exact proportion of asymptomatic peptic ulcer is unknown, it has been estimated that a fifth of general population may have some evidence of peptic ulcer disease including active ulcer, scars or previous surgery (Dew 1987). Asymptomatic peptic ulcer has been intensively studied in 1980s. Recently, a very important study from Lu et al reported the results of endoscopic screening in a large Chinese population as a part of self-paid routine health examination, and found that around 11% of 6,500 people had peptic ulcer disease (Lu, Chang et al. 2004). Additionally, up to two third of patients with peptic ulcer disease were asymptomatic.

At the present, the pathophysiology of asymptomatic peptic ulcer is unknown, and even the pathogenesis of dyspepsia in patients with peptic ulcer disease or the mechanism of ulcer pain is still unclear (Wilcox and Clark 1997). It is well known that the extent and severity of erosions is not directly associated with an increased risk for dyspeptic symptoms or severity of symptoms (Collins, Davies et al. 1986; Holtmann, Gschossmann et al. 2002). However, several factors have been shown to be associated with silent peptic ulceration, for example, age (Wilcox and Clark 1997), NSAIDs (Pounder 1989) and ulcer size (Wilcox and Clark 1997; Lu, Chang et al. 2004).

Bleeding peptic ulcer occurs predominantly in elderly patients (Hernandez-Diaz and Rodriguez 2002; Targownik and Nabalamba 2006) and older age is often associated with asymptomatic peptic ulcer (Clinch, Banerjee et al. 1984; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997; Hilton, Iman et al. 2001). A study from Clinch et al showed that up to 35% of patients older than 60 years with peptic ulceration were asymptomatic compared with only 8% of patients aged 20-50 years (Clinch, Banerjee et al. 1984). Similarly, Hilton et al found that up to 30% of patients older than 60 years were asymptomatic compared with 7% in patients younger than 50 years old. Wilcox and Clark focused on the factors which were associated with asymptomatic peptic ulcer, and found that age less than 80 years old was a determinant of ulcer pain. In addition, Matthewson et al reported that patients who presented with bleeding as a first presentation were significantly older than patients who presented with pain (Matthewson, Pugh et al. 1988). Interestingly, in this study, 40% of asymptomatic patients had a history of peptic ulcer disease and had previously presented with abdominal pain, not bleeding, as a first presentation. Thus it seems that increasing age was associated with having less ulcer pain. Conversely, Lu et al did not find age difference between patients with symptomatic and asymptomatic peptic ulcer (Lu, Chang et al. 2004).

Decreasing symptoms with age can be explained by several reasons. Moore and Clinch stated that (Moore and Clinch 2004) elderly patients show evidence of decreased visceral sensation. Cutaneous and visceral pain decrease with age, and

painless peptic perforation (Coleman and Denham 1980) and acute myocardial infarction (Pathy 1967) occur more common in elderly patients. The reasons for this include impaired A-delta fibre function, altered serotonin metabolism, increased responsiveness of older individuals to non-opioid analgesic pathways at the spinal cord level, or decreased nitric oxide responsiveness (Moore and Clinch 2004). Consequently, there might be abnormality of gastric sensory function in patients with asymptomatic bleeding peptic ulcer because majority of patients with bleeding peptic ulcer are elderly patients.

A number of patients with asymptomatic bleeding peptic ulcer take NSAIDs. Thus NSAIDs may also be associated with asymptomatic peptic ulcer (Clinch, Banerjee et al. 1984; Mellem, Stave et al. 1985; Dew 1987), through pain relief effects masking the abdominal symptoms. However, this notion has been challenged by studies from Wilcox and Clark, Lu et al and Matthewson et al who did not find association between asymptomatic peptic ulcer and history of NSAID use (Matthewson, Pugh et al. 1988; Wilcox and Clark 1997; Lu, Chang et al. 2004). Most of the patients who are prescribed NSAIDs are elderly patients because of underlying diseases such as cardiovascular disease and arthritis and the apparent association of NSAID use and asymptomatic use may therefore be co-incidental and related to the age of the patients. The mechanism of NSAIDs masking peptic ulcer is unknown. It might be because elderly patients are more tolerant to dyspepsia than young people or they might be suffering pain from arthritis, therefore they were not concerned about their abdominal symptoms.

Another possible factor associated with painless peptic ulcer is ulcer size. A study from Wilcox and Clark reported that majority (77%) of patients who had a peptic ulcer larger than 2 cm in diameter had peptic ulcer symptoms, compared with less than half of the patients who had an ulcer smaller than 1 cm (Wilcox and Clark 1997). Lu et al reported that having ulcer smaller than 1 cm in diameter and at healing stage was a determinant of peptic ulcer symptoms (Lu, Chang et al. 2004).

Several other factors have been reported to the association with peptic ulcer symptoms. Studies from Wilcox and Clark (Wilcox and Clark 1997), and Matthewson et al (Matthewson, Pugh et al. 1988) found that patients with a history of tobacco used were associated with peptic ulcer symptoms, but the findings contrast with those of Lu et al (Lu, Chang et al. 2004) who could not find such an association. Other factors that have been reported to be associated with asymptomatic peptic ulcer disease include a history of drinking tea, and having a large body mass index (Lu, Chang et al. 2004). Most of the studies have found that symptomatic or asymptomatic peptic ulcer was not associated with gender, race, blood group, history of drinking coffee, alcohol consumption, ulcer location, history of previous ulcer, *H. pylori* infection, and co-morbidities such as hypertension and diabetes (Wilcox and Clark 1997; Lu, Chang et al. 2004).

Another possible explanation for the lack of dyspeptic symptoms in peptic ulcer disease is abnormally altered visceral sensory function. Increased visceral sensitivity may present as abdominal pain in the absence of mucosal injury, such as occurs in functional dyspepsia and irritable bowel syndrome (Greydanus, Vassallo et al. 1991; Mayer and Gebhart 1994; Tack, Bisschops et al. 2004). The lack of dyspeptic symptoms in bleeding peptic ulcer could potentially reflect diminished visceral sensitivity. However, visceral sensory function has not been tested in patients with uncomplicated peptic ulcer disease versus bleeding peptic ulcer disease. Difference in visceral sensory function may potentially explain why a lot of patients with bleeding peptic ulcer are asymptomatic. This issue is addressed in the study reported in Chapter 11 of this thesis.

6.3.13 Gastric motor function in patients with peptic ulcer

Gastric motor function in peptic ulcer disease was extensively studied in 1980s, to uncover the pathophysiology of peptic ulcer disease, before the discovery of *H. pylori* infection.

Active gastric ulcer is associated with a reduction in the frequency and amplitude of phasic contractions in antrum, and delayed gastric emptying (Harasawa, Tani et al. 1979; Brooks 1985). Studies of indomethacin-induced gastric ulceration in rats showed that the degree of delayed gastric emptying was related to the

severity of lesion (Takeuchi, Ueki et al. 1986). Most studies in patients were done when the ulcer was active and found altered gastric emptying. For example, Harasawa et al (Harasawa, Tani et al. 1979) reported that gastric emptying was delayed in 52 patients with active gastric ulcer compared with healthy controls. However, a study from Moore et al reported that gastric ulcer was associated with antral hypomotility even after the ulcer had healed as confirmed by endoscopy (Moore, Malagelada et al. 1986). Kanaizumi et al investigated the relationship between gastric emptying and the location of the ulcers, and found that gastric emptying was accelerated in patients with proximal active gastric ulcer, but delayed with distal gastric ulcer. This study also found that the emptying rates returned to nearly normal when the ulcers had healed compared with healthy controls (Kanaizumi, Nakano et al. 1989).

Rapid gastric emptying has been reported in patients with active duodenal ulcer (Kanaizumi, Nakano et al. 1989). This finding has been confirmed by others (Malagelada, Longstreth et al. 1977; Harasawa, Tani et al. 1979; Dubois and Castell 1981; Lam, Isenberg et al. 1982; Maddern, Horowitz et al. 1985; Parr, Grime et al. 1988) and appears to correlate with gastric acid hyper-secretion (Harasawa, Tani et al. 1979).

The mechanism underlying the disturbance to gastric emptying in peptic ulcer disease is still unknown. Delayed gastric emptying may play in aetiology of gastric ulcer, as a result of the gastric mucosa being exposed to acid longer than

usual period. The pathogenesis of duodenal ulcer may partly be explained by rapid gastric emptying, as rapid gastric emptying increases the acid load from the stomach delivered to the duodenum and thereby exposes the duodenal mucosa to more acid than usual, especially in patients who have hyper-secretion of gastric acid. Conversely, abnormal gastric emptying may be a consequence of peptic ulcer disease.

6.3.14 Gastric sensory function in patients with peptic ulcer

The relationship between gastric motor and sensory function and clinical manifestation of peptic ulcer disease, however, is not known. This issue is addressed in the study reported in Chapter 11 and Chapter 12 of this thesis.

Research on gastric sensory function in peptic ulcer disease is rare. Most of the studies focus on functional dyspepsia, not organic dyspepsia. Mertz et al compared the symptoms and visceral perception in functional and organic dyspepsia and found that both groups reported very similar symptoms in term of type and severity of symptoms. However, with respect to visceral perception, functional dyspepsia had significantly lower pain threshold or hyper-visceral sensitivity compared with organic dyspepsia. Nevertheless, there were only 4 out of 10 patients with organic dyspepsia had peptic ulcer disease (Mertz, Fullerton et al. 1998).

Although *H. pylori* is an important pathogenetic factor in peptic ulcer disease, it has been shown that *Helicobacter pylori* has no influence on gastric motor and sensory function (Mearin, de Ribot et al. 1995; Perri, Clemente et al. 1998; Saslow, Thumshirn et al. 1998; Chiloiro, Russo et al. 2001).

6.3.15 Summary

Peptic ulcer disease is common. The exact pathophysiology of peptic ulcer disease is still unclear. Major causes of peptic ulcer disease are *H. pylori* infection and use of NSAIDs. While *H. pylori* infects half of the world's population, it is not known why only 20 % develop peptic ulcer disease at some point in their life; therefore individual genetic polymorphism might be one of the factors. It is well known that *H. pylori* cause gastric hypo secretion in gastric ulcer and hyper secretion in duodenal ulcer. Up to 20-30% of patients taking long term NSAIDs experience adverse gastrointestinal effects. NSAIDs cause ulcers by disrupting the gastric mucosal barrier mainly via prostaglandin synthesis pathway, which result to the mucosa are vulnerable to its own acid.

Gastric ulcer is associated with increased levels of pepsinogen II, increased duodenogastric reflux, decreased mass of gastric parietal cells, and decreased maximal acid output (Feldman, Friedman et al. 2002). On the other hand,

duodenal ulcer is associated with increased parietal cell mass, serum pepsinogen I concentration, capacity to secrete acid and pepsin, increased drive to secrete acid and pepsin, parietal cell sensitivity to gastrin, increased duodenal acid and pepsin loads and increased rate of gastric emptying (Holtermuller and Malagelada 1980).

Several factors contribute to the pathogenesis of peptic ulceration, but not all are present in every patient. Only 20-50% of duodenal ulcer patients have one of these mechanisms and the majority of peptic ulcer patients have normal ranges of gastric acid, secretion, acid homeostasis and gastroduodenal motility (Grossman 1981).

The incidence of uncomplicated peptic ulcer disease has been declining over the last few decades, largely as a result of common use of PPIs and eradication of *H. pylori*. On the other hand, the incidence of complications of peptic ulcer disease, including bleeding peptic ulcer and perforated peptic ulcer disease, has remained relatively stable over the same period of time, which might be caused by increased use of NSAIDs, and a large number of patients with complicated peptic are asymptomatic before the bleeding occurs.

The mechanism of peptic ulcer pain is largely unknown. So far, studies suggest that acid is the important stimulant of ulcer pain, when inflammation or injury take place. The cause of asymptomatic peptic ulcer is also unknown. Ageing,

NSAIDs used and ulcer size have been reported to be associated with asymptomatic peptic ulcer.

Altered gastric motor function has been proposed to be associated with the pathogenesis of peptic ulcer disease, though this idea is not well acknowledged. While, no study has reported in relate to visceral sensory function in patients with peptic ulcer disease.

Patients with peptic ulcer disease usually present with either dyspepsia or acute bleeding. Most of the patients with uncomplicated peptic ulcer disease present with abdominal pain. On the other hand, many of patients with bleeding peptic ulcer present with bleeding as a first presentation without previous peptic ulcer symptoms. Gastric motor function and visceral sensory function may be the key responsible for the difference between clinical manifestations of complicated and uncomplicated peptic ulcer. These issues will be discussed in this thesis.

6.4 FUNCTIONAL DYSPEPSIA

Functional gastrointestinal disorders are one of the most common medical conditions (Chang 2004). Functional gastrointestinal disorders may present with variety of symptoms, for example fullness, bloating, nausea, vomiting, constipation, diarrhoea, weight loss and dyspepsia. There are two common

syndromes in functional gastrointestinal disorders, functional dyspepsia and irritable bowel syndrome.

Functional dyspepsia is defined as persistent or recurrent upper abdominal pain or discomfort, with or without other abdominal symptoms such as nausea, vomiting, bloating, fullness and early satiety, which lasts for at least 3 out of 12 months (Shearman, Finlayson et al. 1997; Thumshirn 2002). The diagnosis of functional dyspepsia is commonly based on the Rome II criteria:

- Persistent or recurrent upper abdominal pain or discomfort
- Symptoms lasting for at least 12 weeks in the past 12 month
- No evidence of any structural lesion or biochemical abnormality which is related to abdominal symptoms
- Abdominal symptoms are not relieved by defecation and not associated with altered frequency or form of stool (i.e. not irritable bowel syndrome)

More recently, Rome III criteria have been formulated (Tack, Talley et al. 2006) which have less restriction compared with the Rome II criteria:

1. One or more of the following:
 - Bothersome postprandial fullness
 - Early satiation
 - Epigastric pain
 - Epigastric burning

And

2. No evidence of structural disease (including at upper endoscopy) that is likely to explain the symptoms

Criteria must be fulfilled for the last 3 months with the onset of symptoms at least 6 months prior to the diagnosis.

6.4.1 Epidemiology functional dyspepsia

Functional dyspepsia is the most common cause of dyspeptic symptoms (Chang 2004), and occurs in around 20-30 % of population (Jones, Lydeard et al. 1990; Agreus, Svardsudd et al. 1995). Also 20% of this population meets doctors because of their symptoms (Mertz, Fullerton et al. 1998). Approximately half of patients with functional dyspepsia seek medical attention at some point in their life time. Up to 50% of patients who undergo upper gastrointestinal endoscopy due to dyspepsia have normal endoscopic findings and are termed functional dyspepsia. The prevalence decreases with advancing age (Jones, Lydeard et al. 1990), and has not changed much in the last decades. Approximately one third of patients with functional dyspepsia are free of symptoms spontaneously (Agreus, Svardsudd et al. 1995).

6.4.2 Pathophysiology of functional dyspepsia

At present, the aetiology and pathophysiology of functional dyspepsia remain unclear (Mertz, Fullerton et al. 1998; Feldman, Friedman et al. 2002; Haag, Talley et al. 2004). Functional dyspepsia is a heterogeneous disorder which characterised by a number of pathophysiologic mechanisms. There is evidence to suggest that the main pathophysiological factors are visceral sensation and alterations in motility and psychosocial factors (Thumshirn 2002). Moreover, there is strong evidence from family and twin studies to suggest that genetic factors are related to functional dyspepsia. Recently, G-protein polymorphisms (GNB3 825 CC genotype) have been associated with functional dyspepsia (Holtmann, Siffert et al. 2004).

Alterations in motility usually includes delayed gastric emptying, impaired initial distribution of a meal within the stomach, impaired gastric accommodation to a meal, antral hypomotility, gastric dysrhythmias and altered duodeno-jejunal motility. Alterations of motor function are found around 50% of patients with functional dyspepsia. These abnormalities include impaired fundic relaxation, antral dilatation, hypomotility, gastroparesis, small bowel dysmotility and abnormal duodenogastric reflexes (Thumshirn 2002).

Alterations of visceral sensation in patients with functional dyspepsia include hypersensitivity to physiological and minor noxious stimuli. Moreover, there is

evidence to suggest that gastric sensory threshold of patients with functional dyspepsia is lower than healthy controls (Thumshirn 2002).

6.4.2.1 Altered Motility

Delayed gastric emptying has been shown to be one of the pathogenesis of functional dyspepsia. Studies showed that up to 30-50% of patients with functional dyspepsia have delayed gastric emptying of solid (Stanghellini, Tosetti et al. 1996; Maes, Ghoo et al. 1997) and liquid (Sarnelli, Caenepeel et al. 2003). Nevertheless, most studies fail to show the correlation between delayed gastric emptying and upper gastrointestinal symptoms in patients with functional dyspepsia who had delayed gastric emptying. A study from Talley et al showed that patients with functional dyspepsia are associated with significantly delayed gastric emptying and impaired quality of life, however, upper gastrointestinal symptoms and impaired quality of life is unlikely explained by delayed gastric emptying (Talley, Locke et al. 2006). Thus, it is still unknown whether delayed gastric emptying really causes the symptoms in patients with functional dyspepsia.

Normally proximal stomach functions as a reservoir then the food is ingested, and slowly delivers to distal stomach. Normal proximal stomach is generally able to increase the volume without increase in pressure to accommodate the meals,

which is called gastric accommodation. Impaired gastric accommodation has been shown in a subgroup of patients with functional dyspepsia. Gilja et al showed that impaired gastric accommodation of the stomach occurred in patients with functional dyspepsia measured by ultrasound after ingested 500 ml meat soup (intra-gastric meal distribution). A study from Tack et al showed that up to 40% of patients with functional dyspepsia have impaired gastric accommodation measured by gastric barostat and correlated with early satiety symptom (Tack, Piessevaux et al. 1998). Symptoms occur from impaired gastric accommodation may be explained by abnormally increase pressure in the proximal stomach, due to the stomach fails to increase volume to hold the meal, which activate gastric mechanoreceptors in the gastric walls and induce symptoms.

6.4.2.2 Altered sensation

It is well known that patients with functional dyspepsia have enhanced sensitivity to gastric distension and associated with abdominal symptoms especially epigastric pain (Tack, Caenepeel et al. 2001). Tack et al reported that 34-66% of patients with functional dyspepsia had hypersensitivity to gastric distension (Tack, Bisschops et al. 2004). A study from Mertz et al also showed that most of the patients with functional dyspepsia had enhanced visceral sensitivity compared with only 20% of patients with organic dyspepsia (Mertz, Fullerton et al. 1998).

Patients with functional dyspepsia reported nausea symptoms when acid was infused at the duodenal bulb, which means they also have enhanced visceral sensitivity to chemical stimuli (Samsom, Verhagen et al. 1999).

Psychological stress may influence visceral sensitivity. A study from Geeraerts et al showed that experimentally induced anxiety can alter gastric motor and sensory function in healthy subjects (Geeraerts, Vandenberghe et al. 2005). Moreover, a study in healthy human showed that anxiety induced by mental stress increase the sensation of gas and pain during sigmoid distension (Ford, Camilleri et al. 1995).

6.4.2.3 Molecular mechanisms

The link between molecular mechanisms and functional dyspepsia has been recently established. Holtmann et al showed that G-protein $\beta 3$ (GNB3) subunit 825 CC genotype is associated with functional dyspepsia (Holtmann, Siffert et al. 2004). While Camilleri et al reported an association between GNB3 CC and TT with dyspepsia (Camilleri, Carlson et al. 2006). Moreover, GNB3 has been shown to be associated with gastro-oesophageal reflux disease (de Vries, ter Linde et al. 2009). However, there is no association between GNB3 and IBS (Andresen, Camilleri et al. 2006). G-protein will be described in detail in Chapter 16.

6.4.2.4 Psychological disorders

Psychological factors influence gut physiology, symptom experience, health behaviour and outcome. It is now widely believed that psychological, especially anxiety and depression are factors that are closely linked to functional gastrointestinal disorders; conditions that are believed to be associated with augmented perception of visceral sensations. Nevertheless, the role of psychological problems in functional gastrointestinal disorders could be vary. Psychosocial factors may simply drive people to seek medical attention more often than other organic diseases. On the other hand, psychosocial could be one of the main pathogenesis of functional gastrointestinal disorders (Locke, Weaver et al. 2004). Studies in brain-gut axis perhaps could explain better in physiology between psychological factors and gut.

6.4.2.5 Inflammation

Inflammation has a role in functional gastrointestinal disorders in particular post-infectious functional dyspepsia and post-infectious IBS. Up to a quarter of patients with functional dyspepsia develop gastrointestinal symptom acutely after an infectious illness (Spiller 2004). Interestingly, patients with post-infectious functional dyspepsia commonly have impaired gastric accommodation (up to 67%) (Tack, Demedts et al. 2002).

Although it is generally recommended to eradicate *H. pylori* in patients who have dyspeptic symptoms, the link between *H. pylori* and symptoms in functional dyspepsia is controversial (Talley and Hunt 1997). Blum et al showed that *H. pylori* eradication did not improve upper gastrointestinal symptoms in patients with functional dyspepsia after 12 months (Blum, Talley et al. 1998). In addition, there was no significant difference in terms of symptoms relief and quality of life between patients treated with *H. pylori* eradication with Omeprazole and Omeprazole alone. Another study from Talley et al reported that gastrointestinal symptoms reported from patients with functional dyspepsia was not different between patients who had *H. pylori* eradication and placebo during 12 months follow up (Talley, Janssens et al. 1999). Sarnelli et al found no significant difference in gastric emptying, accommodation and gastric sensitivity in patients with functional dyspepsia between *H. pylori* positive and negative measured by gastric barostat (Sarnelli, Cuomo et al. 2003).

Immune activation has been studied recently in patients with functional gastrointestinal disorders. Patients with irritable bowel syndrome, particularly post-infectious IBS, produced significantly higher TNF- α , IL-1 β , and IL-6 compared with healthy control, though the cytokines were measured from peripheral blood mononuclear cells (PBMC) which could indirectly reflect lamina propria mononuclear cells (LPMC) (Liebrechts, Adam et al. 2007). PBMC from

patients with functional dyspepsia has also been shown to be associated with severity of abdominal symptoms (Liebregts, Adam et al. 2008).

Recent studies showed that patients with functional dyspepsia are associated with significantly higher number of eosinophil found in the duodenum compared with asymptomatic controls, and suggested that duodenal eosinophilia may characterise a subset of patients with functional dyspepsia (Talley, Walker et al. 2007).

6.4.3 Summary

Functional dyspepsia is a heterogeneous disorder which characterised by a number of pathophysiologic mechanisms, including delayed gastric emptying, impaired gastric accommodation, gastric hypersensitivity, psychological disorders, inflammation and genetics or molecular mechanisms. Approximately one third of the patients with functional dyspepsia have delayed gastric emptying, one-third has impaired gastric accommodation, one-third has gastric hypersensitivity, one third has psychological problems and one fourth have history of infectious illness. Certainly, individual may have only one or two characteristics of these disorders. Patients with functional dyspepsia may also present with variety of upper gastrointestinal symptoms, such as fullness, bloating, pain, belching, nausea, early satiety, heartburn, weight loss and

vomiting. Fullness, bloating and pain are the most common symptoms complaint (Tack, Demedts et al. 2002).

CHAPTER 7: MOLECULAR MECHANISM IN VISCERAL SENSITIVITY

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7.1 INTRODUCTION

G-proteins are second messengers which are important for cell- to-cell communication. They are expressed in all cells of the human body, and triggered by G-protein-coupled-receptors (GPCRs) which are located on the cell surface. This chapter will review the association between G-proteins, particularly GNB3, and gastrointestinal diseases.

7.2 G-PROTEIN-COUPLED-RECEPTORS (GPCRS)

G-protein-coupled-receptors (GPCRs) are one of the cell surface receptors which are important for cell-to-cell communication. They are the largest family of cell surface molecules involved in signal transduction. GPCRs trigger intracellular response through G-proteins.

7.3 G-PROTEINS

G-proteins are composed of α , β , and γ subunits and the α -subunit interacts with a $\beta\gamma$ complex. At the present, there are at least 23 α , 6 β , and 12 γ subunits, and the

number of these subunits that is being identified is increasing (Hermans 2003). Each of the heterotrimeric G-proteins transduces their signal in specific receptors.

G-proteins are second messengers which are essential for stimulus-response coupling of receptors such as adenylyclases, phosphoinositide systems, ion channels, various protein kinases and transcription factors (Holtmann, Liebrechts et al. 2004). G-proteins mediate signalling from a superfamily of heptahelical receptor (Holtmann, Siffert et al. 2004), also they transduce signals from receptors with tyrosine kinase activity (Siffert 2005). They are expressed in all cells of the human body (Holtmann, Liebrechts et al. 2004) and are located on the cell membrane, and effects on the membrane-cytoplasm interface (Zhang, Melia et al. 2004). Their main role is to translate signals from the cell surface in to a cellular response (Holtmann, Siffert et al. 2004), via GPCRs. Eighty percent of all known membrane receptors transmit their signal via heterotrimeric G-proteins (Holtmann, Liebrechts et al. 2004). Therefore, G-protein dysfunction could potentially block intracellular signal transduction. On activation of the receptor, G-proteins transform the receptor and, in turn, modulate a large variety of intracellular effector systems. Therefore, abnormalities of G-proteins might be associated with different disorders (Holtmann, Siffert et al. 2004). In fact, diseases such as diabetes and certain forms of cancer, among other pathologies, are thought to arise due to derangement of G protein signalling. Recently, a few studies have shown that G-protein signalling may be one of the factors in the pathogenesis of gastrointestinal diseases (Siffert 2005).

7.4 GNB3 C825T

GNB3 (Guanine Nucleotide binding protein), beta polypeptide 3 is also one of the G-proteins which involved in second messenger cascades. A common C825T polymorphism in exon 10 of the gene GNB3 that encodes the β_3 subunit of heterotrimeric G-proteins has been recently identified. G-protein β_3 825 is therefore composed of three genotypes, CC, CT and TT. In general, the GNB3 825TT allele is predictive of enhanced G-proteins activation and increased cellular or physiologic responses, or signal transduction responses. In contrast, the GNB3 825CC genotype forms only minute amounts of the β_3 splice variant, and is characterized by diminished signal transduction responses (Holtmann, Siffert et al. 2004).

7.4.1 GNB3 C825T and gastrointestinal diseases

Mutation of the G-protein α subunit mutation but less so of the β and γ mutation has been associated with human disease (Spiegel and Weinstein 2004). A variety of hormones and neurotransmitters also use specific receptors that interact with G-proteins in the transmembrane signalling process. For example, G-proteins are also involved with insulin, glucose transport and vasodilation. Polymorphisms of G-protein β_3 subunit (GNB3 C825T) have been shown to be associated variety of

disorders, especially multifactorial and polygenetic disorders, such as hypertension, diabetes, atherosclerosis (Siffert 2005), depression and obesity (Siffert 2000). GNB3 status is also associated with increased immune activation (Lindemann, Virchow et al. 2001) and altered activation of α_2 -adrenoreceptors (Baumgart, Naber et al. 1999). Recently, GNB3 825CC has been associated with functional dyspepsia (Holtmann, Siffert et al. 2004).

The association between GNB3 C825T and gastrointestinal diseases has been the subject of recent interest, mainly in patients with functional dyspepsia and irritable bowel syndrome. Siffert et al reported that distribution of GNB3 C825T varies depending on the ethnic background. Europeans may have CC approximately 23-45%, CT 45-55% TT 7-22%, while Asian may have CC 15-25%, CT 50-55%, TT 20-35%, and African may have CC 0-3%, CT 20-30% and TT 70-80% (Siffert, Forster et al. 1999). Therefore different ethnic background may be an important confounder factor in the studies.

An association between GNB3 C825T and function dyspepsia was shown initially by Holtmann et al who demonstrated that GNB3 825CC genotype is associated with functional dyspepsia (Holtmann, Siffert et al. 2004). This study showed that GNB3 825CC was more common in patients with functional dyspepsia (58.2% CC, 28.4% CT, and 13.4% TT) compared with healthy asymptomatic controls (41.6% CC, 47.8 CT, and 10.6% TT). Holtmann et al suggested that G-proteins may play a role in functional GI disorders through altered activation of α_2 –

adrenoreceptors (Baumgart, Naber et al. 1999), and impaired adrenoreceptor function, since α_2 – adrenoreceptors, have an impact on colonic motor and sensory function (Bharucha, Camilleri et al. 1997). Since functional dyspepsia is a heterogeneous disorder which characterised by a number of pathophysiologic mechanisms, including impaired gastric motor and sensory function, therefore, patients who have GNB3 825CC, which is associated with decreased signal transduction, may be associated with impaired gastric motor function which leads to dyspepsia, especially patients with dysmotility-like dyspepsia. In contrast, Van Lelyveld et al found that only GNB3 825TT is associated with functional dyspepsia (van Lelyveld, Linde et al. 2008). On the other hand, Tahara et al did not find any association in Japanese dyspeptic population (Tahara, Arisawa et al. 2008).

Grudell et al looked at the association between GNB3 C825T and gastric motor function, as assessed by gastric emptying, in patients with functional dyspepsia, IBS and healthy controls, and found that patients with functional dyspepsia and IBS-diarrhoea who have GNB3 825CT/TT have delayed gastric emptying compared with CC (Grudell, Camilleri et al. 2008). In contrast, healthy volunteers who have GNB3 825CC have significantly delayed gastric emptying compared with CT/TT, suggesting that GNB3 C825T have a different role in gastric motor function in different disorders.

Camilleri et al showed that the GNB3 825CC and GNB3 825TT variants are associated with meal-unrelated dyspepsia patients (Camilleri, Carlson et al. 2006). This study found that 67% of patients with dyspepsia have GNB3 825CC compared with 43% of healthy controls, while up to 20% of patients with dyspepsia have GNB3 825TT compared with only 3% of controls.

In contrast to those reports in functional dyspepsia, there is no association between GNB3 C825T and irritable bowel syndrome (Andresen, Camilleri et al. 2006). Andresen et al looked at the distribution of GNB3 C825T in 233 patients with lower functional GI disorder and 152 healthy controls, and found that they were similar. The study also showed that there was no significant association between GNB3 C825T polymorphisms and overall patients with lower functional GI disorder and each subgroup, including irritable bowel syndrome, functional abdominal pain and lower functional GI disorder-functional dyspepsia overlap subgroup(Andresen, Camilleri et al. 2006).

Interestingly, a study from Netherlands reported recently an association between GORD and GNB3 825CT, including reflux oesophagitis, NERD and functional heartburn (de Vries, ter Linde et al. 2009). The study showed that 47.1% of patients with GORD have CT (46% CC and 6.9 TT), compared with only 37% of healthy controls. Nevertheless, distribution of GNB3 C825T of healthy controls in this study is different from other studies, only 37% of healthy have CT compared

with 47% and 68% from study of Holtmann et al and Camilleri et al (Camilleri, Carlson et al. 2006) respectively.

7.5 SUMMARY

G-proteins are second messengers which are important for cell- to-cell communication. They are expressed in all cells of the human body, and triggered by G-protein-coupled-receptors (GPCRs) which are located on the cell surface. Most of the gastrointestinal hormones, neurotransmitters, chemokines, local mediators, and sensory stimuli are stimulated and released through GPCRs and G-proteins; it is likely that G-proteins play a role in gastric motor and sensory function.

Symptoms associated with functional dyspepsia are complex, including epigastric pain, bloating, early satiety, fullness, epigastric burning, belching, nausea and vomiting. Functional dyspepsia is a very heterogeneous disorder, including delayed gastric emptying, impaired gastric accommodation to a meal, hypersensitivity to gastric distension, *H. pylori* infection, altered response to duodenal lipids or acid, abnormal duodenojejunal motility, central nervous system dysfunction, or psychological issues (Tack, Bisschops et al. 2004).

As a polymorphism of a G-protein may lead to a wide number of pathophysiological effects in gastrointestinal tract, it is conceivable that G-protein play a role or in part in functional gastrointestinal disorder. It has been shown that GNB3 C825T is associated with functional dyspepsia and GORD. Moreover, it also has been shown that GNB3 C825T is associated with gastric motor function. However, more studies are required to elucidate further information the role of GNB3 in gastrointestinal system. The association between GNB3 C825T and gastric motor and sensory function will be discussed in Chapter 16 of this thesis.

CHAPTER 8: EPIDEMIOLOGY OF UPPER GASTROINTESTINAL BLEEDING

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Upper gastrointestinal bleeding (UGIB) is an important emergency medical condition. Epidemiologic information is beneficial to doctors as the information might facilitate doctors' evaluation of the problem or disease which can prevent the illnesses. Furthermore, epidemiology provides guidance for doctors to manage the problem of patients when the illnesses occur (Coggon 1997). This chapter will review the epidemiology of upper gastrointestinal bleeding, including incidence, age, sex, mortality and aetiology.

8.1 INCIDENCE

The incidence of upper gastrointestinal bleeding in England, Netherlands, Scotland (Rockall, Logan et al. 1995; Czernichow, Hochain et al. 2000), France (Czernichow, Hochain et al. 2000), Italy, Spain, Northern Europe, the United States of America (Paspatis, Metrella et al. 2000) and Mediterranean region (Burillo, Melero et al. 2001) is approximately 50-150 cases per 100,000 population per year, which amount to an annual hospital admission rate of 100 per 100,000 hospital admissions.

Overall, the incidence of UGIB has been gradually decreasing over the past decades (van Leerdam, Vreeburg et al. 2003; van Leerdam 2008) (Table 1).

8.2 AGE

One of the most important changes in the epidemiology of upper gastrointestinal bleeding in last 50 years is age. In 1975, the number of patients with upper gastrointestinal bleeding age more than 60 years old was around one-half of total. This proportion had increased to two-thirds by 1993 (Crocker 1991; Shearman, Finlayson et al. 1997). Also the incidence of upper gastrointestinal bleeding is more common in the older age group (Crocker 1991; Longstreth 1995; Rockall, Logan et al. 1995; Blatchford, Davidson et al. 1997; Shearman, Finlayson et al. 1997; Paspatis, Metrella et al. 2000; Arlt and Leyh 2001; Burillo, Melero et al. 2001; Kaplan, Heckbert et al. 2001; Huang and Lichtenstein 2003), and approaches 1% per year (Kaplan, Heckbert et al. 2001). In addition, evidence indicates that older patients with upper gastrointestinal bleeding have a worse prognosis (Antler, Pitchumoni et al. 1981; Rockall, Logan et al. 1995; Huang and Lichtenstein 2003). The main reason that old age patients are the most common patients with upper gastrointestinal bleeding is probably the general increase in age population (Paspatis, Metrella et al. 2000; Leerdam, Vreeburg et al. 2003) and the reduction in the prevalence of *H. pylori* in younger patients (Paspatis, Metrella et al. 2000; Leerdam, Vreeburg et al. 2003). Moreover, NSAIDs and aspirin, which are the main risk factors for bleeding peptic ulcer, are frequently used in elderly patients (Czernichow, Hochain et al. 2000) for their analgesic and anti-inflammatory effect, as well as antithrombotic effect.

8.3 SEX

The proportion of male patients has always been greater than that of patient female (Longstreth 1995; Rockall, Logan et al. 1995; Paspatis, Metrella et al. 2000; Arlt and Leyh 2001). Many studies have stated that number of males is almost double of females (Blatchford, Davidson et al. 1997; Paspatis, Metrella et al. 2000; Arlt and Leyh 2001).

8.4 SEASON

Seasonal change is also involved with incidence of upper gastrointestinal bleeding, the number of patients with upper gastrointestinal bleeding increases in winter (Arlt and Leyh 2001) and decreases in summer (Csendes, Medina et al. 1995; Shearman, Finlayson et al. 1997; Arlt and Leyh 2001; Burillo, Melero et al. 2001).

8.5 MORTALITY

The mortality rate of upper gastrointestinal bleeding has been approximately 10% over the past 50 years (Rockall and Logan 1995; Huang and Lichtenstein 2003).

Whilst some studies have reported that the mortality rate has slightly declined in the last 20 years (Crocker 1991), in most studies it has remained steady at around 10 % (Sandel, Kolkman et al. 2000; Behrman 2005; Kasper, Braunwald et al. 2005). Multivariate analyses has shown that increased age, severe and life-threatening co-morbidity and rebleeding are associated with a higher mortality rate (Leerdam, Vreeburg et al. 2003). Rockall (Rockall, Logan et al. 1996) found that age, shock, co-morbidity, diagnosis, major stigmata of recent haemorrhage and rebleeding are all predictors for mortality. Inpatients who bleed had higher fatality rate than patients who were admitted with bleeding from emergency department (Blatchford, Davidson et al. 1997; Czernichow, Hochain et al. 2000; Paspatis, Metrella et al. 2000). Nevertheless, most of the patients with upper gastrointestinal bleeding who died had severe co-morbidities and were over 60 years of age. Patients who die from exsanguination are rare and mortality rate in patients without organ failure or malignant disease is very low (Rockall and Logan 1995). Rockal et al reported that the mortality rate of patients with UGIB who were younger than 60 years old and had no co-morbidity was only less than 1% (Rockall, Logan et al. 1995).

8.6 RECURRENT BLEEDING

Recurrent bleeding is associated with high mortality rate. A proportion of recurrent bleeding accounts around 5-12% per patient-year (Arlt and Leyh 2001).

The risk is especially high in patients with bleeding peptic ulcer (20-22%) and variceal bleeding (25-29%) (van Leerdam, Vreeburg et al. 2003). Also patients with recurrent bleeding have higher risk for operation compare to those who do not (Paspatis, Metrella et al. 2000). The risk of recurrent bleeding can be predicted by endoscopy, by the presence of high risk stigmata of recent haemorrhage such as oozing bleeding, non-bleeding visible vessels and adherent clots (Leerdam, Vreeburg et al. 2003). Therefore, patients with high risk stigmata of recent haemorrhage should receive endoscopic treatment (Leerdam, Vreeburg et al. 2003).

8.7 AETIOLOGIES OF UPPER GASTROINTESTINAL BLEEDING

Aetiologies of UGIB could be divided into 2 categories, non-variceal bleeding and variceal bleeding. Common causes of non-variceal bleeding are peptic ulcer, reflux oesophagitis, gastric erosion and Mallory-Weiss tear. Overall, peptic ulcer is the most common cause of bleeding and accounts for approximately 50%, followed by oesophagitis, and gastric erosion. There are many factors which predispose of bleeding, for instance, Non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, ethanol, and anticoagulant therapy (Feldman, Friedman et al. 2002). Variceal bleeding occurs up to 50% of patients with cirrhosis.

8.7.1 Peptic ulcer

Epidemiology and incidence of peptic ulcer disease have been described in previous chapter. Basically, the incidence and admission rates for uncomplicated peptic ulcer disease have decreased dramatically over the past few decades. In contrast, the incidence of bleeding peptic ulcer has remained unchanged. Peptic ulcer disease is the most common cause of UGIB, accounts for 50%. It is not uncommon that patients with bleeding peptic ulcer present with bleeding as their first presentation without previous abdominal symptoms.

8.7.2 Gastric erosions

Gastric erosions are responsible for 20-30% of bleeding cases (Shearman, Finlayson et al. 1997; Wolfson, Allan et al. 2005). Gastric erosion sometimes define as stress ulceration, stress ulceration is the erosion of mucosa in gastrointestinal which is oesophagus, stomach and duodenum. Stress ulcers are found in every type of critical ill patients, for example, septic shock, burn, malignancies, severe medical illness, etc... (Crocker 1991). Also, gastric erosions are frequently correlated with patients who are prescribed non-steroidal anti-inflammatory drugs. Normally, gastric erosions do not cause massive bleeding

because they involve only superficial mucosal of the stomach (Shearman, Finlayson et al. 1997).

8.7.3 Oesophageal Varices

Bleeding from oesophageal varices accounts around 2-7% of cases of upper gastrointestinal bleeding (Shearman, Finlayson et al. 1997). Most of the variceal bleeding is associated with portal hypertension, especially in cirrhosis patients, and is therefore associated with a high mortality rate (Shearman, Finlayson et al. 1997).

8.7.4 Mallory-Weiss tear

Mallory-Weiss tear is the cause of upper gastrointestinal bleeding around 5% (Shearman, Finlayson et al. 1997; Wolfson, Allan et al. 2005). Mallory-Weiss syndrome is the mucosal laceration of the cardia or gastro-oesophageal junction, which is usually caused by retching or vomiting. Hence, Mallory-Weiss tear is associated with low mortality because most of the patients with this syndrome usually have no underlying disease and any co-morbidity.

Other uncommon causes of upper gastrointestinal bleeding are carcinoma, Dieulafoy abnormality, gastroduodenal vascular malformations and arteriovenous malformations (Shearman, Finlayson et al. 1997).

8.8 TREATMENT

Initial evaluation for patients with UGIB is very important. Haemodynamic instability (heart rate and blood pressure), and blood transfusion before endoscopy are usually signs of massive bleeding. The most important treatment for patients with UGIB is to stabilise haemodynamic (vital signs) and followed by endoscopic assessment and therapy.

8.9 SUMMARY

In summary, upper gastrointestinal bleeding is a common emergency situation which requires immediate assessment and treatment. The incidence of UGIB has progressively decreased over the past few decades. However, the mortality rate has remained unchanged at 10%. The mortality rate is significantly higher in elderly patients and patients with co-morbidity. The most common cause of UGIB is peptic ulcer disease. Most patients with bleeding peptic ulcer appear to be asymptomatic, however, the incidence and proportion of patients between

symptomatic and asymptomatic peptic ulcer is unknown. Few studies have shown the factors that could be associated with asymptomatic peptic ulcer such as age, NSAIDs and ulcer size.

The incidence and proportion between patients with symptomatic and asymptomatic peptic ulcer disease, including bleeding peptic ulcer and uncomplicated peptic ulcer will be discussed in Chapter 14. In addition, Chapter 14 will reveal the potential factors that could influence symptoms of peptic ulcer.

Author	Country	Study type	Year period	Number	Incidence (1/100,000)	Decrease	Rebleeding	Mortality
Di Fiore*	France	Prospective	1996 vs 2000	712 vs 453	NA	36%	NA	11.7% vs 7.2%
Leerdam [#]	The Netherlands	Prospective	1993-94 vs 2000	951 vs 769	61 vs 48	23%	16% vs 16%	14 % vs 13%
Thomopoulos [†]	Greece	Retrospective	1986-87 vs 2000-01	668 vs 636	NA	NA	NA	5.2% vs 3.1%
Ohmann [‡]	Germany	Prospective	1989-90 vs 1999-2000	295 vs 277	NA	NA	NA	NA

* (Chassaignon, Letoumelin et al. 2003)

(van Leerdam, Vreeburg et al. 2003)

† (Thomopoulos, Vagenas et al. 2004)

‡ (Ohmann, Imhof et al. 2005)

Table 1: Overview of time trend studies in acute upper gastrointestinal bleeding (van Leerdam 2008).

SECTION 2:

COMMON

METHODOLOGIES

CHAPTER 9: COMMON METHODOLOGIES

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9.1 INTRODUCTION

This chapter reviews the common methodologies used for upper gastrointestinal motor and sensory testing. Moreover, this chapter will explain the methodologies used in the basic science laboratory including cell isolation, cell culture, ELISA, DNA isolation and PCR. Methodologies used in this thesis are based on previous publications. In addition, this chapter will also discuss the standardised methods used for recording, scoring and analysis in this thesis.

9.2 SYMPTOM QUESTIONNAIRES

Reflux symptoms, gastrointestinal symptoms and psychiatric co-morbidities in subjects participated in the studies were assessed utilising validated questionnaires: Gastrointestinal symptom score (GIS), Bowel Disease Questionnaire (BDQ), Nepean Dyspepsia Index (NDI), Hospital Anxiety and Depression Scale (HADS), and reflux questionnaire (PAGI-SYM).

9.2.1 Gastrointestinal Symptom score (GIS)

The GIS assesses the intensity of gastrointestinal symptoms in patients with functional dyspepsia, and addresses patient's gastrointestinal symptoms in the past 1 week, including epigastric pain, abdominal cramps, fullness, early satiety, loss of appetite, sickness, nausea, vomiting, retrosternal discomfort, and acid eructation/heartburn. Each symptom, is scored using a 5 point Likert scale (no problem (0) - very severe problem (4)). The total score for all symptoms ranges from 0-40, and a higher score reflects more severe symptoms (Adam, Liebrechts et al. 2005).

9.2.2 Bowel Disease Questionnaire (BDQ)

The BDQ assesses various types of symptoms including upper abdominal symptoms, bowel symptoms, reflux symptoms and lifestyle over the previous 12 months (Talley, Phillips et al. 1990; Talley, Boyce et al. 1995). Questions about gastrointestinal symptoms are categorised into 5 subgroups: ulcer type dyspepsia, dysmotility type dyspepsia, reflux type dyspepsia, irritable bowel syndrome (IBS) - diarrhoea, and IBS - constipation. Subjects are asked to locate their abdominal symptom (upper or lower), and report the frequency and intensity of their symptoms.

9.2.3 Nepean Dyspepsia Index (NDI)

The NDI assesses symptoms of dyspepsia and health-related quality of life. Quality of life is categorised into 4 groups that assess the impact of dyspepsia on: the subject's daily lifestyle and activities, knowledge and control of abdominal symptoms, ability and enjoyment of eating and drinking, and sleepiness (Talley, Haque et al. 1999; Talley, Verlinden et al. 1999) in which low scores indicate poor quality of life. Additionally, there are symptom checklists that measure the frequency, intensity, and bothersome nature of 15 upper gastrointestinal symptoms over the period of 2 weeks prior the study using 4 or 5-point Likert scales.

9.2.4 Hospital Anxiety and Depression Scale (HADS)

The HADS is a validated tool for the assessment of anxiety and/or depression. There are 7 questions on anxiety and 7 on depression scored on a 4-point Likert scale (0-not at all to 3-always present). Participants who rate between scores of 8-10 in either category are categorised as mild anxiety or depression, while scores of 11-14 indicate moderate, and 15-21 severe, anxiety or depression (Herrmann 1997).

9.2.5 The Patient Assessment of upper GastroIntestinal disorders-SYMptom severity index (PAGI-SYM)

PAGI-SYM assesses the intensity of upper gastrointestinal symptoms, especially reflux symptoms, in patients with GORD, dyspepsia and gastroparesis, and addresses patient's upper GI symptoms in the past 1 week. The questionnaire consists of 20 questions in 6 categories, including 7 questions on heartburn, regurgitation or reflux, 3 questions on nausea or vomiting, 4 questions on postprandial fullness or early satiety, 2 questions on bloating, 2 questions on upper abdominal pain and 2 questions on lower abdominal pain. Each symptom is scored using a 6 point Likert scale (none (0) – very severe (5)) and a higher score reflects more severe symptoms. The overall score were then analysed by averaging across categories (Rentz, Kahrilas et al. 2004; Revicki, Rentz et al. 2004).

9.3 TESTING FOR HELICOBACTER *PYLORI* STATUS

All subjects were tested for the presence of *Helicobacter pylori* by 14C Urea breath test. This test was performed before the gastric motor and sensory tests. Acid suppressing medications, for example proton pump inhibitors and histamine-2 receptor blockers were stopped for at least 1 week before the test. All subjects were fasted overnight, at least 8 hours before the test. Subjects ingested 37 kBq of

¹⁴C urea. If *Helicobacter pylori* are present, urease from *Helicobacter pylori* transforms the urea to ammonia and carbon dioxide. The labelled carbon dioxide is then excreted by the lungs and detected in the breath with a liquid scintillation counter 15 min after of ingestion. The normal range of urea breath test is less than 150 dpm. The test is claimed positive when the urea breath test detects carbon dioxide more than 250 dpm. At the end of their participation, subjects who were *H. pylori* positive were counselled about their *H. pylori* status and were offered eradication therapy for *H. pylori* if clinically appropriate.

9.4 NUTRIENT CHALLENGE TEST

The barostat is generally regarded as the gold standard for the assessment of gastric sensitivity. However, it is invasive and poorly tolerated by subjects. In this study, we wanted to assess visceral sensory function non-invasively. Drinking tests, with water or nutrients (Jones, Hoffman et al. 2003; Haag, Talley et al. 2004), have been developed as a potential non-invasive approach. A standardised nutrient challenge is believed to be an effective visceral sensory stimulus in healthy subjects (Choung, Talley et al. 2007) and patients with functional dyspepsia (Haag, Talley et al. 2004). The nutrient challenge test has been used in various studies (Holtmann, Talley et al. 1996; Boeckxstaens, Hirsch et al. 1999; Kim, Myung et al. 2000; Mulak 2003; Delgado-Aros, Camilleri et al. 2004; Haag, Talley et al. 2004; Choung, Talley et al. 2007) in patients with functional

dyspepsia, irritable bowel syndrome and healthy subjects although volumes and rates of ingestion have varied among the studies. It correlates well with mechanosensory thresholds as measured by the barostat (Holtmann, Talley et al. 1996). It has been used to assess the symptom of early satiety and predicts gastric accommodation (Tack, Caenepeel et al. 2003). We therefore chose to use a 200 ml of nutrient challenge every 5 minutes because this reflects real life and should reflect mechanosensory function, even though the nutrient drink may also assess global gastric function.

Visceral sensitivity was assessed by a standardised nutrient challenge test. After an 8-hour fast, subjects were asked to drink 200 ml of a standardised nutrient liquid (Ensure®, Abbott Laboratories, Australia) every 5 minutes up to a cumulative volume of 800 ml. Before and 5 min after each 200 ml drink, symptoms were assessed using a visual analogue scale (range 0-100 mm) with 0 = no symptom and 100 = unbearably severe. This tool assesses five symptoms; fullness, abdominal pain, nausea, retrosternal/abdominal burning and acid regurgitation. The peak and cumulative symptom responses were determined and the cumulative scores for each symptom individually and for all symptoms combined were used as the primary outcome variables (Holtmann, Talley et al. 1996). Subjects were familiarised with these scales before commenced the study. Subjects were asked to report their GI symptoms before each drink at minute 0, 5, 10 and 15. Subjects reported symptoms only at minute 20 without any drink.

9.5 GASTRIC EMPTYING NUTRIENT CHALLENGE TEST

Gastric emptying was also measured in some subjects and patients as part of the nutrient challenge test. After an 8-hour fast, subjects were asked to drink 200 ml of a standardised nutrient liquid (Ensure®, Abbott Laboratories, Australia), containing 5 MBq ^{99m}Tc - rhenium sulphide colloid (RAH radiopharmacy, Adelaide, South Australia), every 5 minutes up to a cumulative volume of 800 ml (Holtmann, Talley et al. 1996).

Gastric emptying was measured by scintigraphy. Posterior dynamic images were acquired every 3 minutes for 2 hours on a GE Millennium MPR single head gamma camera with the patients sitting upright. Data collection began at the start of the meal as minute 0. Data were acquired for 120 minutes, with 1-minute frames for the first 30 minutes and at 3-minute frames intervals thereafter. Radionuclide data were corrected for subject movement. For analysis, regions of interest were drawn around the proximal stomach, distal stomach and total stomach. For each region, percentage retention was assessed in each region at different time points. The percentage of label remaining in the proximal, distal and total stomach at 100 minutes was calculated.

9.6 PERIPHERAL BLOOD MONONUCLEAR CELL ISOLATION

A 32 ml sample of fresh blood was taken and kept in 4 heparin gel tubes. All processes were sterile. Peripheral blood mononuclear cells (PBMCs) were freshly isolated from the samples by density gradient centrifugation (Liebregts, Adam et al. 2007). Blood was diluted with sterile phosphate buffered saline (PBS) and slowly layered onto Ficoll-Hypaque (Sigma, Castle Hill, NSW, Australia) and centrifuged at 800 g for 20 minutes. The white blood cell ring fraction (PBMCs) is clearly visible after the centrifugation, and is transferred to a new tube. PBMCs were washed twice with PBS, the first wash was centrifuged with 600 g for 10 minutes, and second wash with 300 g for 15 minutes. The pellet (PBMCs) was resuspended in 5ml PBS, and viability was assessed by Trypan blue exclusion (True Blue Stain [0.4%]; Gibco, Karlsruhe, Germany). PBMCs were then counted in Trypan Blue with a Neubauer Counting Chamber. Ten x 10⁶ PBMCs then were aliquoted in 10-ml tubes, and centrifuged with 600 g for 10 minutes. The pellet then was loosen and resuspended to 1 x 10⁶ cells/ml in foetal calf serum (FCS) and Dimethyl sulfoxide (DMSO) in cryovials. PBMCs then were stored in a cryobox at -80 °C for at least 24 hours before transfer into liquid nitrogen for long term storage.

After white blood cell ring fraction (PBMCs) were transferred to a new tube, the rest of the blood component was also kept for DNA isolation process. However,

the Ficoll-Hypaque was removed and the blood was then diluted with PBS before the blood was stored at -20 °C.

9.7 CELL CULTURE

For cell culture, the selected cryovials were taken from liquid nitrogen, and then resuspended with 8 ml of RPMI-1640 in 10 ml tubes. RPMI-1640, developed by Moore et. al. (Moore, Gerner et al. 1967; Moore and Kitamura 1968) at Roswell Park Memorial Institute for the culture of human normal leukocytes, consists of liquid, with sodium bicarbonate and L-glutamine. The samples were centrifuged with 600 g for 10 minutes (first wash), and washed again with RPMI 1640 (Gibco), centrifuged with 300 g for 15 minutes (second wash). The supernatant was then discarded, and pellet was resuspended with 1 ml RPMI 1640. The viability of PBMCs was also assessed by trypan blue exclusion (cells were counted in the Neubauer counting chamber). The PBMCs were then aliquoted to a 24-well plate at 1×10^6 cells/well, and resuspended with RPMI 1640 cell culture media, which was supplemented with 10% foetal calf serum (FCS), 100 U/ml penicillin, 0.1 mg/ml streptomycin, L-glutamine, sodium pyruvate, MEM non-essential amino acids, and kanamycin. The cells were incubated at 37 °C for exactly 24 hours in a humidified 5% CO₂ atmosphere. After 24 hours incubation, the cells in the supernatant again were assessed for viability by counting with Neubauer counting chamber, and transferred to 10 ml tubes, centrifuged with 600

g for 5 minutes. Finally, 230 μ l of the supernatant was transferred into eppendorf tubes, and stored at -80 °C.

9.8 CYTOKINE ASSAY

Cell-free supernatant fractions were collected as described and stored at -80 °C until assayed. TNF- α , IL-1 β , IL-6, and IL-10 were quantified using ELISA kits (BD Bioscience, San Diego, CA, USA) according to the manufacturer's instructions. Each well of a 96-well plastic tray was coated with 100 μ l diluted capture antibody and incubated overnight at 4°C. The following day, the wells were washed 3 times with washing buffer (PBS with 0.05% Tween), and blocked with 200 μ l/well of Assay Diluent, and incubated at room temperature for 1 hour. The wells were washed 3 times, added 100 μ l of standard, diluted samples and control, and incubated at room temperature for 2 hours. The wells were again washed 5 times, added 100 μ l of Working Detector, and incubated for 1 hour. The wells were then washed 7 times, 100 μ l of Substrate Solution added, and were then incubated for 30 minutes in the dark. Finally, 50 μ l of Stop Solution was added into each well, and the density of the solution was read by a micro plate reader at a wavelength of 450 nm and a reference wavelength of 590. Density values were linearly correlated with the concentrations of cytokine standards. The limit of sensitivity of the assays is 5pg/ml.

9.9 DNA ISOLATION

The blood component which was left from the cell isolation process was utilized for genomic DNA isolation. The principle of this process is to destroy all red cells in the buffy coat or granulocytes samples by addition of a hypotonic solution prior to DNA extraction from the white cells and dendritic cells. The end result is a white cell pellet, which is rendered free of red cells and haemoglobin because residual haemoglobin in the DNA preparation may inhibit the DNA polymerase in the PCR. Therefore, Red Cell Lysis Buffer (RCLB), composed of 10 ml 1M TrisCl pH 7.5, 5 ml 1M MgCl₂, 2 ml 5M NaCl, and 983 ml sterile H₂O was required.

Initially, PBS and residual Ficoll-Hypaque in the blood component were removed. The pellet was washed twice with RCLB by centrifugation at 2,500 rpm for 10 minutes. Then the pellet was resuspended with 2 ml of Nuclei Lysis Buffer (NLB) (10 ml 1M TrisCl pH 8.0, 4 ml 0.5 EDTA pH 8.0, 80 ml 5 M NaCl, and 906 ml sterile H₂O) + 10% SDS, to which was added 60µl of 10 mg/ml Proteinase K, and incubated in water bath at 42 °C overnight. The next day, tubes were removed from water bath, and 1 ml of 6 M NaCl added and mixed vigorously to precipitate the cellular proteins. The tubes were then stored in a refrigerator at -20 °C for 30 minutes, and then centrifuged at 3,600 rpm for 25 minutes.

Only supernatant (contains DNA, approximately 3 ml) was transferred to 10 ml tubes. The supernatant then was combined with 7 ml of 100% ethanol. DNA precipitates and becomes visible. The DNA pellet was transferred by a glass DNA hook to eppendorf tubes and mixed and dissolved in TE-buffer. The DNA was stored at 4 °C for 12 hours and eventually stored at -80 °C.

9.10 PCR-GENOTYPE

This methodology was based on previously published reports (Holtmann, Siffert et al. 2004). The genotype of C825T polymorphism was isolated by polymerase chain reaction (PCR). PCR was performed in a 15 µl volume, in 96 wells plastic tray, containing 2 µl of genomic DNA, 1.5 µl of deoxynucleoside triphosphate (dNTP), 0.12 µl of Taq DNA polymerase, 1.2 µl of 25 mM MgCl₂, 1.5 µl of 10xPCR buffer, 3 µl of 1M Betaine, 5.38 µl of H₂O, and 0.15 µl of 0.3 µmol/L of the forward 5'TGACCCACTTGCCACCCGTGC-3' primer, and 5'GCAGCAGCCAGGGCTGGC-3' primer. After an initial denaturation step at 95 °C for 5 minutes, there were 35 cycles of denaturation at 95 °C for 1 minute, annealing at 60 °C for 45 seconds and extension at 60 °C for 1 minute. And the final step was performed at 72 °C for 7 minutes. The PCR products were then digested with 2.5 units of BseDI mixed with 10xPCR buffer, and H₂O for 4 hours. The final products were separated by electrophoresis in 2.5% agarose gels and

visualized by ethidium bromide staining under UV light (Holtmann, Siffert et al. 2004). All laboratory procedures were performed blinded to case-control status. The genotypes of GNB3 C825T were read by at least 2 technicians.

9.11 ETHICAL CONSIDERATIONS

The procedures outlined in this research project did not pose any major ethical concerns for the subjects. The Urea breath test for *H. pylori* is a routine non-invasive diagnostic test. Moreover, detecting *H. pylori* is related to subject's future health. These implications were discussed with the subjects and eradication was offered where clinically appropriate to patients who were *H. pylori* positive. The radionuclide gastric emptying study was modified from the method currently used for the diagnosis of gastric emptying disorders. Whilst the breath test and the gastric emptying test both involve use of radionuclides, the total calculated dose of radiation used on each subject was kept to the minimum to ensure a low exposure to radiation. The Bernstein test has been a routine diagnosis test in the past although it has been superseded for diagnostic purposes by oesophageal pH monitoring.

The studies were explained to the subjects in easy terms. They also received a patient information sheet which explained the procedures involved and the rights to their confidentiality. Then subjects were asked to sign consent form before

study participation. Subjects were assured that they may withdraw from the study at any time for any reason.

All human subjects gave written informed consent, and each study in this thesis was approved by the Royal Adelaide Hospital Human Ethics Committee.

SECTION 3:

RESULTS

CHAPTER 10: INCIDENCE AND RISK FACTORS OF UNCOMPLICATED PEPTIC ULCER AND BLEEDING PEPTIC ULCER OVER A 10-YEAR PERIOD

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10.1 INTRODUCTION

Upper gastrointestinal bleeding is a common medical emergency. The most common cause of upper gastrointestinal bleeding is bleeding peptic ulcer (BPU), which accounts for 50% of cases. Patients with peptic ulcer disease (PUD) usually present either with discomfort and pain or with life threatening ulcer bleeding.

Significant changes have occurred in the epidemiology of peptic ulcer disease over time. Recent studies have reported that the incidence of uncomplicated peptic ulcer disease (uPUD) has decreased substantially over the past 20 years (Lassen, Hallas et al. 2006; Post, Kuipers et al. 2006), presumably as a result of decrease in the prevalence of *Helicobacter pylori* infection due to effective *H. pylori* eradication (Rauws and Tytgat 1990) and improving living conditions (Roosendaal, Kuipers et al. 1997; Loffeld and van der Putten 2003), and widely used of proton pump inhibitors (PPI) (Van Soest, Siersema et al. 2006). On the other hand, the incidence of BPU has remained unchanged, and the mortality rate remains approximately 10% (Morgan and Clamp 1984; Branicki, Coleman et al. 1990; Katschinski, Logan et al. 1994). It is relatively common that patients with life threatening peptic ulcer bleeding are asymptomatic until the bleeding occurs and this may explain the unchanging incidence of BPU. Studies have shown a number of factors that might influence symptoms of peptic ulcer disease (Wilcox

and Clark 1997). However, precise data are lacking on the proportion and the change of proportion of patients with and without symptoms prior to the presentation of ulcer bleeding, and factors that might influence these.

The aims of this study, therefore, were to determine the incidence and risk factors of uPUD, BPU and asymptomatic peptic ulcer, and changes of epidemiology of peptic ulcer disease over a 10-year period, between 1997 to 2007, at the Royal Adelaide Hospital.

10.2 SUBJECTS AND METHODS

We retrieved data from the Royal Adelaide Hospital which is the largest tertiary hospital of South Australia. Since 1997, data on patients with upper gastrointestinal bleeding and the results of endoscopies performed have been registered in a haematemesis and melaena database held by the Department of Gastroenterology and Hepatology, Royal Adelaide Hospital.

All of the patients with PUD presenting to the Royal Adelaide Hospital in 1997, 2002 and 2007 were diagnosed by endoscopy. Patients with BPU were identified by hematemesis and melena database, whilst patients with uPUD were identified from the Departmental endoscopic database. A peptic ulcer was defined as a mucosal break at least 3 mm in diameter with visible depth.(Hawkey, Karrasch et

al. 1998; Yeomans and Naesdal 2008) Peptic ulcer patients were excluded from the study if they had a diagnosis of gastric cancer.

Data on demographics, symptomatic status, type of ulcer, *Helicobacter pylori* infection, medications and co-morbidities at diagnosis with PUD were extracted by review of the case notes of all patients with PUD. An asymptomatic ulcer was defined as an ulcer in the absence of any dyspeptic symptom, including epigastric pain or discomfort, or minimal symptoms which did not affect the subject's daily lifestyle which recorded in the case notes. The study was approved by the Royal Adelaide Hospital Human Ethics Committee.

10.2.1 Statistical analysis

Proportions and 95 % confidence intervals for patients with and without symptoms were calculated. Demographics, age and sex, were analysed between patients with BPU and uPUD, and with and without symptoms. Univariate associations with symptoms were initially explored by the chi-square test with the Yates correction for continuity, where appropriate. p -values ≤ 0.05 were considered significant. Symptomatic status, type of ulcer, age, sex and medication use were the primary variables. The risk of bleeding and symptoms were assessed by univariate and multivariate analyses. Data are present as mean \pm standard deviation of the mean. For the statistical analysis SAS Version 6.12 (SAS

Institute Inc., Cary, North Carolina, USA) and SPSS Version 12 (Statistical Package for Social Sciences, SPSS Inc., Chicago, Illinois, USA) were used.

10.3 RESULTS

10.3.1 Trends in patients with uncomplicated peptic ulcer disease and bleeding peptic ulcer

In 1997, 2002 and 2007, a total of 562 patients with PUD were seen at the Royal Adelaide Hospital (345 males, 217 females, mean age 65 ± 18 yr), 325 with BPU and 237 with uPUD. The number of patients with uPUD decreased progressively from 1997 to 2007 in both males (70 in 1997, 35 in 2002, and 26 in 2007) and females (58 in 1997, 31 in 2002, and 17 in 2007) (Figure 1). In contrast, the number of patients with BPU with over the same time period remained unchanged for both males (69 in 1997, 79 in 2002, and 66 in 2007) and females (37 in 1997, 41 in 2002, and 33 in 2007).

In uncomplicated peptic ulcer, the proportion of gastric ulcer increased significantly from 43% in 1997 to 72% in 2007 ($p=0.01$, Figure 2). In contrast, the proportion of duodenal ulcer fell significantly from 47% in 1997 to 21% in 2007 ($p<0.01$). In bleeding peptic ulcer, the proportion of gastric ulcer also

increased significantly from 45% in 1997 to 59% in 2007 ($p<0.01$, Figure 3). In contrast, the proportion of duodenal ulcer fell significantly from 51% in 1997 to 36% in 2007 ($p<0.05$).

10.3.2 Trends in patients with asymptomatic peptic ulcer disease

Overall, 79% of patients with uPUD presented with peptic ulcer symptoms, 21% were asymptomatic. In contrast, 74% of patients with BPU were asymptomatic, 26% had dyspeptic symptoms before the bleeding occurred ($p<0.001$). The proportion of asymptomatic ulcers remained stable in BPU patients over a 10-year period (74% in 1997, 78% in 2002, and 70% in 2007, Figure 4) while it increased in uPUD patients from 9% in 1997 to 29% in 2002 and 47% in 2007 ($p<0.001$).

10.3.3 Demographics of patients with peptic ulcer disease

Patients with BPU were significantly older than uPUD (70 vs 56 yr, $p=0.001$) and the number of patients with BPU increased with age. However, the mean age of patients with BPU and uPUD did not change over a 10-year period (BPU: 1997=69, 2002=72, 2007=70; uPUD: 1997=56, 2002=56, 2007=60).

Males were significantly more common than females amongst patients with BPU (M:F=1.9:1), and there was no change in this ratio over time (1997=1.9:1, 2002=1.9:1, 2007=2:1). In contrast, while males were slightly more common than females amongst patients with uPUD (M:F=1.2:1), the ratio increased over time, from 1.2:1 in 1997 to 1.5:1 in 2007. Among patients with BPU, males were significantly younger than females (67 vs 77, $p=0.001$), while male patients with uPUD had similar age to females (55 vs 58, $p=0.027$).

The proportion of possible NSAIDs/aspirin-related ulcer in the BPU group increased over 10 years from 53% to 69% ($p<0.02$) and was significantly higher than in uPUD patients (17%, $p<0.001$).

10.3.4 Demographics of patients with asymptomatic peptic ulcer disease

Overall, asymptomatic PUD patients were significantly older than symptomatic patients (71 yr vs 58 yr, $p<0.001$). This relationship hold true for both BPU patients (73 vs 67 yr, $p=0.001$) and uPUD patients (66 vs 55 yr, $p<0.001$). Among patients with BPU, asymptomatic males were relatively older than symptomatic male patients (70 vs 65, $p=0.058$), while asymptomatic females were significantly older than symptomatic female patients (79 vs 70, $p=0.002$). Among patients with uPUD, asymptomatic patients were significantly older than patients with

dyspeptic symptoms in both males and females (male: 64 vs 53, $p=0.002$; female: 68 vs 57, $p=0.006$).

10.3.5 *Helicobacter pylori* and peptic ulcer disease

Overall, 42% of patients with peptic ulcer disease had *H. pylori* infection. Patients with BPU had significantly higher rates of *H. pylori* infection than patients with uPUD (46% vs 36%, $p=0.035$). The proportion of *H. pylori* associated with BPU has decreased from 59% in 1997 to 32% in 2002 and increased to 49% in 2007. Similarly, proportion of patient with uPUD who had *H. pylori* infection has decreased from 46% in 1997 to 21% in 2002 and increased to 30% in 2007.

10.3.6 NSAIDs/aspirin and peptic ulcer disease

Use of NSAIDs/aspirin was more common in patients with BPU than in patients with uPUD (64% vs 17%, $p<0.001$). The proportion of BPU associated with NSAIDs/aspirin use increased over 10 years from 53% in 1997 to 68% in 2002 and 70% in 2007 ($p<0.02$). In contrast, the proportion was significantly smaller in patients with uPUD and did not change over time (18% in 1997, 16% in 2002 and 16% in 2007).

10.3.7 Non-NSAIDs/aspirin non-*Helicobacter pylori* peptic ulcer disease

The proportion of uPUD not associated with either NSAIDs/aspirin or *H. pylori* increased over 10 years from 45% in 1998 to 64% in 2002 and 63% in 2007. In contrast, the proportion of BPU not associated with either NSAIDs/aspirin or *H. pylori* decreased from 24% in 1997, and 25 % in 2002 to 14% in 2007.

10.3.8 Factors associated with peptic ulcer bleeding

On univariate analysis, use of aspirin (RR 1.79), NSAIDs (RR 1.43), anticoagulants (RR 1.46), calcium channel blockers (CCB) (RR 1.41), COX-II inhibitors (RR 1.27), clopidogrel (RR 1.4) or the presence of *H. pylori* infected (RR 1.27) were associated with peptic ulcer bleeding. However, on multivariate analysis, only aspirin was a significant associated with BPU (relative risk 1.23).

10.3.9 Factors associated with ulcer symptoms

On univariate analysis, patients who were on aspirin were more often asymptomatic (RR 0.612). In contrast, patients who were on clopidogrel (RR 1.31) and smoking (RR 1.33) were more likely to report dyspeptic symptoms.

On multivariate analysis, the presence of symptoms reduced the risk for BPU (RR=0.41, CI 0.33-0.49, $p<0.001$). Aspirin was significantly associated with asymptomatic ulcers (RR=0.649, $p<0.001$), whereas smoking was significantly associated with symptomatic ulcers. (RR=1.255, $p=0.013$)

10.4 DISCUSSION

Significant changes in the diagnosis and treatment of peptic ulcer disease have occurred over the past three decades, which have led to changes in the epidemiology of peptic ulcer disease. Our study shows that the number of patients with uncomplicated peptic ulcer disease (uPUD) has decreased progressively over the past 10 years, in both males and females. In contrast, the number of patients with bleeding peptic ulcer (BPU) has remained unchanged over the same period of time. These findings are similar to studies in the Netherlands (Post, Kuipers et al. 2006), and Danish county (Lassen, Hallas et al. 2006).

Several factors may have contributed to the decline in number of patients with uPUD. First, the prevalence of *H. pylori* infection, a major pathogenetic factor, has decreased since World War II as a result of improving living conditions (Roosendaal, Kuipers et al. 1997; Loffeld and van der Putten 2003). Second, the development of effective *H. pylori* eradication therapy in patients with peptic

ulcer disease (Rauws and Tytgat 1990), which has significantly reduced the rate of recurrent peptic ulcer disease, and the number of patients with non-ulcer dyspepsia. Finally, increasing use of PPI over the past two decades for problem such as gastro-oesophageal reflux disease may also contributed to the decline in number of patients with PUD (Van Soest, Siersema et al. 2006).

More importantly, this study shows that the number of patients with bleeding peptic ulcer (BPU) has remained unchanged over the same period of time, which is similar to previous studies (van Leerdam, Vreeburg et al. 2003; Lassen, Hallas et al. 2006; Post, Kuipers et al. 2006). Several factors may have contributed to this finding. The majority of patients with an acute BPU were asymptomatic until bleeding occurred. Therefore, it is difficult to treat PUD and subsequently prevent the complications of PUD. An increased used of NSAIDs as shown in this study and previous studies (Higham, Kang et al. 2002; Lassen, Hallas et al. 2006) could also explain the stable number of BPU patients as NSAIDs used associated with substantially increased risk of peptic ulcer bleeding (Stack, Atherton et al. 2002).

This study demonstrates that up to 74% of patients with BPU were asymptomatic, while only 21 % of uPUD. Data on the prevalence of ulcer symptoms prior to ulcer bleeding are few. The proportion of PUD patients without symptoms has been reported to range from 43%-87% (Coggon, Langman et al. 1982; Mellem, Stave et al. 1985; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997). The findings of this epidemiological survey are consistent with those of the systematic

survey of our patients in previous study using validated questionnaires which also showed that the majority (83%) of 30 patients with BPU were asymptomatic (Chapter 12).

This study is the first to demonstrate the trends of asymptomatic ulcer over the last decade. The proportion of asymptomatic ulcer remained stable in BPU patients over a 10-year period (70%-78%), while it dramatically increased in uPUD patients (9% to 47%). Precisely what determines whether a patient with peptic ulcer will have dyspeptic symptoms is unclear as the mechanism of peptic ulcer pain is still unknown. However, lack of symptoms in PUD could be explained by several reasons. First, differences in the processing of upper gastrointestinal visceral afferents may play an important role in the clinical presentation of patients with BPU, as symptomatic PUD patients had a significantly augmented symptom response to a standardised nutrient challenge test compared to patients with asymptomatic PUD (see Chapter 12). Second, asymptomatic PUD were significantly older than symptomatic PUD in both males and females, and for both BPU and uPUD. These findings add further support to the notion that age may be one of the factors that determines dyspeptic symptoms in PUD (Clinch, Banerjee et al. 1984; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997). Elderly subjects have been reported to exhibit a decreased symptom response to a standardized nutrient challenge test (see Chapter 11), and gastric balloon distension (Rayner, MacIntosh et al. 2000), and older age is also associated with diminished visceral sensation in the oesophagus (Lasch, Castell et

al. 1997) and rectum (Lagier, Delvaux et al. 1999). Third, NSAIDs used may also mask ulcer symptoms by their analgesic effect (Armstrong and Blower 1987; Stack, Atherton et al. 2002).

This study shows that the proportion of gastric ulcer in patients with uPUD and BPU increased significantly over time, as a result of the greater reduction in number of patients with duodenal ulcer compared with gastric ulcer. This trend is consistent with the trends in the US study (Wang, Richter et al. 2010). The decreased proportion of DU could possibly be explained by the decrease in the prevalence of *H. pylori* infection over the last decades, as at least 80% of duodenal ulcers are associated with *H. pylori* infection (Graham, Klein et al. 1988).

The proportion of BPU associated with *H. pylori* has progressively decreased over time, and consistent with a recent study in the US (Feinstein, Holman et al. 2010), which could be explained by the decrease in the prevalence of *H. pylori* infection in general populations (Roosendaal, Kuipers et al. 1997; Loffeld and van der Putten 2003). As a result of decrease in *H. pylori*-associated uPUD and stable NSAIDs use, the proportion of non NSAID, non *H. pylori*-associated uPUD has increased over time. On the other hand, the proportion of BPU associated with NSAIDs/ aspirin use has increased over 10 years. Increase use of NSAIDs/ aspirin may cause asymptomatic ulcer bleeding which leads to a presentation of BPU.

Aspirin, NSAIDs, anticoagulants, calcium channel blockers (CCB), COX-II inhibitors, clopidogrel and *H. pylori* infected were all more often associated with bleeding peptic ulcer on univariate analysis. However, aspirin was the only medication associated with increased risk of BPU on multivariate analysis in our study, which is consistent with other previous studies. Stack et al (Stack, Atherton et al. 2002) and Sakamoto et al (Sakamoto, Sugano et al. 2006) have shown that low dose aspirin was associated with BPU with odd ratio of 7.7, Kawai et al also showed that 15.8% of patients taking low dose aspirin developed gastroduodenal ulcer (Kawai, Watanabe et al. 2010). We could not find any significant association between SSRIs and BPU.

Aspirin was significantly associated with asymptomatic ulcers on both univariate and multivariate analysis in our study, while smoking was significantly associated with symptomatic ulcers. Kawai et al showed that no difference was seen in gastrointestinal symptoms reported between patients with and without peptic ulcer who were taking low dose aspirin (Kawai, Watanabe et al. 2010). However the notion that aspirin mask the ulcer symptoms is unclear.

In conclusion, over the last 10 years, the incidence of uPUD has decreased whereas BPU has remained stable. The majority of patients with BPU are asymptomatic whilst the majority of patients with uPUD had dyspeptic symptoms. Age and use of aspirin appear to be risk factors for the manifestation

of ulcers without dyspepsia that ultimately manifest with life threatening BPU. Therefore, bleeding peptic ulcer is difficult to prevent. More effective identification, elimination and/or management of risk factors will remain essential strategies for reducing the incidence of peptic ulcer complications.

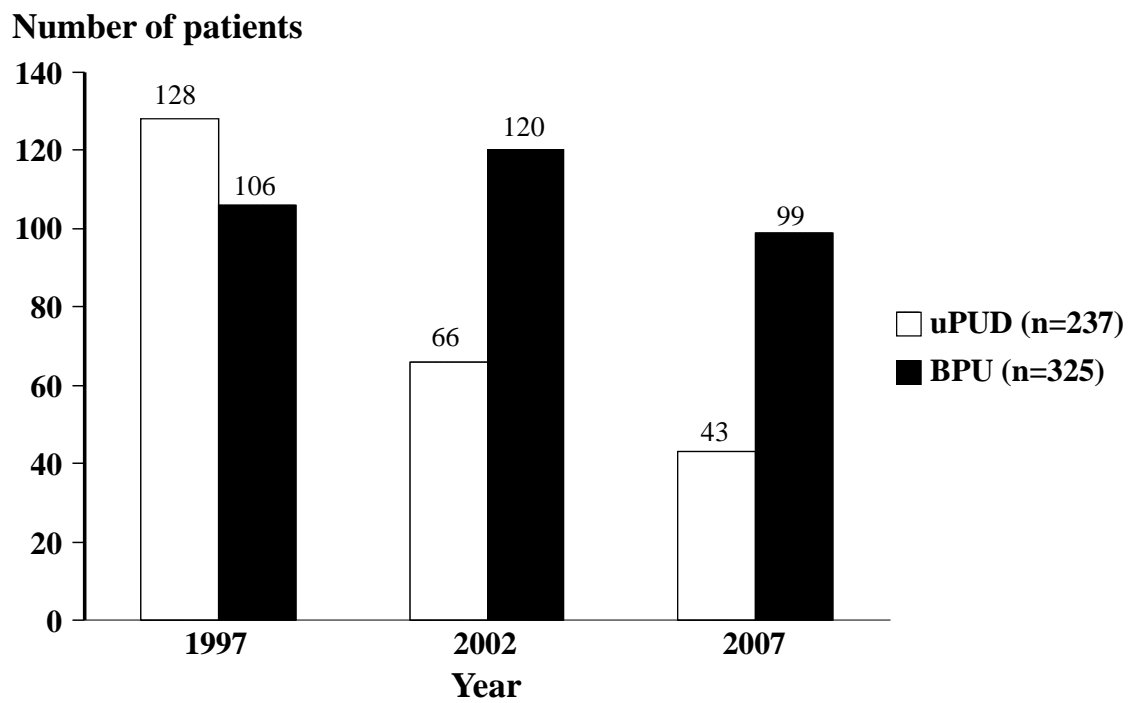


Figure 1: Number of patients with peptic ulcer disease.

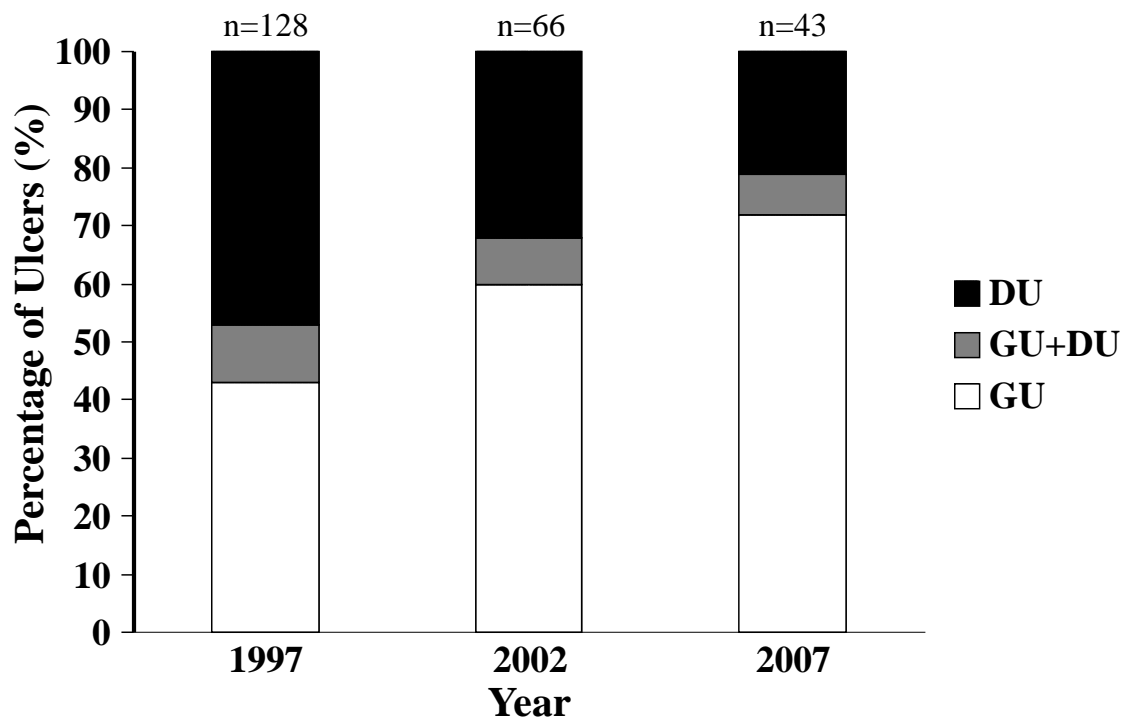


Figure 2: Proportion of gastric and duodenal ulcer in uncomplicated peptic ulcer.

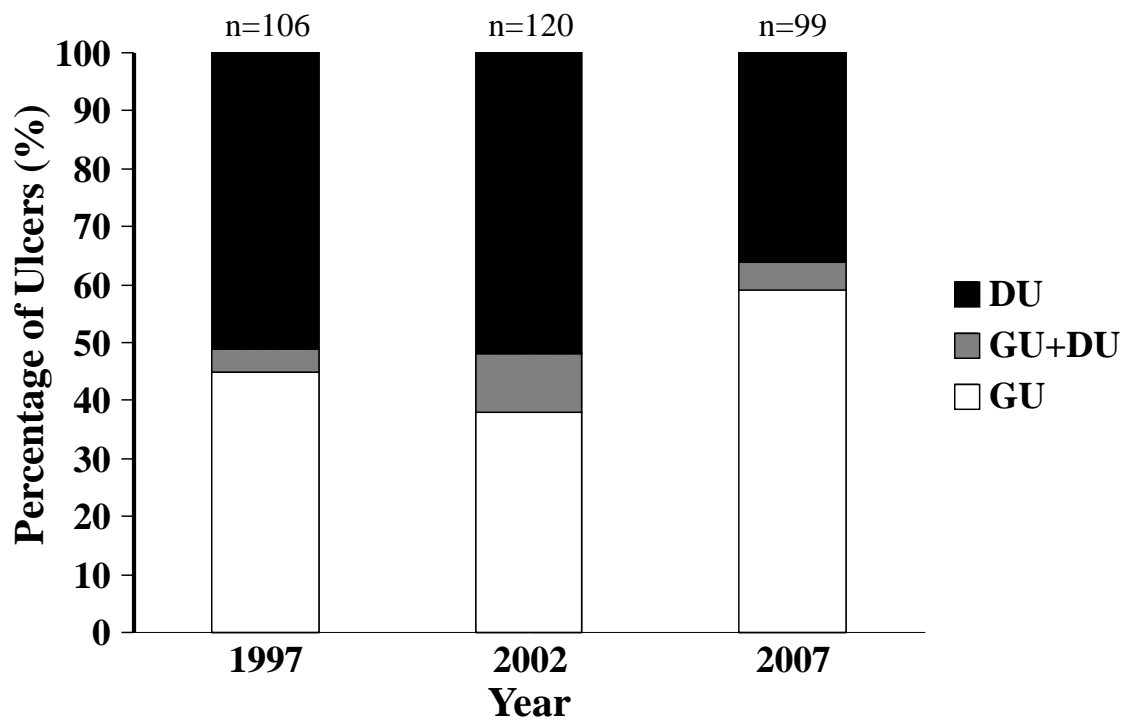


Figure 3: Proportion of gastric and duodenal ulcer in bleeding peptic ulcer.

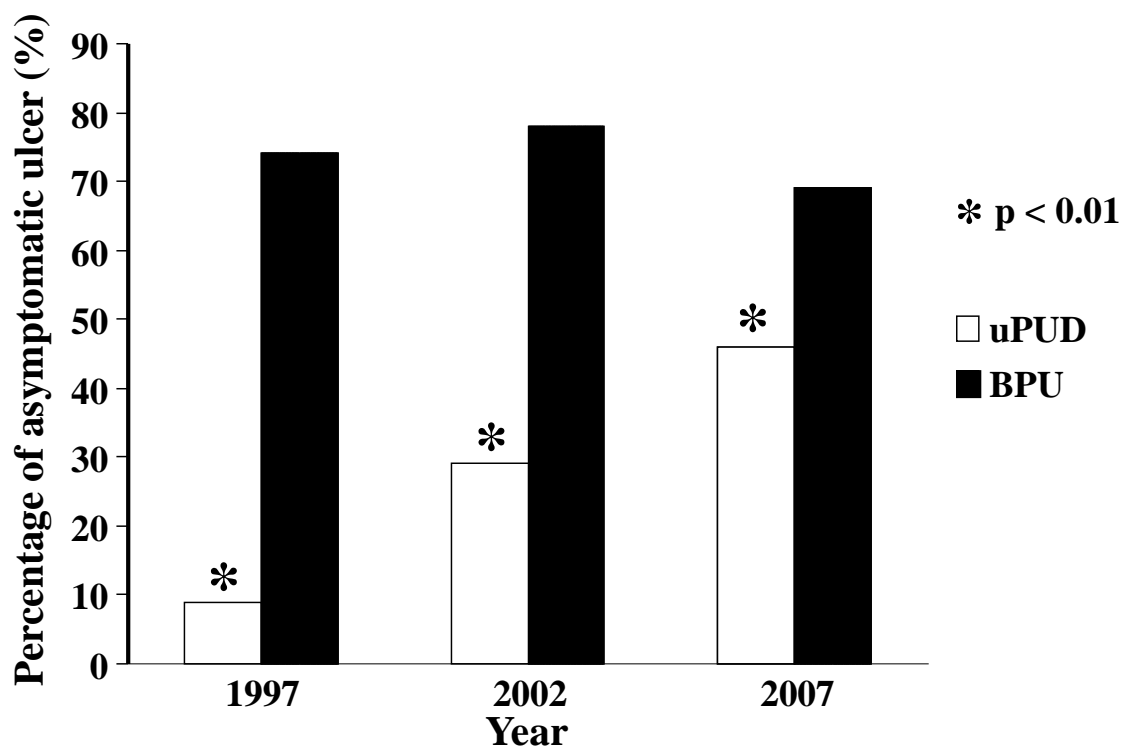


Figure 4: The proportion of asymptomatic ulcer remained stable in BPU patients while it increased in uPUD patients from 11% in 1997 to 48% in 2007.

CHAPTER 11: THE AGEING GUT: DIMINISHED SYMPTOM RESPONSE TO A STANDARDISED NUTRIENT STIMULUS

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11.1 INTRODUCTION

Many gastrointestinal diseases manifest by symptoms such as abdominal pain or discomfort. The prevalence of dyspepsia (Agréus, Svardsudd et al. 2001) abdominal pain (Kay, Jorgensen et al. 1994) and the severity of reflux symptoms (Johnson and Fennerty 2004) decreases with advancing age. Ageing is also associated with diminished perception of pharyngeal (Aviv, Martin et al. 1994) oesophageal (Lasch, Castell et al. 1997) and rectal sensations (Lagier, Delvaux et al. 1999). However, data on the effect of age on the visceral sensory function of the stomach are lacking.

A standardised nutrient challenge test is a simple and non-invasive test and is a validated and reproducible test for assessing gastric visceral sensory function (Holtmann, Talley et al. 1996; Haag, Talley et al. 2004). The aim of this study was to assess the effect of age on gastric sensory function using the nutrient challenge test. We hypothesised that there would be an inverse relationship between age and the symptom response to a standardised nutrient challenge. In order to control for potential confounders, we also assessed baseline gastrointestinal symptoms and psychological factors such as depression and anxiety that are believed to be associated with disorders of sensory function in the gastrointestinal tract (Delvaux 1999).

11.2 SUBJECTS AND METHODS

11.2.1 Subjects

Fifty three healthy volunteers (24 females, mean age 46.2 years, range 19-78 years) were recruited consecutively by advertisement. No subject had a history of gastrointestinal, significant systemic disease or was taking medication which is known to influence gastrointestinal tract. Six out of 53 subjects (age range 26-78 years) were *H. pylori* positive on ¹⁴C urea breath test. Subjects who had a history of functional gastrointestinal disorders or psychiatric co-morbidities were excluded. The study was approved by the Royal Adelaide Hospital Human Ethics Committee and all of the volunteers gave written informed consent before completing the questionnaires and standardised nutrient challenge test.

11.2.2 Assessment of gastrointestinal symptoms

The presence and severity of gastrointestinal symptoms and psychiatric co-morbidities were assessed utilising validated questionnaires: the Gastrointestinal Symptom (GIS) score (Adam, Liebrechts et al. 2005), the Bowel Disease Questionnaire (BDQ) (Talley, Phillips et al. 1990; Talley, Boyce et al. 1995), the

Nepean Dyspepsia Index (NDI) (Talley, Haque et al. 1999; Talley, Verlinden et al. 1999), and the Hospital Anxiety and Depression Scale (HADS) (Herrmann 1997).

The GIS assesses the intensity of gastrointestinal symptoms in patients with functional dyspepsia, and addresses patient's gastrointestinal symptoms in the past 1 week. The BDQ assesses various types of symptoms including upper abdominal symptoms, bowel symptoms, reflux symptoms and lifestyle over the previous 12 months. The NDI assesses symptoms of dyspepsia and health-related quality of life. The HADS is a validated tool for the assessment of anxiety and/or depression.

11.2.3 Standardised nutrient challenge test

Visceral sensitivity was assessed by a standardised nutrient challenge test performed on the same day following completion of the questionnaires.

11.2.4 Statistical analysis

The relationships between age and both baseline symptoms as well as individual and global cumulative symptom responses during the nutrient challenge test were

assessed by Spearman rank correlation. Symptom responses were also assessed in two subgroups of subjects: those deemed to be elderly (> 60 yr, 15 subjects: 11 males, mean age 65.7 ± 1.3 yr) and those deemed to be young (< 40 yr, 19 subjects: 7 males, mean age 24.6 ± 1 yr). p – values ≤ 0.05 were considered significant. Data are present as mean \pm standard error of the mean. For the statistical analysis SAS Version 6.12 (SAS Institute Inc., Cary, North Carolina, USA) and SPSS Version 12 (Statistical Package for Social Sciences, SPSS Inc., Chicago, Illinois, USA) were used.

11.3 RESULTS

11.3.1 Standardised nutrient challenge test:

All subjects were able to ingest the intended target volume of 800 ml enteral feeding solution within the specified time. Forty-eight out of 53 subjects (91%, 95% CI 83-98) reported some symptoms during the nutrient load. The mean global cumulative symptom score was 106.6 ± 14.8 . All subjects with symptoms experienced fullness as the most dominant symptom with a maximum reported score of 271 (mean score 79.8 ± 9.5); 17 reported nausea as a symptom while 36 did not report nausea. The maximum reported score for nausea was 189 (mean score 14.9 ± 4.9) and that for pain 189 (mean score 9.8 ± 4.5). These three items

accounted for more than 90 % of the global symptom load. Only 5 subjects reported burning (maximum reported score was 20) and 10 reported regurgitation (maximum score 31).

Base-line scores for perception in any category were unrelated to age. In response to the nutrient challenge, however, there was a significant inverse correlation between age and the cumulative scores for pain ($r=-0.426$, $p=0.002$, Figure 1) and nausea ($r=-0.28$, $p=0.045$, Figure 2). Subjects who were below 40 years of age reported significantly higher cumulative scores for pain and nausea (pain: 22 ± 11.9 ; nausea: 29.3 ± 12) during the standardised nutrient challenge test than did subjects who were older than 60 years (pain: 0.9 ± 0.9 ; nausea: 4.5 ± 3.9) (pain: $p=0.002$, nausea: $p=0.043$, Figure 3). The difference between the groups increased progressively during the nutrient challenge test (Figure 4). The significant difference in pain and nausea scores between the age groups remained when controlled for levels of anxiety and depression by multivariable analysis. There was no significant difference between age and either the cumulative scores for the other individual symptoms (fullness, heartburn and regurgitation), or the global cumulative symptom score ($r=-0.03$, $p=0.81$). In addition, there was no significant difference in either individual symptom scores or the cumulative symptom score between the genders.

11.3.2 Baseline symptom scores

11.3.2.1 Gastrointestinal Symptom score

Based upon the GIS, 16 out of 53 (30%, 95% CI 18-43) subjects had pre-existing mild levels of dyspepsia with a low mean score of 0.75 and a maximum score of 9 out of 40. Eight subjects reported scores of greater than 1 (very mild) all of whom were under 50 years of age. Eight subjects reported total score of 1 and the rest reported no symptoms. Subjects who reported any kind of gastrointestinal symptom were significantly younger (mean age 39.1 ± 4.5 yr) than subjects who reported none (mean age 49.2 ± 2.8 yr; $p < 0.05$). The GIS score was inversely correlated with age ($r = -0.31$, $p = 0.026$, Figure 5).

11.3.2.2 Bowel Disease Questionnaire and Nepean Dyspepsia Index

Abdominal pain or discomfort in the past twelve months, was reported by 8/53 (15%, 95% CI 5-25) patients on the BDQ; 4 subjects reported very mild symptom, while 3 subjects and 1 subjects reported mild and moderate symptom respectively. Nausea was reported by 20/53 subjects. Overall scores ranged from 0-3. Age did not correlate significantly with symptom severity reported on the BDQ for any symptom type (Table 1).

On the NDI, no subject reported an impaired quality of life. Age had no significant effect on the impact of abdominal complaints on subject's quality of life. However, subjects who reported a greater severity of abdominal pain or discomfort on the BDQ also reported a lower score, i.e. worse health-related quality of life, on the NDI ($r=-0.29$, $p=0.035$). Also subjects who reported a higher frequency of abdominal pain or discomfort on the BDQ ($r=-0.31$, $p=0.023$), and higher score on the GIS ($r=-0.32$, $p=0.019$) reported a significantly lower score on the NDI.

11.3.2.3 Hospital Anxiety and Depression Scale (HADS)

No subject had clinically relevant anxiety or depression. However, of the 53 subjects, 6 subjects (11%, 95% CI 3-20) were categorised as having mild anxiety and 2 (4%, 95% CI 0.5-13) moderate anxiety, while 2 subjects were categorised as having mild depression. Overall, the score for anxiety was inversely related to age ($r=-0.27$, $p<0.05$). However, there was no significant association between age and the score for depression.

When the data from the baseline symptom questionnaires were correlated with the symptom response to a standardised nutrient challenge test, subjects who reported a higher of score on the GIS had a higher pain symptom score response to the

nutrient challenge test. ($r=0.30$, $p=0.031$). However, no correlation was found between symptom scores on the other questionnaires and those during the nutrient challenge test.

Table 1: Demographic data and symptoms reported by questionnaires in subjects

< 40 years and > 60 years. Data presented as mean \pm SEM.

	Age < 40 yrs (n=19)	Age > 60 yrs (n=15)	<i>p</i> value
Demographic			
- Age	24.6 \pm 1	65.7 \pm 1.3	0.0001
- Gender (M)	7/19	11/15	0.077
- <i>H. pylori</i> infection	1/19	4/15	0.207
Symptoms			
GIS (1wk)	1.3 \pm 0.5	0.2 \pm 0.1	0.111
HADS score			
- Anxiety	4.9 \pm 0.8	3.6 \pm 0.8	0.215
- Depression	1.6 \pm 0.5	2.2 \pm 0.5	0.228
NDI			
- Interference daily activities/work	98.8 \pm 1	99.7 \pm 0.3	0.837
BDQ (12 months)			
- Severity of abd pain	0.5 \pm 0.2	0.1 \pm 0.1	0.451
- Frequency of abd pain per wk	0.5 \pm 0.3	0.3 \pm 0.3	0.537

11.4 DISCUSSION

The main finding of this study is that an inverse relationship exists between the symptom responses to a standardised nutrient challenge and age in healthy volunteers, even when controlled for underlying levels of anxiety and depression. These data are consistent with the notion that elderly people have decreased gastric visceral sensation compared with younger people. The age relationship was evident for pain and nausea but not for fullness suggesting either that pain/nausea and fullness are influenced differently by age or that different pathways are involved. Our data suggest that elderly people have decreased gastric visceral sensation compared with younger people. Our findings could help to explain in part why younger subjects may perceive symptoms in the presence of lesions while the elderly may develop severe lesions or even complications such as peptic ulcer bleeding without having symptoms, and why the prevalence of dyspepsia decreases with advancing age.

In the selection of subjects for the study, care was taken to exclude those with history of relevant gastrointestinal disease or functional gastrointestinal disorders that might influence their response to the nutrient challenge test. Nevertheless, on questionnaire, 30% of our subjects reported some gastrointestinal symptoms in the past. Such findings are consistent with previous reports. Adam et al. have

reported that healthy subjects without dyspepsia had a GIS mean score of 2.1 (Adam, Liebrechts et al. 2005), and other studies have reported that between 7 and 41% of healthy subjects have some symptoms consistent with a functional gastrointestinal disorder (Jones and Lydeard 1989; Holtmann, Goebell et al. 1994). A similar prevalence of background symptoms has also been reported in other populations of healthy subjects such as blood donors (Holtmann, Goebell et al. 1994) and population based samples (Talley, Holtmann et al. 2000). While it might be argued these symptoms are due to underlying structural abnormalities, it is well recognised that there is no association of these symptoms with *H. Pylori* (Holtmann, Goebell et al. 1994) and all subjects with NSAIDs intake were excluded from our study.

The GIS score was also significantly and inversely correlated with age. This suggests that even within normal subjects there is a higher sensitivity of gastrointestinal symptom in younger subjects, which might help them to perceive gastrointestinal diseases while the elderly cannot. Moreover, according to BDQ, GIS and NDI questionnaire, subjects who reported a higher frequency of abdominal pain or discomfort on the BDQ and higher score on the GIS reported a significantly lower score on the quality of life measured by NDI, supporting the validity of these questionnaires. We found no significant impact of age on symptoms assessed by the BDQ or NDI.

Previous studies have suggested that anxiety and depression influence gastric sensory function (Geeraerts, Vandenberghe et al. 2005). We therefore assessed depression and anxiety, using well established and valid instruments (Herrmann 1997). Although all of our subjects were healthy, and subjects who had psychiatric co-morbidities were excluded, our data show that age was inversely related to levels of anxiety. This finding is consistent with the studies of Bland et al (Bland, Newman et al. 1988), Regier et al (Regier, Farmer et al. 1993), and from the Australian Bureau of Statistics (1998) (McLennan) which showed that the prevalence of anxiety disorders decreased in people over 65 years of age, and those of Henderson et al (Henderson, Jorm et al. 1998), who reported that the mean scores for anxiety in the general population decline with age. There are several possibly reasons to explain this prevalence, for example; elderly people are less exposed to problems with relationships and work or less likely to be seriously short of money (Henderson, Jorm et al. 1998). Age may also be correlated with decreased emotional responsiveness, and increased emotional control (Jorm 2000). However, in the present study in healthy subjects no clear association between the anxiety score and sensory function was found.

Importantly, the present study showed that age is inversely correlated with symptom responses, particularly pain and nausea, to the standardised nutrient challenge. Ageing has also been shown to be associated with increased visceral pain thresholds in other part of gastrointestinal tract as well as somatic tissues. Lasch et al (Lasch, Castell et al. 1997) reported an age-related decrease in

oesophageal pain sensation response to intraoesophageal balloon distension, and Lagier et al (Lagier, Delvaux et al. 1999) showed that healthy aged subjects had significantly higher rectal sensory thresholds than did young subjects. In addition, healthy aged subjects have been reported to have higher somatosensory thresholds than do young subjects (Lautenbacher, Kunz et al. 2005).

We did not find any significant association between ageing and other symptoms such as fullness, heartburn and regurgitation, or the cumulative symptom score during the nutrient challenge test. Heartburn and regurgitation are symptoms of reflux and not expected in healthy subjects. Moreover, they are only an indirect consequence of gastric filling. Nausea and pain are more clearly nociceptive responses. Fullness was the most dominant symptom reported and did not differ between younger and older patients. The substantial contribution of fullness to the global cumulative score may have obscured the significant effects from other symptom components. Pain perception from oesophageal (Lasch, Castell et al. 1997) and rectal balloon distension (Lagier, Delvaux et al. 1999) decreases with age. Possibly age has a greater effect on nociception than on sensation in general which could explain why perceptions of fullness, heartburn and regurgitation were apparently not affected by age. Alternatively, elderly patients may have more serious (objective) reflux but the symptom severity is not different from younger subjects (Johnson and Fennerty 2004).

At present, the mechanism of ageing on visceral sensation is unclear. Aged Long-Evans rats who were injected with formalin into their hind paws had less response to the pain than did middle-aged rats (Gagliese and Melzack 1999). Possibly the function of gastric mechanoreceptors, afferent pathways or central processing of visceral signals might be impaired in elderly subjects. Previous studies have shown a relationship between age and a reduction in the number of myenteric oesophageal neurons in humans (Meciano Filho, Carvalho et al. 1995) and peripheral afferent fibre function in human (Chakour, Gibson et al. 1996).

Our findings have implications for the understanding of differing presentations of disease among patients of differing ages. Decreased visceral sensation with age may explain the decreasing prevalence of abdominal pain (Kay, Jorgensen et al. 1994) and functional dyspepsia with increasing age (Jones, Lydeard et al. 1990; Tibblin, Bengtsson et al. 1990). Older patients with functional dyspepsia have higher pain, discomfort and fullness thresholds than do younger patients in response to gastric balloon distension (Mertz, Fullerton et al. 1998). In addition, our findings may explain why peritonitis and abdominal complaints are more occult in older persons (Wroblewski and Mikulowski 1991). A study from Japan showed that only 46.5% of older patients (60-69 years old) with gastric cancer who initially presented with symptoms had epigastric pain compared with 71.6% of young patients (less than 40 years old) (Eguchi, Takahashi et al. 1999). Complicated ulcers (e.g. ulcers with life threatening bleeding) appear to occur predominantly in elderly patients (Hernandez-Diaz and Rodriguez 2002). An age-

related reduction in visceral sensory function may result in elderly patients being less symptomatic such that they do not present with their ulcer disease until life threatening bleeding occurs. Hilton and colleagues (Hilton, Iman et al. 2001) showed that 30% of patients over 60 years old with peptic ulceration were asymptomatic compared with only 7% of younger patients. Whilst it might be argued that NSAID use, which is more likely cause ulcers in the elderly, could reduce ulcer pain, this notion has been challenged (Lu, Chang et al. 2004).

In conclusion, the symptom response, in particular pain and nausea, to a standardised nutrient challenge is inversely correlated with age. This finding suggests that age related changes of visceral sensory function occur and may contribute to differing presentations of gastrointestinal illness in patients of different ages. The mechanisms underlying this effect remain to be elucidated.

**CHAPTER 12: ASSOCIATION BETWEEN CLINICAL
MANIFESTATIONS OF COMPLICATED AND
UNCOMPLICATED PEPTIC ULCER AND VISCERAL
SENSORY DYSFUNCTION**

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12.1 INTRODUCTION

Peptic ulcer disease (PUD) usually manifests as either dyspepsia or, less commonly, with life threatening complications such as bleeding and perforation (Linder and Wilcox 2001). Over the past two decades the incidence of uncomplicated peptic ulcer disease (uPUD) has dropped substantially, whilst the incidence of peptic ulcer bleeding seems to have remained unchanged (Lassen, Hallas et al. 2006; Post, Kuipers et al. 2006). Approximately 30% to 50% of patients with bleeding peptic ulcer (BPU) are asymptomatic until bleeding occurs (Crocker 1991) even though the endoscopic assessment may reveal multiple ulcer scars suggestive of previous ulceration. Moreover, the majority of patients dying from peptic ulceration have no symptoms of ulcer disease until the presentation of their final, fatal illness (Pounder 1989).

The mechanism of ulcer pain is still unclear. The extent and severity of erosions are not directly associated with an increased risk for dyspeptic symptoms (Collins, Davies et al. 1986; Holtmann, Gschossmann et al. 2002). However, several factors have been associated with silent peptic ulceration. BPU occurs predominantly in elderly patients (Hernandez-Diaz and Rodriguez 2002; Targownik and Nabalamba 2006) and older age is often associated with asymptomatic peptic ulcer (Clinch, Banerjee et al. 1984; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997; Hilton, Iman et al. 2001). Non-steroidal anti-inflammatory drugs (NSAIDs) may also be associated with asymptomatic peptic

ulcer (Clinch, Banerjee et al. 1984; Mellem, Stave et al. 1985; Dew 1987), although this notion has been challenged (Wilcox and Clark 1997; Lu, Chang et al. 2004).

Another possible explanation for the lack of dyspeptic symptoms in PUD is abnormally altered visceral sensory function. Increased visceral sensitivity may present as abdominal pain in the absence of mucosal injury, such as occurs in functional dyspepsia and irritable bowel syndrome (Greydanus, Vassallo et al. 1991; Mayer and Gebhart 1994; Tack, Bisschops et al. 2004). A lack of dyspeptic symptoms in BPU could potentially reflect diminished visceral sensitivity.

The aims of this study, therefore, were to assess symptom profiles and compare visceral sensory thresholds in patients with BPU, uPUD and healthy controls (HC).

12.2 SUBJECTS AND METHODS

12.2.1 Patients and healthy subjects

Consecutive patients with BPU and uPUD who were diagnosed by endoscopy were screened. Patients with pyloric stenosis, malignant ulcers, previous

abdominal surgery or gastrointestinal cancer, who were over the age of 80 years old, or who had diabetes mellitus controlled by insulin were excluded. Healthy volunteers were recruited by public advertisement. Thirty patients with BPU (24 men, mean age 64 ± 1.6 years), 25 patients with uPUD (14 men, mean age 53.3 ± 3.2 years) and 32 healthy asymptomatic volunteers (22 men, mean age 58.9 ± 1.4 years) were recruited for participation in this study. The study was approved by the Royal Adelaide Hospital Human Ethics Committee and all of the volunteers gave written informed consent.

A total of 184 patients with uPUD were screened, 93 were excluded due to serious co-morbidities, 91 patients were asked to participate the study, and 25 patients (17 GU, 7 DU, 1 GU and DU, 14 males, mean age 53.3 ± 3.2) agreed to participate the study. 18 patients had been prescribed proton pump inhibitor (PPI) therapy before the endoscopy. A total of 212 patients with BPU were screened (114 GU, 80 DU, 18 GU and DU). Of these, 123 patients were excluded due to serious co-morbidities, 89 patients were asked to participate in the study, and 30 patients (14 GU, 11 DU, 5 GU and DU, 24 males, mean age 64 ± 1.6) agreed. All 30 patients with BPU, 25 uPUD and 32 HC completed all 4 questionnaires and the standardised nutrient challenge test.

12.2.2 Protocol

At the time of diagnosis of PUD, patients were informed about the study provided that the clinical circumstances allowed, and the endoscopy findings were recorded (ulcer number, size, and location). A peptic ulcer was defined as a mucosal break at least 3 mm in diameter with visible depth (Hawkey, Karrasch et al. 1998; Yeomans and Naesdal 2008). An asymptomatic ulcer was defined as an ulcer in the absence of any dyspeptic symptom, including epigastric pain or discomfort, or minimal symptoms which did not affect the subject's daily lifestyle as reported on the Bowel Disease Questionnaire as very mild or mild abdominal pain or discomfort. Demographic, social, and clinical data, including gender, race, age, body weight, and height, co-morbidities, history of NSAIDs used, *Helicobacter pylori* infection, alcohol ingestion, and smoking habits were also recorded. *H. pylori* infection was tested by serology or a biopsy-based rapid urease test in all patients. All patients who had *H. pylori* infection received esomeprazole 20 mg, amoxicillin 1000 mg, and clarithromycin 500 mg twice daily for 7 days either after the index endoscopy or at the time of discharge from hospital. In patients with GU, ulcer healing was confirmed by endoscopy (as defined by complete epithelialisation) whereas duodenal ulcers were assumed to have healed after 8 weeks of PPI therapy and *H. pylori* eradication where appropriate.

At least 8 weeks after diagnosis and after healing of GU was confirmed by endoscopy, the subjects underwent the study tests at one visit. First, the subjects were asked to complete symptoms questionnaires. Second, subjects were tested for *H. pylori* by ¹⁴C urea breath test. Third, subjects were assessed for gastric visceral sensitivity by a standardised nutrient challenge test. All subjects were asked to stop taking medication which are known to influence gastrointestinal tract, including NSAIDs, at least 7 days prior to the study.

12.2.3 Assessment of gastrointestinal symptoms

The presence and severity of gastrointestinal symptoms and psychiatric comorbidities were assessed utilising validated questionnaires: the Gastrointestinal Symptom (GIS) score (Adam, Liebrechts et al. 2005), the Bowel Disease Questionnaire (BDQ) (Talley, Phillips et al. 1990; Talley, Boyce et al. 1995), the Nepean Dyspepsia Index (NDI) (Talley, Haque et al. 1999; Talley, Verlinden et al. 1999), and the Hospital Anxiety and Depression Scale (HADS) (Herrmann 1997). The GIS assesses the intensity of gastrointestinal symptoms in patients with functional dyspepsia, and addresses patient's gastrointestinal symptoms in the past 1 week. The BDQ assesses various types of symptoms including upper abdominal symptoms, bowel symptoms, reflux symptoms and lifestyle over the previous 12 months. The NDI assesses symptoms of dyspepsia and health-related

quality of life. The HADS is a validated tool for the assessment of anxiety and/or depression.

12.2.4 Standardised nutrient challenge test

Visceral sensitivity was assessed by a standardised nutrient challenge test performed on the same day following completion of the questionnaires. After an 8-hour fast, subjects were asked to perform a standardised nutrient challenge test (Holtmann, Talley et al. 1996). The detail of nutrient challenge test has been described in Methodology section.

12.2.5 Statistical analysis

Proportions and 95 % confidence intervals for patients with and without symptoms were calculated. Demographics, ulcer size and location of ulcers were analysed between patients with and without symptoms, and between uPUD and BPU. Univariate associations with symptoms were initially explored by the chi-square test with the Yates correction for continuity, where appropriate, and a Kruskal–Wallis one-way ANOVA was used to determine differences in cumulated symptom response to nutrient challenge test among 3 groups where appropriate at a significance level of $p \leq 0.05$. The primary hypothesis tested was

that there is a difference between patients with BPU and uPUD for the cumulated symptom response to a standardised 800 ml nutrient challenge. We thus compared the response to a standardised nutrient challenge for patients with BPU, uPUD and HC and between patients with and without symptoms. Analysis of variance adjusting for age, gender and BMI was used to compare the cumulative symptom response. p values ≤ 0.05 were considered significant. Data are present as mean \pm standard error of the mean (SEM). For the statistical analysis SAS Version 6.12 (SAS Institute Inc., Cary, North Carolina, USA) and SPSS Version 12 (Statistical Package for Social Sciences, SPSS Inc., Chicago, Illinois, USA) were used.

12.3 RESULTS

12.3.1 Demographics

Demographic data, the characteristics of the ulcers and the various factors that could potentially determine the symptoms of patients with BPU and uPUD are shown in Table 1. All patients had peptic ulcer with a mucosal break at least 3 mm in diameter with visible depth confirmed by endoscopy. Most (15/25) of the patients with uPUD had been treated with PPI and in most instances, the ulcer had the appearances of being in the healing phase. Eighty three percent of the patients with BPU were asymptomatic prior the bleeding event. In contrast, all patients

with uPUD presented with abdominal pain ($p<0.0001$). Patients with BPU were significantly older than patients with uPUD ($p=0.01$). There was no significant difference in mean age between HC and uPUD or BPU. Patients with BPU had significantly larger ulcers ($p<0.01$). Two patients with BPU had diabetes whose blood sugar has been well controlled by medication. There were no significant differences in gender, BMI, location of ulcers, number of ulcers, use of NSAIDs, or smoking between the groups.

At the time of diagnosis, rates of *H. pylori* infection were not significantly different among the groups (uPUD=48%, BPU=57%). On the study day, two of patients with BPU, 2 uPUD patients and 6 HC tested positive to *H. pylori* (after receiving *H. pylori* eradication therapy). However, there were no significant differences in *H. pylori* infection among the groups ($p>0.99$ between BPU and uPUD).

12.3.2 Symptom profiles

After at least 8 weeks of treatment of the ulcer, and confirmation of healing of GU, most patients were asymptomatic. Twenty five out of 30 (83%, 95% CI 65-94%) patients with BPU and 13/25 (52%, 95% CI 32-72%) patients with uPUD reported no symptoms on GIS and NDI. Patients with uPUD reported significantly higher symptom scores on the GIS questionnaire than did patients

with BPU ($p=0.016$, Table 2) and HC ($p=0.0001$). Patients with uPUD also reported significantly higher levels of abdominal symptoms on the NDI questionnaire, and had significantly poorer quality of life affected by dyspepsia than patients with BPU. On the BDQ, uPUD patients reported significantly more severe and more frequent abdominal pain than BPU patients. Four healthy subjects were categorised as having mild anxiety. Two patients with BPU were categorised as having mild anxiety, 1 moderate anxiety, and 1 severe anxiety, and 6 mild depression, whereas 5 patients with uPUD had mild anxiety, 5 moderate anxiety, and 2 moderate depression. In addition, uPUD patients reported significantly higher levels of anxiety but not depression than did patients with BPU.

Healthy volunteers reported very few symptoms on all questionnaires and significantly less than both BPU and uPUD patients ($p<0.01$). However, the volunteers reported similar scores for anxiety to BPU patients, but significantly lower than for uPUD patients ($p<0.02$).

12.3.3 Standardised nutrient challenge test

All subjects were able to ingest the intended target volume of 800 ml within the specified time. Seventy-four out of 87 subjects reported some symptoms during the nutrient load, while 8 BPU patients and 5 HC reported no symptoms. Fullness

was the most prominent symptom reported (mean score 101.8 ± 9.2) followed by nausea (mean score 25.1 ± 6.2) and pain (mean score 18 ± 4.5) and these three items accounted for more than 90 % of the overall symptom load.

Patients with uPUD had significantly higher peak and cumulative symptom responses to the standardised nutrient challenge test than did HC and BPU for most of individual symptoms, as well as a higher total symptom score ($p < 0.0001$, Figure 1). However, patients with BPU had similar symptom response to HC (no significantly difference in individual symptoms and total symptom score) but significantly lower symptom responses than those patients with uPUD.

12.3.4 Asymptomatic vs symptomatic ulcers

Patients were also grouped into those with and without dyspeptic symptoms, irrespective of whether the ulcer had bled (Table 3). More than 85% of patients with asymptomatic PUD were male, compared with 50% of those with symptomatic ulcers ($p < 0.02$). Asymptomatic patients were significantly older than patients who experienced dyspeptic symptoms ($p < 0.01$). Patients who had no abdominal pain had significantly larger ulcers than patients who experienced pain ($p < 0.02$). There were no significant differences in BMI, location of ulcers, number of ulcers, use of NSAIDs, *H. pylori* infection, or smoking between symptomatic and asymptomatic peptic ulcer patients.

Symptomatic peptic ulcer patients had significantly higher peak and cumulative symptom responses to the nutrient challenge test than did asymptomatic ulcers for most of the symptom items (Figure 2). Moreover, symptomatic ulcer patients reported significantly higher anxiety scores ($p < 0.01$, Table 4) but not depression scores than did asymptomatic patients.

Table 1: Demographics, characteristics of ulcers and symptom reported by questionnaires in healthy controls, patients with uncomplicated peptic ulcer disease, and bleeding peptic ulcer at the time of diagnosis.

	Healthy controls (n=32)	uPUD (n=25)	BPU (n=30)	p value (uPUD vs BPU)
Symptomatic (n (%))	NA	25 (100)	5 (17)	
Asymptomatic (n (%))		0 (0)	25 (83)	<0.0001
Gender (M/F)	22 /10	14/11	24/6	ns
Age (yrs)	58.9 ± 1.4	53.3 ± 3.2	64 ± 1.6	0.01
BMI (kg/m ²)	28 ± 1	29.4 ± 1.2	26.9 ± 0.8	ns
Ulcer type				
(GU/DU/GU+DU)	NA	17/7/1	14/11/5	ns
NSAID usage	0	8	15	ns
<i>H. pylori</i> infection	NA	12	17	ns
<i>H. pylori</i> infection (on the study day)	6	2	2	ns
Smoking	0	6	9	ns
Number of ulcers				
- Single / Multiple		17/8	16/14	ns
- Mean number	NA	1.5 ± 0.2	1.7 ± 0.2	ns
Size of ulcer				
- <1cm / ≥1cm		22/3	16/14	0.013
- Mean (cm)	NA	0.5 ± 0.1	1 ± 0.1	0.0001

Table 2: Demographics, characteristics of ulcers and symptom reported by questionnaires in healthy controls, patients with uncomplicated peptic ulcer disease, and bleeding peptic ulcer on the study day.

	Healthy controls (n=32)	uPUD (n=25)	BPU (n=30)	p value (uPUD vs BPU)
GIS (1wk)	0.3 ± 0.1	7.1 ± 1.2	3.9 ± 1.1	0.016
NDI (2wks)				
- Pain	0.2 ± 0.2	3.1 ± 0.8	1.2 ± 0.5	0.023
- Discomfort	0.1 ± 0.1	4 ± 0.8	1.5 ± 0.5	0.004
- Burning	0	1.5 ± 0.5	0.8 ± 0.5	0.032
- Cramp	0.1 ± 0.1	1.5 ± 0.6	0.6 ± 0.4	ns
- Fullness	0.2 ± 0.1	3.9 ± 0.9	1.3 ± 0.4	0.014
- Pressure	0	2.6 ± 0.7	0.5 ± 0.4	0.002
- Bloating	0.1 ± 0.1	4 ± 0.9	0.9 ± 0.5	0.001
- Interference daily activities/work	99.9 ± 0.1	85.1 ± 4.5	89.7 ± 7	0.039
BDQ (12 months)				
- Severity of abd. pain	0.1 ± 0.1	2.8 ± 0.3	1 ± 0.3	0.0001
- Frequency of abd pain per wk	0.2±0.1	3.3±0.4	1.1±0.3	0.0001
HADS score				
- Anxiety	3.8 ± 0.5	6.6 ± 0.9	3.8 ± 0.7	0.012
- Depression	2.2 ± 0.3	4 ± 0.6	3.7 ± 0.6	ns

Table 3: Demographics and characteristics of ulcers between symptomatic and asymptomatic peptic ulcer patients at the time of diagnosis.

	Symptomatic ulcer	Asymptomatic	
	(n=30)	(n=25)	p value
BPU/uPUD (n (%))	5 (17)/25 (83)	25 (100)/0 (0)	<0.0001
Gender (m/f) (n (%))	14 (47)/16 (53)	22 (88)/3 (12)	0.013
Age (yrs)	54.2 ± 2.8	65.2 ± 1.7	0.004
BMI (kg/m ²)	29.2 ± 1	26.6 ± 0.9	ns
Ulcer GU/DU/GU+DU	20/9/1	11/9/5	ns
NSAID usage (%)	10 (33)	13 (52)	ns
<i>H. pylori</i> infection (%)	14 (47)	15(60)	ns
Smoking	8 (27)	7 (28)	ns
Number of ulcer			
- Single/Multiple	18 (60)/12 (40)	14 (56)/11 (44)	ns
- Mean number	1.5 ± 0.1	1.8 ± 0.2	ns
Size of ulcer			
- <1cm/≥1cm	26 (87)/4 (13)	13 (52)/12 (48)	0.012
- Mean cm	0.6 ± 0.1	1.1 ± 0.1	0.013

Table 4: Symptom reported by questionnaires between symptomatic and asymptomatic peptic ulcer patients on the study day.

	Symptomatic (n=30)	Asymptomatic (n=25)	p value
GIS (1wk)	7.5 ± 01.1	2.8 ± 0.9	0.001
NDI (2wks)			
- Pain	3 ± 0.7	1 ± 0.5	0.017
- Discomfort	3.8 ± 0.7	1.2 ± 0.5	0.002
- Burning	1.8 ± 0.6	0.3 ± 0.3	0.005
- Cramp	1.6 ± 0.5	0.3 ± 0.3	0.018
- Fullness	3.8 ± 0.7	0.9 ± 0.4	0.004
- Pressure	2.6 ± 0.8	0	<0.0001
- Bloating	6.5 ± 0.8	0.5 ± 0.3	<0.0001
- Interference daily activities/work	84.9 ± 4.2	90.9 ± 4.2	0.023
BDQ (12 months)			
- Severity of abd. pain	2.7 ± 0.3	0.7 ± 0.2	<0.0001
- Frequency of abd pain per wk	3.2 ± 0.4	0.8 ± 0.3	<0.0001
HADS score			
- Anxiety	6.5 ± 0.8	3.3 ± 0.5	0.009
- Depression	3.9 ± 0.6	3.8 ± 0.7	ns

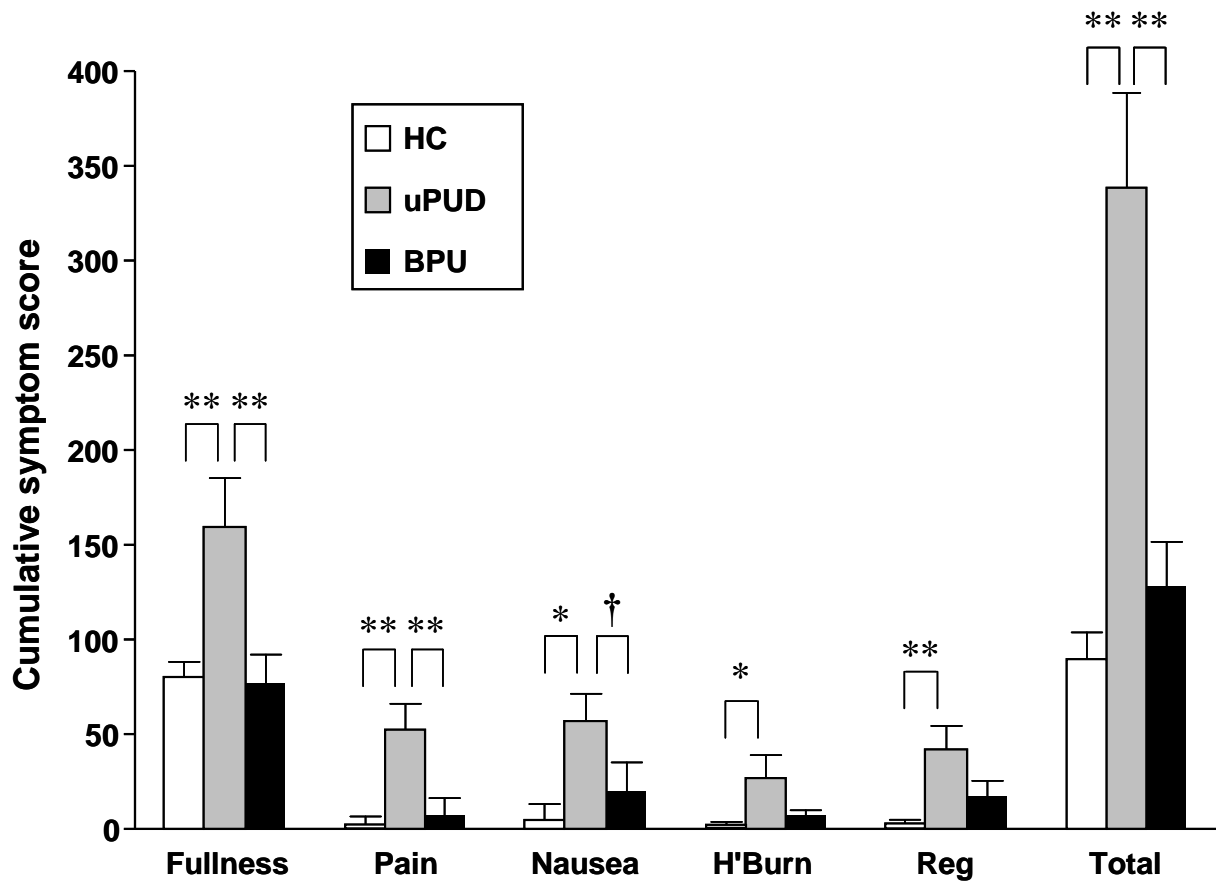


Figure 1: Cumulative symptom response to standardised nutrient challenge test among healthy controls (HC), patients with uncomplicated peptic ulcer (uPUD) and bleeding peptic ulcer (BPU). Data presented as mean + SEM († $p < 0.05$; * $p < 0.01$; ** $p < 0.001$).

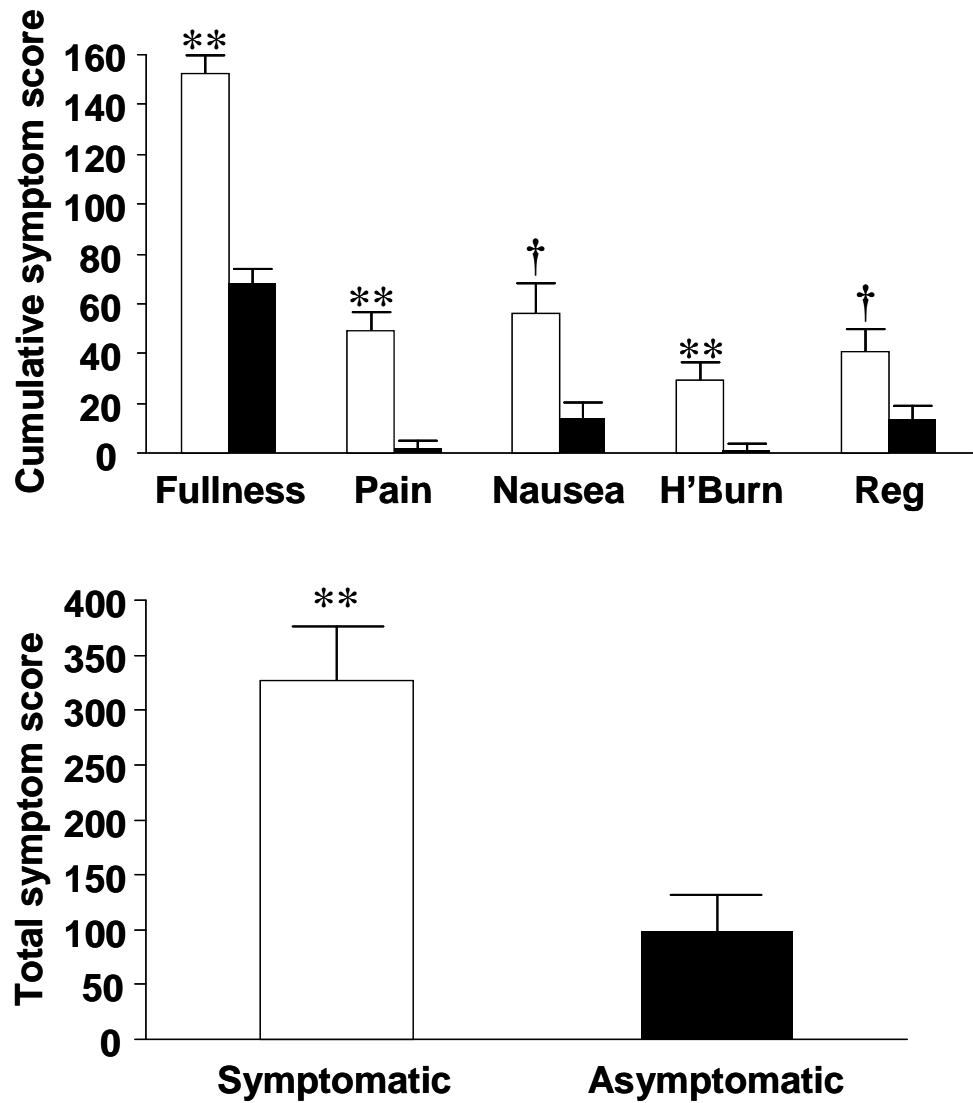


Figure 2: Cumulative symptom response to standardised nutrient challenge test between symptomatic (open bars) and asymptomatic peptic ulcer patients (filled bars). Data presented as mean + SEM († $p < 0.05$; ** $p < 0.001$).

12.4 DISCUSSION

The observation that the majority of patients with an acute BPU are asymptomatic until the life threatening severe bleeding occurs remains a hitherto unexplained yet potentially important observation (Coggon, Langman et al. 1982). The findings from our current study provide a potential explanation. While we had hypothesised that patients with BPU might be less sensitive as compared to healthy controls, our data suggest that patients with uPUD are actually more sensitive than HC. Using a standardised test of visceral sensation, our findings show that, patients with uPUD have an augmented symptom response whilst patients with BPU have a symptom response to a test meal that is not different from that in HC.

Data on the prevalence of ulcer symptoms prior to ulcer bleeding are few. The proportion of patients without symptoms has been reported to range from 43%-87% (Coggon, Langman et al. 1982; Mellem, Stave et al. 1985; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997). The systematic survey of our patients using validated questionnaires shows that the majority (83%) of patients with BPU are asymptomatic. In contrast, the majority of patients with uPUD usually present with abdominal pain. Our study also shows that after ulcer healing, or treatment that would reasonably be expected to have healed the ulcer, patients with uPUD continued to report symptoms that were more severe than those in

patients with BPU. Persistence symptoms after healing of ulcers might be the result of post inflammatory hyperalgesia, as occurs in an animal model of transient colitis (Adam, Liebrechts et al. 2006). The patients were studied after cessation of PPI therapy and, potentially, rebound acid secretion might have contributed to persistence of symptoms (Reimer, Sondergaard et al. 2009). However, such effects would not explain the difference between patients with bleeding and uncomplicated ulcer disease.

The major finding of this study is that patients with BPU have diminished gastric visceral sensation compared with uPUD. Similarly asymptomatic PUD patients, irrespective of bleeding status, have diminished visceral sensation compared with patients who experienced peptic ulcer symptoms. These differences were present after ulcer healing suggesting a fundamental difference in visceral sensitivity between patients with bleeding or asymptomatic ulcers and those with symptomatic or uncomplicated ulcers. Lowered visceral sensitivity and asymptomatic status is a plausible explanation for the presentation of ulcers with complications such as bleeding. Conversely, visceral hyperalgesia, higher degrees of psychological distress, more concomitant bowel symptoms and persistent dyspepsia after medical treatment in patients with uPUD may explain earlier presentation and diagnosis of the ulcers.

The augmented symptom response to the test meal and the higher level of background symptoms after ulcer healing suggest that patients with symptomatic

but uncomplicated peptic ulcers share similarities with patients with functional dyspepsia (Haag, Talley et al. 2004). Potentially, the uncomplicated ulcers could have been incidental findings in patients with functional dyspepsia. However, the rapid and substantial relief of symptoms in 52% of patients with anti-ulcer therapy in this group argues against this notion as the response to either *H. pylori* eradication or PPI therapy is relatively poor in functional dyspepsia (Talley, Janssens et al. 1999; Wong, Wong et al. 2002).

Precisely how the differences in symptom response to the meal relate to the occurrence of ulcer symptoms, however, is unclear as the mechanism of peptic ulcer pain is still unknown. The relevance of gastric acid bathing the ulcer crater is controversial (Ruffin 1959; Kang, Yap et al. 1986). Disordered gastric motility has also been proposed to be a cause of ulcer pain (Ruffin 1959). Diminished symptom responses for fullness, abdominal pain, nausea and heartburn in BPU patients suggest diminished spinal afferent function but impairment of pain pathways in patients with asymptomatic PUD remains to be directly tested.

In this study we used a standardised nutrient challenge test to assess visceral sensitivity. This test has been used in various studies of patients with functional dyspepsia, IBS and healthy subjects (Holtmann, Talley et al. 1996; Kim, Myung et al. 2000; Mulak 2003; Delgado-Aros, Camilleri et al. 2004; Haag, Talley et al. 2004; Choung, Talley et al. 2007) and correlates well with mechanosensory thresholds as measured by the barostat (Holtmann, Talley et al. 1996) that is

currently the gold standard for testing gastric visceral sensation. The test meal did not reproduce the ulcer symptoms in the patients. Whilst it could be argued that a nutrient challenge test may not be the most appropriate test for ulcer pain, it was not the aim of this study to reproduce ulcer pain but rather to assess underlying levels of visceral sensitivity.

We have reported preliminary data suggesting that patients with uPUD have slower gastric emptying than patients with BPU (Gururatsakul, Bellon et al. 2008) and have suggested that this may contribute to symptoms. However, such differences are unlikely to have contributed to the differences in sensory response to the meal in the current study as visceral sensation was assessed during the accumulation phase of the meal and not during emptying. Nevertheless, the differences in symptom responses to a standardised nutrient challenge could have resulted from differences in gastric accommodation, as has been reported in patients with functional dyspepsia (Tack, Piessevaux et al. 1998), although this variable was not assessed.

Patients with BPU were significantly older and had significantly larger ulcers than uPUD patients. When patients were grouped into those with and without dyspeptic symptoms, again asymptomatic patients were significantly older and had larger ulcers compared with dyspeptic patients. These findings add further support to the notion that age may be one of the factors that determines dyspeptic symptoms in PUD (Clinch, Banerjee et al. 1984; Matthewson, Pugh et al. 1988;

Wilcox and Clark 1997). Elderly subjects have been reported to exhibit a decreased symptom response to a standardised nutrient challenge test (Gururatsakul, Holloway et al. 2009) and gastric balloon distension (Rayner, MacIntosh et al. 2000), and older age is also associated with diminished visceral sensation in the oesophagus (Lasch, Castell et al. 1997) and rectum (Lagier, Delvaux et al. 1999). However, the aged subjects in these studies were on average 50 years older than the younger controls (Lasch, Castell et al. 1997; Lagier, Delvaux et al. 1999; Rayner, MacIntosh et al. 2000). In the present study, the age difference between patients with BPU and uPUD, or symptomatic and asymptomatic PUD was only 10 years. Thus it is unlikely that age is a major determinant of differences in symptom status in our patients. Male gender may also be associated with asymptomatic PUD. In the present study, males formed the majority of asymptomatic patients and were significantly less common in symptomatic group. Whilst ulcer size has been reported to be a determinant of symptoms (Wilcox and Clark 1997; Lu, Chang et al. 2004), we did not detect an effect of either ulcer size or location on symptoms after a meal challenge in this study.

The study also shows that patients with uncomplicated or symptomatic ulcers reported significantly higher scores for anxiety but not depression than did patients with BPU and HC. Psychological factors, especially anxiety, are associated with gastrointestinal symptoms in patients with functional gastrointestinal disorders (Mayer, Craske et al. 2001) and in the general

population (Haug, Mykletun et al. 2002). It also has been shown that self-reported PUD, presumably due to symptomatic ulcers, is associated with a generalised anxiety disorder (Goodwin and Stein 2002). Gastric sensorimotor function can be altered by experimentally-induced anxiety in healthy subjects, which suggested that psychological factors may play a role in dyspeptic symptoms even though those subjects did not have psychological disorders (Geeraerts, Vandenberghe et al. 2005). Such observations may explain why patients with dyspeptic symptoms reported anxiety scores higher than asymptomatic peptic ulcer patients. However, the mechanism of the association between psychological factors and dyspeptic symptoms remains unclear.

This study has some unavoidable limitations. First, we were not able to assess visceral sensation in patients with asymptomatic uPUD as these patients do not seek any medical attention and the ulcers are found only serendipitously or when complications occur. Second it might be argued that the size of ulcers in the uPUD group was relatively small and, in some cases, were over-diagnosed erosions. However, we set criteria based on those previously published and accepted (Hawkey, Karrasch et al. 1998; Yeomans and Naesdal 2008) and all, in contrast to erosions, had depth. The small size of the ulcers in some patients can be explained by the use of PPI therapy before the endoscopy. Third, the questionnaires were completed 8 weeks after the diagnosis. However, it appeared problematic to assess the dyspeptic symptoms immediately after the ulcer presentation with in some cases very life threatening manifestations.

Nevertheless, the BDQ addressed the patients' symptoms over the previous 12 months, which included the period before PUD was diagnosed.

The findings of the study have implications for our understanding and management of PUD. Normally visceral pain is one of the reasons patients seek medical attention.(Cervero and Laird 2004) Failure to identify bleeding ulcers at an earlier stage through symptoms could explain the failure of the incidence of BPU to fall over the past 20 years (Ruigomez, Garcia Rodriguez et al. 2000) despite better treatment and cure of ulcer disease with proton pump inhibitors and eradication of *H. pylori*. More effective identification, elimination and/or management of risk factors will remain essential strategies for reducing the incidence of peptic ulcer complications.

In conclusion, the majority of patients with BPU present without dyspeptic symptoms. In contrast, even after healing of the ulcer, patients with a history of uncomplicated ulcers and ulcer symptoms have a significantly augmented symptom response to a standardised nutrient challenge test compared to patients with BPU. Lack of dyspeptic symptom in patients with life threatening BPU may reflect failure of augmented visceral sensation and result in them presenting late with complications rather than earlier with their primary disease. The data suggest that differences in the processing of upper gastrointestinal visceral afferents may play an important role in the clinical presentation of patients with complicated peptic ulcer. Based upon our data it might be speculated that mechanisms that are

involved into the manifestation of symptoms in patients with functional dyspepsia may actually prevent the manifestation of ulcer complications since ulcers manifest with symptoms that trigger health care seeking and treatment before complications occur.

**CHAPTER 13: COMPLICATED AND UNCOMPLICATED
PEPTIC ULCER DISEASE: ALTERED SYMPTOM
RESPONSE TO A NUTRIENT CHALLENGE LINKED TO
GASTRIC MOTOR DYSFUNCTION**

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13.1 INTRODUCTION

Bleeding peptic ulcer disease remains a potentially life-threatening disorder. As demonstrated earlier that approximately 30% to 50% of patients with bleeding peptic ulcer are asymptomatic until bleeding occurs (Crocker 1991) even though the endoscopic assessment may reveal multiple ulcer scars suggestive of previous ulceration.

The mechanism of ulcer pain is still unclear. It is well known that the extent and severity of erosions is not directly associated with an increased risk for dyspeptic symptoms or more severe symptoms (Collins, Davies et al. 1986; Holtmann, Gschossmann et al. 2002). However, several factors including older age (Hernandez-Diaz and Rodriguez 2002; Targownik and Nabalamba 2006) and non-steroidal anti-inflammatory drugs (NSAIDs) (Clinch, Banerjee et al. 1984; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997; Hilton, Iman et al. 2001) have been associated with silent peptic ulceration. Using a nutrient challenge test, we have shown recently that the lack of dyspeptic symptoms in peptic ulcer disease is associated with a failure to augment visceral sensitivity (Gururatsakul, Holloway et al. 2010). In addition to assessing visceral sensation, the nutrient challenge test may be influenced by a number of other physiological elements including gastric emptying. Previous studies have identified altered gastric emptying in peptic ulcer disease. Gastric emptying has been reported to be delayed in patients with active gastric ulceration (Harasawa, Tani et al. 1979) and

accelerated in patients with duodenal ulcer (Lam, Isenberg et al. 1982; Maddern, Horowitz et al. 1985). The relationship between gastric motor function and clinical manifestation of peptic ulcer disease, however, is not known. The aim of this study, therefore, was to assess gastric emptying in patients with bleeding peptic ulcer (BPU), uncomplicated peptic ulcer (uPUD) and healthy controls (HC), and the relationship between symptoms and gastric emptying.

13.2 SUBJECTS AND METHODS

13.2.1 Patients and healthy subjects

The selection criteria of patients with BPU, uPUD and healthy control have been described in the methodology section. Seventeen patients with BPU (16 men, mean age 65.9 ± 2.2 years), 10 patients with uPUD (3 men, mean age 50.9 ± 3.8 years) and 15 healthy asymptomatic volunteers (10 men, mean age 59.6 ± 1.4 years) were recruited for participation in this study.

13.2.2 Protocol:

The protocol has already been described in Chapter 11, section 11.2.2.

13.2.3 Assessment of gastrointestinal symptoms

The presence and severity of gastrointestinal symptoms and psychiatric comorbidities were assessed utilising validated questionnaires: the Gastrointestinal Symptom (GIS) score (Adam, Liebrechts et al. 2005), the Bowel Disease Questionnaire (BDQ) (Talley, Phillips et al. 1990; Talley, Boyce et al. 1995), the Nepean Dyspepsia Index (NDI) (Talley, Haque et al. 1999; Talley, Verlinden et al. 1999), and the Hospital Anxiety and Depression Scale (HADS) (Herrmann 1997), as have been described in methodology chapter.

13.2.4 Standardised nutrient challenge gastric emptying test

Visceral sensitivity and gastric accommodation were assessed by a standardised nutrient challenge test performed on the same day following completion of the questionnaires. After an 8-hour fast, subjects were asked to drink 200 ml of a standardised nutrient liquid (Ensure®, Abbott Australasia Pty. Ltd., Botany, NSW, Australia), containing 5 MBq ^{99m}Tc-rhenium sulphide colloid (RAH radiopharmacy, Adelaide, South Australia), every 5 minutes up to a cumulative volume of 800 ml. Before and 5 min after each 200 ml drink, symptoms were assessed using a visual analogue scale (range 0-100 mm) with 0 = no symptom and 100 = unbearably severe. This tool assesses five symptoms; fullness,

abdominal pain, nausea, retrosternal/abdominal burning and acid regurgitation. The cumulative symptom responses were determined and used as the primary outcome variables (Holtmann, Talley et al. 1996).

Gastric emptying was measured by scintigraphy. Posterior dynamic images were acquired for 2 hrs on a GE Millennium MPR single head gamma camera with the patients sitting upright. Data collection began at the start of the meal consumption. Data were acquired for 120 minutes, with 1-minute frame intervals for the first 30 minutes and at 3-minute frame intervals thereafter. Radionuclide data were corrected for subject movement and radioactive decay. For analysis, regions of interest were drawn around the proximal, distal and total stomach. For each region, the percentage retention was assessed at different time points. The percentage of label remaining in the proximal, distal and total stomach at 100 minutes was calculated.

13.2.5 Statistical analysis

Statistical analysis was performed using SAS Version 6.12 (SAS Institute Inc., Cary, North Carolina, USA) and SPSS Version 12 (Statistical Package for Social Sciences, SPSS Inc., Chicago, Illinois, USA). Proportions and 95 % confidence intervals for patients with and without symptoms were calculated. Demographics, ulcer size and location of ulcers were analysed between patients with

uncomplicated peptic ulcer and bleeding peptic ulcer. Univariate associations with demographics, location of ulcers and symptom reported were initially explored by the chi-square test with the Yates correction for continuity where appropriate. The primary hypothesis tested was that there is a difference between patients with BPU and uPUD for the cumulated symptom response to a standardised 800 ml nutrient challenge and the regional retentions in the proximal and total stomach. We thus compared the response to a standardised nutrient challenge and gastric emptying retention for patients with BPU, uPUD and healthy control. A Kruskal-Wallis analysis of variance was used to compare the cumulative symptom response to nutrient challenge test and one-way ANOVA was used to determine differences in gastric emptying in proximal, distal and total stomach at 100 minutes among 3 groups where appropriate at a significance level of $p \leq 0.05$. Differences in gastric emptying in the proximal, distal and total stomach at 100 minutes among 3 groups was analysed using analysis of covariance adjusting for age, gender and BMI. In addition, the relationship between symptoms reported on questionnaires, individual cumulative symptom responses during the nutrient challenge test and gastric emptying test was assessed by Pearson correlation. Data are present as mean \pm standard error of the mean.

13.3 RESULTS

13.3.1 Demographics

Demographic data, the characteristics of the ulcers and the various factors that could potentially determine the symptoms of patients with bleeding peptic ulcer and uncomplicated peptic ulcer disease are shown in Table 1. Sixteen of the 17 patients with bleeding peptic ulcer who participated this study were asymptomatic prior the bleeding event. In contrast, all patients with uncomplicated peptic ulcer presented with abdominal pain ($p < 0.001$). Patients with bleeding peptic ulcer were significantly older than patients with uPUD ($p = 0.001$). Also BPU patients were predominantly males, while uPUD patients were predominantly females ($p = 0.001$). There was no significant difference in mean age and gender between HC and uPUD or BPU. Only one patient with BPU was a diabetic patient and blood sugar has been well controlled by medication. There were no significant differences in BMI, location of ulcers, number of ulcers, use of NSAIDs, or smoking between the groups.

At the time of diagnosis, rates of *H. pylori* infection were not significantly different among the groups (HC = 40%, uPUD = 40%, and BPU = 70%). On the study day, 1 BPU, 1 uPUD patients and 3 HC tested positive to *H. pylori* on ^{14}C

urea breath test (after received *H. pylori*. eradication therapy). However, there were no significant differences in *H. pylori* infection among the groups.

13.3.2 Symptom profiles

After at least 8 weeks of treatment of the ulcer, and confirmation of healing of gastric ulcers, patients with uPUD reported significantly higher symptom scores on the GIS questionnaire than did patients with BPU ($p=0.018$, Table 2) and HC ($p=0.001$). Patients with uPUD also reported significantly higher levels of abdominal symptoms in the past 2 weeks on the NDI questionnaire. In terms of quality of life affected by dyspepsia, patients with uPUD had poorer quality of life than patients with BPU, however, the difference did not reach statistical significance. On the BDQ, uPUD patients reported significantly more severe and more frequent abdominal pain than did BPU patients. Two patients with BPU were categorised as having mild anxiety and 1 mild depression whereas 3 patients with uPUD had mild anxiety, 1 moderate anxiety, and 1 mild depression. In addition, uPUD patients reported higher levels of anxiety but not depression than did patients with BPU but the difference did not reach statistical significance.

Healthy volunteers reported very few symptoms on all questionnaires and significantly less than uPUD patients ($p<0.01$). While they also reported fewer

symptoms on all questionnaires than BPU patients, the difference did not reach statistical significance.

13.3.3 Standardised nutrient challenge gastric emptying test

Patients with uPUD had significantly higher percent retention in the total stomach at 100 minutes compared with HC (p=0.006, Figure 1) and BPU (p=0.008). Patients with BPU had similar percent retention to HC but significantly lower percent retention compared with uPUD. Patients with uPUD had significantly higher percent retention in the proximal stomach at 100 minutes compared with HC (p=0.005, Figure 2) and BPU (p=0.024). Patients with BPU had similar percent retention to HC but significantly lower than patients with uPUD. There was no significant difference in percent retention in the distal stomach among the groups (p=0.60). On analysis of covariance, adjusting for age, gender and BMI, only HC, not BPU, had significantly lower percent retention in the proximal stomach (p=0.02) and total stomach (p=0.03) compared with uPUD.

All subjects were able to ingest the intended target volume of 800 ml within the specified time. Thirty-four out of 42 subjects reported some symptoms during the nutrient load, while 6 BPU patients and 2 HC reported no symptoms. Fullness was the most prominent symptom reported (mean score 84.5 ± 13.9) followed by

nausea (mean score 22.5 ± 8.8) and pain (mean score 14.6 ± 6.3) and these three items accounted for more than 90 % of the overall symptom load.

Patients with uPUD had significantly higher peak and cumulative symptom responses to the standardised nutrient challenge test than did HC ($p=0.026$, Figure 3) and BPU ($p=0.007$). However, patients with BPU had similar symptom response to HC (no significant difference in cumulative symptom score) but significantly lower symptom responses than those patients with uPUD. There was a significant correlation only between the cumulative scores for pain and percent retention in the total stomach ($r=0.30$, $p=0.05$).

There was a significant correlation between the percent retention in the total stomach and both the severity of abdominal pain or discomfort reported on the BDQ ($r=0.33$, $p=0.032$) and symptom score reported on the NDI (abdominal discomfort: $r=0.40$, $p=0.01$; bloating: $r=0.33$, $p=0.035$). However, percent retention in the total stomach was not correlated with symptom score reported on the GIS ($r=0.28$, $p=0.072$).

13.4 DISCUSSION

The observation that the majority of patients with an acute bleeding peptic ulcer are asymptomatic until the life threatening severe bleeding occurs remains a

hitherto unexplained yet potentially important observation (Coggon, Langman et al. 1982). Our findings show that, after ulcer healing, patients with bleeding peptic ulcer have similar gastric emptying to healthy controls. In contrast, patients with uncomplicated peptic ulcer have delayed gastric emptying compared with HC and patients with BPU. In addition, there was a modest direct correlation between gastric emptying and symptoms reported on the questionnaires. We have shown previously that patients with a history of uPUD and ulcer symptoms had a significantly higher augmented symptom response to the standardised nutrient challenge test compared with patients with bleeding peptic ulcer, suggesting that differences in the processing of upper gastrointestinal visceral afferents may play an important role in the clinical presentation of patients with complicated peptic ulcer (Gururatsakul, Holloway et al. 2010). The findings from the present study further suggest that differences in gastric motor function may also contribute.

This is the first study to assess gastric motor function in patients with uncomplicated peptic ulcer and bleeding peptic ulcer after ulcer healing. Our findings show that patients with uncomplicated peptic ulcer disease have significantly and abnormally delayed gastric emptying compared with healthy controls and BPU. These differences were present after ulcer healing suggesting a fundamental difference in gastric emptying between patients with bleeding or asymptomatic ulcers and those with symptomatic or uncomplicated ulcers. The delay in gastric emptying was limited to the proximal stomach. Whilst this may reflect a true regional selectivity in the disturbance to gastric motility, it might

also be a consequence of using a liquid test meal. The proximal stomach is the major determinant of liquid gastric emptying. Although the antral pump also helps to drive liquid emptying (Camilleri, Malagelada et al. 1985), a liquid meal may not adequately assess distal gastric motility and subtle differences might have been missed.

In the present study, only 60% of patients with BPU had gastric ulcer compared with 90% of patients with uPUD although this disparity was not statistically significant. Previous studies of gastric emptying in peptic ulcer disease have shown that active gastric ulcer is associated with delayed gastric emptying (Harasawa, Tani et al. 1979; Miller, Malagelada et al. 1980), while active duodenal ulcer is associated with accelerated gastric emptying (Harasawa, Tani et al. 1979; Lam, Isenberg et al. 1982; Maddern, Horowitz et al. 1985) compared with healthy subjects. However, Kanaizumi et al has shown that for both gastric and duodenal ulcer, gastric emptying generally returned to normal when ulcers had healed (Kanaizumi, Nakano et al. 1989).

Gastric emptying was assessed scintigraphically as part of a nutrient challenge test. Scintigraphy is generally considered a gold standard test for evaluating gastric emptying. However, the technique usually involves the reasonably rapid ingestion of the meal rather than relatively slow and stepwise ingestion as used in the present study. The technique of nutrient challenge gastric emptying with analysis of gastric retention at 100 minute has been used in previous studies

(Horowitz, McNeil et al. 1986; Pilichiewicz, Horowitz et al. 2007). Although most studies have used only 100 ml of nutrient liquid to test gastric emptying (Nguyen, Chapman et al. 2008), the method used in the current study was an adaptation to account for slow ingestion of a real-life large meal (Haag, Talley et al. 2004). Controls were included to establish a normal range

The precise association between gastric emptying and the symptomatic status of peptic ulcer disease is unclear, as the mechanism of peptic ulcer pain is still unknown. Our study was not designed optimally to test this as symptoms were assessed during and immediately after ingestion rather than during the emptying phase. Nevertheless, there was a significant relationship between gastric retention and symptom scores on the questionnaires as well as in response to the meal suggesting that gastric emptying might influence background symptoms in peptic ulcer disease.

It would appear that patients with symptomatic but uncomplicated peptic ulcers share similarities with patients with functional dyspepsia, including increased visceral sensitivity and delayed gastric emptying. Up to 25-50% of patients with functional dyspepsia have delayed gastric emptying (Stanghellini, Tosetti et al. 1996; Maes, Ghoois et al. 1997; Sarnelli, Caenepeel et al. 2003; Tack, Bisschops et al. 2004) and 34-66% have gastric hypersensitivity (Tack, Bisschops et al. 2004). Our study also shows that after ulcer healing, patients with uPUD continued to report symptoms that were more severe than those in patients with

BPU. Having delayed gastric emptying at first or failure to improve gastric emptying in patients with uPUD may explain why they continue to have abdominal symptoms after ulcers had healed. Thus, it seems that delayed gastric emptying may be associated with the pathophysiology of abdominal symptoms, but not peptic ulcer disease. Persistence symptoms after healing of ulcers also might be the result of post inflammatory hyperalgesia, as occurs in an animal model of transient colitis (Adam, Liebrechts et al. 2006). Or perhaps patients with uPUD begin as non-consulters with non-ulcer dyspepsia until the development of an ulcer crater leads them to develop more severe dyspeptic symptoms and eventually presentation.

Several factors have been suggested to influence the symptoms of peptic ulcer disease, including age (Clinch, Banerjee et al. 1984; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997), gender, BMI (Lu, Chang et al. 2004) and size of the ulcer (Wilcox and Clark 1997; Lu, Chang et al. 2004). Patients with BPU, not HC, were significantly older than uPUD patients. This finding add further support to the notion that age is possibly one of the factors that drives dyspeptic symptoms in peptic ulcer disease. However, it has been shown that age does not have a major influence on gastric emptying (Moore, Tweedy et al. 1983; Horowitz, Maddern et al. 1984; Madsen and Graff 2004) and patients with uPUD were similar in age to the controls yet had significantly delayed gastric emptying. Thus age differences in this study are unlikely to affect the results of gastric emptying studies.

Females formed the majority of symptomatic uncomplicated peptic ulcer patients and were significantly less common in the asymptomatic bleeding peptic ulcer group. Female gender has been shown to be associated with delayed gastric emptying (Hermansson and Sivertsson 1996; Mearadji, Penning et al. 2001). Differences in the gender mix of the ulcer groups might therefore partly explain the reason why the differences in gastric emptying became attenuated between BPU and uPUD groups after adjusting for age, gender and BMI. However, there was no significant difference in gender between HC and patients with uPUD and yet patients with healed uPUD had significantly delayed gastric emptying compared with healthy controls after adjusting for age, gender and BMI.

After 8 weeks of treatment, *H. pylori* was eradicated in 92% of patients with BPU and 75% of patients with uPUD. Although 1 BPU, 1 uPUD and 3 HC had *H. pylori* infection on a study day, it has been shown that *H. pylori* has no influence on gastric emptying (Perri, Clemente et al. 1998; Saslow, Thumshirn et al. 1998; Chiloiro, Russo et al. 2001) or gastric sensation (Mearin, de Ribot et al. 1995; Saslow, Thumshirn et al. 1998).

This study has other unavoidable limitations. For example, we were not able to assess gastric emptying in patients with asymptomatic uncomplicated peptic ulcer, which surely occurs in the general population, as these patients do not seek

any medical attention and the ulcers may be found only serendipitously or when complications occur.

The findings of the study have implications for our understanding and management of peptic ulcer disease. It appears that patients with asymptomatic bleeding peptic ulcer share similarities with healthy controls. This could explain the failure of the incidence of BPU to fall over the past 20 years (Ruigomez, Garcia Rodriguez et al. 2000) despite better treatment and cure of ulcer disease with proton pump inhibitors and eradication of *H. pylori*. Of major interest, patients with symptomatic uncomplicated peptic ulcers appear to share similarities with patients with functional dyspepsia, including visceral hypersensitivity and delayed gastric emptying. Thus more effective identification, elimination and/or management of risk factors will remain essential strategies for reducing the incidence of peptic ulcer complications.

In conclusion, even after ulcer healing, in addition to alterations of visceral sensory function, patients with a history of uncomplicated and ulcer symptoms have significantly delayed gastric emptying compared with healthy controls and patients with asymptomatic bleeding peptic ulcer. Abnormal gastric motor function may contribute to dyspeptic symptom in patients with uPUD as they still continue to have dyspeptic symptom after the ulcer had healed.

Table 1: Demographics, characteristics of ulcers and symptoms reported by questionnaires in healthy controls, patients with uncomplicated peptic ulcer disease, and bleeding peptic ulcer at the time of diagnosis.

	Healthy controls (n=15)	uPUD (n=10)	BPU (n=17)	p value (uPUD vs BPU)
Symptomatic (n (%))	0 (0)	10 (100)	1 (6)	
Asymptomatic (n (%))	100 (100)	0 (0)	16 (94)	<0.0001
Gender (M/F)	10 /5	3/7	16/1	0.001
Age (yrs)	59.6 ± 1.4	50.9 ± 3.8	65.9 ± 2.2	0.001
BMI (kg/m ²)	28.7 ± 1.7	31 ± 2.4	26.5 ± 1.1	ns
Ulcer type				
(GU/DU/GU+DU)	NA	8/1/1	6/7/4	ns
NSAID usage	0	1	8	ns
<i>H. pylori</i> infection	3	4	12	ns
<i>H. pylori</i> infection (on the study day)	3	1	1	ns
Smoking	0	2	6	ns
Number of ulcers				
- Single / Multiple		5/5	9/8	ns
- Mean number	NA	1.8 ± 0.3	1.8 ± 0.3	ns
Size of ulcer				
- <1cm / ≥1cm		9/1	10/7	ns
- Mean (cm)	NA	0.4 ± 0.1	0.9 ± 0.1	ns

Table 2: Symptoms reported by uncomplicated and bleeding peptic ulcer patients after ulcer healing on the study day

	Healthy controls (n=15)	uPUD (n=10)	BPU (n=17)	p value (uPUD vs BPU)
GIS (previous 1wk)	0.1 ± 0.1	9.2 ± 1.6	4.2 ± 1.6	0.018
NDI (previous 2wks)				
- Pain	0	4.2 ± 1.5	0.9 ± 0.6	0.046
- Discomfort	0	4.8 ± 1.4	1.2 ± 0.6	0.018
- Burning	0	1.6 ± 0.9	0.6 ± 0.4	ns
- Cramping	0.2 ± 0.2	2.1 ± 1.1	0.6 ± 0.5	ns
- Fullness	0.3 ± 0.2	4.5 ± 1.3	1.3 ± 0.5	ns
- Pressure	0	3.7 ± 1.3	0.2 ± 0.2	0.015
- Bloating	0.1 ± 0.1	6.5 ± 1.5	0.9 ± 0.5	0.002
- Interference daily activities/work	100	85.2 ± 5.1	91.6 ± 4.1	ns
BDQ (previous 12 months)				
- Severity of abd. pain	0.1 ± 0.1	2.9 ± 0.4	0.8 ± 0.3	0.01
- Frequency of abd pain per wk	0.1±0.1	3.2±0.7	1.1±0.4	0.013
HADS score				
- Anxiety	3.2 ± 0.6	6.1 ± 1.3	3.7 ± 0.7	ns
- Depression	2.5 ± 0.5	3.4 ± 1.1	3.8 ± 0.8	ns

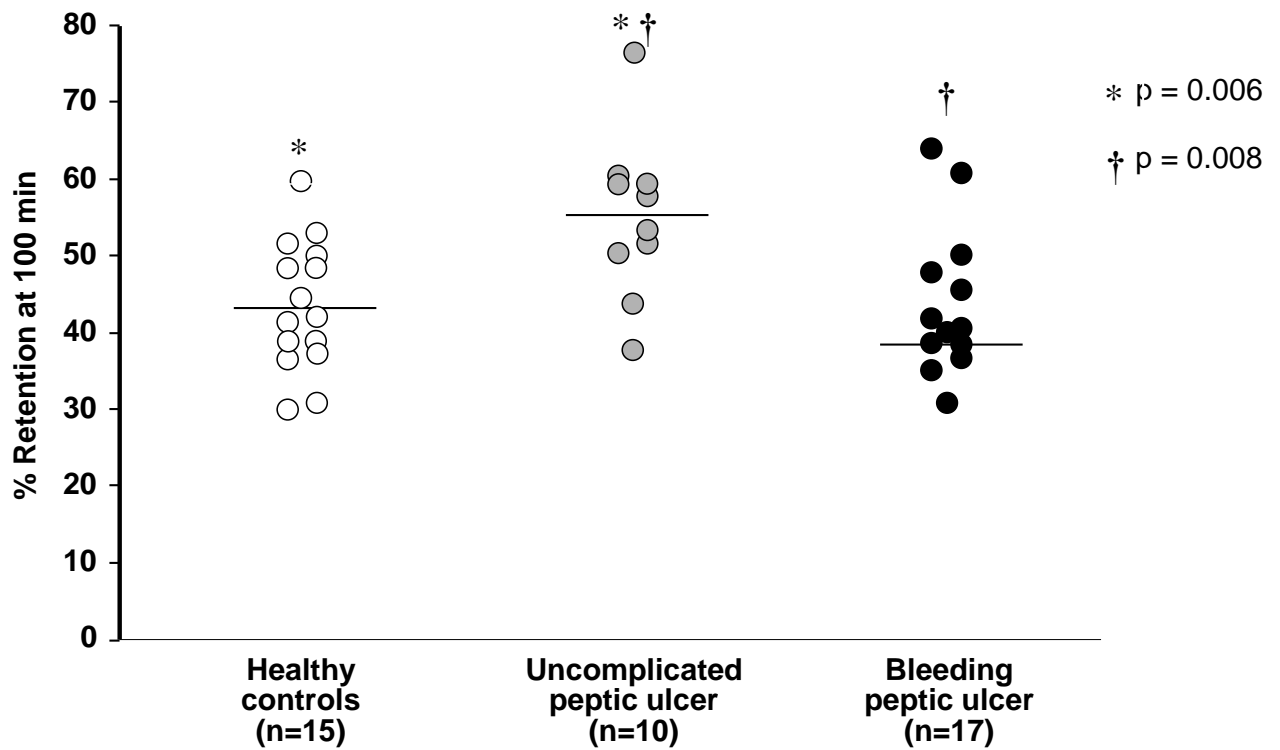


Figure 1: Gastric emptying of a nutrient liquid from the total stomach among healthy controls, patients with uncomplicated peptic ulcer and bleeding peptic ulcer (* = p value between uncomplicated peptic ulcer vs healthy controls; † = p value between uncomplicated peptic ulcer vs bleeding peptic ulcer).

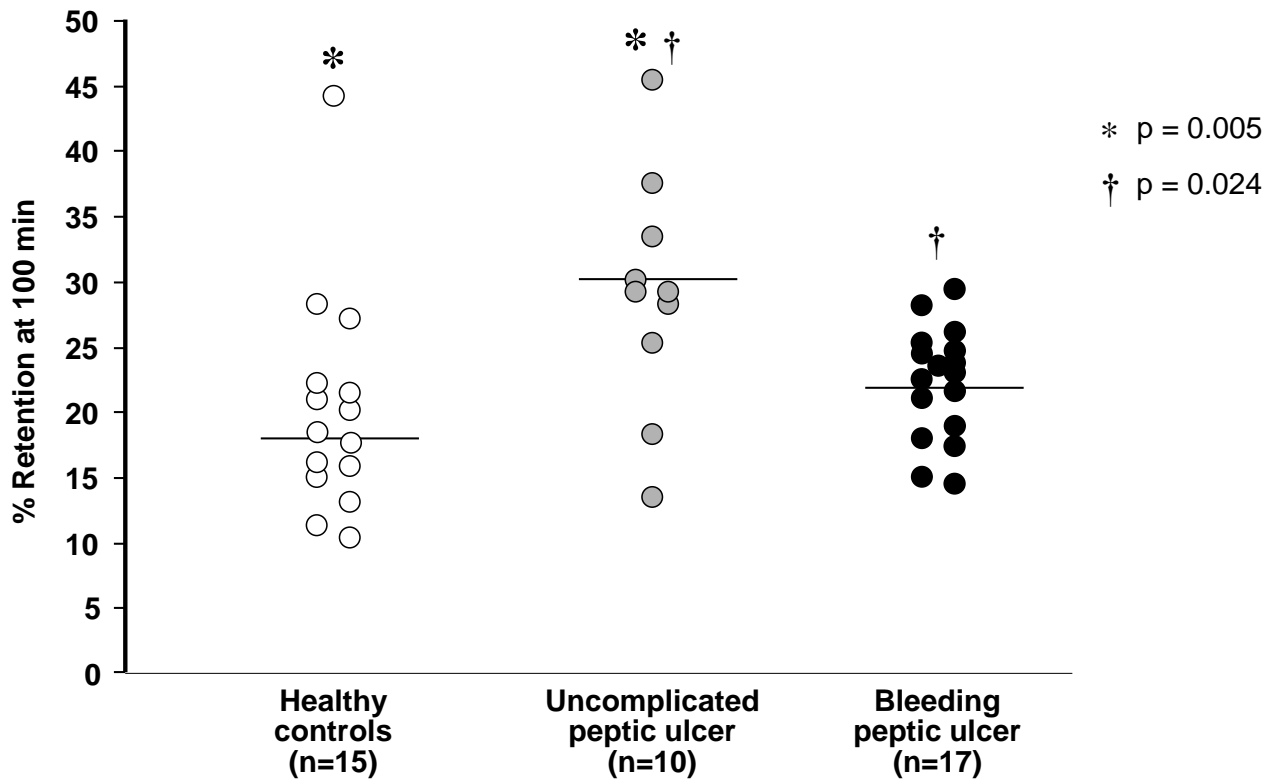


Figure 2: Gastric emptying of a nutrient liquid meal from the proximal stomach among healthy controls, patients with uncomplicated peptic ulcer and bleeding peptic ulcer (* = p value between uncomplicated peptic ulcer vs healthy controls; † = p value between uncomplicated peptic ulcer vs bleeding peptic ulcer).

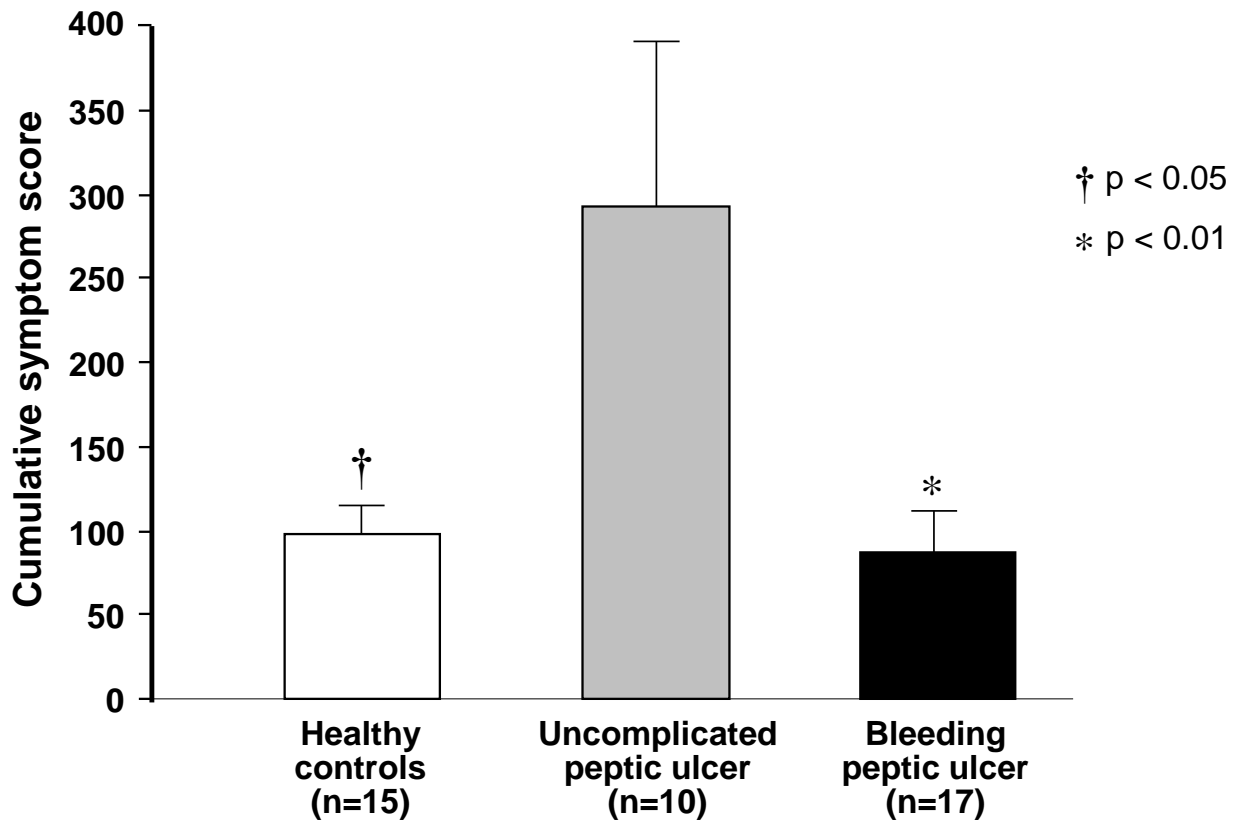


Figure 3: Cumulative symptom response to a standardised nutrient challenge test among healthy controls (HC), patients with uncomplicated peptic ulcer (uPUD) and bleeding peptic ulcer (BPU). Data presented as mean + SEM. († p<0.05: healthy controls vs uncomplicated peptic ulcer; * p<0.01: uncomplicated peptic ulcer vs bleeding peptic ulcer).

CHAPTER 14: IMMUNE ACTIVATION AND CLINICAL MANIFESTATION OF PEPTIC ULCER DISEASE

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14.1 INTRODUCTION

Peptic ulcer disease (PUD) usually manifests as either dyspepsia or life threatening complications such as bleeding and perforation (Linder and Wilcox 2001). We have shown previously that most of patients with uPUD have dyspeptic symptoms, in contrast, majority of patients with bleeding peptic ulcer (BPU) are asymptomatic (Gururatsakul, Holloway et al. 2010). The mechanism of ulcer pain is still unclear. However, several factors including older age (Hernandez-Diaz and Rodriguez 2002; Targownik and Nabalamba 2006) and non-steroidal anti-inflammatory drugs (NSAIDs) (Clinch, Banerjee et al. 1984; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997; Hilton, Iman et al. 2001) have been associated with silent peptic ulceration. We have shown recently that patients with uPUD have an augmented symptom response to a standardised nutrient challenge and delayed gastric emptying compared with patients with BPU and healthy controls (HC) (Gururatsakul, Bellon et al. 2008; Gururatsakul, Holloway et al. 2010).

Immune activation may predict the abdominal symptom pattern and severity as a result of the intensity and duration of an acute inflammation is associated with the development of post inflammatory abdominal symptoms (Adam, Liebrechts et al. 2006). Recent studies have shown that systemic immune activation (cytokine production) was enhanced and associated with abdominal symptoms in patients

with irritable bowel syndrome (IBS) (Liebregts, Adam et al. 2007) and functional dyspepsia (Liebregts, Adam et al. 2008). Thus, immune activation might be associated with abdominal symptoms in patients with PUD or even healthy people. The aim of this study, therefore, was to study the link between immune activation and clinical manifestation of patients with PUD. Proinflammatory cytokine production was shown to be associated with anxiety in patients with IBS (Liebregts, Adam et al. 2007). We, therefore, also aimed to explore the link between anxiety or depression and the release of inflammatory cytokine.

14.2 SUBJECTS AND METHODS

14.2.1 Patients and healthy subjects

The selection criteria of patients with BPU, uPUD and healthy control have been described in the methodology section. Twenty three patients with BPU (19 men, mean age 66.2 ± 1.3 years), 20 patients with uPUD (12 men, mean age 52.2 ± 3.6 years) and 20 healthy asymptomatic volunteers (14 men, mean age 59 ± 1.8 years) were recruited for participation in this study.

14.2.2 Protocol

The protocol has been described in Chapter 11, section 11.2.2. At least 8 weeks after diagnosis and after healing of gastric ulcer was confirmed by endoscopy, the subjects underwent the study tests at one visit. First, the subjects were asked to complete symptoms questionnaires. Second, subjects were tested for *H. pylori* by ¹⁴C urea breath test. Third, subjects were taken blood sample. Subjects who had *H. pylori* positive on ¹⁴C urea breath test were excluded.

14.2.3 Assessment of gastrointestinal symptoms

The presence and severity of gastrointestinal symptoms and psychiatric comorbidities were assessed utilising validated questionnaires: the Gastrointestinal Symptom (GIS) score (Adam, Liebrechts et al. 2005), the Bowel Disease Questionnaire (BDQ) (Talley, Phillips et al. 1990; Talley, Boyce et al. 1995), the Nepean Dyspepsia Index (NDI) (Talley, Haque et al. 1999; Talley, Verlinden et al. 1999), and the Hospital Anxiety and Depression Scale (HADS) (Herrmann 1997), as has been described in methodology section.

14.2.4 Peripheral Blood Mononuclear Cell Isolation and Cell Culture

Isolation of Peripheral blood mononuclear cells (PBMCs) and cell culture have already been described in methodology section 9.9 and 9.10.

14.2.5 Enzyme-Link Immunosorbent Assay (ELISA)

Cell-free supernatant fractions were collected and stored at -80 °C until assayed. TNF- α , IL-1 β , IL-6, and IL-10 were quantified using ELISA kits (BD Bioscience, San Diego, CA, USA) according to the manufacturer's instructions. The detail of ELISA has been described in methodology section.

14.2.6 Statistical analysis

Proportions and 95 % confidence intervals for patients with and without symptoms were calculated. Univariate associations with symptoms were initially explored by the chi-square test with the Yates correction for continuity, where appropriate. Analysis of variance was used to determine differences in cytokine levels among patients with uPUD, BPU and HC. Additionally, analysis of covariance adjusting for age, gender and BMI was used to compare cytokine production among 3 groups. A p value ≤ 0.05 was considered significant. Data are

present as mean \pm standard error of the mean. In a second step, the correlation between cytokine productions and abdominal symptoms, anxiety and depression score reported from questionnaires was analysed by Pearson correlation test. For the statistical analysis SAS Version 6.12 (SAS Institute Inc., Cary, North Carolina, USA) and SPSS Version 12 (Statistical Package for Social Sciences, SPSS Inc., Chicago, Illinois, USA) were used.

14.3 RESULTS

14.3.1 Demographics

Eighteen of the 23 patients with BPU who participated this study were asymptomatic prior the bleeding event. In contrast, all patients with uPUD presented with abdominal pain ($p < 0.001$, table 1). There was no significant difference in gender between HC, uPUD and BPU. Patients with BPU were significantly older than patients with uPUD ($p < 0.001$). However, there was no significant difference in mean age between HC and uPUD or BPU. All subjects were confirmed to be *H. pylori* negative.

14.3.2 Symptom profiles

After at least 8 weeks of treatment of the ulcer, and confirmation of healing of gastric ulcers, patients with uPUD reported significantly higher symptom scores on the GIS questionnaire than did patients with BPU ($p=0.037$, Table 1) and HC ($p=0.001$). Patients with uPUD also reported significantly higher levels of abdominal symptoms in the past 2 weeks on the NDI questionnaire (discomfort and bloating in upper abdomen: $p=0.05$). In terms of quality of life affected by dyspepsia, patients with uPUD had significantly poorer quality of life affected by abdominal symptoms than patients with BPU ($p=0.01$). On the BDQ, uPUD patients reported significantly more severe and more frequent abdominal pain than did BPU patients ($p<0.01$).

Healthy volunteers reported very few symptoms on all questionnaires and significantly less than uPUD patients ($p<0.01$). They also reported fewer symptoms on all questionnaires than BPU patients; however, the difference did not reach statistical significance. There was no significant difference in anxiety and depression scale among the groups.

14.3.3 Cytokine production

Patients with uPUD released significantly higher TNF- α , IL-1 β and IL-6 than did patients with BPU and HC (Figure 1 and 2), while there was no significant difference between patients with BPU and HC. The significant difference in TNF- α , IL-1 β and IL-6 remained when adjusted for age, gender and BMI. However, there was no significant difference in IL-10 production among the groups (Figure 2).

14.3.4 Asymptomatic vs symptomatic ulcers

Patients were also grouped into those with and without dyspeptic symptoms, irrespective of whether the ulcer had bled. More than 85% of patients with asymptomatic peptic ulcer were male, compared with 60% of those with symptomatic ulcers ($p < 0.082$). Asymptomatic patients were significantly older than patients who experienced dyspeptic symptoms ($p < 0.01$).

Symptomatic peptic ulcer patients (25/43) had significantly higher TNF- α , IL-1 β and IL-6 production than did asymptomatic ulcer patients (18/43) (TNF- α : $p = 0.037$; IL-1 β : $p = 0.029$; IL-6: 0.015). However, there was no significant difference in IL-10 production between the two groups.

14.3.5 Association between cytokine levels, abdominal symptoms, and psychiatric comorbidity

Overall, TNF- α , IL-1B and IL-6 production was significantly associated with anxiety and gastrointestinal symptoms. Proinflammatory cytokine TNF- α , IL-1 β , and IL-6 production was significantly correlated with GIS score, severity and frequency of abdominal pain, on the BDQ, and NDI (table 2). In addition, TNF- α , IL-1 β and IL-10 production was significantly associated with anxiety score, while there was no significant association with depression. However, there was no significant association between anti-inflammatory cytokine IL-10 production and gastrointestinal symptoms and depression.

Among patients, TNF- α , IL-1B, and IL-6 production was significantly correlated with severity of abdominal symptoms on GIS score (TNF- α : $r=0.42$, $p=0.013$; IL-1B: $r=0.53$, $p=0.001$; IL-6: $r=0.36$, $p=0.033$), BDQ (TNF- α : $r=0.52$, $p=0.001$; IL-1B: $r=0.46$, $p=0.004$; IL-6: $r=0.47$, $p=0.004$), and anxiety score (TNF- α : $r=0.41$, $p=0.015$; IL-1B: $r=0.33$, $p=0.046$; IL-6: $r=0.29$, $p=0.082$). There was no significant association between IL-10 and gastrointestinal symptoms and anxiety or depression.

14.4 DISCUSSION

The observation that the majority of patients with an acute bleeding peptic ulcer are asymptomatic until the life threatening severe bleeding occurs remains a hitherto unexplained yet potentially important observation (Coggon, Langman et al. 1982). This study is the first to demonstrate an association between systemic cellular immune activation and symptom manifestation in patients with peptic ulcer disease. While all of the patients with uPUD presented with peptic ulcer symptoms, most of the patients with BPU were asymptomatic. The important finding of this study is increased levels of systemic proinflammatory cytokines TNF- α , IL-1 β , and IL-6 in patients with uPUD, despite the ulcer had healed, compared with patients with BPU and HC. Findings are consistent with previous studies which showed the increased level of immune activation in patients with functional gastrointestinal disorders (Liebregts, Adam et al. 2007; Liebregts, Adam et al. 2008; Kindt, Van Oudenhove et al. 2009). While patients with bleeding peptic ulcer had similar cytokine production to healthy controls, we have shown previously that patients with a history of symptomatic uPUD had an augmented symptom response to the standardised nutrient challenge test (Gururatsakul, Holloway et al. 2010) and delayed gastric emptying (Gururatsakul, Bellon et al. 2008) compared with patients with BPU and HC, suggesting that patients with uPUD share similarities with patients with functional dyspepsia. Thus, the findings from this study provide an additional potential explanation for this phenomenon, and may help to explain the different clinical presentation

between patients with uPUD and BPU as patients with uPUD tend to have earlier diagnosis from small ulcer but higher demand of early endoscopy from severe symptoms.

The findings also show the association between proinflammatory cytokine production and severity of abdominal symptoms across all subjects. These findings are consistent with previous study from our group which showed the correlation between immune activation and gastrointestinal symptoms in patients with functional dyspepsia (Holtmann, Gschossmann et al. 2001; Liebrechts, Adam et al. 2008). Previous studies also showed that the level of IL-1 β and IL-6 from PBMC reflect disease activity in inflammatory bowel disease (Nakamura, Saito et al. 1992; Holtkamp, Stollberg et al. 1995). Therefore, the findings give further support to the link between immune activation and abdominal symptoms. Therefore, might be minor inflammation in the stomach, which may explains why patients with uPUD still have abdominal symptoms even though the ulcer had healed. Furthermore, minor inflammation may explain why patients with healed uPUD had visceral hypersensitivity response to the standardised nutrient challenge test in the previous study (Gururatsakul, Holloway et al. 2010), as inflammation may cause visceral hypersensitivity in animal models (Adam, Liebrechts et al. 2006).

Our study also shows that after ulcer healing, or treatment that would reasonably be expected to have healed the ulcer, patients with uPUD continued to report

symptoms that were more severe than those in patients with BPU. Persistence symptoms after healing of ulcers might be the result of post inflammatory hyperalgesia, as occurs in an animal model of transient colitis (Adam, Liebrechts et al. 2006). In addition, although the ulcer had healed, enhanced immune activation may reflect minor inflammatory process and explain the persistent symptoms in patients with uPUD.

The cytokine level we measured were base line cytokine which has not been stimulated by bacteria such as *H. pylori* or *E. coli* lipopolysachharide, this may explain why the differences were not obvious among the groups although there were statistically significant differences.

The findings show the association between psychological stress, anxiety, and level of proinflammatory (TNF- α and IL-1 β) and IL-10 production. These findings are consistent with our previous study in IBS patients which demonstrated the association between TNF- α and anxiety score (Liebrechts, Adam et al. 2007). Psychological stress and anxiety may potentially stimulate cytokine production (Maes, Song et al. 1998; Kaestner, Hettich et al. 2005). In addition, psychological factors, especially anxiety, are associated with gastrointestinal symptoms in patients with functional gastrointestinal disorders (Mayer, Craske et al. 2001) and in the general population (Haug, Mykletun et al. 2002). In contrast, it has been shown that immune activation (IL-6) can activate hypothalamic-pituitary-adrenal axis (Spath-Schwalbe, Born et al. 1994), which consequently

may modulate psychological symptoms (Rybakowski and Twardowska 1999). Therefore, it is controversial whether cytokine modulate psychological symptoms or vice versa.

Immune activation is generally enhanced in patients with chronic inflammatory diseases, especially rheumatoid arthritis (Kutukculer, Caglayan et al. 1998). In addition, *H. pylori* infection is associated with increased cytokine production (Lindholm, Quiding-Jarbrink et al. 1998). We thus excluded patients with known case chronic inflammatory diseases and *H. pylori* positive in this study.

As a result of IL-10 is an anti-inflammatory cytokine, this may explain why there was no association between IL-10 and gastrointestinal symptoms and clinical manifestation of PUD. However, the correlation between IL-10 production and anxiety score may be explained by the body system try to inhibit proinflammatory cytokine production.

This study has some unavoidable limitations. First, we were not able to measure cytokine production in patients with asymptomatic uPUD, which surely occurs in the general population, as these patients do not seek any medical attention until the complications occur. Second, we were unable to assess immune activation when the ulcers were active, but measure after the ulcer had healed, which may not be truly associated with clinical manifestation of peptic ulcer disease. However, there were number of patients with PUD had *H. pylori* positive at the

time of diagnosis, which may confound the cytokine production. In addition, there were number of patients with BPU had blood transfusion at admission, which may alter systemic immune activation. Finally, cytokine production we measured was from PBMCs, which indirectly reflect gastric immune response, however, systemic cytokine production has been shown to reflect disease activity in inflammatory bowel disease (Nakamura, Saito et al. 1992).

In conclusion, proinflammatory cytokine production is linked to the clinical manifestation of peptic ulcer disease. Also there is an association between immune activation and abdominal symptoms and anxiety.

Table 1: Demographics and symptom reported by questionnaires in healthy controls, patients with uncomplicated peptic ulcer disease, and bleeding peptic ulcer on the study day.

	Healthy controls (n=20)	uPUD (n=20)	BPU (n=23)	p value (uPUD vs BPU)
Symptomatic (n (%))		20 (100)	5 (22)	
Asymptomatic (n (%))	NA	0 (0)	18 (78)	<0.0001
Gender (M/F)	14/6	12/8	19/4	ns
Age (yrs)	59 ± 1.8	52.2 ± 3.6	66.2 ± 1.3	0.01
GIS (1wk)	0.1 ± 0.1	6.7 ± 1.4	3.3 ± 1.1	0.037
NDI (2wks)				
- Discomfort	0	4.1 ± 0.9	1.8 ± 0.8	0.05
- Bloating	0.1 ± 0.1	3.6 ± 0.9	1.3 ± 0.7	0.05
- Interference daily	99.9 ± 0.1	86.8 ± 4.1	93.7 ± 3.4	0.013
BDQ (12 months)				
- Severity of abd. pain	0.1 ± 0.1	2.6 ± 0.4	1.1 ± 0.3	0.01
- Frequency of abd pain	0.3 ± 0.2	3.3 ± 0.5	1.2 ± 0.4	0.01
per wk HADS score				
- Anxiety	4.7 ± 0.7	6.3 ± 1.1	4.5 ± 0.8	ns
- Depression	2.4 ± 0.4	3.9 ± 0.7	4.2 ± 0.8	ns

Table 2: Correlation of TNF- α , IL-1 β , IL-6 and IL-10 with abdominal symptoms and anxiety and depression score across all subjects (* p<0.05, ** p<0.01).

	GIS	Severity of abd pain (BDQ)	Frequency of abd pain (BDQ)	Abd pain (NDI)	Abd discomfort (NDI)	Abd cramp (NDI)	Anxiety (HADS)	Depression (HADS)
TNF-α	r=0.432 p=0.001**	r=0.502 p=0.001**	r=0.363 p=0.004**	r=0.233 p=0.090	r=0.300 p=0.027*	r=0.318 p=0.019*	r=0.400 p=0.003**	r=0.188 p=0.173
IL-1β	r=0.517 p=0.001**	r=0.449 p=0.001**	r=0.301 p=0.024*	r=0.365 p=0.007**	r=0.334 p=0.015*	r=0.425 p=0.001**	r=0.311 p=0.020*	r=0.136 p=0.316
IL-6	r=0.403 p=0.002**	r=0.489 p=0.001**	r=0.352 p=0.008**	r=0.241 p=0.086	r=0.304 p=0.028*	r=0.237 p=0.091	r=0.259 p=0.065	r=0.110 p=0.426
IL-10	r=0.241 p=0.068	r=0.208 p=0.118	r=0.149 p=0.264	r=0.493 p=0.001**	r=0.401 p=0.002**	r=0.510 p=0.001**	r=0.313 p=0.017*	r=0.193 p=0.147

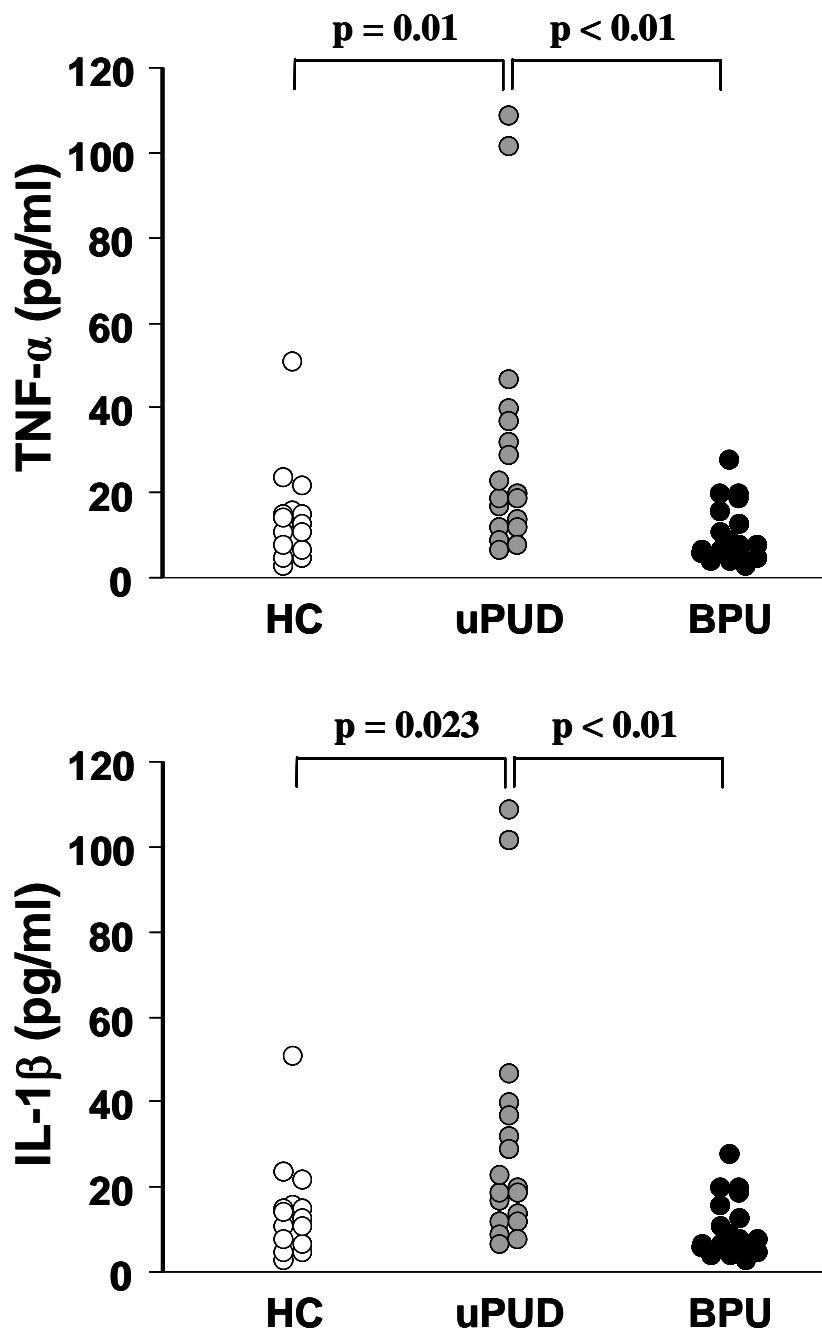


Figure 1: TNF- α and IL-1 β production (pg/ml) by PBMC from health controls (HC), uncomplicated peptic ulcer (uPUD), and bleeding peptic ulcer (BPU).

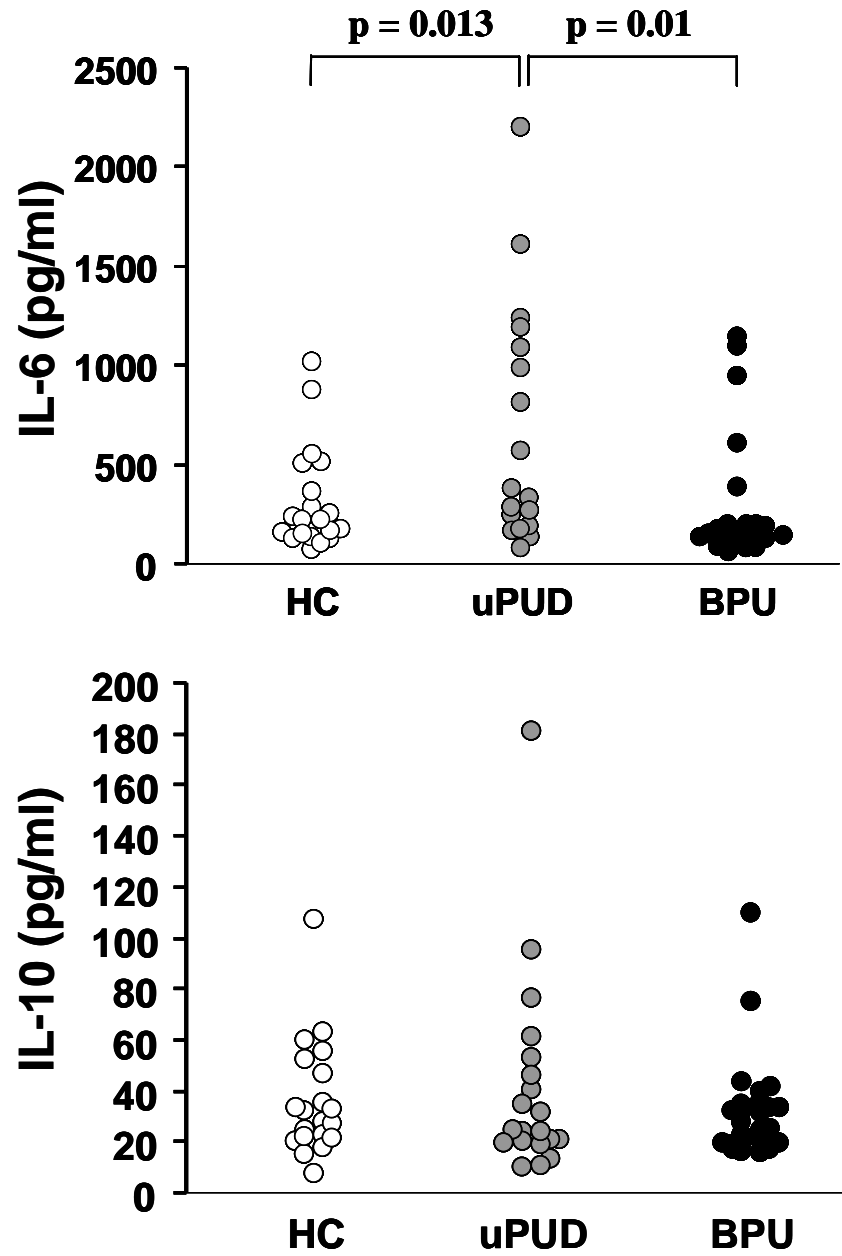


Figure 2: IL-6 and IL-10 production (pg/ml) by PBMC from health controls (HC), uncomplicated peptic ulcer (uPUD), and bleeding peptic ulcer (BPU).

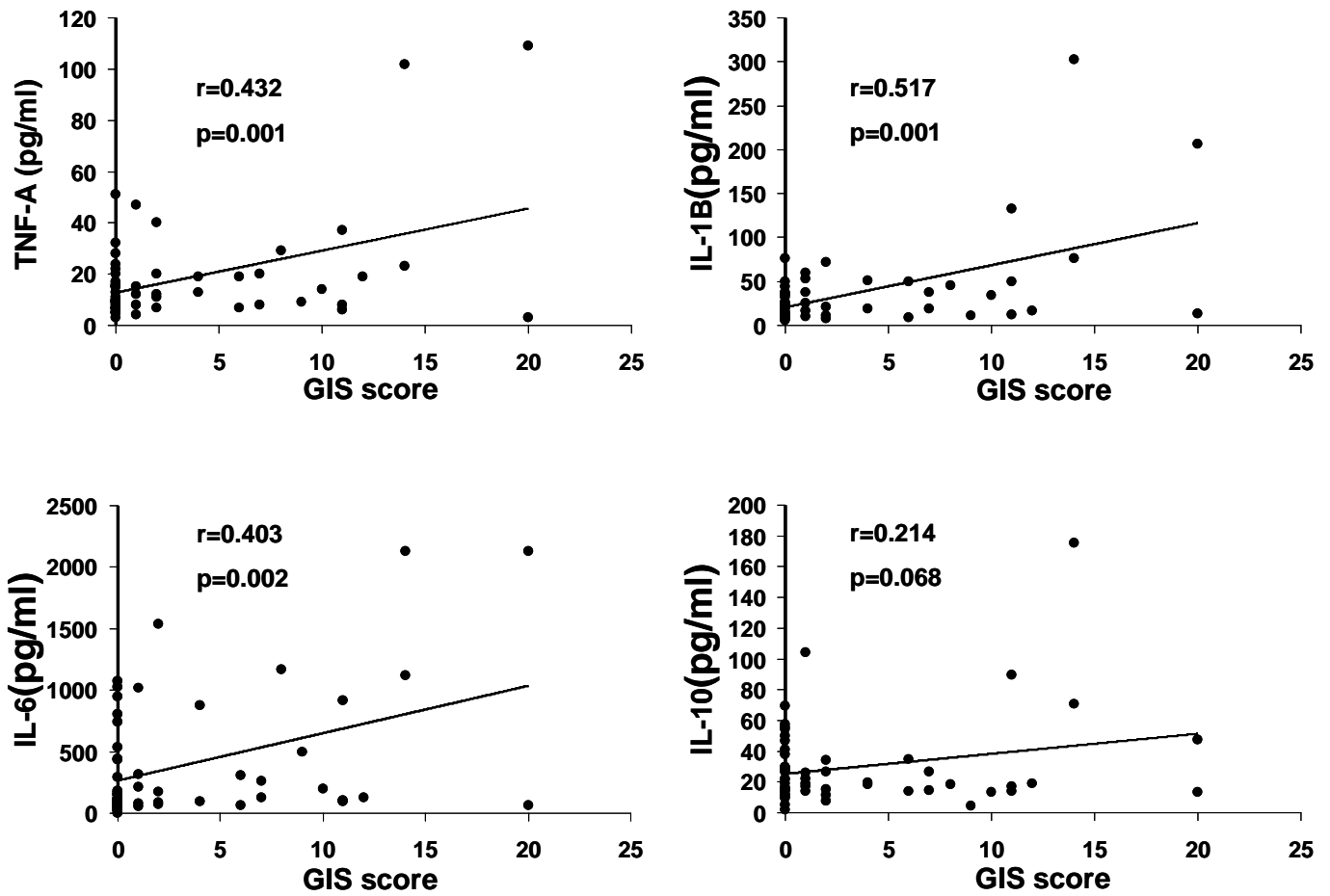


Figure 3: A significant correlation between TNF- α , IL-1 β , IL-6 and IL-10 production (pg/ml) and gastrointestinal symptom scores reported by GIS.

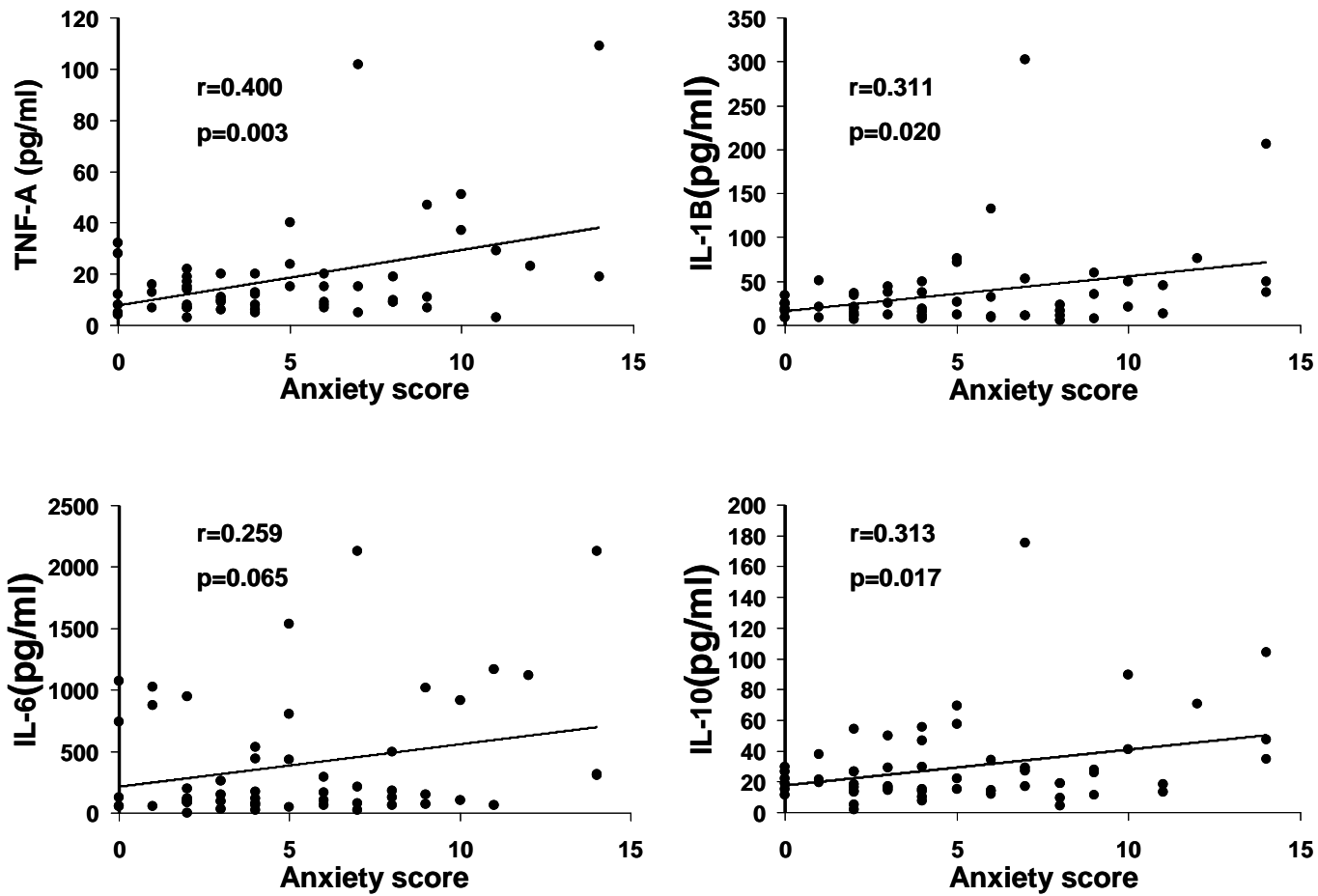


Figure 4: A significant correlation between TNF- α , IL-1 β , IL-6 and IL-10 production (pg/ml) and anxiety score assessed by Hospital Anxiety and Depression Scale.

**CHAPTER 15: SYMPTOMATIC UNCOMPLICATED
PEPTIC ULCER DISEASE: INPART FUNCTIONAL
DYSPEPSIA?**

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15.1 INTRODUCTION

We have previously reported lower sensory thresholds (Chapter 12) and delayed gastric emptying (Chapter 13) in patients with uPUD compared to both patients with BPU and healthy controls, features more typically associated with functional dyspepsia. In previous studies, we have also reported that 8 weeks after ulcer healing patients with uPUD continued to report symptoms that were more severe than those in patients with BPU (Chapter 12 and 13). However, the long term report symptoms in patients with uPUD and BPU is not known. Therefore, the aim of this study is to compare the symptoms reported in patients with uPUD compared with BPU after 1 year of ulcer healing. We hypothesised that 1 year after ulcer healing and *H. pylori* eradication patients with uPUD are more likely to have ongoing dyspeptic symptoms compared to patients with BPU.

15.2 SUBJECTS AND METHOD

The Gastrointestinal Symptom (GIS) score (Adam, Liebrechts et al. 2005), the Bowel Disease Questionnaire (BDQ) (Talley, Phillips et al. 1990; Talley, Boyce et al. 1995), the Nepean Dyspepsia Index (NDI) (Talley, Haque et al. 1999; Talley, Verlinden et al. 1999), and the Hospital Anxiety and Depression Scale (HADS) (Herrmann 1997) questionnaires were sent out to patients with

uncomplicated and bleeding peptic ulcer 12 months after diagnosis and treatment. After the diagnosis of peptic ulcer disease, *Helicobacter pylori* eradication was performed where *H. pylori* positive. At diagnosis of peptic ulcer disease, symptomatic status was determined by case note review. Seventeen patients (6 uPUD, 11 BPU) had been included in the earlier study of symptoms and PUD (Chapter 12).

15.2.1 Assessment of gastrointestinal symptoms

The presence and severity of gastrointestinal symptoms and psychiatric comorbidities at diagnosis and 12 months after presentation and treatment were assessed utilising validated questionnaires: the Gastrointestinal Symptom (GIS) score (Adam, Liebrechts et al. 2005), the Bowel Disease Questionnaire (BDQ) (Talley, Phillips et al. 1990; Talley, Boyce et al. 1995), the Nepean Dyspepsia Index (NDI) (Talley, Haque et al. 1999; Talley, Verlinden et al. 1999), and the Hospital Anxiety and Depression Scale (HADS) (Herrmann 1997), as have been described in methodology chapter.

15.2.2 Statistical analysis:

The proportion of patients with and without upper gastrointestinal symptoms, and psychological co-morbidity (ies) were calculated at baseline and after 12 months. Proportions and 95 % confidence intervals for patients with and without symptoms were calculated. Comparison were made between groups and between time-points using contingency tables with a $p < 0.05$ regarded as significant. Symptoms reported were initially explored by the chi-square test with the Yates correction for continuity where appropriate. Symptoms reported on questionnaires were then explored by one-way ANOVA. p values ≤ 0.05 were considered significant. Data are present as mean \pm standard error of the mean. For the statistical analysis SAS Version 6.12 (SAS Institute Inc., Cary, North Carolina, USA) and SPSS Version 12 (Statistical Package for Social Sciences, SPSS Inc., Chicago, Illinois, USA) were used.

15.3 RESULTS

15.3.1 Demographics

Questionnaires were sent out to 64 patients with uPUD and 117 patients with BPU 12 months after diagnosis and treatment. Forty four patients completed the questionnaires and were available for analysis (Table 1), including 16 patients

with uPUD (9 males, mean age 59.4 ± 3 yr) and 28 BPU (19 males, mean age 63.7 ± 2 yr). Of these, 6 patients with uPUD and 11 BPU had been included in the previous study of symptoms and PUD (Chapter 12).

Out of 16 patients with uPUD, 9 patients had gastric ulcers, 6 patients had 6 duodenal ulcers, and 1 patient had both gastric and duodenal ulcer at diagnosis. Out of 28 patients with BPU, 19 patients had gastric ulcer, 8 patients had duodenal ulcer, and 1 patient had both gastric and duodenal ulcer.

Nine out of 16 patients with uPUD were taking Proton Pump Inhibitors (PPI) after 12 months of diagnosis of peptic ulcer disease, while 8 out of 28 patients with BPU were continuously taking PPI after 12 months.

15.3.2 Symptomatic status at diagnosis

At the time of diagnosis, 14 out of 16 patients with uPUD presented had dyspeptic symptoms compared with only 6 out of 28 patients with BPU ($p<0.001$, Table 2). Patient who symptomatic were significantly younger than asymptomatic peptic ulcer patients ($p=0.004$). Eleven out of 20 patients (55%) with symptomatic peptic ulcer at diagnosis were taking PPI, while 6 out of 24 asymptomatic patients (25%) were taking PPI.

15.3.3 Symptomatic status 12 months after ulcer healing

Fifteen out of 20 patients who had dyspeptic at diagnosis had ongoing symptoms after 12 months of treatment with peptic ulcer disease.

After 12 months, 9 out of 14 patients with symptomatic uPUD and 6 out of 6 patients with originally symptomatic BPU reported persistent symptoms, whereas symptoms had resolved in 5 uPUD ($p < 0.02$, table 3). New symptoms did not develop in any asymptomatic patient after healing. Thus a greater proportion (9/16) of patients with uPUD had persistent symptoms than patients with BPU (6/28, $p = 0.02$). Ten out of 15 patients with persistent symptoms were taking PPI, while 7 out of 29 patients with asymptomatic ulcer were taking PPI after 12 months.

15.3.4 Impact of age on symptomatic status

At diagnosis, when patients were divided into symptomatic and asymptomatic ulcer, patients with dyspeptic symptoms were significantly younger than asymptomatic patients (mean 57 vs 67 yrs, $p = 0.004$, table 2). After 12 months follow up, patients with persistent symptoms remained significantly younger than symptom-free patients (57 vs 65, $p = 0.018$).

15.3.5 Impact of anxiety and depression

Patients with persistent symptoms reported significantly higher scores for anxiety (9.2 ± 1.4 vs 3.8 ± 0.6 , $p=0.002$) and depression (7.2 ± 1.1 vs 3 ± 0.6 , $p=0.003$) compared with asymptomatic patients. In addition, patients with persistent symptoms also reported significantly impaired quality of life affected by dyspeptic symptoms ($p < 0.001$) compared with asymptomatic patients.

15.4 DISCUSSION

The observation that the majority of patients with an acute bleeding peptic ulcer are asymptomatic until the life threatening severe bleeding occurs remains a hitherto unexplained yet potentially important observation (Coggon, Langman et al. 1982). Data on the prevalence of ulcer symptoms prior to ulcer bleeding are few. The proportion of patients without symptoms has been reported to range from 43%-87% (Coggon, Langman et al. 1982; Mellem, Stave et al. 1985; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997). We have shown the proportion of asymptomatic bleeding peptic ulcer, and asymptomatic uncomplicated peptic ulcer disease in a large cohort in the previous retrospective study (Chapter 10). Our study shows that the majority of patients with BPU are

asymptomatic at the time of diagnosis and presentation. In contrast, the majority of patients with uPUD usually present with abdominal pain.

The previous study (Chapter 12) showed that after ulcer healing, or treatment that would reasonably be expected to have healed the ulcer, 48% of patients with uPUD continued to report symptoms that were more severe than those in patients with BPU. The present study extends these observations and shows that 12 months after treatment of peptic ulcer disease, including *H. pylori* eradication and taking PPI 12 months, 56% of patients with uPUD, and 64% of patients with originally symptomatic peptic ulcer disease continued to have symptoms.

This is the first study to report the symptomatic status of patients with complicated and uncomplicated peptic ulcer after 12 months of follow-up. Of the patients who had dyspeptic symptoms at the time of diagnosis, irrespective whether the ulcer had bled, 75% continued to have dyspeptic symptoms after 12 months unless the symptoms were the result of recurrent ulcers, unlikely since risk factors were eliminated, persistent symptoms suggest that the patients may have had underlying functional dyspepsia and that perhaps this was the cause of the symptoms, not the ulcer found at endoscopy. Persistence symptoms after healing of ulcers might be the result of post inflammatory hyperalgesia, as occurs in an animal model of transient colitis (Adam, Liebrechts et al. 2006). Moreover, persistence of symptoms after healing of ulcers might be the result of visceral hypersensitivity or delayed gastric emptying as was shown in the previous studies

(Gururatsakul, Holloway et al. 2010). In addition, patients with uncomplicated peptic ulcer disease had significantly and abnormally delayed gastric emptying compared with healthy controls and BPU, suggesting that different in gastric motor function may play an important role in clinical presentation of patients with peptic ulcer disease.

Patients with persistent symptoms reported significantly higher anxiety and depression scores compared with asymptomatic patients, and also reported significantly impaired quality of life affected by dyspeptic symptoms compared with asymptomatic patients. It would appear that patients with symptomatic peptic ulcers share similarities with patients with functional dyspepsia, including hyper-visceral sensitivity, delayed gastric emptying, and ongoing dyspeptic symptoms despite had peptic ulcer treatment and taking PPI. Up to 25-50% of patients with functional dyspepsia have delayed gastric emptying (Stanghellini, Tosetti et al. 1996; Maes, Ghooos et al. 1997; Sarnelli, Caenepeel et al. 2003; Tack, Bisschops et al. 2004) and 34-66% have gastric hypersensitivity (Tack, Bisschops et al. 2004) and Psychological factors, especially anxiety, are associated with gastrointestinal symptoms in patients with functional gastrointestinal disorders (Mayer, Craske et al. 2001).

Patients with symptomatic peptic ulcer were significantly younger than asymptomatic patients. These findings add further support to the notion that age may be one of the factors that determines dyspeptic symptoms in PUD (Clinch,

Banerjee et al. 1984; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997). Elderly subjects have been reported to exhibit a decreased symptom response to a standardised nutrient challenge test (Chapter 11) and gastric balloon distension (Rayner, MacIntosh et al. 2000), and older age is also associated with diminished visceral sensation in the oesophagus (Lasch, Castell et al. 1997) and rectum (Lagier, Delvaux et al. 1999).

This study has some unavoidable limitations. The patients who had ongoing dyspeptic symptoms may have pathological finding in their gastrointestinal system after 12 months period. However, patients who had gastric ulcers already had follow up endoscopy to confirm ulcer healing, while patients with duodenal ulcer were assumed to have ulcer healing after a course of treatment. Moreover, risk factor such as *H. pylori*, NSAIDs and aspirin were eliminated at the time of diagnosis.

We did not question the patients with peptic ulcer retrospectively whether how long they suffered from dyspepsia before they were diagnosed peptic ulcer disease. Knowing the time period of dyspepsia before presentation may add future support to the notion that patients with uncomplicated peptic ulcer disease share similarities with patients with functional dyspepsia.

In conclusion, most patients with dyspeptic symptoms prior to the diagnosis of peptic ulcer disease continue to have dyspeptic symptoms 12 months after ulcer

healing and *H. pylori* eradication. Patients with persistent dyspeptic symptoms have higher level of anxiety and depression score than patients without symptoms. The data suggest that most patients with symptomatic peptic ulcer disease have concomitant functional dyspepsia, which may have led to the diagnostic endoscopy being performed that probably prevented the development of a life threatening ulcer bleed.

Table 1: Demographics and symptoms in patients with uncomplicated peptic ulcer disease, and bleeding peptic ulcer.

	uPUD (n=16)	BPU (n=28)	p value (uPUD vs BPU)
Symptoms at diagnosis	14/16 (87.5%)	6/28 (21.4%)	0.001
Symptoms after 12 months	9/14 (64.3%)	6/6 (100%)	0.04
Symptoms after 12 months (total)	9/16 (56.3%)	6/28 (21.4%)	0.02
Symptoms improved	5/14 (35.7%)	0/6 (0%)	0.04
Age	59.4±2.9	63.7±2.1	0.225
On PPI	9/16 (56.3%)	8/28 (28.6%)	0.136
Male	9/16 (56.3%)	19/28 (67.9%)	0.657

Table 2: Demographics and symptoms reported by questionnaires in symptomatic and asymptomatic peptic ulcer disease patients at diagnosis.

	Symptoms at diagnosis (n=20)	No symptoms at diagnosis (n=24)	<i>p</i> value
Age	56.9±2.6	66.5±1.8	0.004
Male	11/20 (55%)	17/24 (70.8%)	0.44
Symptoms after 12 months	15/20 (75%)	0/24 (0%)	0.001
PPI	11/20 (55%)	6/24 (25%)	0.085
Anxiety score	7.6±1.2	3.8±0.8	0.013
Depression score	5.9±1	3.1±0.7	0.04
GIS	107±2.7	1±0.3	0.001
NDI – Interference	82.1±5.5	98.1±1.4	0.004

Table 3: Demographics and symptoms reported by questionnaires in symptomatic and asymptomatic peptic ulcer disease patients after 12 months.

	Symptoms after 12 months (n=15)	No symptoms after 12 months (n=29)	p value
Age	56.3±3.3	65±1.7	0.018
Male	9/15 (60%)	19/29 (66%)	0.976
PPI	10/15 (67%)	7/29 (24%)	0.016
Anxiety score	9.2±1.4	3.8±0.6	0.002
Depression score	7.2±1.1	3±0.6	0.003
GIS	14.8±3.1	0.8±0.3	0.001
NDI-Interference	76.1±6.6	98.4±1.2	0.001

**CHAPTER 16: SYMPTOM RESPONSE TO A
STANDARDISED NUTRIENT CHALLENGE TEST IS
LINKED TO G-PROTEIN β SUBUNIT C825T**

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16.1 INTRODUCTION

It is believed that GNB3 C825T polymorphisms may be associated with variations in signal transduction response which could lead to variations in sensory or motor function of the gut, although the role in irritable bowel syndrome is less clear (Andresen, Camilleri et al. 2006). However, so far it is not known whether there is any difference among healthy subjects who had CC, CT, or TT genotype in terms of minor abdominal complaints or meal-related symptoms. We hypothesised that GNB3 C825T polymorphisms are associated with meal-related symptoms in healthy subjects. The aims of this study, therefore, were to assess the distribution of GNB3 C825T polymorphisms and the association between GNB825 3 polymorphisms and symptoms during a nutrient challenge in healthy subjects.

16.2 SUBJECTS AND METHODS

16.2.1 Subjects

43 healthy volunteers (22 females, mean age 48 years, range 19-78 years) were recruited consecutively by advertisement. No subject had a history of

gastrointestinal, significant systemic disease or was taking medication which is known to influence gastrointestinal tract. Six out of 43 subjects were *H. pylori* positive on ^{14}C urea breath test. Subjects who had a history of functional gastrointestinal disorders or psychiatric co-morbidities were excluded. The study was approved by the Royal Adelaide Hospital Human Ethics Committee and all of the volunteers gave written informed consent before completing the questionnaires and standardised nutrient challenge test.

16.2.2 Assessment of gastrointestinal symptoms

The presence and severity of gastrointestinal symptoms and psychiatric co-morbidities were assessed utilising validated questionnaires: the Gastrointestinal Symptom (GIS) score (Adam, Liebrechts et al. 2005), the Bowel Disease Questionnaire (BDQ) (Talley, Phillips et al. 1990; Talley, Boyce et al. 1995), the Nepean Dyspepsia Index (NDI) (Talley, Haque et al. 1999; Talley, Verlinden et al. 1999), and the Hospital Anxiety and Depression Scale (HADS) (Herrmann 1997). The GIS assesses the intensity of gastrointestinal symptoms in patients with functional dyspepsia, and addresses patient's gastrointestinal symptoms in the past 1 week. The BDQ assesses various types of symptoms including upper abdominal symptoms, bowel symptoms, reflux symptoms and lifestyle over the previous 12 months. The NDI assesses symptoms of dyspepsia and health-related

quality of life. The HADS is a validated tool for the assessment of anxiety and/or depression.

16.2.3 Standardised nutrient challenge test

Visceral sensitivity was assessed by a standardised nutrient challenge test performed on the same day following completion of the questionnaires. After an 8-hour fast, subjects were asked to perform the standardised nutrient challenge test (Chapter 9).

16.2.4 Genotyping

The genotype of C825T polymorphism was isolated by polymerase chain reaction (PCR) as described in Chapter 9.

16.2.5 Statistical analysis

Baseline symptoms reported on questionnaires, individual and global cumulative symptom responses during the nutrient challenge test were assessed in 3 subgroups of subjects: CC, CT and TT, adjusting for age, gender and BMI, using

analysis of variance. p -values ≤ 0.05 were considered significant. Data are present as mean \pm standard error of the mean. For the statistical analysis SAS Version 6.12 (SAS Institute Inc., Cary, North Carolina, USA) and SPSS Version 12 (Statistical Package for Social Sciences, SPSS Inc., Chicago, Illinois, USA) were used.

16.3 RESULTS

GNB3 825-TT was found in 5 subjects (12%), CT in 17 (37%) and CC in 22 (51%). When gastrointestinal symptoms were assessed by questionnaires, only minimal levels of background gastrointestinal symptoms were evident in this group of healthy volunteers. Although background symptoms were rare and mild, subjects with TT genotype reported significantly higher symptom score than subjects with CC and CT genotype ($p < 0.02$, Figure 1).

During the standardised nutrient challenge, the highest scores were reported for fullness, followed by nausea and pain. The cumulative pain symptom response to the standardised nutrient challenge test was significantly higher in subjects with TT genotype compared with CT and CC (TT=16.8 \pm 20.8; CT=1.9 \pm 3.6; CC=0.4 \pm 1.3, $P < 0.005$, Figure 2). In contrast fullness score was significantly higher in subjects with CC compared with CT (CC=99.6 \pm 75.2; CT=52.7 \pm 41.7, $p < 0.02$, Figure 3). No significant differences were found in nausea, regurgitation

and heartburn symptom score during a standardised nutrient challenge test according to genotype (Figure 4).

16.4 DISCUSSION

There are data that have associated GNB3 C825T polymorphism with obesity, hypertension, coronary heart disease, stroke, and depression, and also differential responses to diuretics, antidepressants, sildenafil, clonidine, angiotensin II, and endothelin-1 modulators. Recent studies suggest an association between the GNB3 C825T genotype and functional gastrointestinal disorders (Holtmann, Siffert et al. 2004; Camilleri, Carlson et al. 2006). While the overall risk associated with GNB3 C825T is small, very little is known about potential mechanisms.

This study explored the association between genetic variations in GNB3 C825T and background gastrointestinal symptoms and gastric sensory function in healthy subjects. The major finding was that subjects with GNB3 825 TT genotype had higher background gastrointestinal symptom scores compared with CT and CC and had higher pain scores during a standardised nutrient challenge compared with CT and CC, which are consistent with a study of Camilleri, et al (Camilleri, Carlson et al. 2006) who showed that GNB3 825 CC and TT are associated with patients with meal-unrelated dyspepsia. In contrast, subjects with GNB3 825 CC

genotype had higher fullness score during a standardised nutrient challenge compared with CT. These findings are consistent with a study of Holtmann, et al (Holtmann, Siffert et al. 2004) who showed the association between patient with functional dyspepsia and GNB3 825 CC genotype. These findings suggest that GNB3 C825T may play a different role in gastric motor and sensory function.

Precisely how the differences in symptom response to the meal relate to GNB3 C825T, however, is unclear. Having higher fullness score in subjects with GNB3 825 CC during the test meal in this study may reflect delayed gastric emptying which consistent with a previous study of Grudell et al (Grudell, Camilleri et al. 2008). CC genotype is usually associated with diminished signal transduction responses, which may be associated with decreased gastric motor function and could potentially explain the higher fullness score in subjects with GNB3 825 CC genotype. On the other hand, TT genotype is usually associated with increased signal transduction responses, which may be associated with increased pain signalling pathway and could explain higher pain score reported in subjects with GNB3 825 TT.

This study has some unavoidable limitations. First, we explored only one particular GNB3 genotype, on the basis that this gene has some clear biological rationale given the role of these mechanism to the control of gut motility (Kleuss, Hescheler et al. 1991; Kleuss, Scherubl et al. 1992; Gollasch, Kleuss et al. 1993; Kleuss, Scherubl et al. 1993; Bharucha, Camilleri et al. 1997; Baumgart, Naber et

al. 1999). In addition, GNB3 C825T has been shown to be associated with functional dyspepsia and gastro-oesophageal reflux disease (Baumgart, Naber et al. 1999; Holtmann, Siffert et al. 2004; de Vries, ter Linde et al. 2009). However, in this study, we mainly assessed the association between gastric visceral sensation response to standardise nutrient challenge test rather than gastric motor function, and this is the first study demonstrate the association between GNB3 C82T genotype and meal-related symptoms in healthy subjects. Second, the sample size of this study is small, however, it is worth noting that the distribution of GNB3 C825T in our study (51% CT, 37% CC and 12% TT), similar to distributions of healthy controls in a previous study (Grudell, Camilleri et al. 2008).

In conclusion, while the data require confirmation in a larger sample, they are consistent with the concept that GNB3 825T-CC plays a role in the processing of visceral sensory information or the gastrointestinal motor responses to a nutrient challenge, and possibly plays a role in the physiological and clinical manifestations of functional gastrointestinal disorder.

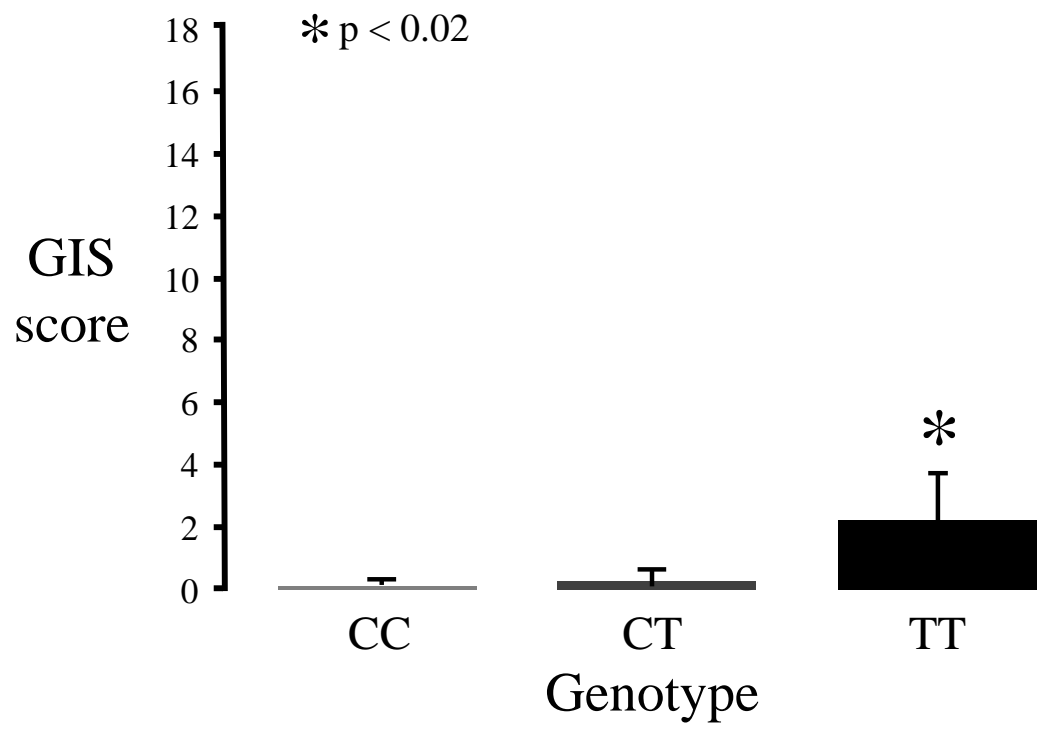


Figure 1: Background Gastrointestinal Symptoms according to genotype.

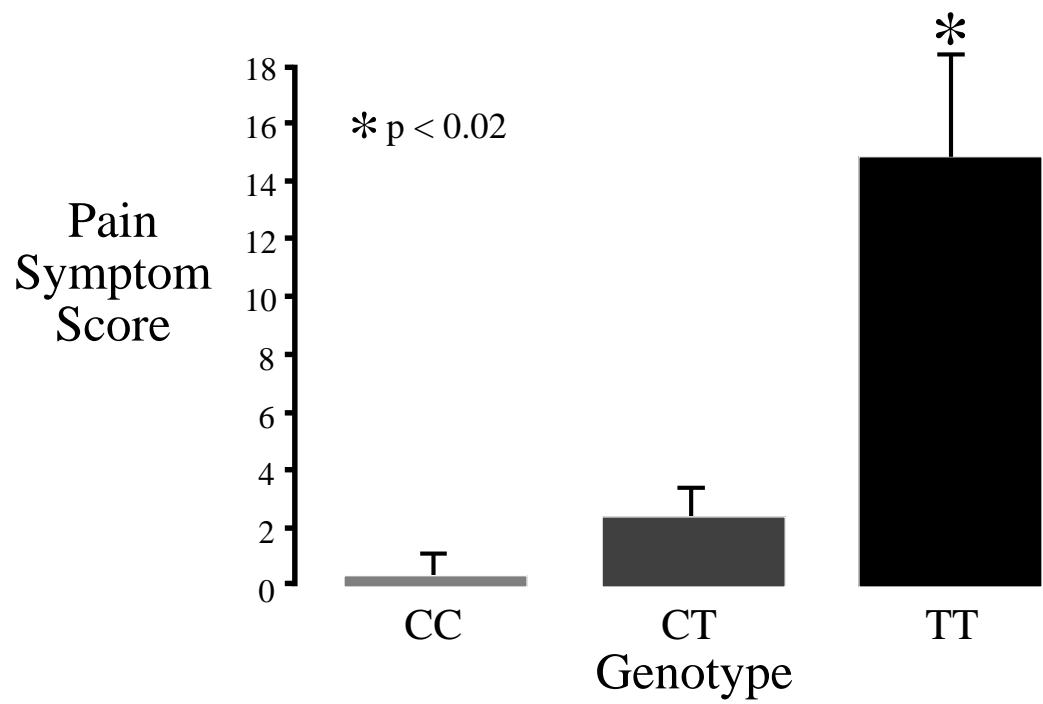


Figure 2: Effect of GNB3 genotype on cumulative pain score during standardised nutrient challenge test.

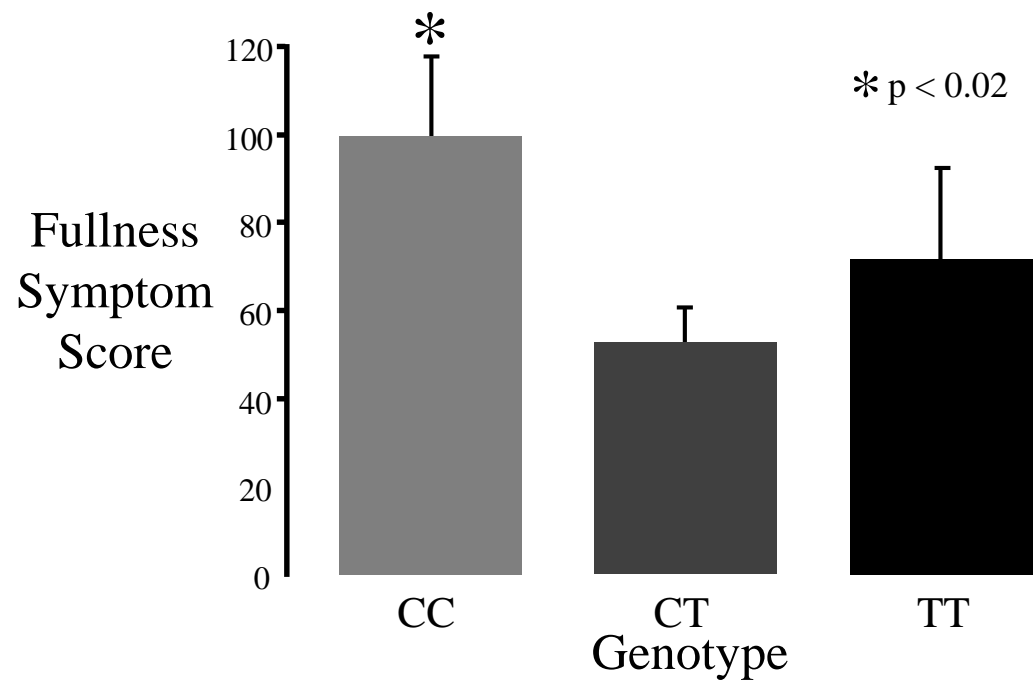


Figure 3: Effect of GNB3 genotype on cumulative fullness score during standardised nutrient challenge test.

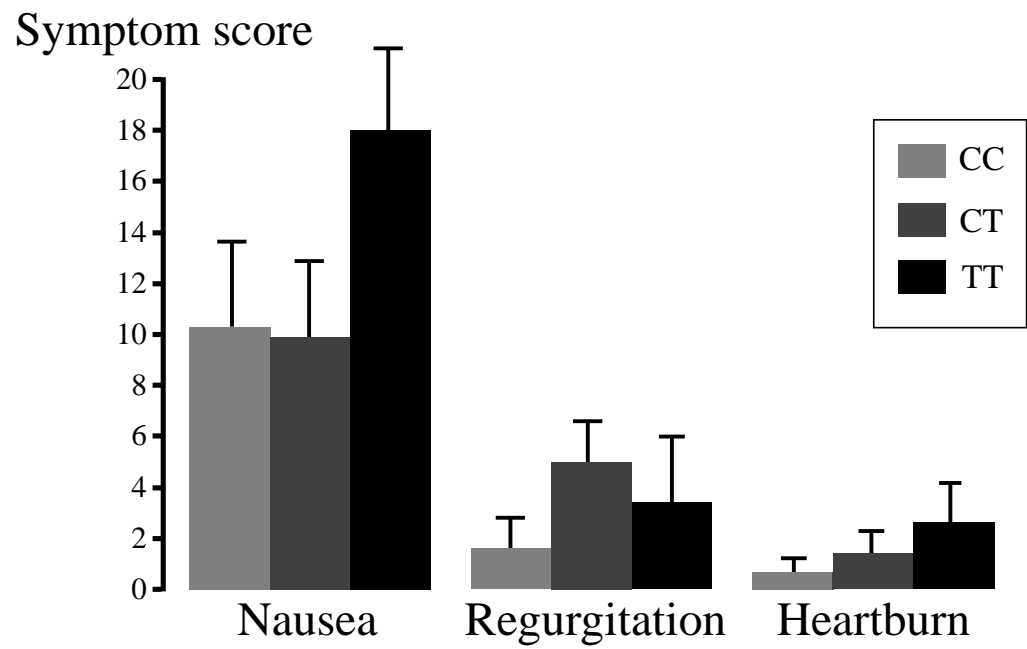


Figure 4: Effect of GNB3 genotype on nausea, regurgitation and heartburn score during standardised nutrient challenge test.

CHAPTER 17: CONCLUSIONS AND FUTURE DIRECTION

The work described in this thesis has contributed significant new information to our current understanding of the nature, pathogenesis, epidemiology, risk factors and treatment of peptic ulcer disease, especially complicated peptic ulcer disease. The findings have significantly improved and added new knowledge in the fields of gastric motor and sensory function in peptic ulcer disease, which identify the aggressive management of peptic ulcer disease and should also stimulate further research into the mechanisms of peptic ulcer pain.

Peptic ulcer disease is common. The exact pathophysiology of peptic ulcer disease is still unclear. However, major causes of peptic ulcer disease are *H. pylori* infection and NSAIDs used. It is well known that *H. pylori* cause gastric hypo secretion in gastric ulcer and hyper secretion in duodenal ulcer. Up to 20-30% of patients taking long term NSAIDs experience adverse gastrointestinal effects. Gastric ulcer is associated with increased levels of pepsinogen II, increased duodenogastric reflux, decreased mass of gastric parietal cells, and decreased maximal acid output [206]. On the other hand, duodenal ulcer is associated with increased parietal cell mass, serum pepsinogen I concentration, capacity to secrete acid and pepsin, increased drive to secrete acid and pepsin, parietal cell sensitivity to gastrin, increased duodenal acid and pepsin loads and

increased rate of gastric emptying [366]. Only 20-50% of duodenal ulcer patients have one of these mechanisms and the majority of peptic ulcer patients have normal ranges of gastric acid, secretion, acid homeostasis and gastroduodenal motility.

Peptic ulcer disease (PUD) usually manifests as either dyspepsia or, less commonly, with life threatening complications such as bleeding and perforation (Linder and Wilcox 2001). Over the past two decades the incidence of uncomplicated peptic ulcer disease (uPUD) has dropped substantially, whilst the incidence of peptic ulcer bleeding seems to have remained unchanged (Lassen, Hallas et al. 2006; Post, Kuipers et al. 2006). Approximately 30% to 50% of patients with bleeding peptic ulcer (BPU) are asymptomatic until bleeding occurs (Crocker 1991) even though the endoscopic assessment may reveal multiple ulcer scars suggestive of previous ulceration. Moreover, the majority of patients dying from peptic ulceration have no symptoms of ulcer disease until the presentation of their final, fatal illness (Pounder 1989).

The mechanism of ulcer pain is still unclear. The extent and severity of erosions are not directly associated with an increased risk for dyspeptic symptoms (Collins, Davies et al. 1986; Holtmann, Gschossmann et al. 2002). However, several factors have been associated with silent peptic ulceration. BPU occurs predominantly in elderly patients (Hernandez-Diaz and Rodriguez 2002; Targownik and Nabalamba 2006) and older age is often associated with

asymptomatic peptic ulcer (Clinch, Banerjee et al. 1984; Matthewson, Pugh et al. 1988; Wilcox and Clark 1997; Hilton, Iman et al. 2001). Non-steroidal anti-inflammatory drugs (NSAIDs) may also be associated with asymptomatic peptic ulcer (Clinch, Banerjee et al. 1984; Mellem, Stave et al. 1985; Dew 1987), although this notion has been challenged (Wilcox and Clark 1997; Lu, Chang et al. 2004). While, no study has reported in relate to visceral sensory function in patients with peptic ulcer disease. Altered gastric motor function has been proposed to be associated with the pathogenesis of peptic ulcer disease, though this idea is not well acknowledged. Gastric motor function and visceral sensory function may be the key responsible for the difference between clinical manifestations of complicated and uncomplicated peptic ulcer.

The incidence and proportion of patients between symptomatic and asymptomatic peptic ulcer is unknown. Relatively few studies have shown the factors that could be associated with asymptomatic peptic ulcer such as age, NSAIDs and ulcer size. The incidence and proportion of patients with symptomatic and asymptomatic peptic ulcer disease, including bleeding peptic ulcer and uncomplicated peptic ulcer were investigated in Chapter 10. This chapter shows that over the last 10 years, the incidence of uPUD has decreased whereas BPU has remained stable. The majority of patients with BPU are asymptomatic whilst the majority of patients with uPUD had dyspeptic symptoms. This chapter also revealed the potential factors that could influence symptoms of peptic ulcer. Age and use of

aspirin appear to be risk factors for the manifestation of ulcers without dyspepsia that ultimately manifest with life threatening BPU.

The prevalence of dyspepsia (Agréus, Svardstam et al. 2001) abdominal pain (Kay, Jorgensen et al. 1994) and the severity of reflux symptoms (Johnson and Fennerty 2004) decreases with advancing age. Ageing is also associated with diminished perception of pharyngeal (Aviv, Martin et al. 1994) oesophageal (Lasch, Castell et al. 1997) and rectal sensations (Lagier, Delvaux et al. 1999). However, data on the effect of age on the visceral sensory function of the stomach are lacking. The work described in Chapter 11 is consistent with the notion that elderly people have decreased gastric visceral sensation compared with younger people. The findings could help to explain in part why younger subjects may perceive symptoms in the presence of lesions while the elderly may develop severe lesions or even complications such as peptic ulcer bleeding without having symptoms, and why the prevalence of dyspepsia decreases with advancing age.

The work presented in Chapter 12 provided substantial insights into the different clinical manifestation of uncomplicated and complicated peptic ulcer disease. Using a standardised test of visceral sensation, our findings show that, patients with uPUD have an augmented symptom response whilst patients with BPU have a symptom response to a test meal that is not different from that in HC, suggesting a fundamental difference in visceral sensitivity between patients with bleeding or asymptomatic ulcers and those with symptomatic or uncomplicated

ulcers. Lowered visceral sensitivity and asymptomatic status is a plausible explanation for the presentation of ulcers with complications such as bleeding. Conversely, visceral hyperalgesia, higher degrees of psychological distress, more concomitant bowel symptoms and persistent dyspepsia after medical treatment in patients with uPUD may explain earlier presentation and diagnosis of the ulcers.

The finding described in Chapter 13 support the findings of Chapter 12. These findings show that, even after ulcer healing, in addition to alterations of visceral sensory function, patients with a history of uncomplicated and ulcer symptoms have significantly delayed gastric emptying compared with healthy controls and patients with asymptomatic bleeding peptic ulcer. Abnormal gastric motor function may contribute to dyspeptic symptom in patients with uPUD as they still continue to have dyspeptic symptom after the ulcer had healed.

Immune activation may predict the abdominal symptom pattern and severity as a result of the intensity and duration of an acute inflammation is associated with the development of post inflammatory abdominal symptoms (Adam, Liebrechts et al. 2006). The work presented in Chapter 14 shows an association between systemic cellular immune activation and symptom manifestation in patients with peptic ulcer disease. There were increased levels of systemic proinflammatory cytokines TNF- α , IL-1 β , and IL-6 in patients with uPUD, despite the ulcer had healed, compared with patients with BPU and HC. The findings further support the notion that patients with uPUD share similarities with patients with functional

dyspepsia. Thus, the findings from this study provide an additional potential explanation for this phenomenon.

The work described in Chapter 15 demonstrates that most patients with dyspeptic symptoms prior to the diagnosis of peptic ulcer disease continue to have dyspeptic symptoms 12 months after ulcer healing and *H. pylori* eradication. Patients with persistent dyspeptic symptoms have higher level of anxiety and depression score than patients without symptoms. The data suggest that many, perhaps, most patients with symptomatic peptic ulcer disease have concomitant functional dyspepsia, which may have led to the diagnostic endoscopy being performed that may have prevented the development of a life threatening ulcer bleed.

Based upon our data it might be speculated that mechanisms that are involved into the manifestation of symptoms in patients with functional dyspepsia may actually prevent the manifestation of ulcer complications since ulcers manifest with symptoms that trigger health care seeking and treatment before complications occur.

Chapter 16 of this thesis might potentially explain one of the mechanisms of abdominal pain. The work described in this study shows that GNB3 825T-CC plays a role in the processing of visceral sensory information or the gastrointestinal motor responses to a nutrient challenge.

In conclusion, the thesis has highlighted the differences in gastric motor function, gastric sensory function and immune activation between patients with uncomplicated peptic ulcer disease and bleeding peptic ulcer. This thesis also showed the epidemiologic data of patients with symptomatic and asymptomatic peptic ulcer disease, including the risk factors of being asymptomatic peptic ulcer. The results have important therapeutic implications, and suggest aggressive management of patients with peptic ulcer disease. In addition, the research study also suggests further studies in the areas of the mechanism and pathogenesis of peptic ulcer pain, and abdominal pain, which are likely to result in better strategies to manage and prevent this important clinical condition.

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