GASTRIC REMNANT CARCINOMA: HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL PROFILE

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Dissertation submitted in fulfilment of part III of the requirements for the degree of Master of Medicine (Anatomical Pathology), University of Cape Town.

August 2004

DECLARATION

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14-08-2004

ACKNOWLEDGMENTS

I wish to express my sincere thanks and appreciation to the following people.

My supervisors Professor P. Hall and Associate Professor A. Mall, for their ideas, guidance and patience.

Professor David Dent and Dr Eugenio Panieri for assistance with the cases and the clinical data.

Mrs Heather McCleod for technical assistance and advice on immunohistochemical stains.

Gail Henderson for doing the histochemical staining.

Ray Kriel and Craig Jamieson for capturing images and technical assistance.

Prof P Cruse for providing a supernumerary post for pathology training and for the use of facilities to undertake the research.

My wife Soaad for her care, support and encouragement.

ABBREVIATIONS

GRC	Gastric remnant carcinoma
PGC	Primary gastric carcinoma
GU	Gastric Ulcer
DU	Duodenal ulcer
IM	Intestinal metaplasia
Type I IM	Complete intestinal metaplasia
Type II IM	Incomplete intestinal metaplasia
H&E	Haematoxylin and eosin
PAS	Periodic acid-Schiff
PAB/AB	Periodic acid-Schiff / Alcian Blue 2.5
HID	High iron diamine
NOS	Not otherwise specified
WHO	World Health Organization
EBV	Epstein-Barr Virus

LIST OF TABLES

Page no

LIST OF FIGURES

TABLE OF CONTENTS

GASTRIC REMNANT CARCINOMA: HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL PROFILE

ABSTRACT

Gastric remnant carcinoma (GRC) is a gastric cancer that develops in gastric remnant more than five years after resection for benign disease. GRC comprises 1%-9% of all gastric cancers. Partial gastrectomy for peptic ulcer is thought to be a risk factor for GRC. Pancreato-duodenal and bile reflux may play an important part in the aetiology of GRC. Primary gastric carcinoma (PGC) is a gastric cancer that arises in un-operated stomach and chronic gastritis is a well-known risk factor. Consequently there appear to be differences in the aetiology of GRC and PGC. According to many studies, surgical treatment of early GRC (Stage I or II) resulted in the same or better prognosis with similar stage PGC. However if diagnosed late, GRC has a worse prognosis than PGC at the same stage. In this study haematoxylin and eosin, alcian blue pH 2.5, periodic acid Schiff, high iron diamine and Giemsa stains as well as immunohistochemical methods (eight antibodies against MUC1 to MUC6) were used to determine the type of mucin and the pattern of staining in twenty cases of GRC and twenty PGC (ten cases of intestinal type PGC, ten diffuse type PGC) and ten normal gastric mucosal biopsies. The aim of the study was to describe the morphology of GRC and the adjacent gastric mucosa, as well as to determine the histochemical and immunohistochemical mucin profile of GRC and to compare this with that of PGC and normal mucosa.

In general, morphologically, histochemically and imunohistochemically, gastric remnant carcinoma closely resembles primary gastric carcinoma. This includes the findings of a high association of Type I and IIB intestinal metaplasia in the intestinal types of both GRC and PGC compared to the diffuse types of GRC and PGC, although a smaller percentage of GRCs expressed MUC1 and MUC2 than PGCs.

CHAPTER ONE

INTRODUCTION

1.1 NORMAL STOMACH

The stomach is a dilated portion of the alimentary canal, located in the left hypochondrium and epigastric region of the abdomen. It has the cardia and the pyloric orifices. It has the greater and lesser curvatures and two surfaces, an anterior and posterior surface respectively.

The stomach is an organ of storage and digestion. Embryologically, the stomach arises from the foregut. The blood supply is mainly by the coeliac artery. It can accommodate more than two litres of fluid (Snell 1992).

The stomach is anatomically divided into four regions.

1) The gastric cardia: is the part of the stomach located adjacent to the gastro-oesophageal junction.
2) The fundus: is dome-shaped and located above the level of the gastro-oesophageal junction.
3) The corpus or body: constitutes the major portion of the stomach.
4) The pylorus: located distally and subdivided into pyloric antrum, pyloric canal and pyloric sphincter.

The wall of the stomach is divided into mucosa, submucosa, muscularis propria and adventitia. The mucosa of the stomach is thick and thrown into numerous folds or rugae. The mucosa consists of an epithelial layer, lamina propria and muscularis mucosa. The gastric glands in the body and fundus are relatively straight and not coiled. The mucus secreting cells are limited to the upper portions of the glands. The mucus surface cells produces mucus that is chemically distinct from that produced by the mucus neck cells. Gastric glands of both fundus and body contain parietal cells that secrete hydrochloric acid. The body of the stomach is the main special secretory area and it contains parietal and chief cells. The parietal cells are responsible for the secretion of intrinsic factor, which facilitate the absorption of vitamin B_{12}. Chief cells secrete large amounts of zymogen. Mucosal glands of the cardia and pylorus are

mostly devoid of acid secretory cells. Gastric pits in the cardiac and pyloric regions lead to glands that are coiled, lined mostly by mucus cells. Gastric mucosa also contains cells that are part of the enterochromaffin system. G cells are found in the region of the antrum and secrete gastrin, which promotes parietal cell secretion of hydrochloric acid. Gastrin also stimulates antral motor activity. Gastrin activity is inhibited by acidity. Cells termed I cells, found in much of the stomach secrete cholecystokinin which stimulates the release of pancreatic secretions rich in protein. S cells are found in the distal stomach and secrete secretin that stimulates alkaline secretions from the pancreas (Snell 1992, Wastell 1991).

Gastric surgery has many complications, (Cuschieri 1995) which include: (Palmer 1991):

1) Small stomach syndrome associated with a feeling of fullness after only a moderately sized meal.
2) Bilious vomiting.
3) Anemia due to iron deficiency anemia or occasionally vitamin B_{12} deficiency due to loss of intrinsic factor.
4) The dumping syndrome comprises attacks of fainting and sweating after food intake. This is probably an osmotic effect due to the high osmolarity of the gastric content, which passes rapidly into the jejunum, absorbing fluid into the gut lumen and producing a temporary reduction in the circulating blood volume.
5) Steatorrhoea, the production of fatty stool.
6) Stomal ulceration, which is more common after duodenal ulceration and very rare after gastric ulcer surgery.
7) Gastric remnant carcinoma.

1.2 MUCINS

1.2.1 MUCIN SECRETION, STRUCTURE, DISTRIBUTION AND FUNCTION

Mucus is a slimy, sticky secretion that coats epithelial surfaces in 99% of invertebrates (Denny 1989, Devine et al. 1992, Kim et al. 1995, Gevers 1987). Mucins are glycoproteins of large molecular weight that are the main components of mucus and impart gel-forming properties to it (Allen 1981, Mall et al. 1990). Mucins consist of a non-globular thread-like protein core, which contains a high level of serine, theonine, alanine, glycine and proline (Devine et al. 1992, De Kretser et al. 1986, Seregni et al. 1997) and contains both highly glycosylated and naked regions. (Allen 1981). The cores of different mucins vary in size, amino acid sequence and

the number of tandem repeats. Each tandem repeat is specific to a specific mucin type (Jass 2000).

The primary function of mucus is to form a protective cover over epithelial surfaces in the stomach, colon, gall bladder and tracheobronchial tree (Allen 1981). In the stomach the mucous barrier is a continuous layer of water insoluble gel, of median thickness 180 um in humans, which adheres to the epithelium and protects it from the high shear forces associated with digestion (Allen 1981). In gastric ulcer patients, the gel is substantially weaker, containing less polymeric mucin (Allen 1990, Younan et al 1982). Alteration of mucins such as altered glycosylation patterns have been reported to occur in gastric and colonic adenocarcinoma (Feizi 1985).

1.2.2 HISTOCHEMISTRY OF GASTRIC MUCIN

Gastric mucins have been classified as neutral or acidic mucins, the latter of which is also subdivided into sulphomucin and sialomucins according to the reaction with periodic acid Schiff (PAS), alcian blue and high iron diamine (Segura et al. 1983). Normal gastric epithelium shows predominantly neutral mucins that stain positively for PAS. Sulphomucin and sialomucin are not expressed in a significant amount in the normal stomach. Sialomucin can be weakly expressed in the base of the fundus pits, while sulphomucin is faintly expressed in the mucus cells of the normal stomach. Cardiac glands at the gastro-esophageal junction can secrete small amount of sulphomucins and sialomucin as well as the normal neutral mucin (Jass 1996). Intestinal metaplasia shows positive staining for the acidic mucins with or without the expression of neutral mucins. Intestinal adenocarcinoma expresses neutral, acidic and some sulphomucin (Taylor et al. 1998).

1.2.3 MUCIN GENES

Human mucins are derived from a heterogeneous family of genes. There are at least 15 different mucins genes that have now been identified and described (MUC1, 2, 3A, 3B, 4, 5AC, 5B, 6, 7, 8, 9, 10, 11, 12 and MUC13).

MUC1 is a membrane-associated mucin (Kim et al. 1995) that consists of an extracellular and a membrane-associated domain (Silverman et al. 2001). The gene is located on chromosome 1q21-24 and it is fully sequenced (Swallow et al. 1987a, 1987b). MUC1 glycoprotein is primarily a mammary-type apomucin, that is also expressed in bronchial epithelium, salivary gland, pancreas, and prostatic and uterine epithelium. In the gastrointestinal tract, MUC1 is strongly expressed in the base of

colonic crypts, in colonic goblet cells and columnar cells (Tashiro et al. 1994). In the stomach MUC1 is expressed in the apical and cytoplasmic region of foveolar epithelium and mucus neck cells. Parietal cells also show diffuse cytoplasmic staining of MUC1 (Winterford et al. 1999). Ultrastructural studies showed that MUC1 is located in the secretory vesicles and along the microvillous brush border of columnar cells and the cytoplasmic remnants of the goblet cells (Winterford et al. 1999). MUC1 core peptide is highly expressed on apical membranes of the bronchus, breast, salivary gland, pancreas, prostate, and uterus and is sparsely expressed in the gastric surface cells, gallbladder, small intestine, and colonic epithelium. Increased expression of MUC1 was observed in adenocarcinoma of the breast and ovary (Tashiro et al. 1994), with the expression of MUC1 in breast carcinoma found to be associated with better differentiation and a better prognosis (Rahn et al. 2001).

MUC2 is a secretory gel-forming mucin (Kim et al. 1995). The gene is located on chromosome 11p15.5. It is present in a cluster that also includes MUC5AC, MUC5B and MUC6. MUC2 is the predominant form of mucin in human intestinal and colonic tissue and is considered to be specific to goblet cells (Gum et al. 1992). MUC2 is weakly expressed in the tracheobronchial tree (Gum et al. 1992).

MUC3 is a membrane bound mucin, encoded on chromosome 7q22 and expressed in the small intestine and colorectum, salivary gland and gall bladder (Shekels et al. 1998). MUC3 antibody stains both goblet and colonic epithelial cells, but is not expressed in the normal gastric mucosa (Ho et al. 1993). More recent evidence suggests that MUC3 consists of two genes, MUC3A and MUC3B, both of which encode membrane-bound mucins (Kyo et al 2001)

The MUC4 gene is localized to chromosome 3q29 (Porchet et al 1995). It is a trans-membrane protein with two extra cellular epidermal growth factor like domains that may bind to c-erb-2 (Moniaux et al. 1999). It is expressed in the respiratory and reproductive tracts and gastric mucosa, and to a lesser extent by goblet cells of the colorectum (Winterford et al. 1999, Seregni et al. 1997). Colorectal cancer shows loss of expression of MUC4 (Biemer-Huttman et al. 2000).

MUC5AC is a secretory gel-forming mucin, normally expressed in the superficial and foveolar epithelium of gastric mucosa and neck cells and absent in the deep glands of the gastric body and pyloric glands of the antrum (Machado et al. 2000).

MUC5B is highly expressed in the respiratory epithelium (Seregni et al. 1997).

MUC6 is a major mucin in the stomach (Kim et al. 1995). It is predominantly expressed in the neck mucus cells of gastric antrum, pyloric glands, and principle cells of the gastric body (Machado et al. 2000). It is also expressed in the gall bladder, pancreatic ductules and seminal vesicle.

MUC7 gene is located in chromosome 4q13-q21, which has been fully sequenced and has previously been called MG2. It is approximately10.0 kb in length. It encodes a low molecular weight mucin (Bobek et al. 1993) and is expressed in the salivary glands (Bobek et al. 1996).

MUC8 has recently been cloned. It is localized to chromosome 12q24.3 and is normally expressed by the submucosal glands of the human trachea (Shankar et al. 1997).

MUC9 is also known as ovicductin, and has been identified in the rabbit endocervix (Hendrix et al. 2001). The gene for MUC9 is localized to chromosome 1p13 and its likely functions include protection of the early embryo and the fallopian tube (Lapensee et al. 1997).

MUC10 has been described in the submandibular salivary glands of embryonic mouse and it is possibly involved in the gland development (Melnick et al. 2001).

MUC11 and MUC12 were described by Williams et al. (1999). They are down regulated in colorectal cancer and both are mapped to chromosome band 7q22 (Williams et al. 1999). MUC11 is a 2.8 kb long. MUC12 encodes a trans-membrane mucin and it is a homologous with epidermal growth factor-like growth factors, suggesting that MUC12 may be involved in epithelial cell growth regulation (Williams et al. 1999).

MUC13 is localized to chromosome band 3q13.3. It encodes a 512 amino acid protein. It is a novel human cell surface transmembrane mucin, which is expressed by epithelial and hemopoietic cells. MUC13 is highly expressed in the large intestine and trachea. MUC13 is expressed at a mild level in small intestine, kidney, appendix and stomach. Immunohistochemical staining has shown that MUC13 is expressed on

the apical membrane of gastrointestinal columnar cells and cytoplasm of goblet cells (Williams et al. 2001).

1.2.4 MUCIN EXPRESSION IN NORMAL AND DISEASED STOMACH

1.2.4.1 Normal Stomach

There are some conflicting and controversial results arising from studies of mucin expression in the normal stomach. Buisine et al. (2000) demonstrated that MUC1 and MUC5AC are strongly expressed in normal stomach with weak or negative MUC6, MUC2, MUC3 and MUC4 staining and negative for MUC5B and MUC7. Taylor et al. (1998) showed strong expression of MUC4, MUC5 and MUC6 and weak or no expression of MUC1 in the normal stomach. Ho et al. (1995) showed strong expression of MUC5 and MUC6, but weak faint positivity of MUC1 and absence of MUC2, MUC3 and MUC4 staining in the normal stomach.

1.2.4.2 Intestinal Metaplasia

Histochemically, intestinal metaplasia is classified into the complete type (type I), incomplete type (type IIA) and incomplete (type IIB) that is positive for sulphomucins. Complete intestinal metaplasia is negative for MUC5AC and positive for MUC2. In incomplete intestinal metaplasia there is a combined positivity of MUC5AC and MUC2 (Jass et al. 2000). Intestinal metaplasia shows strong expression of MUC2 in the supra-nuclear area of goblet cells and strong MUC3 reactivity in columnar cells with no expression of MUC4, MUC5 and MUC6 (Buisine et al. 2000, Ho et al. 1995).

1.2.4.3 Effect of *H.pylori* on Mucins Expression in the Stomach

Three human mucin genes are expressed strongly in the normal stomach: MUC1 a membrane-bound mucin, MUC5AC and MUC6, which are expressed in surface mucous cells and mucous glands respectively (Byrd et al. 1997, de Bolos et al. 1995, Ho et al. 1995). *H.pylori* can bind to gastric mucins and results in either inhibition, or potentiation of bacterial adhesion to the gastric epithelium (Murata et al. 1992). *H.pylori* infection alters the expression of gastric mucin on the surface epithelial cells. In *H.pylori* infected patients, MUC6 mucin is focally expressed in the surface mucous cells as well as mucous glands whilst MUC5 is expressed weakly on the surface. There is no alteration in the expression of the membrane bound MUC1 (Byrd et al. 1997). Eradication of *H.pylori* results in the reversal of surface MUC5AC and MUC6 expression to normal patterns (Byrd et al. 1997).

Molecular mimicry between *H.pylori* lipopolysaccharide structures and host mucin-associated antigens could lead to protection from host immune surveillance and promote colonization (Appelmelk et al. 1996, Byrd et al. 1997).

1.2.4.4 Gastric carcinoma

Acidic mucins are found in the majority of gastric cancers, but intestinal and diffuse cancers differed in the proportions of acid and neutral mucins secreted (Jass 1996). Jass (1996) reported that acidic mucin and particularly sulphomucin predominated in intestinal cancer, whereas neutral mucins were more abundant in cancers of the diffuse type. There are striking differences in both quality and quantity of mucin gene expression in PGC compared with normal gastric mucosa. Gastric cancer is usually associated with a decrease in mucin of the normal gastric type and the additional expression of mucin genes, which are normally expressed in the intestine (Ho et al. 1995). Early cancer and poorly differentiated carcinoma are associated with decrease and loss of mucin, but the advanced stage of well-differentiated adenocarcinoma is associated with multiple mucin gene expression (Torrado et al. 1992). PGC shows altered mucin expression with loss of MUC5AC and MUC6 in some tumor glands. It also shows abnormal expression of MUC2 and MUC3 with the appearance of MUC5B mRNA. MUC1 expression has been observed to be expressed diffusely in the cytoplasm of the malignant cells, in addition to the expected normal apical membrane reactivity (Ho et al. 1995). MUC3 is increased in gastric adenocarcinoma. Marked differences in the mucin gene expression were noted between the different histological types of gastric cancer. Intestinal type carcinoma is more likely to express aberrant mucins compared with the diffuse type gastric carcinoma. PGC and fetal gastric epithelium may show similar mucin expression (Buisine et al. 2000, Ho et al. 1995).

1.2.5 MUCIN EXPRESSION IN THE INTESTINE

1.2.5.1 Normal Intestine and Tumours

Since gastric carcinomas may be of intestinal type and can express intestinal type mucins that resemble mucins expressed in small bowel, it is important to know the types of mucin normally expressed in the normal bowel. Normal small bowel mucosa mainly shows the expression of MUC2 and MUC3 with less expression of MUC1 and MUC4, and no expression of MUC5AC, MUC5B, or MUC7. MUC6 is expressed mainly in the duodenum. MUC2 is expressed in the goblet cells and MUC3 by goblet and columnar cells of the bowel (Buisine et al 2001). Colonic

adenoma and carcinoma show an increase in the expression of MUC1 and a decrease of the expression of MUC2 with increasing grades of epithelial dysplasia in adenomas (Jass 2000). MUC2 and MUC5AC are markedly increased in hyperplastic polyps and serrated adenoma (Jass et al. 2000). Colonic carcinomas show distinct patterns of MUC2 and MUC3 expression, although the expression of each were reduced compared with the levels in normal colonic mucosa (Chung et al. 1994).

1.2.6 SIGNIFICANCE OF THE STUDY OF MUCIN GENES

Advanced stage carcinoma and poorly differentiated tumors are associated with the expression of aberrant multiple mucin core peptides (Bresalier et al. 1990). Increase mucin gene expression may contribute to tumor cell growth and metastatic change. Highly mucinous tumors are associated with worse prognosis in carcinomas of the colon (Nakamori et al. 1994). Mucin genes may provide a good target in cancer treatment (Hoff et al. 1989, Kuan et al. 1987).

1.3 GASTRITIS

1.3.1 Introduction

Gastritis is the inflammation of the gastric mucosa and can be acute, chronic or a mixed gastritis. The mixed form is a combination of an acute and chronic inflammation, referred to as active chronic gastritis. Acute gastritis is diagnosed when there is neutrophil infiltration of the mucosa and glands, whilst in chronic gastritis, there is a chronic inflammatory cell infiltrate in the mucosa, of plasma cells and lymphocytes. Inflammation is a complex reaction involving an inflammatory cell infiltrate with alteration of the epithelial cells and the release of various mediators (Geboes 1992). The most common causes of acute gastritis are the heavy ingestion of non-steroidal anti-inflammatory drugs (NSAIDs), heavy smoking, excessive consumption of alcohol and or drugs, chemotherapy, radiotherapy, infection, shock, stress and uraemia. Acute gastritis can also be the result of the reflux of duodenal content. The most common known causes of chronic gastritis are *H.pylori*, autoimmune gastritis, chemicals, tuberculosis and Crohn 's disease. Gastritis can be classified according to etiological, morphological or topographical information (Dixon et al. 1996).

1.3.2 CLASSIFICATION OF GASTRITIS

1.3.2.1 Aetiological Classification:

1.3.2.1.1 *H.pylori* Associated Gastritis: H pylori is a slender gram-negative bacillus. However they are occasionally present in the stomach in coccoid forms (Chan et al. 1994). *H. pylori* can be identified by histological and serological methods or by breath tests. *H. pylori* infection is very common throughout the world, but most infected individuals remain asymptomatic for years or even for decades (Blaser 1992). *H. pylori* is the major cause of gastritis, especially chronic diffuse superficial gastritis (Graham 1989). *H. pylori* has also been linked to gastric ulcer, gastric carcinoma and gastric lymphoma (Veenedaal et al. 1996). *H. pylori* has been identified as a gastric carcinogen (Correa 1995, Hansson et al. 1995) but the exact mechanism of carcinogenesis is not known. *H. pylori* infection induces apoptosis in gastric epithelium by producing many cytotoxins (VacA), lipopolysaccharides and nitric oxide. *H.pylori* induces the release of host inflammatory and immune responses, which lead to the release of cytokines that potentiate apoptosis. Increased apoptosis is seen in all pre-cancerous conditions of the stomach and is associated with the development of gastric carcinoma, including intestinal and diffuse types (Xia et al. 2001). Chronic superficial gastritis may ultimately progress to involve gastric epithelium and glands, leading to chronic atrophic gastritis and to gastric atrophy (Morris et al.1987). *H. pylori* induced acute gastritis cause transient hypochlorhydria (Morris et al.1987, Sobala et al.1991). The exact mechanism remains unclear, but one hypothesis claims that the function of the parietal cell is directly affected (Cave et al.1989). However most studies show that basal acid secretion does not seem to differ markedly between infected and uninfected controls (Morris et al. 1998). When no *H. pylori* can be identified and changes are short of other diagnostic categories, the gastric biopsy is reported as chronic gastritis without further qualification. Crohn's disease should be suspected in a case of *H. pylori* negative focal gastritis according to Correa et al. (1992).

1.3.2.1.2 Chemical Induced Gastritis

This disease is also known as reactive gastropathy and reflux gastritis. The causes of chemical gastritis include alcohol, non-steroidal anti-inflammatory drugs (NSAID) and bile reflux (Dixon et al.1996). Partial gastrectomy is also a cause for reflux gastritis.

Mechanism of Chemical Induced Gastritis.

Bile acid damages the gastric mucosal barrier, allowing hydrogen ions to diffuse back into the cells, which are subsequently damaged resulting in gastritis (Davenport 1968). Chronic reflux of duodenal content and bile lead to chronic superficial gastritis and eventually to chronic atrophic gastritis (Dewar et al.1983). The affected mucosa is susceptible to ulceration and there is also evidence that it may predispose to gastric carcinoma (Domellof et al. 1976, du Plessis 1965, Gear et al. 1971, Keighley et al. 1975). Billroth I partial gastrectomy and truncal vagotomy and drainage, result in greater enterogastric reflux because they either destroy or bypass the pylorus respectively (Keighley et al.1975). Long term follow up of patients who have undergone partial gastrectomy for benign gastric diseases revealed an increase in the incidence of gastric carcinoma (Gear et al. 1971, Stalsberg et al.1971). Reflux gastritis in an intact stomach is a controversial entity, with some authors stating that the gastritis results from NSAIDs induced damage (Frezza et al 2001, Sobala et al. 1990). There is a significant correlation between the severity of reflux gastritis and both hypochlorhydria and high bile acid concentration in the stomach (Dixon et al.1986). It might be argued that increased alkalinity is more likely to result from long term reduction in G cells and parietal cell activity brought about by chronic atrophic gastritis, rather than a short term effect of alkaline reflux (Dixon et al.1986). Cases of hypochlorhydria due to bile reflux do not show intestinal metaplasia, which is a feature of chronic atrophic gastritis.

Histological Changes of Chemical Gastritis

The gastric mucosa shows foveolar hyperplasia, oedema, congestion and smooth muscle proliferation in the lamina propria (Dixon et al.1986). A slight increase in the number of chronic inflammatory cells may be observed. If there are no erosions, neutrophil infiltration will not be a feature. However these changes are not entirely agreed on. Niemela et al (1987) suggested that gastroduodenal reflux may be the cause of pre-pyloric ulceration in a mucosa already damaged by *H.pylori* (Karttunen et al.1988). Foveolar hyperplasia appears to be the result of excessive cell exfoliation from the surface epithelium or of stimulation by cytokines or other inflammatory mediators. These changes were first reported in patients with bile reflux and subsequently observed after gastric surgery (Dewar et al 1984). Some investigators found a poor correlation between bile reflux and the histological changes described above (Hoare et al.1978).

1.3.2.2 Morphological classification: (Correa et al.1992)

1.3.2.2.1 Non-atrophic gastritis

a) Chronic superficial gastritis (SG): The inflammation is in the superficial mucosa, predominantly in portions of the mucosa occupied by pits (Correa et al.1988, 1992).

b) Diffuse antral gastritis (DAG): Typically seen in North America and Western Europe. It is confined to the antrum, with easily identified *H. pylori*. DAG may result in duodenal ulcers, probably as a result of increased basal acid output and the high response of the parietal cells to stimulation (El Omar et al.1995).

1.3.2.2.2 Chronic atrophic gastritis.

a) Diffuse corporal gastritis (DCG): The inflammation involves the whole mucosal thickness and is associated with loss of glandular elements.

b) Multifocal atrophic gastritis (MAG). MAG is prevalent mainly in under-developed countries. Typically it is patchy in distribution and involves both pyloric and fundal mucosa (Correa et al.1992). *H. pylori* are sparse. It may associated with benign gastric ulcer and carcinoma (Correa et al.1995). The explanation for the difference in the geographic variations is unclear, but one possibility is the early age of infection in the developing countries. However dietary deficiencies such as vitamin C and high salt consumption are also believed to play an important role (Caygill et al.1996).

1.3.2.3 Topographical classification:

Chronic gastritis can be classified as either antral predominant or corpus predominant, or by another classification as multifocal or diffuse in distribution.

1.3.3 Grading Of Gastritis

H. pylori should be evaluated on the areas where it is usually resides, such as in the surface mucus and areas that do not show intestinal metaplasia. A neutrophil infiltrate is a universal feature of *H.pylori* infection. Neutrophils are seen in the lamina propria, within the epithelium and in the lumen of glands and foveolae. The density of neutophils has been correlated with the extent of mucosal damage and *H. pylori* infection (Correa et al 1992). Neutrophils are a sensitive indicator of the presence of *H.pylori*, and they disappear within days after eradication of the

infection (Correa et al 1992). A few mononuclear leukocytes are normal within the gastric mucosa. The normal stomach contains 2-5 mononuclear inflammatory cells (lymphocytes, plasma cells and macrophages) per high power field (HPF= field diameter of 0.44 mm). Plasma cells are absent in the normal gastric mucosa. Some observers consider the presence of two plasma cells per high field to be the upper limit for normal gastric mucosa (Correa et al 1992). Glandular atrophy is manifested by an increase in the distance between individual glands. Grading of minor glandular atrophy in the antrum is difficult, because the antrum normally contains more connective tissue, than other regions of the stomach. Intestinal metaplasia is an indicator for the presence of atrophy. Gastric atrophy usually represents an end stage of chronic gastritis (Dixon et al. 1996). Gastric atrophy is a precursor for the genesis of carcinoma.

When reporting gastric biopsies, it is recommended that *H. pylori*, chronic inflammation, neutrophil activity, gland atrophy and intestinal metaplasia be evaluated. Chronic inflammation, active inflammation, intestinal metaplasia and atrophy can be graded into three grades, mild, moderate and severe (Correa et al.1992).

1.4 COMPLICATION OF CHRONIC GASTRITIS.

1.4.1 Intestinal Metaplasia

Intestinal metaplasia refers to the progressive replacement of gastric mucosa by intestinal epithelium and having the light and electron microscopic features of intestinal epithelium, of either small or large bowel type, including goblet cells, absorptive cells (with brush border), Paneth cells and endocrine cells (Mingazzini et al.1984, RubinbW 1969). Chronic gastritis may be associated with two types of metaplasia, intestinal or pyloric metaplasia. Metaplasia is common in chronic gastritis and it increases in prevalence with the duration of the chronic gastritis.

Intestinal metaplasia can be classified into three types according to the type of mucin expressed (Appelman 1994). Intestinal metaplasia has been divided into complete (type I) and incomplete (type II).

Complete intestinal metaplasia (Type 1). The histological features are nearly identical to that of the small intestine epithelium with villi and crypts lined by goblet cells and absorptive cells with a brush border. Histochemically the mucin present is

predominantly sialomucin with or without a small amount of sulfomucin and or neutral mucin. (Barwick 1987, Dixon et al 1996, Rothery GA et al. 1985).

Incomplete intestinal metaplasia. The absorptive cells are absent in this condition. Columnar cells with the appearance of gastric foveolar cells are retained.
Incomplete IM can be divided histologically into two subtypes:

a) Type IIa or II is characterized by the predominance of neutral mucin. There are a few absorptive columnar cells secreting neutral and sialomucin. Goblet cells containing predominantly sialomucin are also present.

b) Type IIb or III is characterized by crypts lined by tall columnar cells which contain abundant sulphomucin, with a small number of goblet cells that have either sialomucin or sulfomucin. (Jass et al. 1981, Silva at al. 1990).

Sulphomucin differs from sialomucin by positivity for high iron diamine (Born et al. 1977). Type III (IIB) metaplasia is believed by some to be closely linked to cancer while type I IM has the lowest risk of gastric carcinoma (El-Zimmaity et al. 2001, Futoshi et al.1982, Iida et al.1982, Jass et al.1979). Others have reported no such link between type IIB and carcinoma (Dixon et al. 1986). Some studies showed that intestinal metaplasia is not a consistent change, that the type of metaplasia appeared to change in both directions and that intestinal metaplasia regresses after *H. pylori* eradication. (Maaroon et al. 1985, Silva et al.1990). One study showed that on follow-up, typing intestinal metaplasia did not predict the pattern in subsequent biopsies or the outcome of the patients (Jass et al.1981). The available data suggest that the pattern, extent, and srverity of atrophy with or without intestinal metaplasia, are better predictors of the risk of gastric cancer than the subtypes of intestinal metaplasia (Jass et al. 1981).

1.4.2 Peptic Ulcer

Peptic ulcer is a breach in the epithelial surface of the stomach that has penetrated through the muscularis mucosa into the wall of the stomach. Peptic ulcers can be acute or chronic. All gastric ulcers, except those which result from treatment with NSAIDs, are surrounded by an area of acute and chronic inflammation (Gear et al. 1971, MacDonald 1973). Most gastric ulcers are accompanied by multifocal atrophic gastritis (MAG), while duodenal ulcers are accompanied by diffuse antral gastritis (DAG) and not atrophic gastritis (Correa et al.1992). Some studies showed that patients with *H. pylori* infection and duodenal ulcer also have a disturbance of gastric function, characterized by an increased release of gastrin by the antral mucosa, and an

exaggerated acid response to stimulation by gastrin compared with infected persons without ulceration (El-Omar et al.1995, McGowan et al.1996). At the edge of all ulcers, reactive epithelial changes can occasionally be confused with dysplasia or carcinoma.

1.4.3 Atrophic Gastritis

Atrophic gastritis is an important pre-cancerous lesion. There are three types of chronic atrophic gastritis, including type A, type B and type AB chronic gastritis (Glass et al.1975, Strickland et al. 1973). Type A atrophic gastritis is located in the body, associated with achlorhydria, and subsequent bacterial overgrowth. This overgrowth enables the Nitrates to be converted into nitritis and N- nitroso compounds, which are potent gastric carcinogens, especially for intestinal type gastric carcinomas (Reed et al. 1981, Tannenbaum et al. 1983). Type B atrophic gastritis is located primarily in the antrum and is associated with normal or hypersecretion of gastric acid and peptic ulceration. The most common causes are *H.pylori*, exogenous and endogenous irritants such as hot drinks, alcohol, tobacco and refluxed bile (Wyatt et al. 1988). Type AB atrophic gastritis consists of patchy gastritis in both the antrum and body of the stomach. This type of gastritis was noted in populations consuming a diet rich in salt, and deficient in fresh fruit and vegetables (Correa 1988). These patients have a gradual decrease in gastric pH, with consequential bacterial overgrowth.

1.4.4 GASTRIC DYSPLASIA AND PRIMARY GASTRIC CARCINOMA

1.4.4.1 Gastric dysplasia.

Gastric dysplasia is a precanerous lesion and defined as a histopathological abnormality in which cancer is more likely to develop than in the normal counterpart (Morson et al. 1980). However, not all pre-cancerous lesions necessarily transform into cancers. In the gastrointestinal tract, the term dysplasia is applied to disorders of growth and differentiation, associated with increased malignant potential. Dysplasia implies a neoplastic but non-invasive process, and should be differentiated from inflammatory atypia (Jass 1983). Dysplasia is graded on the basis of cytological and architectural changes into low and high grade, the latter including carcinoma *in-situ*. A three-tiered system that grades dysplasia into mild, moderate and severe was used in the past (Morson et al. 1980). Gastric dysplasia which seen in gastric adenomas can morphologically resemble dysplasia seen in colorectal adenomas. Gastric dysplasia usually arises in type IIB intestinal metaplasia, but also can arise in type I (complete) intestinal metaplasia or normal gastric mucosa (Jass and Filipe 1981).

There are useful criteria in the differentiation of gastric dysplasia and reactive changes especially at an edge of a peptic ulcer that (Owen et al 1991) include

1) Benign lesion with intestinal metaplasia can show nuclear crowding and increased mitotic activity at the base of the glands, but toward the surface fully mature epithelial cells are identified.
2) Reactive nuclei are vesicular and rounded with single or double enlarged nucleoli, while neoplastic nuclei are hyperchromatic, enlarged, crowded and compressed.
3) In peptic ulceration the inflammation is most severe in the areas of severe atypia. Carcinoma may show severe atypia without the presence of inflammation.
4) The transition between malignant and benign epithelium is abrupt, in comparison to inflammatory atypia, which shows a gradual transition from atypia to normal appearing epithelium.

Two variants of gastric dysplasia have been described (Jass 1983).

1) Type I dysplasia or the adenomatous variant, closely resembles colonic adenomas morphologically. In this type, the dysplastic cells are crowded with elongated nuclei and abundant cytoplasm. Mucin is minimal within the cytoplasm. The dysplasia can be polypoid or flat. The surrounding epithelium may be normal or show intestinal metaplasia. Type I dysplasia can be graded into low and high grade dysplasia. Low grade dysplasia has simple tubular glands with no obvious architectural distortion, lined by cells showing basally located nuclei and sparse mitotic figures. High-grade dysplasia shows marked architectural distortion of the glands with branching and budding form patterns. There is marked mitotic activity and nuclear stratification. This type of dysplasia is usually associated with intestinal type adenocarcinoma.

2) Type II dysplasia (hyperplastic), has both goblet and columnar cells. The cytoplasm is pale and inconspicuous. Nuclei are enlarged, round, vesicular with prominent nucleoli. Loss of polarity is not a prominent feature in this type of dysplasia. Type II dysplasia arises in a background of incomplete intestinal metaplasia. It is associated with high-grade intestinal adenocarcinoma. This type of dysplasia that can be difficult to differentiate from regenerative atypia.

Progression of mild and moderate dysplasia to carcinoma is slow and even rare in mild dysplasia, while on the other hand high grade dysplasia is highly predictive of subsequent cancer. Mild and moderate dysplasia requires an endoscopic follow up,

while severe dysplasia require surgical intervention (Kokkola et al. 1996). Most patients who were diagnosed as having high-grade dysplasia of the gastric mucosa on examination of gastric biopsy specimens, either had synchronous gastric cancer or rapidly developed gastric cancer. Also most cancers that were associated with high-grade dysplasia, were nearly all early gastric carcinoma and thus potentially curable by surgery. (Lansdown et al. 1990).

There are many differences in the diagnosis of gastric carcinoma made by the Japanese and Western pathologists. The Japanese diagnose gastric carcinoma on nuclear and architectural changes, regardless of the presence or absence of invasion into the lamina propria, while Western pathologists require invasion of the lamina propria to diagnose carcinoma (Goldstein and Lewin 1997, Schlemper et al. 1997).

1.4.4.2 Gastric Carcinoma

1.4.4.2.1 Epidemiology and biology

Gastric carcinoma is one of the most common cancers globally (Muir et al. 1987, Parkin et al. 1993). It has a high incidence in the developing countries of the world, including South Africa and endemic especially in the mixed race population of the Western Cape (Dent et al 1981, Wyndham 1985). The disease occurs rarely before the age of 40, but its incidence increases steadily thereafter and peaks in the seventh decade (Fuchs et al. 1995). In the United States the disease is 1.5-2.5 more common in African-Americans than in those of Caucasian origin (Fuchs et al. 1995.). Gastric carinoma is a major killer world-wide and remains the second

most common cause of cancer-related deaths in the world (Whelan et al. 1993). *H.pylori* is a well-known predisposing factor for the development of gastric carcinoma and one study suggested that 60% of gastric adenocarcinomas are attributed to *H.pylori* infection, which implies that the incidence of gastric carcinoma could be reduced by 60% if *H. pylori* was eradicated (Parsonnet et al. 1991). Gastric carcinoma is believed to arise via a multi-step process that includes chronic gastritis, gastric atrophy usually with intestinal metaplasia, and finally dysplasia (Correa et al. 1988, 1992, El-Zmaity et al. 2001). Atrophic gastritis, intestinal metaplasia and dysplasia are considered precancerous lesions (Testoni et al. 1987).

1.4.4.2.2 Pathological features

More than 90% of gastric cancers have been reported to be adenocarcinomas (Fuchs et al. 1995). The most widely used classifications for gastric cancer are that of Lauren (1965), and the World Health Organization (WHO) classifications.

According to the WHO classification of gastric carcinoma the disease includes adenocarcinoma *in-situ*/severe dysplasia, adenocarcinoma, papillary adenocarcinoma, tubular adenocarcinoma, mucinous adenocarcinoma, signet-ring cell adenocarcinoma, adenosquamous carcinoma, squamous carcinoma, small cell carcinoma and undifferentiated carcinoma (Watanabe et all 1991). Lauren classified gastric carcinoma into intestinal and diffuse type (Lauren 1965); each is considered as a separate pathological entity, with different epidemiological, clinical, pathological and biological characteristics (Lauren 1965). Occasionally gastric carcinomas show combined features of diffuse and intestinal carcinomas (Lauren 1965, Oota and Sobin 1977).

Intestinal type adenocarcinoma is usually ulcerated and occurs in the distal stomach. Histologically, the tumour is composed of cohesive neoplastic groups of glands that resemble colonic adenocarcinoma. The majority of gastric carcinomas arise in a non-polypoid mucosa, in contrast to the colonic carcinomas, which arise in adenomatous polyps.

Intestinal type gastric adenocarcinoma is more common in males and affects those of an older age group, and arises in a background of intestinal metaplasia and is closely related to exogenous factors as *H.pylori*, nitrosation and bile reflux (Parsonnet et al. 1991). Intestinal type adenocarcinoma has better prognosis than the diffuse type.

Diffuse type adenocarcinoma occurs in younger patients and spreads diffusely throughout the stomach. The tumour is composed of solitary or small clusters of non-cohesive neoplastic cells without gland formation. It is thought that diffuse type adenocarcinoma is governed by endogenous host-related factors and genetic susceptibility, and is not preceded by known pre-cancerous alteration (Howson et al. 1986).

Early gastric carcinoma is the term applied to invasive carcinoma confined to the mucosa or submucosa (Day and Morson 1978, Seifert et al. 1975, Evans et al. 1987). It differs from carcinoma *in-situ*, which does not penetrate the basement membrane and has no metastatic potential. Early gastric carcinoma is mainly identified in the distal stomach along the lesser curvature. It may have metastatic potential. The five-year survival rate of early gastric cancer is over 90% (de Dombal et al. 1990).

1.4.4.2.3 Risk factors for gastric carcinoma

1) *H. pylori* infection
2) Chronic atrophic gastritis
3) Intestinal metaplasia
4) Cigarette smoking
5) Low socioeconomic status
6) Low consumption of fruit and vegetables
7) Consumption of salted, smoked or poorly preserved foods
8) Pernicious anemia
9) Partial gastrectomy for benign gastric conditions (usually peptic ulcer)
10) Gastric adenomatous polyp
11) Hereditary non-polyposis colon cancer syndrome

Pernicious anemia is associated with a 2-3 folds increased risk of gastric cancer (Hsing et al. 1993). Patients infected with *H.pylori* have a 3-6 fold higher risk of gastric carcinoma (Parsonnet et al. 1991). The malignant potential of adenomatoyus polyps of the stomach is related to size of the polyp and the grade of dysplasia (Nakamura et al. 1985). Consumption of large amount of salted food may lead to atrophic gastritis. The association of *H.pylori* infection with carcinoma is similar regardless of the histological type, although other studies based on the histological detection of *H.pylori* have showed the prevalence to be lower in the diffuse type adenocarcinoma in comparison with the intestinal type adenocarcinoma (Buruk et al. 1993). Gastric carcinomas located in the cardia are less frequently associated with *H.pylori* compared with other tumor locations (Hansson et al. 1993, 1995, Normura et al. 1991) and it has been suggested that environmental factors play a major role in the aetiology of intestinal type gastric carcinoma, while the diffuse type may have a primary genetic etiology (Howson et al. 1986). Type IIB IM is noted to be significantly more frequent in patients with adenocarcinoma than with benign ulcers, as well as more prevalent in

stomach with intestinal type of adenocarcinoma than with diffuse type of adenocarcinoma (Segura and Montero 1983).

1.4.4.2.4 Molecular features of gastric carcinoma.

Gastric carcinomas show allelic deletion of the MCC gene (mutated in colonic cancer) in 33% of patients, APC gene (adenomatous polyposis coli) in 34% of patients and p53 tumor suppressor gene in 64% of patients (Rhyu et al. 1994). Patients with intestinal type of adenocarcinoma have an increased frequency of over expression of epidermal growth factor receptor, erbB-2 and erbB-3 (Wright et al. 1993).

1.4.4.2.5 Prognostic factors for gastric carcinoma

The overall 5-year survival rate for gastric carcinomas was 14% in one American Study (Houben et al. 1995) and less than 10% overall in the United Kingdom (de Dombal et al. 1990).

1) The stagie of gastric carcinoma: is the most important prognostic factor. Gastric carcinoma is staged according to the depth of the invasion of the tumor cells and the presence or absence of metastasis in the regional lymph nodes and other organs. The American Joint Committee incorporates the TNM classification of gastric carcinoma (Felming and Cooper 1997). Intestinal type adenocarcinoma has a 26% five-year survival, while diffuse type adenocarcinoma has 16% five year survival rate (Wanebo et al. 1993). TNM Staging of gastric carcinoma. Please see appendix 1.

2) Degree of differentiation: Tumors with poor differentiation have a diminished survival rate.
3) Genetic alteration. Tumors with abnormal DNA content and proto-oncogenes of tumor suppressor genes are associated with a diminished survival rate (Uchino et al. 1993).
4) Clinical and serology. Adverse prognostic factors include age more than 70 years, Carcinoembryonic antigen (CEA) more than 10ng/ml and CA19-9 more than 37 μ g/ml (Kirkwood et al. 1997).

1.4.4.2.6 Surgical treatment of gastric carcinoma.

Gastrectomy with lymph-node clearance for gastric carcinoma offers the only chance for cure. Partial gastrectomy with resection of the adjacent lymph-nodes is sufficient for distal carcinoma. A randomized comparison between total and partial gastrectomy showed a higher rate of morbidity and mortality after total gastrectomy (Gouzi et al. 1989). Resection of the primary tumor is usually palliative, but offers good symptomatic relief. In the absence of ascites and extensive metastatic disease, patients who are thought to be surgically incurable should be considered for palliative surgery (Thompson et al 1993). This can relieve pain, bleeding and obstruction.

Early gastric carcinoma can be cured surgically, with a cure rate of 89-93% if lymph nodes are free of tumour and 80% - 91% if the lymph nodes are involved (Johansen 1976).

1.5 GASTRIC REMNANT CARCINOMA

1.5.1 Definition

Gastric remnant carcinoma (GRC) was first described by Balfour in 1922. GRC, also known as "gastric stump carcinoma", is defined as gastric carcinoma, which occurs in patients after gastric operation for benign gastric disease (Klarfeld and Resnick 1979). It was defined initially as any carcinoma that had developed in a gastric remnant. Later it was stipulated that at least **five years** must have elapsed after partial resection of the stomach in order to eliminate the possibility that the original resection had been a malignant disease and that the tumour in the gastric remnant was a recurrence (Welvaart and Carsick 1982).

1.5.2 Incidence

GRC occurs in 1-9% of patients operated on for benign gastric disease (Mason et al. 1988, Helsingen and Hillstad 1956, Boren et al. 1980, Fisher et al. 1983, Newman et al. 1997). Some investigators have shown an association between surgery for peptic ulcer and subsequent risk of gastric cancers (Stalsberg and Taksdal 1971, Pickford et al. 1984, Molloy and Sonnenberg 1997, Viste et al. 1986, Schrumpf et al. 1977), while other studies showed no association, and have reported a risk similar to that in the general population (Kalina et al. 1983, Sandler et al. 1984, Fischer et al. 1984, Clark et al. 1983, Schafer et al. 1983, Wlarfeld et al. 1997).

There appears to be a 15-year protective interval of low incidence of carcinoma after which the incidence steadily increases (Domellof et al. 1977). There is a significant

increased risk of GRC twenty years after surgery (Caygill et al. 1987). The time interval since the initial surgery is the most important risk factor for GRC. There is also a geographic difference in the relative risk of GRC. The risk of GRC in Japan, a country that has one of the highest incidences of PGC, is low when compared with the figures that are available in Europe and the United States (Tersmette et al 1991). Some have also observed an increased risk of cancers of the oesophagus, colon, pancreas, breast and lung after peptic ulcer surgery (Wat et al. 1984). Although some studies show no clear relation between site of the original disease (duodenal vs. gastric ulcer) and subsequent development of carcinoma (Stalsberg and Taksdal 1971, Klarfeld and Resnick 1979), others showed that only surgery for gastric ulcer but not for duodenal ulcer was associated with a risk of GRC (Molloy and Sonnenberg 1997). The risk of GRC is greater in patients treated for GU than those treated for duodenal ulcer (Welvaart and Carsick 1982, Molloy and Sonnenberg 1997).

Several observers have studied post surgical patients prior to the development of GRC, and the most common changes seen are atrophic gastritis, intestinal metaplasia and cystic dilatation of gastric glands. These changes occur as early as three months post operatively (Gjeruldsen et al. 1968, Domellof et al. 1977).

1.5.3 Aetiology of GRC

Gastric resection appears to be associated with an increased risk for gastric cancer, and this risk rises with the length of the time since surgery (Molloy and Sonnenberg 1997). Surgery results in gastric hypoacidity, which leads to bacterial overgrowth with a consequent production of carcinogens. A hypothesis involving nitrite and N-nitroso compounds has been suggested (Schlag et al. 1980). Bile reflux is thought to play an important role in the aetiology of GRC and some studies in animal models showed that pancreatico-duodenal secretion reflux is an important factor in the malignant process (Mason et al 1986,1988). Partial gastrectomy creates a new microenvironment, which favours the development of cancer. GRC appears to evolve as the end result of a series of mutoganic cell transformations affecting the gastric mucosa over a prolonged period of time. Antrectomy is thought not only to decrease acid production but also enhances the development of gastric atrophy (Tatsuta et al 1982). Regurgitation of bile and pancreatic enzymes after Billroth II surgery was suggested to be due to the reconstruction, decrease in blood supply and altered enervation at the site of anastomosis (Ruddell et al. 1976, Cheung 1987). Other studies showed no association between reflux and mucosal changes (Domellof et al. 1977). The possible sequence of GRC is bile reflux inducing irritation, decreased resistance of the gastric mucosal

barrier and gastritis, with subsequent absorption of potentially carcinogenic compounds from intestinal contents (Klarfeld and Pesnick 1979). GRC has a similar histogenetic pathway to that for PGC, i.e., via atrophic gastritis and intestinal metaplasia, particularly of type IIB intestinal metaplasia. Operated stomach shows hypochlorhydia and achlorhydia with increased pancreatic and bile reflux resulting in atrophic gastritis and intestinal metaplasia in the gastric stump (Domellof et al. 1977, Pickford et al. 1984, Savage et al. 1979). It is postulated that high gastric acidity associated with Billroth I and II surgery, may be conducive to the proliferation of anaerobic bacteria that colonize the stomach and transform dietary or endogenous nitrates to nitrites. These substances produce additional N-nitroso compounds, which act as carcinogens (Helsingen and Hillestad 1956). Chemical atrophic gastritis in the gastric remnant leads to colonization of the mucosa by bacteria, which may include *H.pylori* (Elder and Knight 1991). *H.pylori* is not found to have a significant association with GRC (Greene 1996, Johannesson et al. 2003). Yamamoto et al. in 1994 showed significant association of Epstein-Barr virus (EBV) and GRC. In-situ hybridization in a study done by Nishikawa et al. (2002) demonstrated that 42% of patient of GRC were positive for EBV and all EBV positive GRC were poorly differentiated carcinomas.

Many studies showed a predilection of the stomal site for subsequent development of GRC. Most studies suggested that GRC is most likely to occur in the middle and antral portion of the stomach near the anastomosis site with the bowel (Freedman and Berne 1977, Morgenstren et al. 1973, Klarfeld and Resnick 1979).

1.5.4 Histological Types and Prognosis of GRC.

GRC can be of the intestinal, diffuse or mixed type according to the classification of Lauren (1965) (Domellof et al. 1977, Pickford et al. 1984, Savage et al. 1979). The histological type and histochemical mucin profile of GRC parallel those recorded in PGC (Stemmerman and Brown 1974). Histologicaly the mucosa of gastric remnant usually shows chronic gastritis, accompanied by atrophy, foveolar hyperplasia, intestinal metaplasia and cystic dilatation of the glands (Harmon et al 1981, Ritchie 1984). The histological features seen after partial gastrectomy resemble those of pre-malignant mucosa in an intact stomach. Follow-up studies may establish the predictive value of these precursor lesions in development of the PGC (Tersmette et al 1995). Prognosis of GRC was recorded as poor in some studies in which an overall survival is reported to be less than 10%; this has been attributed to the late stage at presentation (Morgenstern et al. 1973, Orlando and Welsh 1981). Endoscopic follow up and early diagnosis of GRC resulted in more optimistic survival data (Newman et

al. 1997). Follow up starting 10-15 years after surgery, preferably by endoscopy and multiple biopsies is recommended, because endoscopy is superior to radiology in the diagnosis of early carcinoma (Domellof et al. 1977). Annual screening, especially of patients who are at least 15 years post-gastric resection, using flexible endoscopy and multiple random biopsies may detect early GRC and lead to curative gastrectomy (Greene 1996). One study reported significantly better survival for gastric remnant cancer patients in stage I or II disease compared with similar stage primary proximal gastric carcinoma (Newman et al. 1997). Early diagnosis is emphasized (Klarfeld and Resnick 1979) and the prognosis depends on the stage of the disease when surgery is performed (Bogomoletz et al. 1985, Thorban et al. 2000). A five-year survival rate of up to 47% could be achieved in some studies (Pointner et al 1988, Gianello et al. 1983). Surveillance of patients who had partial gastrectomy, with endoscopy and multipule gastric biopsies may provide the means to diagnose GRC at an early stage (Safatle-Ribeiro et al. 2001), but cost beneficial ratio of the surveillance should be considered and studied.

CHAPTER TWO

MATERIALS AND METHODS

2.1 AIMS OF THE STUDY

1. To describe the morphology of gastric remnant carcinoma (GRC) and the pathology of the adjacent normal mucosa.
2. To determine the histochemical and immunohistochemical mucin profile of GRC.
3. To compare the mucin profile of GRC with the profile of primary gastric carcinoma (PGC) and with data of a previous study performed in this department and with the results available in the literature.
4. To determine any prognostic significance of the mucin expression in GRC.

2.2 METHODS

In this study histochemical and immunohistochemical methods were used to determine the mucin profile in GRC, PGC diffuse type, PGC intestinal type and in normal gastric tissue.

2.2.1 Case selection

Gastric tissue from fifty patients was used in this study. Paraffin embedded tissue specimens were obtained from archival material in the Department of Anatomical Pathology, Groote Schuur Hospital. Twenty cases of GRC diagnosed between 1984 and 2001 were identified from the clinical records (one case was subsequently excluded from this study because the tissue remaining in the paraffin block showed only intraepithelial malignancy but no evidence of the invasive malignancy that was seen in the original sections). The material included twelve biopsies and seven resection specimens. The original haematoxylin and eosin (H&E) stained sections were reviewed and confirmed by a second opinion. The tumours were classified according to the Lauren`s classification (Lauren 1965), into intestinal, diffuse or mixed type of adenocarcinoma.

Twenty cases of PGC were included in the study; of which ten were PGC intestinal type and ten PGC diffuse type. All the intestinal adenocarcinoma specimens and five of the diffuse adenocarcinoma specimens were obtained by partial gastrectomy and total resection respectively, while diffuse tumors were biopsy specimens. The specimens were identified through a computer search between 1999 and 2001 and were retrieved from the Anatomical Pathology Department files; the cases were selected randomly.

Ten cases of the normal histology were selected from gastric biopsies that had been SNOMED coded as normal material. Fifty cases coded as normal gastric tissue were reviewed and ten were selected as a control material. Cases were considered normal after exclusion of acute gastritis, chronic gastritis, *Helicobacter pylori*, metaplasia, atrophy or dysplasia. Six of the biopsies were taken from patients with non-ulcer dyspepsia, and who underwent endoscopy and gastric biopsy. Three of the patients had metastatic carcinoma, for whom endoscopy was used to exclude a PGC; a biopsy was performed despite the absence of tumour and shown to be negative for tumour. This last specimen was also used as a control in a previous carcinoma study (Taylor et al 1998).

This study was approved by the Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town (ERC NO: 026/2002)

2.2.2 Histology.

The updated Sydney Classification System was used for the grading of gastritis, *H.pylori* infection, atrophy and intestinal metaplasia. All carcinomas were classified according to the Lauren classification (1965). Where possible, the protocol for the staging system of gastric carcinoma, as defined by the American Joint Committee on Cancer and the International Union Against Cancer (Compton et al. 1998) was employed.

2.2.3 Histochemistry

Paraffin-embedded tissue blocks were cut to provide four μm thick sections on which the histochemical and immunohistochemical stains were performed. Sections were stained by H&E, Giemsa, periodic acid-Schiff/alcian blue pH 2.5 (PAS/AB 2.5) and high iron diamine (HID).

The combined alcian blue-periodic acid-Schiff (PAS/AB) technique was used to distinguish between acidic and neutral mucins. The sections were washed in distilled water, treated with alcian blue solution for 15 minutes and washed well in distilled water. Sections were then treated with periodic acid solution for 15 minutes and then washed with water. Sections were subsequently treated with Schiff's reagent for 15 minutes and then washed again with water. Sections in the last steps were dehydrated through graded ethanol to xylol and mounted in a synthetic resinous medium and cleaned. PAS stains neutral mucin magenta. AB pH 2.5 stains acidic mucin (sialomucin) and most sulphated mucins blue (Table 2.1).

Sections for HID were de-paraffinized and washed with distilled water and treated with diamine-ferric chloride solution in a Coplin jar for 16-18 hours. The sections were washed well in distilled water and then stained with alcian blue solution for 15 minutes, followed by washing, dehydration and mounted in resin. HID stains sulphated mucin black and non-sulphated mucin blue (Table 2.1) (Bancroft & Cook. 1994).

Giemsa stain was used to detect *H.pylori*. Sections were washed with water and treated with newly prepared fresh 2% Giemsa solution for 30 minutes and followed by washing in water, dehydration and mounting. *H.pylori* appear dark purple against a lighter purple background.

Table 2.1 Histochemical stains for mucin

Stain	Neutral mucin	Acidic mucin	Sulphomucin	Sialomucin
PAS	Red	–	–	–
AB pH 0.5	–	blue	blue	–
AB pH 2.5	–	blue	blue	blue
PAS/AB pH2.5	red	blue	blue	blue
Mucicarmine/AB pH2.5	red	blue	blue	blue
HID/AB pH 2.5	–	–	black/brown	blue
Orcein/AB pH 2.5	–	–	brown	blue
Aldehyde-Fuchsin-AB	–	–	deep purple	blue
Aluminium sulpate-AB	–	–	blue	red

2.2.4 Immunohistochemistry

Antibodies used in this study were against, MUC1, MUC1C, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6 and MUC7.

Table 2.2. Mucin core peptide antibodies

Mucin gene	Type	Clone	Source	Dilution	Method
MUC1	Mouse	Ma695	Novocastra, UK	1/100	Envision
MUC1C	Mouse	Ma552	Novocastra, UK	1/100	Envision
MUC2	Mouse	Ccp58	Novocastra, UK	1/100	Envision
MUC3	Rabbit	pAb*	Biomedia, USA	1/500	Envision
MUC4	Chicken	pAb	Prof. Ho**	1/200	Avidin Biotin
MUC5AC	Mouse	CLH2	Prof. Ho**	1/100	Envision
MUC5B	Chicken	pAb	Prof. Ho**	1/400	Avidin Biotin
MUC6	Mouse	CLH5	Novocastra, UK	1/100	Envision
MUC7	Chicken	pAb	Prof. Ho**		Avidin Biotin

* = Polyclonal antibodies

**= Personal gift from Professor Samuel B. Ho.

Link Antibody = Goat anti-chicken at 1/200 dilution from Vector Laboratories
See (Appendix 2) for the supplier of the antibodies used in this study.

Four mm thick sections were cut and placed on glass slides coated with 3-aminopropyltri-ethoxysilane. Sections were heat-fixed for 30 minutes at 60°C, and then de-waxed and re-hydrated. Endogenous peroxidase activity was quenched with a 1% hydrogen peroxide methanol solution for 15 minutes at room temperature. Sections were washed three times in phosphate buffered saline (PBS) and then micro-waved in citrate buffer (pH 6.00) for 10 minutes. Each section was stained immuno-enzymatically in a humidity chamber using a modified three-step peroxidase conjugated avidin-biotin method. Non-specific binding was blocked with non-immune serum (swine, rabbit or goat)

at a 1:20 dilution for 10 minutes. After incubation with each antibody, sections were washed thoroughly in PBS. Sections were subsequently incubated with 1:250 dilutions of biotinylated rabbit-anti-mouse and swine anti-rabbit (Dako, Copenhagen, Denmark), or 1:500 rabbit-anti-chicken (Biogenesis, Pool, UK) antibody for 30 minutes at room temperature and then with a 1:500 dilution of strep-avidin HRP (Dako, Denmark) for 30 minutes. After washing, peroxidase activity was detected using 3.3 diaminobenzidine (Sigma, St Louis, USA) as a chromogen and hydrogen peroxide as the substrate. After 10 minutes, sections were washed to arrest the reaction and counterstained with haematoxylin, blued in Scott's solution and then washed in tap water. Sections were then dehydrated through graded ethanols to xylol and mounted in a synthetic resinous medium. When the Envision detection system was used, primary antibodies were applied for 30 minutes. Slides were then rinsed with PBS. The Envision reagent was applied for 30 minutes and the peroxidase activity was determined as above.

Positive immunohistochemical staining was either cytoplasmic, or membranous or both. Immunoreactivity was graded as negative (–), weak positive (+), moderate positive (++) and strong positive (+++). A specimen was considered positive if immunoreactive cells were found in at least 5% of the low power field (10 x objective) (Ho et al. 1993). The percentage of cells with positive staining was reported as 0%, 25%, 50%, 75% and 100%. Positive staining was reported as the percentage of positive cells in proportion to the total cell mass. Positive controls were taken from normal tissues known to express the relevant antibody and an internally built positive control was also used.

CHAPTER THREE

RESULTS

3.1 Morphology of the Tumours

Out of the nineteen patients of gastric remnant carcinoma (GRC), fourteen were male and five were female, ranging from 45 to 78 years old. According to Lauren's classification eleven of the tumours were of the intestinal type, six of the diffuse type and two mixed gastric carcinoma (Table 3.1, Figure 3.1A and 3.1 B). The tumours ranged from early to advanced gastric carcinoma and from moderate to poorly differentiated adenocarcinoma.

There were eight male and two female patients with primary gastric carcinoma (PGC) of the intestinal type, and six male and four female patients with PGC of the diffuse type. Patients of both groups were between 48 and 84 years old. The tumours also ranged from early to advanced gastric carcinoma and from moderate to poorly differentiated adenocarcinoma.

Table 3.1 Types of GRC as defined by the classification of Lauren

Lauren's classification	No of cases
Intestinal	11/19 58%
Diffuse	6/19 32%
Mixed	2/19 10%

3.2 *Helicobacter pylori*

The results are summarized in Table 3.3. An assessment of *H pylori* infection could not be done in three cases of GRC because of the absence of gastric mucosa. Two cases of GRC showed mucosa adjacent to the tumour exhibiting intestinal metaplasia with no normal gastric mucosa. Only three of the total number of 19 specimens of GRC showed mild to moderate number of *H. pylori,* of which two cases showed focal intestinal metaplasia. The *H.pylori* organisms were seen on the surface of the non-metaplastic mucosa (Figure 3.2).

Three out of ten cases of PGC intestinal type showed the presence of mild to moderate number of *H. pylori*. Of these three cases, two showed foci of intestinal metaplasia. The organisms were not present on the areas of metaplasia. Two of the ten cases of PGC diffuse type showed the presence of mild to moderate number of *H.pylori*.

3.3 Gastritis

Although most cases of gastric carcinoma showed gastritis, only a small number showed the presence of *H. pylori* (Table 3.2). Gastritis was seen in the non-tumourous gastric mucosa in 12 specimens of GRC, mainly of the chronic or active chronic type. The severity of the gastritis ranged from that of mild to moderate. Two of the *H pylori* negative GRC cases showed the presence of marked reactive lymphoid follicles, highly suggestive of previous infection with *H. pylori* (Genta et al 1993). Nine of the PGC intestinal type and eight of the PGC diffuse type specimens showed gastritis, predominantly of the chronic type. Lymphoid follicles were present in two *H. pylori* negative PGC intestinal type specimens. In accordance with the criteria used in the selection of the normal gastric biopsies, none showed significant inflammatory cell infiltrate or lymphoid follicles.

Table 3.2 Histopathological changes in the different study groups

Histology	GRC	PGC intestinal	PGC diffuse
Intestinal metaplasia	11/19 (58%)	8/10 (80%)	4/10 (40%)
Atrophy	9/19 (47%)	6/10 (60%)	7/10 (70%)
H. pylori	3/19 (16%)	3/10 (30%)	2/10 (20%)
Chronic gastritis	12/19 (63%)	9/10 (90%)	8/10 (80%)

GRC= Gastric remnant carcinoma, PGC= Primary gastric carcinoma

3.4 HISTOPATHOLOGY AND HISTOCHEMICAL MUCIN PROFILE:

3.4.1 Normal gastric mucosa

All of the ten normal gastric mucosa specimens expressed neutral mucin, and none of them expressed acidic mucin.

3.4.2 Intestinal Metaplasia (IM)

Gastric remnant carcinoma. Eleven of the nineteen of these specimens displayed IM, whilst in five specimens there was no IM. Three biopsy specimens consisted only of tumour without adjacent mucosa (Table 3.3). Seven of the eleven cases with IM showed villi, lined by goblet cells positive for alcian blue, and PAS/AB negative columnar cells with brush border, in keeping with the diagnosis of complete intestinal metaplasia (Figure 3.3 a, b). Three of these seven cases also showed small foci of incomplete IM (Type II) of which two were Type IIB IM and the other Type IIA IM. Seven of the eleven IM specimens showed the presence of goblet cells interrupted by columnar cells that stained positive for neutral and acidic mucin, in keeping with Type II IM (Figures 3.4 a, b). Three of the seven cases showed Type IIA IM (Figure 3.4 c), with neutral mucin in the columnar mucosa and no evidence of sulphomucin. The other four cases of incomplete IM showed columnar cells with mucin secretion and significant staining for sulphomucin, indicating the presence Type IIB IM (Figure 3.4 d). Sulphomucin positive IM was present only in the intestinal type GRC (30%), while none of the diffuse type GRC expressed Type IIB IM.

Table 3.3. Intestinal metaplasia in the different study groups.

	GRC			PGC	
	IC	DC	Mixed	IC	DC
Gastric mucosa not present	1/11 9%	2/6 33%	0/2 0%	0/10 (0%)	0/10 (0%)
Intestinal metaplasia	8/10 80%	2/4 50%	1/2 50%	9/10 (90%)	4/10 (40%)
Type I	5/10* 50%	1/4 25%	1/2 50%	5/10** (50%)	3/10 (30%)
Type IIA	2/10 20%	1/4 25%	0/2 0%	3/10 (30%)	1/10 (10%)
Type IIB	3/10 30%	0/4 0%	1/2 50%	3/10 (30%)	0/10 (0%)

* Three cases of GRC showed predominantly Type I with focal Type II IM.
** Two cases of PGC intestinal type showed predominantly Type I and focal Type II IM.

PGC intestinal type. Nine of the ten cases (90%) of PGC intestinal type showed the presence of IM of which five were of the complete type. Two of these five cases also showed small foci of incomplete IM. Six cases of intestinal type adenocarcinoma showed the presence of incomplete IM. Three cases (30%) showed no sulphomucin staining in keeping with Type IIA and three cases (30%) showed the expression of sulphomucin in keeping with Type IIB IM (Table 3.3).

PGC diffuse type. Four of ten cases of PGC diffuse type showed IM; of which three (30%) were complete IM, and one Type IIA IM.

3.4.3 Mucin profile in tumour cells of gastric carcinomas.

GRC. In the GRC group there were eleven intestinal, six diffuse and two mixed type adenocarcinomas in accordance with the Lauren system of classification. Evaluation of the types of mucin expressed by the tumour cells is summarized in Table 3.4. All of the intestinal type adenocarcinoma specimens showed weak neutral mucin staining in tumour cells, while six (55%) were also positive for acidic mucin, three of which strongly expressed sulphomucin within the tumour cells. The expression of neutral mucin was stronger in 5/6 specimens of diffuse adenocarcinoma than that in the intestinal adenocarcinoma. Three of these five specimens were positive for acidic mucin (Figure 3.5), two of which stained strongly for sulphomucin.

Table 3.4 Histochemical type of mucin in tumour cells of GRC- and PGC

	GRC				PGC	
Mucin type	GRC total	intestinal	diffuse	mixed	intestinal	diffuse
Number	19	11	6	2	10	10
Neutral	17 (89%)	11 (100%)*	5 (83%)	1 (50%)	10/10* (100%)	9/10 (90%)
Acidic	9 (47%)	6 (55%)	3 (56%)	0 (0%)	1/10 (10%)	7/10 (70%)
Sulphated	5 (26%)	3 (27%)	2 (33%)	0 (0%)	1/10 (10%)	6/10 (60%)

* all staining was weak

PGC intestinal type. The intestinal type of adenocarcinomas were moderately to poorly differentiated. All cases showed weak neutral mucin staining within the tumour cells (100%). Only one tumour (10%) showed acidic staining, which was positive for sulphomucin (Table 3.4).

PGC diffuse type. Nine of the ten cases (90%) showed moderate to marked neutral mucin staining in the tumour cells while 7/10 (70%) showed the expression of acidic mucin. Six cases (60%) showed moderate to strong sulphomucin staining in the tumour cells (Figure 3.6). In this study, the tumour cells of PGC diffuse type showed higher proportion of strong sulphomucin 6(0%), than in the PGC intestinal type (10%).

3.5 IMMUNOHISTOCHEMISTRY

The results are summarized in Table 3.5

Table 3.5. Immunohistochemistry

	Normal	GRC			PGC	
		IC	DC	mixed	PIC	PDC
no of cases	10	11	6	2	10	10
MUC1	10 (100%)	4 (36%)	1 (17%)	2 (100%)	7 (70%)	3 (30%)
MUC1C	10 (100%)	6 (55%)	0 (0%)	1 (50%)	7 (70%)	4 (40%)
MUC2	0 (0%)	2 (18%)	1 (17%)	0 (0%)	4 (40%)	4 (40%)
MUC4	10 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
MUC5AC	10 (100%)	5 (45%)	2 (33%)	1 (50%)	2 (20%)	6 (60%)
MUC5B	0 (0%)	0 (0%)	1 (17%)	0 (0%)	1 (10%)	0 (0%)
MUC6	5 (50%)	0 (0%)	0 (0%)	0 (0%)	2 (20%)	0 (0%)

Note: because of a problem with the MUC3 antibody the results of staining for MUC3 are not included in this table (see appendix 3).

MUC1: MUC1 consistently showed moderate to strong (+++) positive staining in all ten (10/10) normal gastric biopsies. The staining was in the parietal cells only (25-50% the cells) and was both cytoplasmic and membranous in distribution (Figure 3.7A). The surface mucosa was always negative. The gastric mucosa adjacent to the gastric carcinomas showed positive MUC1

staining only in the parietal cells, similar to the results seen in normal gastric mucosa.

Four out of eleven (36%) of the intestinal type GRC and 1/6 (17%) of the diffuse type GRC showed positive staining for MUC1. MUC1 positivity was strong (+++) and seen in 50-75% of the tumours (Figure 3.7B). Seven out of ten specimens of PGC intestinal type showed strong membranous and occasionally cytoplasmic staining in the tumour cells (Figure 3.7C), whilst in 3/10 cases of PGC diffuse type, there was strong cytoplasmic and membranous staining in 25% of tumour cells.

MUC1C: All normal gastric biopsies showed cytoplasmic and membranous positive staining in the parietal cells. The surface mucosa and the superficial mucus cells of the foveolae were negative for MUC1C. Six cases (55%) of intestinal type showed strong membranous and luminal staining for MUC1C; none (0%) of the diffuse type GRC expressed MUC1C. MUC1C was positive in 7/10 (70%) of PGC intestinal type, the staining being localized in the membrane, cytoplasm and occasionally in the lumen of the glands. Four out of ten (40%) of PGC diffuse type expressed MUC1C.

MUC2: MUC2 staining was absent in all normal gastric biopsies, but consistently showed moderate staining of the cytoplasm in the goblet cells of the IM of the different types of gastric carcinoma (Figure 3.8A). Two out of eleven (18%) of intestinal type GRC (Figure 3.8B) and 1/6 (17%) of diffuse type GRC showed strong cytoplasmic positive staining for MUC2 in 25-50% of the tumour cells. Four out of ten (40%) of PGC intestinal type and 4/10 (40%) of PGC diffuse type showed moderate to strong cytoplasmic staining in 50% of the tumour cells.

MUC3: Immunostaining with an antibody to MUC3 (Biomeda) was tried twice. The results of the first attempt were rejected because of unexplained positivity seen as diffuse and moderately intense cytoplasmic staining in the deep glands of all normal gastric biopsies. Forty percent of PIC showed moderate positivity in less than 25% of the tumour cells, while GRC and PDC

were negative. The staining was repeated using a second antibody that was also supplied by Biomeda; all sections showed unexplained background staining and the stain did not work on a known positive control. Please see Appendix 3.1 for correspondence with the supplier.

MUC4: All (100%) normal gastric biopsies showed mild positive staining in basal mucous glands; positive staining was also seen in the residual normal basal glands in the mucosa adjacent to the different types of gastric carcinoma. Surface and neck epithelium in normal gastric mucosa and in mucosa adjacent to GRCs and PGCs were negative for MUC4. MUC4 was negative in the tumour cells in all GRC, PGC intestinal type and PGC diffuse type.

MUC5AC: MUC5AC staining was positive in 10/10 normal gastric biopsies. The staining was of weak to moderate intensity and expressed in mucous neck cells and the surface mucosa (Figure 3.9A). Sections of the gastric mucosa adjacent to the tumours in the biopsy and resection specimens of the different type of adenocarcinoma all showed the same pattern of staining as the normal gastric biopsies. MUC5AC was diffuse and strongly positive in 5/11 (45%) of the cytoplasm of tumour cells in intestinal GRC (Figure 3.9B) and 2/6 (33%) of diffuse GRC. Two out of ten (20%) of PGC intestinal type showed moderate cytoplasmic positivity in 25% of tumour cells. Six (60%) of PGC diffuse type showed moderate to strong cytoplasmic staining in 25-75% of tumour cells.

MUC5B: There was no MUC5B staining in the normal gastric biopsies or in the gastric mucosa adjacent to the different types of carcinoma. All cases of intestinal GRC were negative for MUC5B, while one case of diffuse GRC showed mild to weak to moderate positive cytoplasmic staining in the tumour cells (Figure 3.10). Only 1/10 (10%) of PGC intestinal type showed mild staining, which was luminal and membranous. All cases of PGC primary type were negative for MUC5B.

MUC6: was positive in 50% of the normal gastric biopsies and in the normal gastric mucosa adjacent to the different gastric carcinomas. The staining was

moderate in intensity and distributed in 75% of deep mucus glands (Figure 3.11). All cases of GRC and PGC diffuse type were negative for MUC6. There was weak cytoplasmic positive staining in the tumour cells in 2/10 (20%) of PGC of the intestinal type.

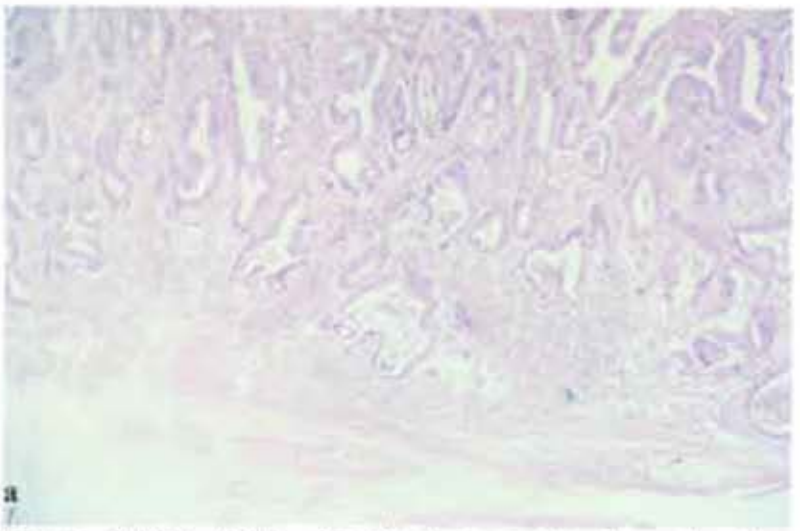


Figure 3.1(a): GRC, intestinal type showing glandular differentiation (H&E, original magnification x 40).

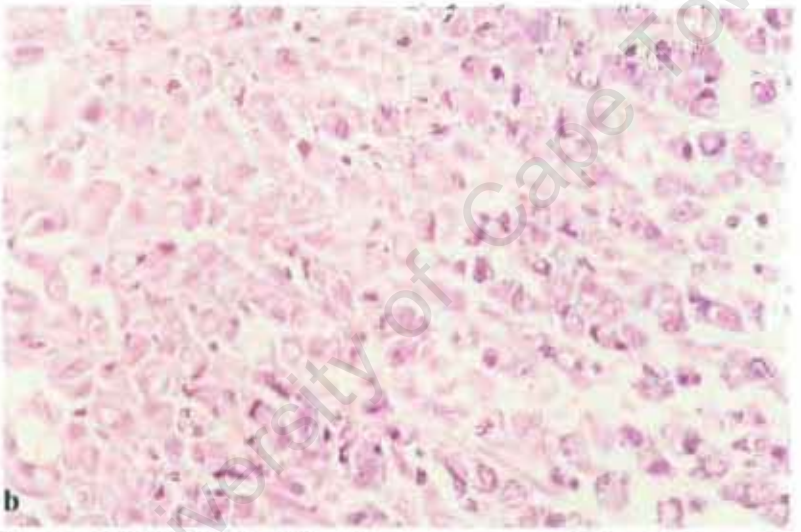


Figure 3.1(b): GRC, diffuse type composed of signet ring cells (H&E, original magnification x 200).

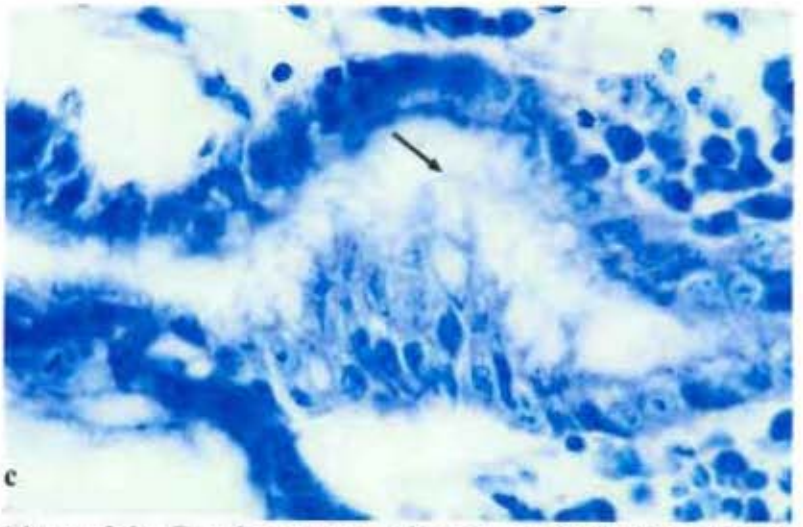


Figure 3.2: Gastric mucosa adjacent to GRC (not shown) showing *H.pylori* organisms (Giemsa, original magnification x 400)

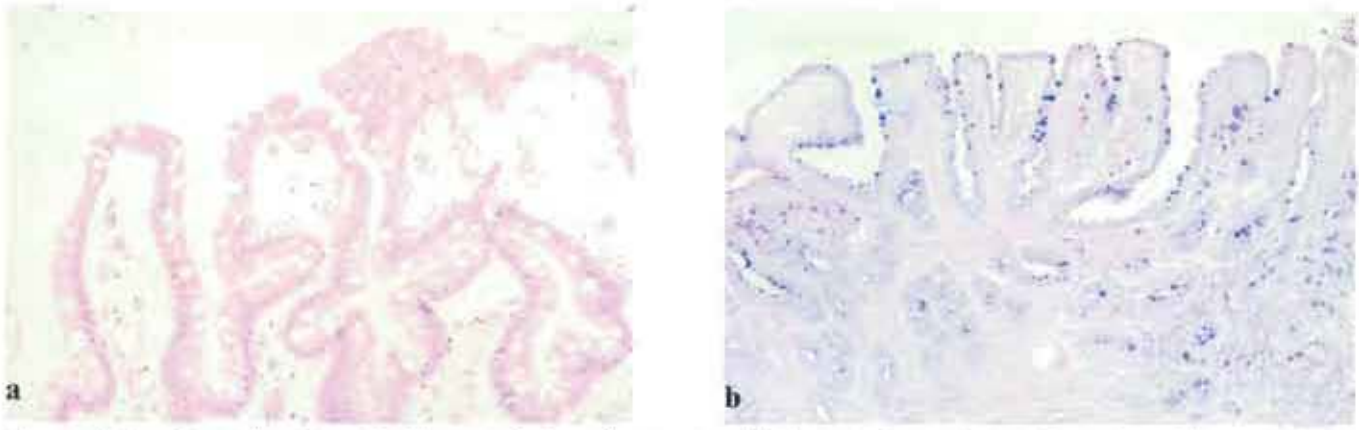


Figure 3.3. Complete intestinal metaplasia adjacent to GRC (not shown). (a) Note the villi with brush border, goblet cells and absorptive cells (H&E, original magnification x 40). (b) Histochemical staining showing acidic type mucin (blue) in the goblet cells with no neutral mucin (PAS/AB, original magnification x 40).

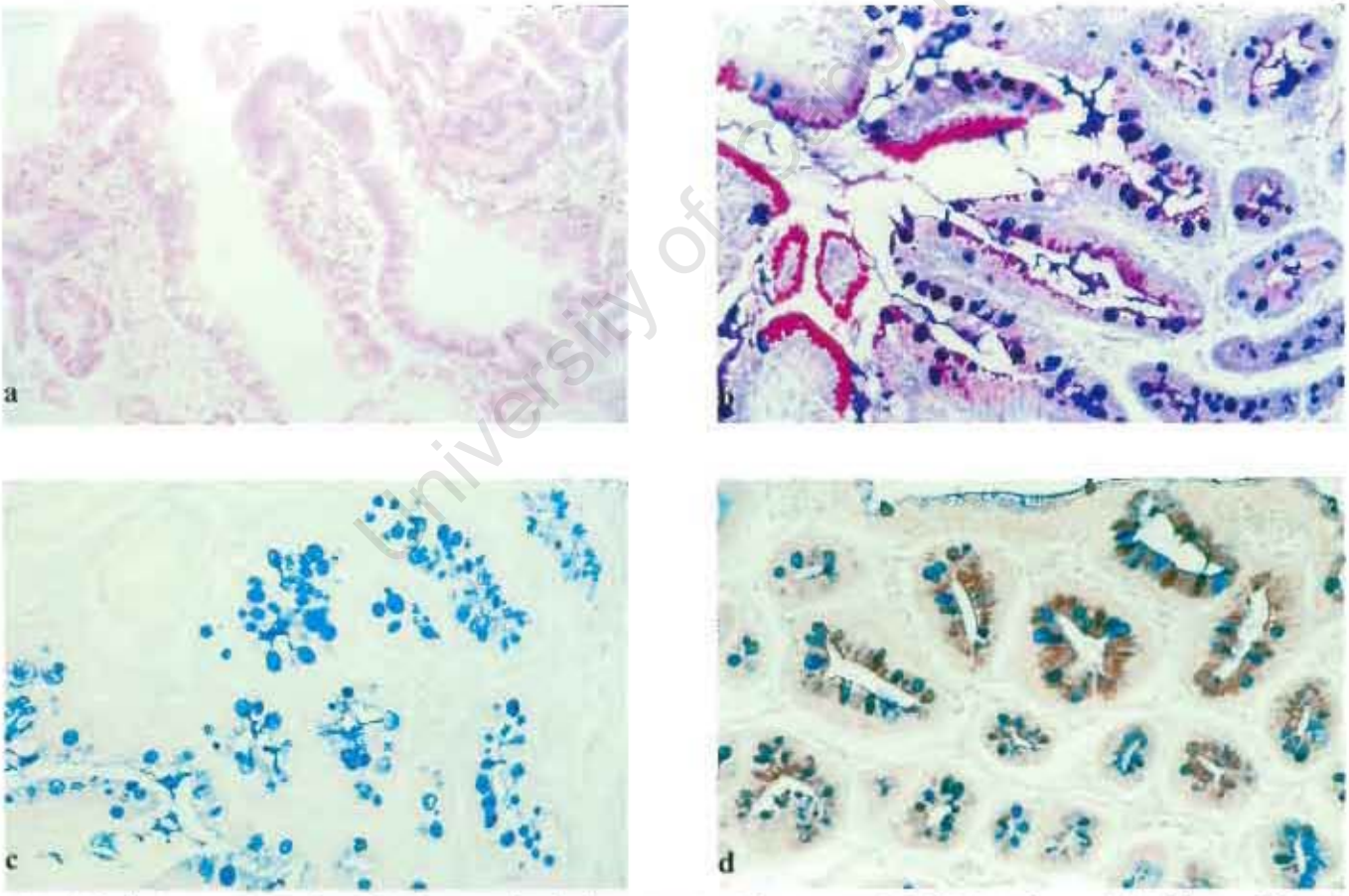


Figure 3.4. Incomplete intestinal metaplasia in mucosa adjacent to GRC (not shown).(a) Note villi and goblet cells with no absorptive cells (H&E, original magnification x 100). (b) Histochemical staining shows acidic mucin in goblet cells (blue) and neutral mucin (red) in columnar cells (PAS/AB, original magnification x 100). (c) Incomplete intestinal metaplasia type A. Only sialomucin (blue) is expressed in goblet cells (HID, original magnification x 100). (d) Incomplete intestinal metaplasia type B. Predominance of sulphomucin (black/brown), compared to sialomucin (blue), expression in goblet cells. (HID, original magnification x 100).

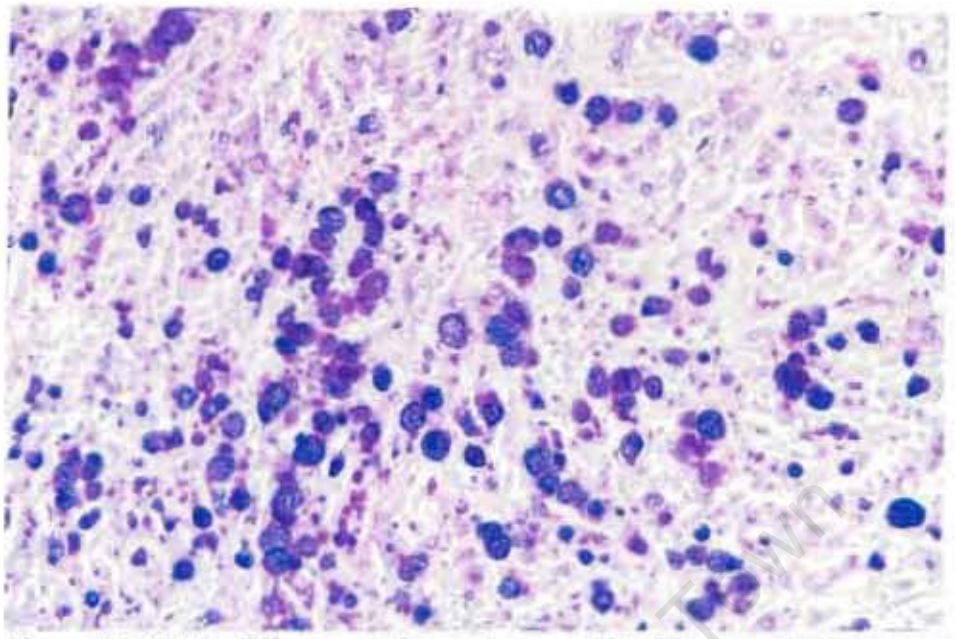


Figure 3.5. GRC, diffuse type shows strong acidic (blue) mucin in tumour cells (PAS/AB, original magnification x 100).

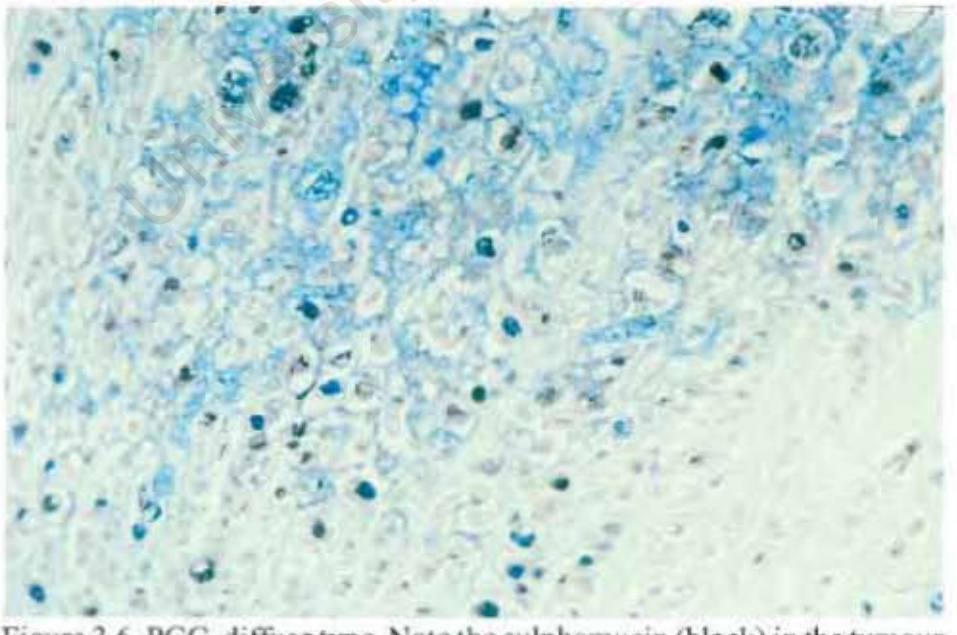


Figure 3.6. PGC, diffuse type. Note the sulphomucin (black) in the tumour cells (HID, original magnification x 100).

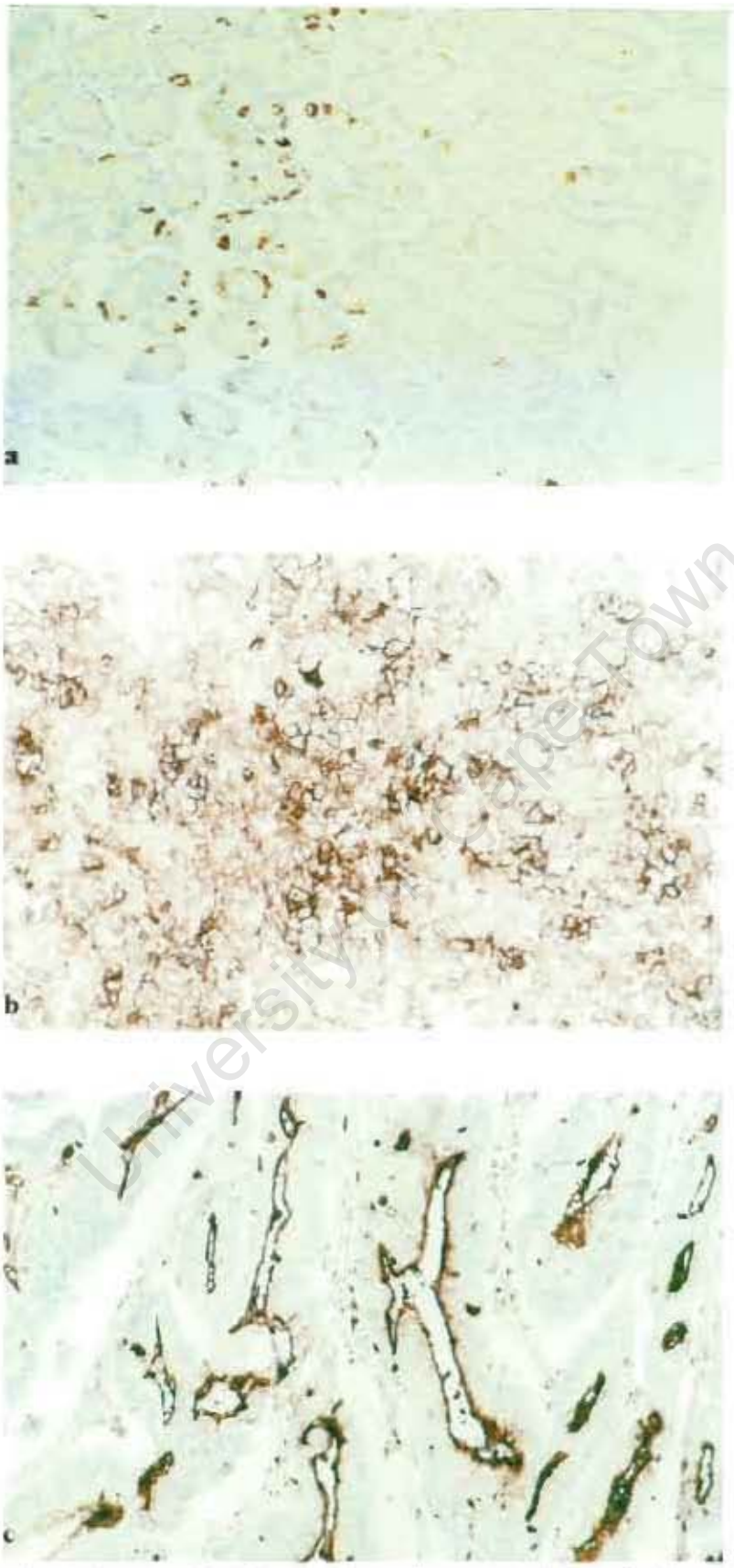
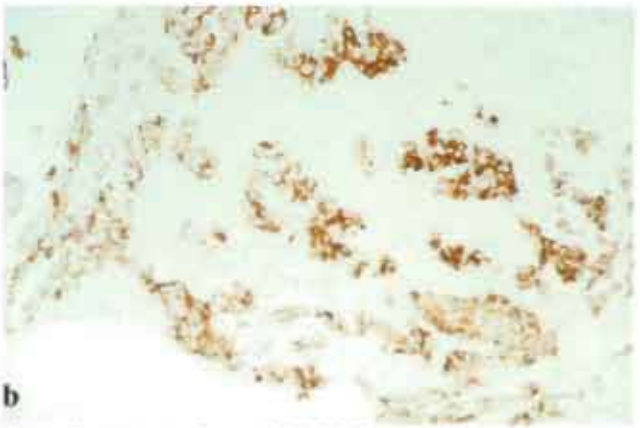


Figure 3.7. MUC1. (a) Normal gastric mucosa (control) shows the expression of MUC1 in the parietal cells (original magnification x 100). (b) GRC, diffuse type shows strong cytoplasmic and membranous expression of MUC1 (original magnification x 100). (c) PGC, intestinal type shows strong membranous expression of MUC1 (original magnification x 100).

b



b

Figure 3.8. MUC2. (a) Intestinal metaplasia shows cytoplasmic expression of MUC2 in goblet cells (original magnification x 100). (b) GRC, intestinal type shows moderate expression of MUC2 in the tumour cells (original magnification x 100).

a

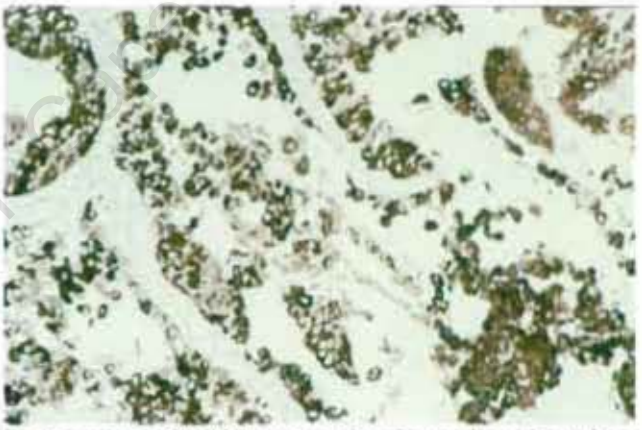


Figure 3.9. MUC5AC. (a) Normal gastric mucosa (control) shows MUC5AC expression in neck and surface epithelium (original magnification x 100). (b) GRC, intestinal type shows strong cytoplasmic expression of MUC5AC (original magnification x 100).

Figure 3.10. MUC5B. GRC, diffuse type showing weak cytoplasmic expression of MUC5B (original magnification x 40).

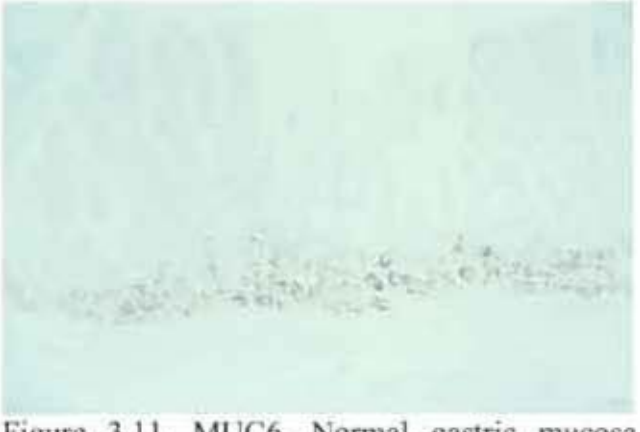


Figure 3.11. MUC6. Normal gastric mucosa (control) shows expression of MUC6 in glands. (original magnification x 40).

CHAPTER FOUR

DISCUSSION

In this study, three cases (16%) of GRC showed the presence of moderate number of *H.pylori* in the mucosa away from the tumour. Fewer cases of GRC showed the presence of *H. pylori* compared to 25% of PGC. *H.pylori* were present on the gastric mucosa that did not show intestinal metaplasia (IM), although IM was present in the same specimens. A similar finding was made in a study performed on PGC specimens by Dixon et al. (1996). In this study on GRC and PGC, *H.pylori* were never seen in incomplete or complete intestinal metaplasia. This result is consistent with the results of Genta et al. (1996), which reported that advanced gastric carcinoma and intestinal metaplasia were considered a hostile environment for *H. pylori* organisms and also that *H. pylori* were seldom seen in the areas of incomplete intestinal metaplasia. The finding of *H.pylori* in this study of GRC was in agreement with the results of Elder et al. (1991), who found *H.pylori* organisms in cases of GRC. They suggested that the finding of *H.pylori* in GRC could be explained by chemical atrophic gastritis in gastric remnant providing a suitable environment for colonization by *H.pylori* (Elder et al. 1991). However Greene et al. (1996) reported that there was no specific association between *H.pylori* and GRC. Two cases in the present study of GRC showed the presence of lymphoid follicles, with no *H. pylori* present. Genta et al. (1993) considered the presence of lymphoid follicles as a feature highly suggestive of previous infection by *H. pylori* organisms. The findings in this study therefore suggest previous *H. pylori* infection with a possible implication of the organism in the pathogenesis of GRC.

Histochemically 80% and 50% of the cases of intestinal type GRC and the diffuse type GRC showed the presence of intestinal metaplasia respectively. The metaplasia was more prevalent in the cases of intestinal type adenocarcinoma. Furthermore type I IM was seen in 50% of the intestinal type GRC and 25% of the diffuse type GRC; 30% of intestinal type GRC had sulphomucin positive incomplete metaplasia (Type IIB) while none of the specimens of diffuse GRC showed type IIB IM. The intestinal type GRC showed higher association with type I IM and type IIB IM than diffuse type GRC. In comparison with the gastric mucins expressed in the normal gastric biopsies

of this study, all normal gastric biopsies expressed neutral with no acidic mucin found, the findings of which are in keeping with those in other studies (Taylor et al 1998). In this study intestinal types GRC and PGC showed similar proportions of Type I, Type IIA and Type IIB IM. Also the diffuse types GRC and PGC showed similar proportions of Type I, Type IIA and Type IIB IM, but there was a difference in the incidence of IM between the intestinal and diffuse types of carcinomas. Intestinal types GRC and PGC showed more association with sulphomucin positive IM (Type IIB). GRC of this study showed higher association with intestinal metaplasia in comparison with the published data; 58% of GRC showed association with IM, and 30% showed Type IIB IM. In other studies, on the other hand, the presence of IM in GRC was reported to be between 22-48% (Domellof et al. 1977, Pickford et al. 1984, Schrumpf et al. 1977, Wladimir et al.1985). Although 30% of the cases of GRC of this study expressed Type IIB IM, Wladimir et al. (1985) reported the presence of IM in 22.5% of GRC, of which none expressed Type IIB (sulphomucin expressing type). In summary, this study showed that IM was more prevalent in the intestinal type carcinomas of GRC or PGC. These results are similar to the other published studies on PGC (Filipe and Potet 1985, Lev 1965, Taylor et al. 1998).

More sulphomucin positivity is expected in intestinal than in the diffuse carcinomas, as Type IIB IM is more common in intestinal types GRC and PGC. However in this study a similar proportion of diffuse type GRC and intestinal type GRC expressed sulphomucin (33% and 27%) respectively, while there was an obvious difference in the PGC cases. In fact more of the diffuse PGC (60%) secreted sulphomucin than the intestinal type PGC (10%). The findings in this study are also different from those in other studies, which showed that sulphomucin tend to predominate in PGC intestinal type, and neutral mucins in the PGC diffuse type (Jass and Filipe 1981). Taylor et al. (1998), in a study of primary gastric carcinomas, showed that sulphomucin was present in 40% of PGC intestinal type and in 14% PGC diffuse type. Whilst our findings could imply differences due to different genetic backgrounds, ethnicity, or even technical problems, it must be remembered that Taylor et al. (1998) performed their studies in the same setting. It is obvious that the question of the incidence of sulphomucin expression in gastric carcinoma of the intestinal and diffuse types needs further investigation.

Immunohistochemically normal gastric mucosa expressed MUC1, MUC4, MUC5AC and MUC6. There was a strong positive MUC1, and MUC1C, weak positive MUC4 and strong positive MUC5AC in 100% of the biopsies and moderately positive MUC6 in 60% of the cases. Taylor et al. (1998) showed positive MUC4, MUC5 and MUC6. Ho et al. (1995) have shown that normal stomach mucosa was characterized by the expression of MUC1, MUC5 and MUC6, while MUC2, MUC3 and MUC4 were negative. In this study, MUC1 and MUC1C were present in the parietal cells of the gastric fundic glands in 100% of the cases and it was cytoplasmic and membranous, while the surface mucus neck cells and antrum type glands were always negative. The results were in agreement with Ho et al. (1995) who showed that MUC1 was expressed in 100% of the normal gastric mucosa. In comparison, Taylor et al. (1998) showed that 20% of the cases of normal gastric tissue were reactive to MUC1 antibodies and it was the parietal, surface mucosa and mucus neck cells that were positive. In this study MUC4 was positive in basal mucus glands in 100% of normal gastric biopsies. The results in this study were similar to a previous study done in the same department by Taylor et al (1998). MUC4 is also expressed in respiratory and reproductive tracts and gastric mucosa and to a lesser extent by goblet cells of the colorectum (Winterford et al. 1999, Seregni et al. 1997). However other studies showed that gastric mucosa did not express MUC4 (Ho et al. 1995). Ho has repeated MUC4 staining on normal gastric mucosa using the same antibody that was used in this study, and has confirmed the presence of MUC4 (personal communication), in gastric tissue. MUC5AC was present in the surface and mucus neck cells of all normal gastric mucosa (control) as well as in surface and mucus neck cells in the gastric mucosa adjacent to the various gastric carcinomas. These findings are similar to other studies (Lopez-Ferrer et al. 2000, Machado et al. 2000). MUC5AC is a secretory gel forming mucin, normally expressed in the superficial and foveolar epithelium of gastric mucosa and neck cells, while its expression is absent in the deep glands of the gastric body and pyloric glands of the antrum (Machado et al. 2000). The expression of MUC6 by the normal gastric mucosa was similar to the findings in other studies (Ho et al. 1995, Machado et al. 2000). MUC2 and MUC5B were negative in the gastric epithelium.

The results of staining with antibody to MUC3 have not been included because of technical problem with the antibody, which gave a false positive reaction with normal gastric mucosa. Please see appendices 3.1 and 3.2.

Intestinal GRC showed strong expression of MUC1 in 36%, MUC2 in18% and MUC5AC in 45% of tumours, while diffuse type GRC showed strong expression of MUC1 in17%, MUC2 in 17%, MUC5AC in 33% and MUC5B in 17% of tumours. In contrast PGC intestinal type showed strong expression of MUC1 in 70%, MUC2 in 40%, MUC5AC in 20% and MUC6 in 20% of tumour cells, while PGC diffuse type showed strong expression of MUC1 in 30%, MUC2 in 40% and MUC5AC in 60% of tumour cells. The expression of MUC2 was aberrant in the intestinal type GRC, while MUC4 and MUC6 were completely absent in contrast to the normal stomach. On the other hand diffuse type GRC showed aberrant expression of MUC2 and MUC5B in comparison to the normal gastric mucosa. Fewer intestinal type GRC cases expressed MUC1 and MUC2 than PGC. Other than that there were no major differences between the mucin profiles of GRC and PGC diffuse type. There were no published results on the immunohistochemical mucin profile concerning GRC to compare with our results. The results in the GRC and PGC resembled, to some extent the published results on PGC, although some differences were also present. Taylor et al. (1998) found considerable difference between PGC diffuse type and PGC intestinal type. The expression of MUC1, MUC5 and MUC6 were strong in late stage PGC intestinal type, while MUC2, MUC5, MUC6 and MUC7 were present in various combinations in a large proportion of PGC diffuse type. More cases of intestinal types GRC and PGC in this study expressed MUC1 than the diffuses types GRC and PGC, the results of which were in agreement with the findings of Taylor et al. (1998) and Ho et al. (1995), who showed that there was an increased expression of mucin gene in intestinal type PGC as compared to the diffuse type. The aberrant expression of MUC2 in the GRC and PGC cases in this study was consistent with the results in the literature, which showed that MUC2 was up regulated in PGC (Cornberg et al. 1999, Lopez-Ferrer et al. 2000). In this study 33% of diffuse GRC and 45% of intestinal GRC were positive to MUC5AC, while 60% of PGC diffuse type and 20% of PGC intestinal type expressed MUC5AC in comparison to 100% in normal gastric mucosa. Ho et al. (1995) showed that the expression of MUC5 levels was decreased in gastric cancer in comparison to the normal gastric mucosa. Reis et al. (1997) found

that PGC diffuse type expressed higher MUC5AC than PGC intestinal type, the finding of which were in agreement with the results of PGC of this study, but not to those of GRC. In a study performed by Wang et al. (2003) they found that patients with MUC1 positive/MUC5AC negative gastric carcinoma showed a low survival rate in comparing with MUC1negative/MUC5AC positive gastric carcinoma patients.

No attempt was made to compare the findings in the study with survival because insufficient follow-up information was available.

It would be of interest to group the GRCs by their location in the stomach to determine whether there are morphological, histochemical and immunohistochemical differences in tumour arising near the site of gastro-jejunal anastomosis compared to those arising in the cardiac and fundic regions. A larger number of cases and more clinical information, including long-term follow-up, will be required. Such a study would permit the mucin in the GRCs be correlated, not only with the location of the tumour in the gastric remnant, but also with survival.

Information about the prevalence of PGC in the general population, in South Africa and other parts of the world, versus the prevalence of GRC is needed to determine whether partial gastrectomy for benign disease is indeed a risk factor for GRC.

CONCLUSION

In general morphologically, histochemically and immunohistochemically gastric remnant carcinoma closely resembles primary gastric carcinoma, this includes the finding of a high association of Type I and IIB intestinal metaplasia in the intestinal types of both GRC and PGC compared to the diffuse types of GRC and PGC, although a smaller percentage of GRCs expressed MUC1 and MUC2 than PGCs.

The similarity of the histochemical and immunohistochemical mucin profile of GRC compared to PGC suggests that they may not be distinct pathological entities.

Future studies are needed to answer some of the unexplained findings such as the more frequent expression of sulphated mucin in diffuse types GRC and PGC compared to intestinal type GRC and PGC, a finding which is different to that of published series.

APPENDIX

Appendix 1: TNM staging of gastric carcinoma

Primary tumor

TX Primary tumour can not be assessed

T0 No evidence of primary tumour

Tis Carcinoma insitu: Intraepithelial tumour without invasion of the lamina propria

T1 Invasion of lamina propria (T1a) or submucosa (T1b)

T2 Invasion of muscularis propria (T2a) or subserosa (T2b)

T3 Tumour penetrates serosa

T4 Invasion of adjacent structures

Regional lymph node metastsis

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in 1 – 6 perigastric lymph nodes

N2 Metastasis in 7 – 15 perigastric lymph nodes

N3 Metastasis in more than 15 perigastric lymph nodes

Distant metastasis

M0 No distant metastasis

M1 Distant metastasis

Staging of gastric carcinoma by the TNM method

0	Tis	N0	M0
IA	T1	N0	M0
I B	T1	N1	M0
	T2	N0	M0
II	T1	N2	M0
	T2	N1	M0
	T3	N0	M0
IIIA	T2	N2	M0
	T3	N1	M0
	T4	N0	M0
IIIB	T3	N2	M0
IV	T4	N1,2,3	M0
	T1-4	N3	M0
	Any T	Any N	M1

Appendix 2: Supplier of antibodies used in the immunohistochemical study.

Biotinylated rabbit-anti-mouse antibody- Dako, Copenhagen, Denmark.
Biotinylated swine-anti-rabbit antibody- Dako, Copenhagen, Denmark.
Biotinylated rabbit-anti-chicken antibody- Biogenesis, Poole, UK.
Strep-avidin HRP- Dako, Copenhagen, Denmark.

Appendix 3.1:

MUC3 is a membrane bound mucin, expressed in small intestine and colorectum. It is secreted by columnar and goblet cells of IM and colorectum (Jass 2000). In this study, all normal gastric biopsies and the gastric mucosa adjacent to the various gastric carcinoma expressed MUC3 in the cytoplasm of mucus secreting cells of the deep glands as well as in the goblet cells of intestinal metaplasia. Taylor et al. (1998) and Ho et al. (1995) did not find MUC3 in the normal gastric mucosa.

The MUC3 antibody used in the first instance was a polyclonal rabbit anti-MUC3 antibody (Biomeda, lot no 8769, cat. no V2056, expiry date 4/04). Upon repeating the staining procedure with another MUC3 antibody from the same company with the same specifications, non-specific and background staining was seen in the tissue. However MUC3 was positive in the goblet cells, which is in agreement with the findings in other studies (Jass 2000, Ho et al. 1995). In this study 40% of intestinal type PGC expressed MUC3 in less than 25% of the tumour cells, while none of GRC or diffuse type PGC expressed MUC3. These results were in contrast to that of Taylor et al. (1998), which showed no expression of MUC3 in the advanced intestinal PGC and positive staining in 29% of the diffuse type PGC. The apparent positive staining for MUC3 in normal gastric mucosa was contrary to published results (Taylor et a. 1998, Ho et al. 1995); similarly the results in PGCs are different from those of Taylor et al. (1998) in a study performed in the same department.

Given the uncertainity about MUC 3 staining in the normal control slides, the immunohistochemical results in the PGCs that conflicted with those of published studies as well as the manufacturer agreeing that there was a problem with the antibody, a decision was made to omit the results of MUC3 for this study.

Appendix 3.2: Letter from the company for MU

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