CHRONIC GASTRITIS, HELICOBACTER PYLORI AND MICRONUTRIENT STUDIES IN PATIENTS AT RISK FOR GASTRIC CARCINOMA

BY

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CONTENTS

STATEMENT OF CANDIDATE

I

I declare that the work on which this dissertation is based is original (except where acknowledgements indicate otherwise), and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

It is a pleasure to record my indebtedness to Professor AG Rose of the Department of Anatomical Pathology, University of Cape Town Medical School, for his encouragement, for granting the facilities to complete this work, for the interest he showed in my work, as well as for his comments and criticisms during the writing of this manuscript.

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For Alma Mater Medica Gedanensis

INTRODUCTION

In spite of falling incidence rates in developed countries, gastric carcinoma is still a common cancer world-wide, and shows an uneven geographical distribution (1). At the end of the forties, gastric cancer was the most common cause of cancer death among Coloured (mixed origin), Asian and White populations in South Africa (2). During the following 30 years the incidence rates among Whites and Asians dropped considerably, as experienced in some European countries, but little change was noted in the Coloured population (3). More recent available estimates based on pathological reports, showed rates as high as 50/100 000 per year in this population group (4). According to these estimates, and our departmental records (5), gastric carcinoma is at least three times more frequent in males, than in females.

The aetiology of gastric carcinoma, despite over a 100 years of investigation in many countries and population groups, is still uncertain. Epidemiological studies have reported a positive association between nitrate intake and gastric carcinoma rates (6, 7). Nitrates have to be reduced to nitrite before carcinogens, such as N-nitroso compounds, can be formed. The formation of these compounds is

influenced by the intragastric pH, catalysers (bacteria, inorganic ions), and the nitrite concentration (8). This is believed to be related to the greater intake of cured foods (salt meat, dried salt fish), fried foods, along with other risk factors. Alcohol appears to be an important factor as well. In the recent Louisiana study of gastric carcinoma and chronic atrophic gastritis, a 2.5-fold risk was associated with intake of beer and total alcohol, a two-fold risk was associated with smoking, and an increase in pH was associated with increasing severity of lesions in gastric mucosa (9). According to Correa (10), this is an endemic or diffuse type of gastric cancer, in which the mucosa becomes injured and necrotised; it differs from the original epidemic type that was responsible for the high cancer rates in the 1930s. The endemic type, more prevalent in lower socio-economic population groups, is associated with heavy drinking of unsophisticated alcoholic beverages, and low intakes of fruit and vegetables may be more important than the nitrate content of foods. The finding regarding nitrate was opposite to the findings in a Colombian population (10).

The introduction and universal use of fibreoptic endoscopy and targeted biopsy has increasingly documented the widespread prevalence of gastritis. Since the routine use of gastroscopy in populations at risk, chronic atrophic gastritis (CAG) was found to be prevalent in some geographical areas (6). A continuing dilemma has been the size of the population suffering from gastritis coupled with the lack of a clearly identifiable pathogenetic mechanism. The recent suggestion that Helicobacter (previously Campylobacter) pylori is the major cause

of chronic inflammation of gastric antral mucosa indicates the possibility of solving, at least partially, this problem. This has potentially very important implications for treatment, and also perhaps for the prevention of peptic ulcer, and may be relevant to the initiation of gastric cancer.

Many of the key classifications of gastritis were devised before better understanding of the epidemiology of H. pylori was obtained.

Schindler (11), in 1947, divided chronic gastritis into superficial, atrophic or hypertrophic. Many classifications that followed were variations of this theme, but were limited by the availability of material which could only be harvested by blind gastric biopsy.

Whitehead et al. (12): 1. distinguished chronic superficial from chronic atrophic gastritis; 2. recognised that antral changes were separate from those affecting the corpus; 3. documented intestinal metaplasia, activity of gastritis and severity of atrophy. This classification is purely morphological, and there is no statement about aetiology.

Strickland and MacKay (13) separated two main types of atrophic gastritis: type A (associated with pernicious anaemia and involving atrophy of the corpus), and type B (unassociated with parietal cell antibodies in the serum and involving atrophy of antral mucosa).

Type A gastritis was reported as "autoimmune" and affects body mucosa, and type B, known as "environmental", affects mainly antral, but may be focally present in body mucosa (14).

The third type, AB gastritis added by Kekki et al (15), is diffuse, but affects mainly the antrum, and may or may not be associated with antibodies to parietal cells (16). The separation of corporal chronic gastritis (type A) from the corresponding type AB is difficult, but has been applied in pernicious anaemia families (16). This type is more common in some European countries, particularly in Scandinavia (15). Multifocal or antral atrophic gastritis not associated with pernicious anaemia is the most frequent type found in various groups of populations, mainly coinciding with the geographic distribution of populations at risk for gastric cancer (14). This type of gastritis is associated with intestinal metaplasia (IM) of gastric mucosa. Incomplete, sulphomucin producing variants of IM have some prognostic value in the development of gastric carcinoma, as pointed out by some authors (14, 18-21). Others (22, 23) have found IM to have no prognostic value with regard to gastric carcinoma.

It is important to stress that the fundus of the stomach is normal or minimally involved in the great majority of cases of type B chronic gastritis.

Another common type of chronic gastritis is the superficial type, characterised by inflammatory infiltration of various activities of the superficial portions of gastric mucosa. Crypt atrophy and IM are absent, or insignificant. This type of gastritis, particularly active, is commonly associated with H. pylori infection

The most recent Sydney System for the Classification of Gastritis, presented during the 9th World Congress of Gastroenterology in Sydney, August 1990 (17), aimed to produce a simple, comprehensive classification.

The histological basis of the Sydney System consists of three parts:

1. aetiology, 2. topography, 3. morphology.

Changes in the gastric antrum and in the gastric corpus are always reported separately. Thus, in order to arrive at a classification, a minimum of two biopsies has to be taken from both the antrum and the corpus. Any specific lesions are biopsied additionally. The large (jumbo or 7 French gauge) biopsy forceps should be used in preference to the other types, in order to harvest muscularis mucosae.

The Sydney System recognises only three basic morphologies:

1. acute gastritis, 2. chronic gastritis, 3. special forms.

Chronic gastritis is the commonest, acute gastritis is transient and rarely biopsied, while special forms are uncommon. The variabilities in the morphological limb pertinent to chronic gastritis are as follows:

1. Normal mucosa. Contains scattered mononuclear cells, lymphocytes and plasma cells. In the corpus, occasional small lymphocytic aggregates may be present at the basis of glands. Granulocytes are absent.

2. Acute versus chronic. If neutrophils are the dominant inflammatory cell, the gastritis is acute. If there is a concomitant increase in chronic inflammatory cells, the gastritis is classified as chronic.

3. Atrophy. Refers to the loss of gastric glands. Thus inflammation and activity are assessed independently.

4. Activity refers to the presence of neutrophils in lamina propria, in intraepithelial sites, or both.

The special forms of gastritis include granulomatous gastritis and its many causes (Crohn's disease, tuberculosis, sarcoidosis, etc), eosinophilic gastritis, vasculitides, lymphocytic and reactive (reflux) gastritis.

Reflux and lymphocytic types of chronic gastritis are uncommon, and are probably not related to gastric cancer (24). Active gastritis, gastric and particularly duodenal ulcers are strongly associated with

H. pylori infestation (24,25,26). Less obvious is the association and role of H. pylori in the aetiology of gastric cancer .

The purpose of this study is to investigate various morphological aspects of chronic gastritis, particularly its chronic atrophic and lymphocytic type, and other gastric mucosal precursor lesions in a population at risk for gastric carcinoma; to investigate the prevalence of H. pylori infection in various lesions of gastric mucosa in population at risk; cellular and immune response of gastric mucosa in H. pylori eradication treatment; nutritional and other factors in aetiology of chronic atrophic gastritis.

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CHAPTER 1

CHRONIC ATROPHIC GASTRITIS IN POPULATION AT RISK FOR GASTRIC CARCINOMA

ABSTRACT

Gastric mucosa of 585 patients at risk for gastric carcinoma and undergoing oesophago-gastroscopy for dyspeptic symptoms, was examined histologically and histochemically. Sixteen (2.7%) of the patients studied, showed gastric carcinoma. Intestinal type of gastric carcinoma was the most common, present in males over 40 yrs old and was found in 1:16.

Early cancers were present in 3 patients. Chronic atrophic gastritis of the A, B and AB types were present in 32.8%. Types A and AB were noted sporadically. Males over 40 and tobacco smokers showed a greater prevalence of the type B (idiopathic chronic pangastritis) and incomplete intestinal metaplasia. No relationship of the mucosal precursor lesions to sulpho- and sialomucins was detected. Helicobacter pylori infestation of gastric mucosa was present in 81% of patients, and was relatively common in young patients with normal gastric mucosa; therefore, this finding confirms a predilection of Helicobacter pylori to the Third World.

INTRODUCTION

Despite the importance of CAG in the epidemiology of gastric carcinoma, very few studies have focused specifically on the aetiology of CAG, and there is a clear need for more studies on gastric cancer precursor lesions in different countries. Such studies are non-existent in Africa. The purpose of this study is to establish the frequency of histological detection of gastric carcinoma, particularly its early type, prevalence of mucosal precursor lesions and the relation of these to IM, sulpho- and sialomucins, H.pylori, sex and age in patients with unspecified dyspepsia

MATERIAL AND METHODS

The study population includes 585 participants who were treated with dyspeptic complaints such as epigastric pain, heartburn, post-prandial pain and bloating, vomiting or nausea with a duration of at least 3 months.

Patients represented various areas in the south- and north-western Cape province, including Namaqualand , and formed part of a programme aimed at screening patients with chronic gastric complaints for early gastric carcinoma. There were 355 males, mean age 45.1 years, and 230 females, mean age 47.2 years. The age range was 19-87 years and was similar for both sexes. One hundred and ninety two males were over 40 years old, in comparison to 133 females (54% and 58% repectively).

At the clinic, the purpose of the examination was explained, and informed consent was obtained. The patients were instructed to fast overnight and to report to the clinic the next morning. Approval from the local hospital ethical committee was given for all aspects of this study.

Endoscopy was carried out using an Olympus GIF-XQ-10 instrument. No sedation was used, but the pharynx was sprayed with xylocaine. Between each endoscopy, the suction channel of the endoscope was washed, and rinsed with Cidex and distilled water. On entering the stomach, gastric juice was aspirated for examination of the pH and nitrites (1).

At the end of endoscopy, two mucosal biopsies were taken from each of the following sites: angulus, 5 cm from the pylorus on the greater curvature, fundus and oesophagus (Fig. 1.1).

The biopsy specimens were collected in 10% buffered formalin. Biopsies were processed routinely, and embedded in paraffin under a dissecting microscope. Sections were cut and stained with haematoxylin and eosin, periodic acid-Schiff (PAS) and alcian blue for neutral and acid mucins, and with high-iron diamine-Alcian blue (HID/AB) for sialo- and sulphomucins. The mucin stains were used for the accurate assessment of intestinal metaplasia, the subtyping of gastritis, the amount and type of mucin produced by the gastric epithelium and the quantity of carbohydrates present. Giemsa stain for spiral organisms was also used. Selected cases were studied immunohistochemically for MT-1, UCHL-1 antibodies and cytoplasmic immunoglobulins. The slides were interpreted without knowledge about source of biopsy, sex, age or other characteristics of the patients.

For histological classification of the mucosal changes, the system devised by others has been used. Patients presenting with gastritis and signs of body and antral crypt atrophy and/or intestinal metaplasia were classified under CAG (idiopathic chronic pangastritis) (Figs. 1.2,1.3) Round cell infiltrates of the lamina propria without loss of crypts and intestinal metaplasia were classified as superficial gastritis (Fig. 1.4).

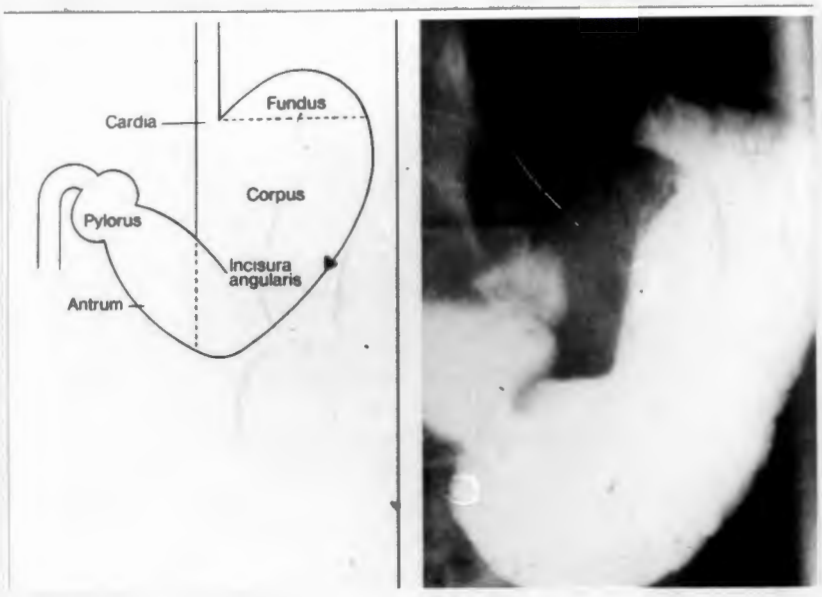

Fig. 1.1: The topography of the stomach. Two mucosal biopsies were taken from each of the following sites: angulus, 5 cm from the pylorus on the greater curvature and fundus.

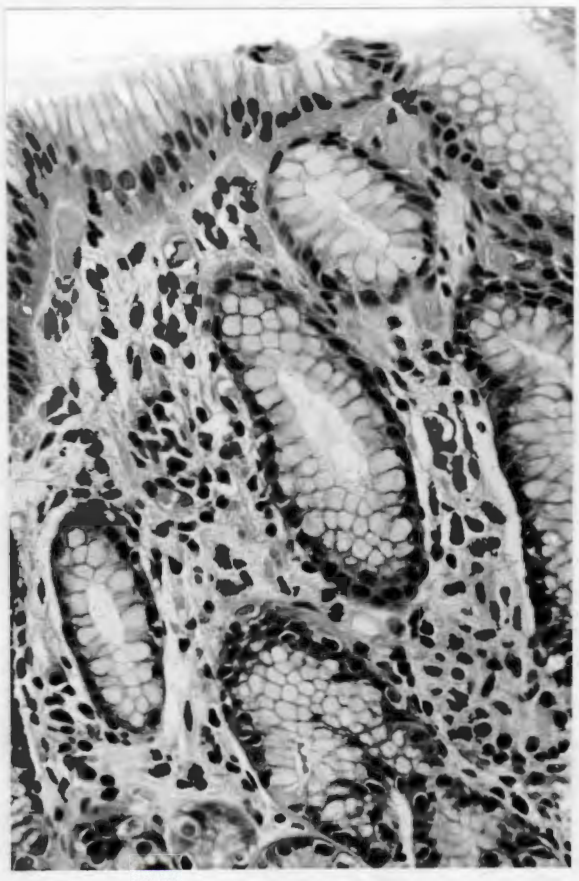

Fig. 1.1a: Normal appearance of gastric glands and lamina propria in the sample from the gastric antrum. No evidence of gastritis. H & E X 420.

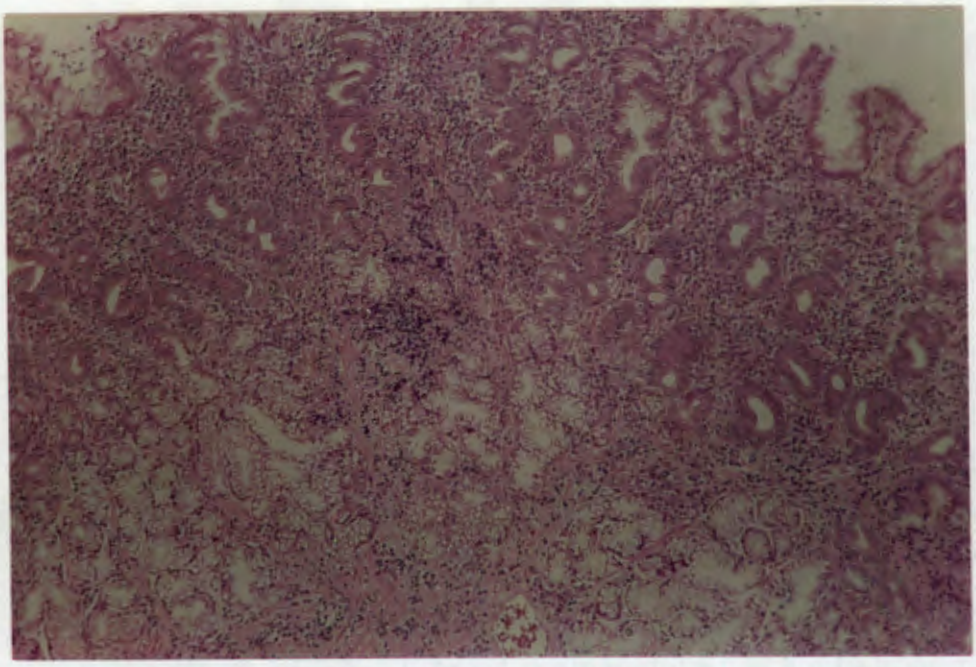

Fig. 1.2: Severe chronic active gastritis. Heavy mixed cellular inflammatory infiltrate of the lamina propria and crypt epithelium. Mild degree of crypt atrophy. H & E X 180.

Fig. 1.3: Chronic atrophic gastritis, inactive. Note marked atrophy of crypts, hypocellular lamina propria and patchy intestinal metaplasia. H & E X 320.

Fig. 1.3a: Chronic atrophic gastritis with extensive intestinal metaplasia. H & E X 320.

Fig. 1.4: Superficial gastritis. Mucosal surface of the gastric antrum. Note the dense, predominantly mononuclear infiltrate of the lamina propria and several neutrophils within the crypt epithelium. H & E X 450.

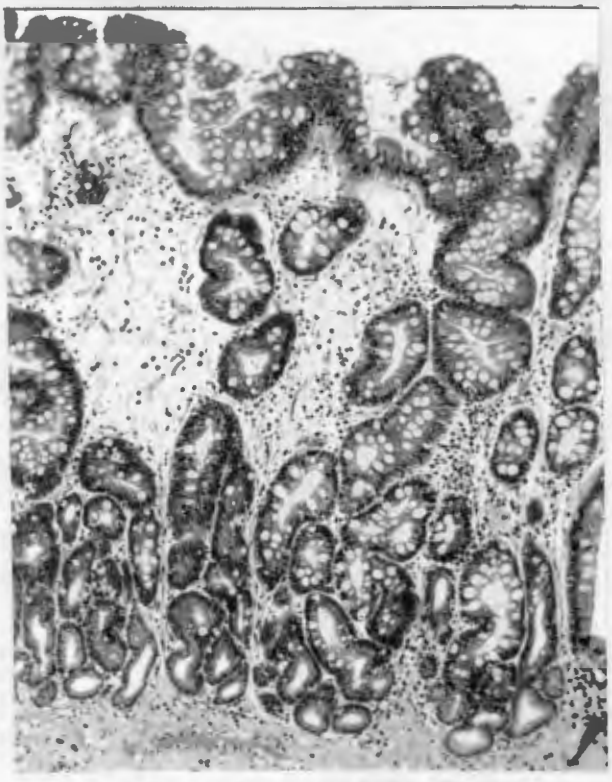

Fig. 1.3a: Chronic atrophic gastritis with extensive intestinal metaplasia. H & E X 320.

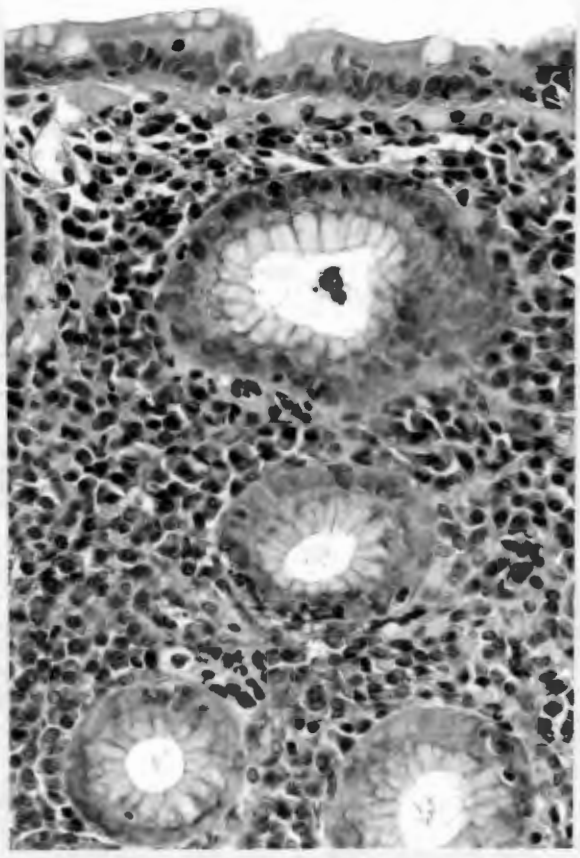

Fig. 1.4: Superficial gastritis. Mucosal surface of the gastric antrum. Note the dense, predominantly mononuclear infiltrate of the lamina propria and several neutrophiles within the crypt epithelium. H & E X 450.

Patients with pathological lesions such as ulcers, tumours etc., were subjected to further investigation and treatment.

From the patients with hypochlorhydria\CAG, and those with normal gastric mucosa, venous blood was collected for vitamins and mineral assessment (1).

Grading of colonisation of gastric mucosa by H.pylori was performed as described in chapter 2. In H. pylori-infected gastric mucosa, the bacteria were seldom seen in the vicinity of the intestinal metaplasia, therefore, the colonisation assessment was done in non-metaplastic mucosal fields. Student's T-test was used for statistical evaluation of results.

RESULTS

Results of the evaluation of gastric mucosa are given in Table 1.1. Sixteen (2.7%) of the 585 patients studied showed gastric carcinoma. Malignancy was most common in males over 40 years old and found in 1:16. Three cases presented early stages of carcinoma, confirmed by consecutive gastrectomy (Figs. 1.5-1.8). Histological classification of the carcinomas, using Lauren's classification (2), revealed that 12 were intestinal and the remainder were diffuse variants. Of 4 affected

females, two had diffuse histological variants (Fig. 1.8) of gastric carcinoma.

There were relatively low numbers of dysplastic lesions (1.8%). All were classified as mild or moderate (Fig. 1.9), and not associated with regenerative changes, commonly seen in specimens taken from ulcers. Ulcers were present in 10% of the patients studied. Mucosal hyperplasia was associated with regeneration or inflammation, and polypoid lesions detected at endoscopy had hyperplastic or/and inflammatory character.

The commonest lesion, chronic gastritis, was diagnosed in 67% of patients with non-ulcer dyspepsia. Reflux gastritis and lymphocytic gastritis were noted in a few cases. Lymphocytic gastritis, a new histological entity, is characterised by a dense T-lymphocyte infiltrate of superficial or foveolar epithelium of gastric mucosa. It was uncommon, and affected 0.8% of the tested population (see chapter 4).

Chronic atrophic and superficial gastritis were the most and equally common lesions of gastric mucosa. Types A and AB of chronic atrophic gastritis were noted sporadically (Table 1.1), therefore, idiopathic chronic pangastritis (CAG) and superficial gastritis deserve special attention.

The relationship of gastritis to the sex and age showed a significant prevalence of superficial gastritis in females, whereas CAG was more prevalent in males (Table 1.2), particularly in those older than 40 years. In males over 40 years, CAG was noted in 35.7%, and younger males showed CAG in 20.6% ($p<0.001$).

Positive association of CAG with tobacco smoking has also been found ($p=0.024$). No such association with the consumption of alcohol existed.

Active forms of CAG and superficial gastritis were 3 times more common than the respective quiescent forms in both sexes, but activity of inflammation in both types of gastritis was mainly mild or moderate. Severe superficial gastritis and severe CAG was seen only in 5 and 6 cases, respectively.

IM distribution in gastric mucosa was the same as the distribution of CAG, and both were more prevalent in the antrum.

Spread of IM in gastric mucosa was predominantly focal in both sexes (Table 1.3), and this pattern of distribution was more characteristic for females ($0.025<p<0.05$), whereas in males IM was patchy or diffuse (Fig. 1.10).

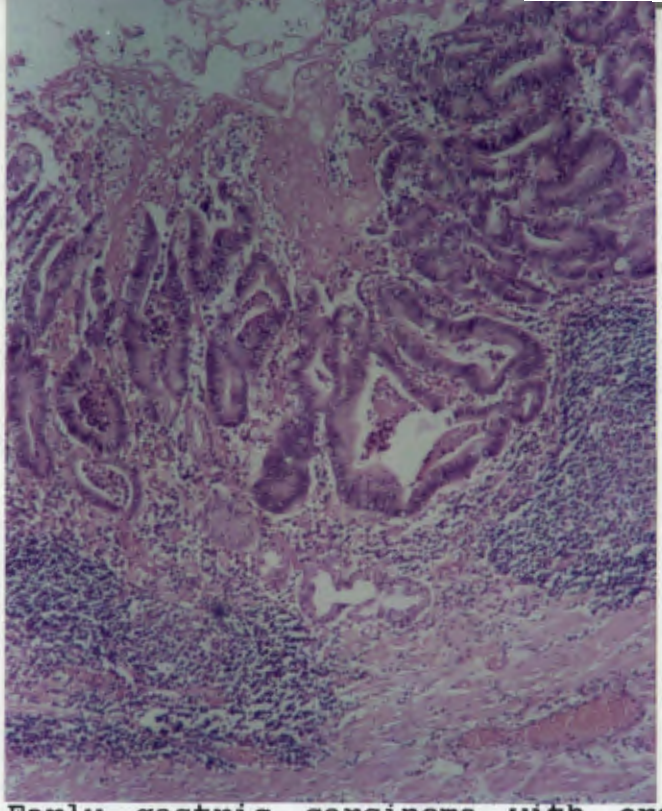

Fig. 1.5: Early gastric carcinoma with eroded surface. Partially preserved crypts are atypical, lined up with densely packed epithelial cells. Note hyperchromatic atypical cellular nuclei. Reactive lymphoid hyperplasia above the muscularis mucosa. H & E X 220.

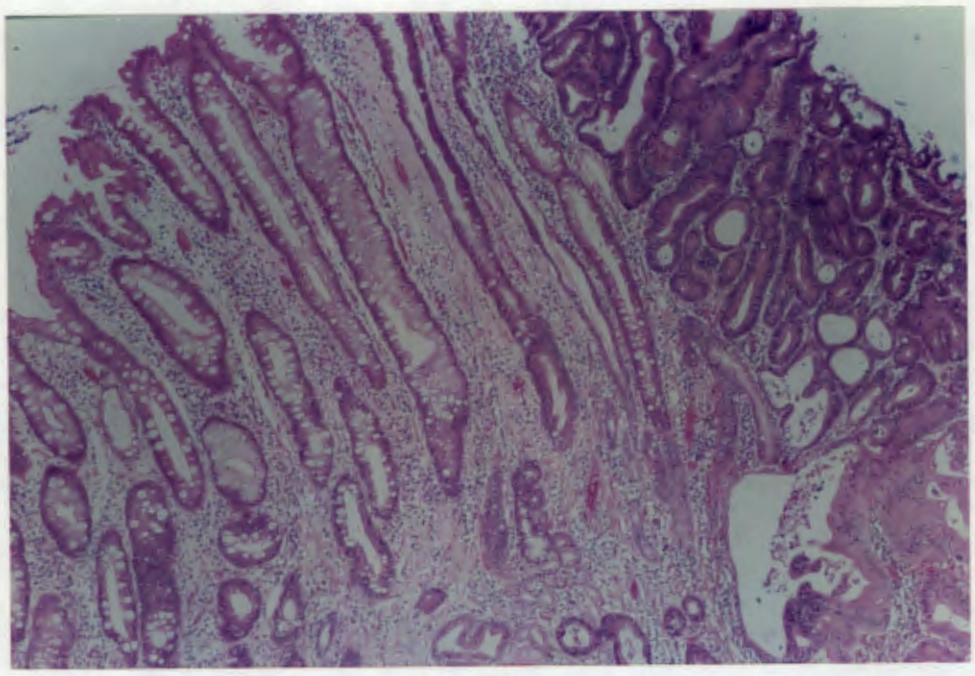

Fig. 1.6: Transitional gastric mucosa with extensive intestinal metaplasia (left) and adjacent superficially spreading gastric carcinoma (right). H & E X 220.

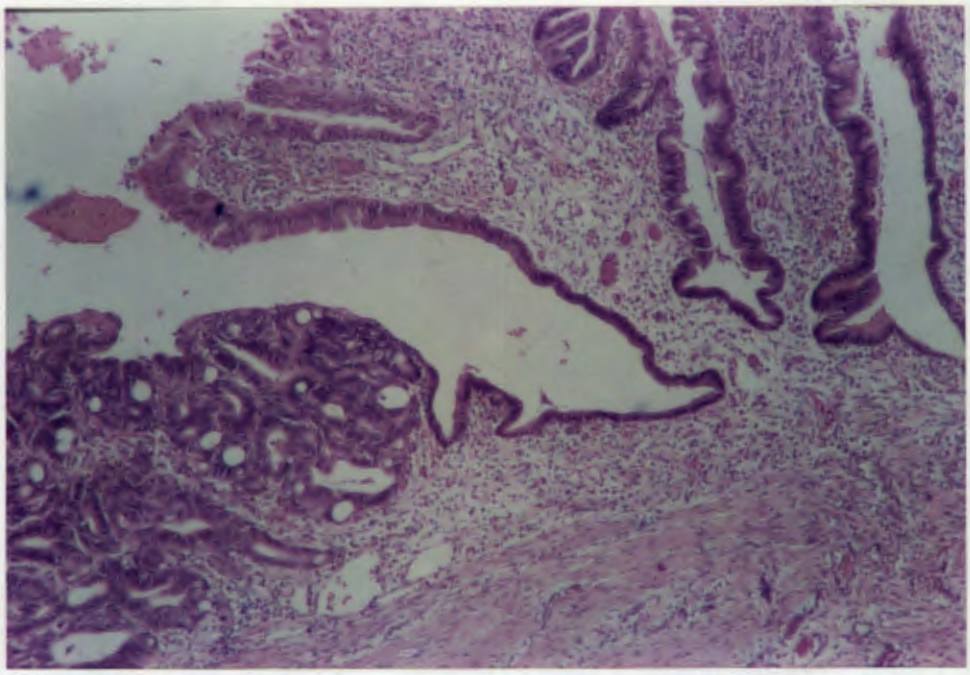

Fig. 1.7: Early gastric carcinoma, intestinal type (left). The dysplastic crypt epithelium can be seen adjacent to the tumour. H & E X 320.

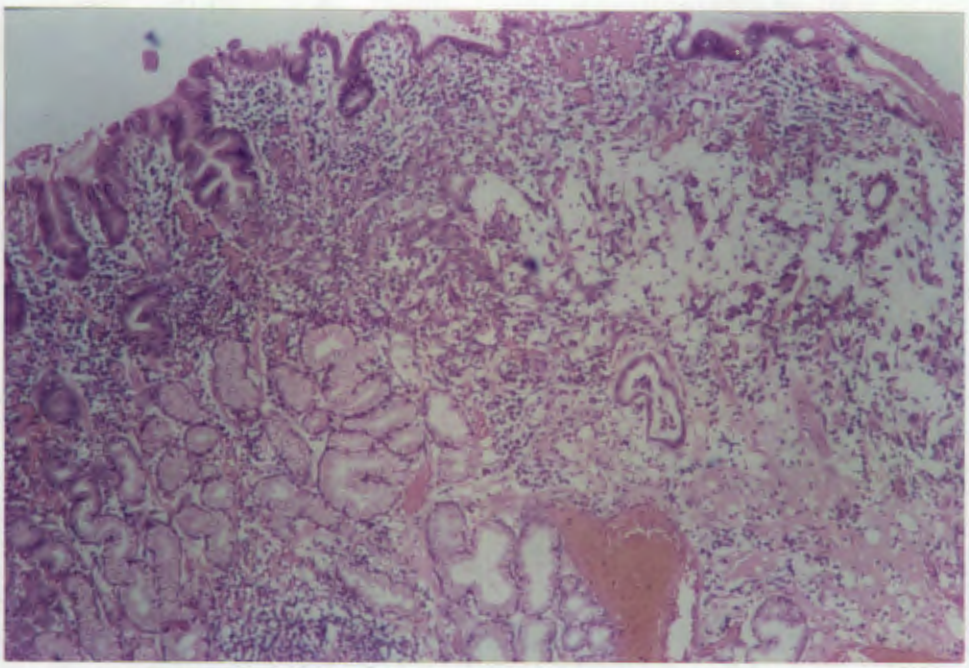

Fig. 1.8: Signet-ring cell early gastric carcinoma. Malignant infiltrate is restricted to the surface and mid-part of gastric mucosa. Cancer cells filled with mucin give a pale appearance to the lesion. H & E X 180.

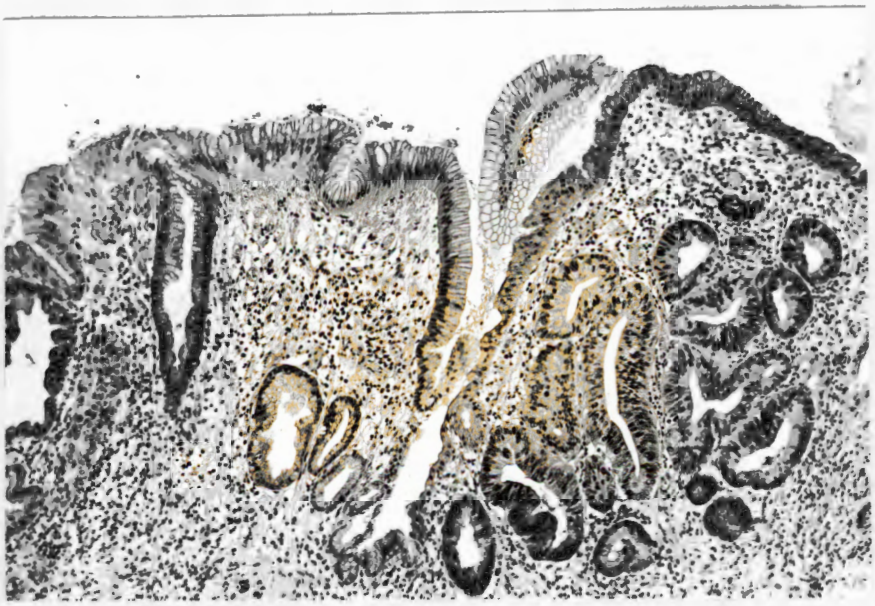

Fig. 1.9: Chronic atrophic gastritis and mild dysplasia (right). H & E X 320.

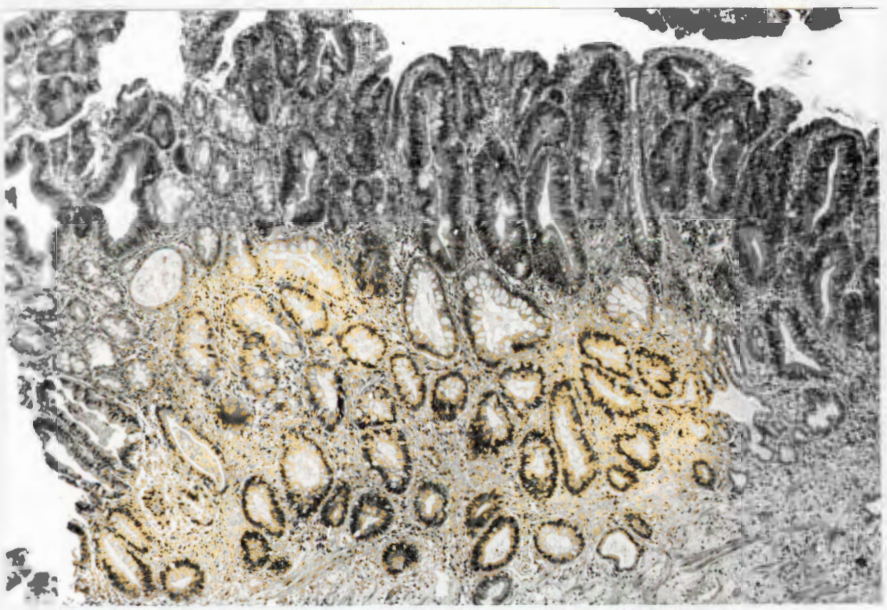

Fig. 1.9a: Chronic atrophic gastritis, intestinal metaplasia (lower left) and moderate to severe dysplasia (upper right corner). H & E X 320.

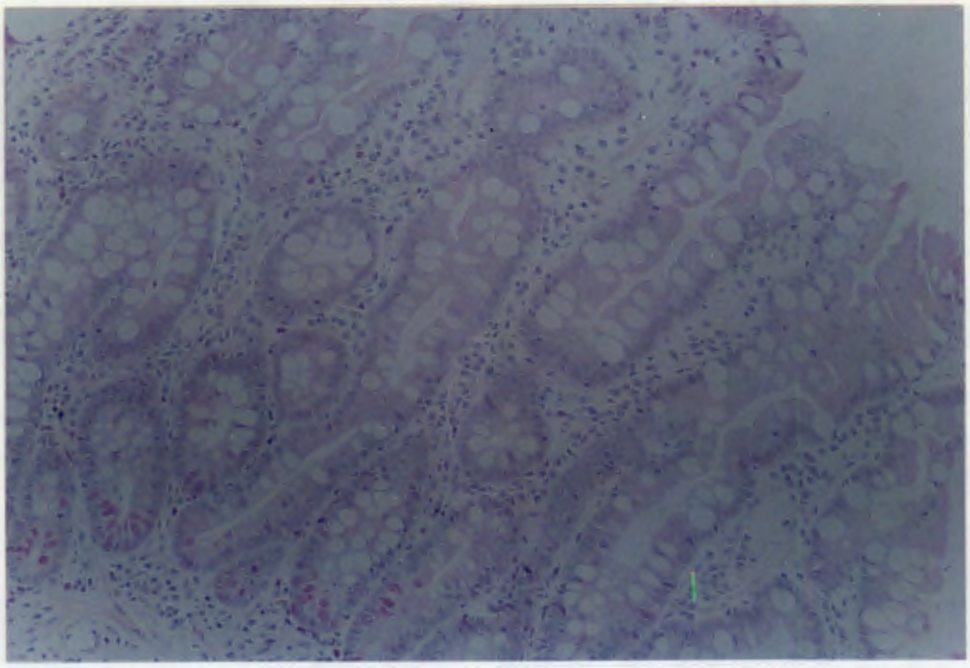

Fig. 1.10: Complete intestinal metaplasia, diffuse. Gastric crypts are regular, with well-formed goblet cells. Brush-border is preserved in absorptive cells. H & E X 450.

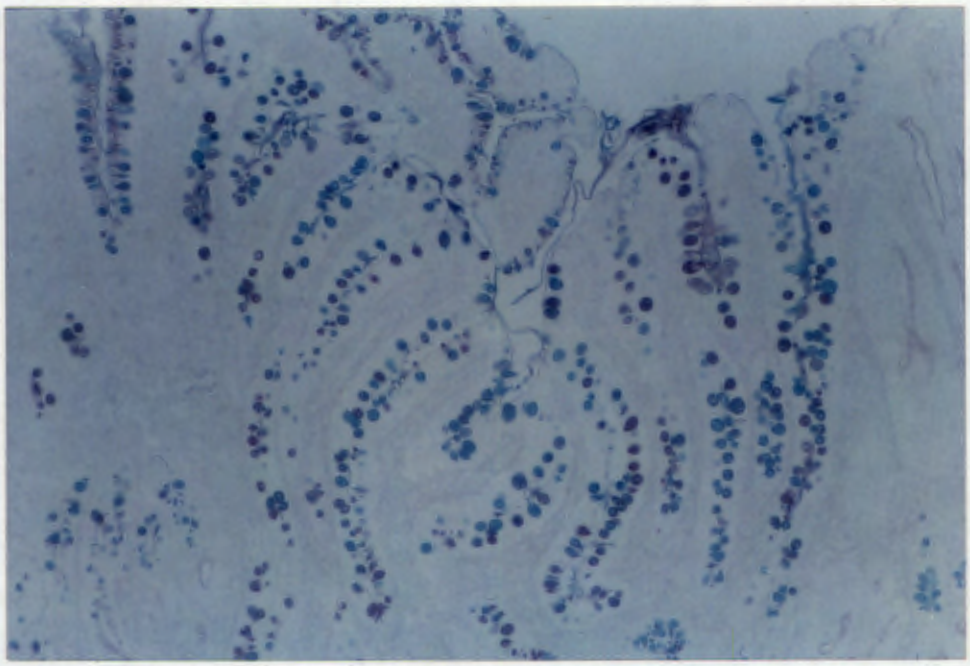

Fig. 1.11: Incomplete intestinal metaplasia. Crypts are irregular, goblet cells are immature, of various sizes, predominant sialomucin production (blue). HID\AB stain X 220.

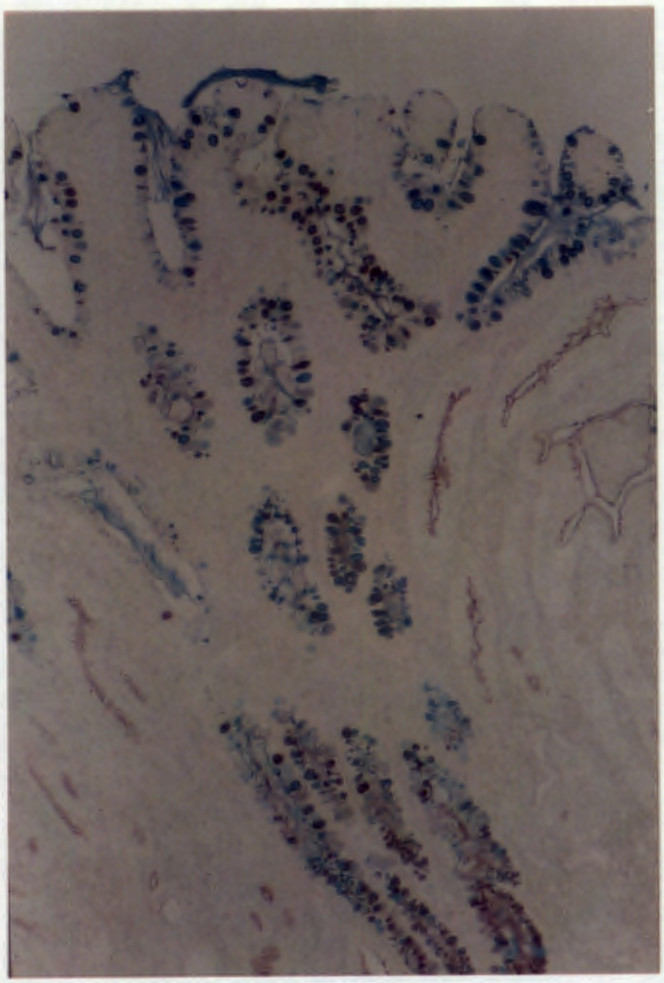

Fig. 1.11a: Patchy, incomplete intestinal metaplasia. Predominant sulphomucin production (dark brown). HID\AB X 220.

The prevalence of complete and incomplete IM and types of secreted mucin (Figs. 1.11, 1.12) is shown in Tables 1.3 and 1.4. Incomplete IM was more common in males ($p<0.001$), and also in those older than 40 years, when compared to the total study population (46% vs 32%, $p<0.001$).

H. pylori infestation of gastric mucosa in the tested population was as high as 81%, therefore, it is difficult to relate it to the specific pathological lesion. Even gastric mucosa, described as normal or with mild quiescent forms of superficial gastritis, showed a high rate of H. pylori infestation (Table 1.5). Within this group of patients, younger subjects showed the presence of H. pylori significantly more often than the older patients, but no difference between males and females in both age groups was noted. More detailed study of H. pylori infestation of gastric mucosa is described in the chapters 2 and 3.

DISCUSSION

The high rate of detected malignancies (2.7%) in patients with unspecified dyspeptic symptoms, does not reflect real cancer incidence in the studied population, but confirms that this population is at high risk for gastric carcinoma. A high incidence of cancer in males, particularly in those over 40 years old (1 in 16), suggests

that a gastric screening programme of this high risk group is very relevant.

The rates of detected malignancies are twice as high as the rates reported in the open access endoscopy clinic in England (3), and three times as high as in unselected patients with dyspepsia in Sweden (4), but lower than the Japanese screening results (5). Only 3 out of 16 detected carcinomas were early and potentially curable, therefore, the cost-benefit ratio for the diagnostic and therapeutic programme has to be carefully evaluated. The prevalence of the intestinal type of gastric carcinoma in males indicates the importance of CAG/IM as precursor lesions in this sex group.

The prevalence of CAG in all studied subjects is comparable to, or higher than, that referred to by others in studies in Finland (6), Poland (7), Colombia (8), England (9), Hungary and Italy (10). Several observations should be made in order to avoid false conclusions.

Diagnostic criteria for CAG have changed since the seventies (when most of the studies referred to were done), and these are still not universally accepted.

The coexistence of IM and hypochlorhydria is most reliable for the diagnosis of CAG, and both criteria, apart from crypt atrophy,

inflammatory infiltrates etc, were used in present study (1). The number of participants in other screening studies were often low (30-50 individuals), and only one study (2) reached 431 unselected subjects from both rural and urban environments. Unfortunately, previous studies related to CAG did not include any information about cancer detection rates.

High rates of CAG in males, more frequent patchy and diffuse distribution of IM and the frequency of incomplete IM in males is suggestive for their causal relationship with gastric carcinoma in the presence of a particularly high rate of malignant lesions in this particular group. No such association was found in the prevalence of sialo- and sulphomucin, confirming the suggestion of Ectors and Dixon (11).

The implication of H. pylori in the aetiology of certain types of gastritis and gastric cancer (12,13) is difficult to assess, since the great majority of patients under endoscopy are infected. Concurrent careful studies in selected groups of patients (chapter 2) showed that H.pylori is associated with active inflammation of the gastric mucosa, gastric and duodenal ulceration, and with gastric carcinoma .

More controversial, is an implication of H. pylori in the aetiology of CAG (9, 14, 15, 16). Lack of this association was found by Siurala et al (17), who explained the absence of H. pylori by a hypochlorhydric gastric environment which was unfavourable for bacterial growth. Our observations showed higher than mean H. pylori infestation rates in CAG, particularly when IM distribution was focal and patchy (12), but prevalence of H. pylori in young patients with normal gastric mucosa and the same occurrence of infestation in both sexes are suggestive against a causal relationship.

A predilection of H. pylori for the Third World, suggested by Sitas and Forman (18), is confirmed in the present study.

Particularly important, is the high infestation rate of gastric mucosa in young individuals with normal or mildly abnormal histology, also observed in over 75% of healthy young individuals in other countries of Africa (18). Young patients in Germany (19), who suffered from epigastric pain, showed active gastritis in 45%, and H. pylori infestation was detected in 66%. Low incidence rates of gastric carcinoma in countries such as Nigeria (18), with a high infestation rate, contradict the causal association of H. pylori.

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TABLE 1.1

GASTRIC MUCOSAL PATHOLOGY IN 585 PATIENTS WITH DYSPEPSIA

Number of patients	585(100)
Normal gastric mucosa	125(21.4)
Superficial gastritis	180(30.8)
Chronic atrophic gastritis type B*	157(26.8)
Chronic atrophic gastritis type A	13 (2.2)
Chronic atrophic gastritis type AB	22 (3.8)
Reflux gastritis	16 (2.7)
Lymphocytic gastritis	4 (0.8)
Mucosal hyperplasia	34 (5.8)
Dysplasia	7 (1.6)
Gastric ulcer	58 (9.9)
Gastric carcinoma	16 (2.7)
Helicobacter pylori infestation	474(81.0)

* =idiopathic chronic pangastritis (Sydney System)

TABLE 1.2

THE PREVALENCE OF HISTOLOGICALLY VERIFIED GASTRITIS IN 585 PATIENTS STUDIED (IN %)

	Males	Females	p value
Superficial gastritis	26.3	35.2	0.016
Chronic atrophic gastritis	35.7	20.6	<0.001
Other types of gastritis	8.5	11.3	NS
Normal mucosa	20.1	24.2	NS

NS = non significant

TABLE 1.3

THE PREVALENCE OF TYPE AND DISTRIBUTION OF INTESTINAL METAPLASIA (IM) IN BOTH SEXES

	IM type			IM distribution		
Sex of patients	Complete	Incomplete	Equivocal	Focal	Patchy	Diffuse
Males	58.2	34.5	6.4	49.6	39.2	11.2
Females	67.2	26.4	7.3	67.3	25.5	7.3
Total	64.4	28.9	6.7	55.0	35.0	10.0

TABLE 1.4

THE PREVALENCE OF SIALO- AND SULPHOMUCIN IN INTESTINAL METAPLASIA (IN %)

	Sialomucin	Sulphomucin	Both
Males	72.0	20.0	8.0
Females	67.3	18.2	14.5
Total	70.6	19.4	10.0

TABLE 1.5

HELICOBACTER PYLORI INFESTATION OF GASTRIC MUCOSA IN 290 PATIENTS WITH NORMAL GASTRIC MUCOSA (IN %)

Age Group	Grade of H. pylori infestation				
	-	+	++	+++	Total positive
20-34					
n = 100	17	25	28	30	83
35-44					
n = 90	16	30	30	24	84
45-75					
n = 100	24	21	24	31	76 *

* statistically significant (p = 0.047) when compared to younger than 45 years.

CHAPTER 2

THE ASSOCIATION OF HELICOBACTER PYLORI WITH MUCOSAL PATHOLOGICAL CHANGES IN A POPULATION AT RISK FOR GASTRIC CANCER

SUMMARY

This study is based on histological, histochemical and ultrastructural investigations of gastric mucosa in two population groups: rural and urban.

1. A group of 178 rural, unselected patients (105 males, 73 females, mean age 44,5 years), from a population with a high risk for gastric carcinoma presenting with chronic dyspepsia, was studied. During upper gastrointestinal endoscopy, a minimum of 8 gastric and oesophageal biopsy specimens were taken and examined histologically, histochemically, and in isolated cases, ultrastructurally, for the presence of Helicobacter pylori (H. pylori) and other pathological lesions.

2. A group of 225 urban patients with non-ulcer dyspepsia, were studied for the presence of antral gastritis and H. pylori colonisation. Sex and age distribution of patients in this group was similar to rural patients.

Gastric colonisation by H. pylori was found in 71.6% of the rural, and in 62.2% of urban patients. 90% of patients with duodenal or gastric ulcers, and 71.6% of patients with non-ulcer and non-cancer dyspepsia, showed a moderate or severe degree of bacterial colonisation. Associations of H. pylori colonisation, with microscopical evidence of type B gastritis, active gastritis, gastric or duodenal ulcer, gastric cancer, oesophagitis and oesophageal glycogenic acanthosis, were found.

INTRODUCTION

The presence of spiral organisms in the stomach of animals and man has been reported in the literature for nearly a century (1,2). Relatively recently Steer and Colin-Jones (3), and Warren and Marshall (4,5) rediscovered and cultured this gram-negative, microaerophobic curved bacillus, and showed an association with gastritis, gastric and duodenal ulcers. The implicated organism has been isolated and identified as Campylobacter pyloridis (6), later renamed as Campylobacter pylori, and finally, the generally accepted name of Helicobacter pylori has been introduced. Unlike most true campylobacters, H. pylori possessed potent urease activity, a property that may have important pathogenic implications.

The organisms may be identified by the non-invasive urea breath test; by detecting raised antibody titres to H. pylori in serum or gastric juice; by culture; by the biopsy-urease test, in which a medium contained urea with pH-sensitive dye is inoculated with the mucosal-biopsy specimen, and by light and electron microscope techniques. It has been reported that light microscopic techniques are at least as sensitive as other techniques, including culture (7,8). H. pylori can be seen in the superficial mucus layer of gastric mucosa,

exhibiting its spiral or "ox-bow" shape, after ordinary haematoxylin and eosin staining. However, the commonly used Warthin-Starry or Giemsa stain is more reliable, and the Gram stain is useful as a rapid diagnostic procedure(7).

H. pylori has been identified as a probable cause of gastritis, and a possible cause of gastric and duodenal ulcer. The original study of Marshall et al(9), involving self-ingestion of culture material, was the first to suggest a causative relationship between H. pylori and gastritis. However, subsequent studies throughout the world have shown widely differing incidences of H. pylori infestation in gastritis e.g. a 42-45% in French and Italian series(7, 10), and an up to 95% incidence in Australian and Canadian(5, 11, 12) reports. Colonisation of gastric mucosa by H. pylori in non-ulcer dyspepsia was noted in 57-61% of cases by Marshall et al(11) and Marcheggiano et al(10); and in 43-45% in cases of other studies(12,13). A closer association of H. pylori was seen with gastric ulcers (70%) and, particularly, with duodenal ulcers (90%)(11,14). Bacterial infestation of normal gastric mucosa was noted in 13-25% by Rokkas et al(13) and O'Connor et al(15), and in 30-45% by others(7,10) in adult patients. No such infestation has been observed in 49 children with gastric symptoms(16).

South African studies in Natal (8,17) showed the presence of H. pylori in culture or histologically in 79% of patients

undergoing routine endoscopy. The organism was seen in 11.5% of patients with normal mucosa, 83-95% of patients with gastritis, 90% of patients with duodenal ulcers, but only in 50% of patients with gastric ulcers. The overall culture rate was lower (53%) in urban Blacks in Johannesburg(18), and in this population there was no correlation between the presence of H. pylori and gastric pathology. Observations made in hospital outpatients by Wright et al(19) in Cape Town, showed an overall culture rate of 73%, and there was no difference in the culture rates in different conditions of gastric and duodenal mucosa.

A possible aetiological link between H. pylori and gastric carcinoma is based on the prevalence studies. Forman et al. (20) indicate a geographic association of H. pylori antibody prevalence and gastric carcinoma mortality in China. Others (21), in a retrospective study of the prevalence of H. pylori in gastric epithelium adjacent to the tumour, suggest a possible aetiological association, especially in the younger age groups. There is no information about any association between H. pylori and oesophagitis.

The aim of the present study was to determine the prevalence of H. pylori in patients with dyspepsia, from a population with a relatively high risk for gastric cancer(22), from the urban population of the different social classes, and to

relate this to microscopic evidence of chronic atrophic gastritis, active gastritis, other types of gastric mucosal pathology, and to oesophagitis.

MATERIALS AND METHODS

This study looked at pretreatment biopsies from 178 unselected rural patients (mean age 44,5 years, range 20-68 years) from thr rural Coloured population in the vicinity of Cape Town (Atlantis, Malmesbury, Paarl, Worcester), and biopsies from 225 patients of various social classes, in Cape Town. Subdivision of this group into subgroups, according to the socio-economic status of patients, is beyond the scope of this paper, and will be studied separately. Patients had had upper gastrointestinal dyspeptic symptoms for at least three months, such as epigastric pain, nausea, vomiting, heartburn, post-prandial pain or bloating. Upper gastrointestinal endoscopy was performed, and 2 mucosal biopsy specimens were taken from each rural patient, from the following sites: angulus; 5 cm from pylorus on the greater curvature; fundus, as well as lower and mid-oesophagus (a total of 8 biopsies). An additional 5-10 biopsies were taken from macroscopically visible lesions.

Rural patients were examined in the nearest local hospital or clinic. Urban patients with similar symptoms were selected in the GIT Clinic of Groote Schuur Hospital and also consisted of dyspeptic patients. Patients with active GI bleeding, with previous acid reducing surgery, or with active ulcers or tumours, were excluded from this studied group. Mucosal biopsies were taken from the gastric antrum only. Mucosal samples were fixed in 10% buffered formalin, processed routinely, and were carefully orientated in paraffin blocks. Sections were cut and stained with H & E, periodic acid Schiff and Alcian blue for neutral and acid mucins, and with high iron diamine-alcian blue for sialo-and sulphomucins. The mucin stains were used for the accurate assessment of intestinal metaplasia, type of gastritis, the amount and type of mucus production by gastric epithelium or the amount of carbohydrates in the oesophageal epithelium. The Warthin-Starry and Giemsa stains were used for spiral organisms.

Mucosal specimens from five random patients were fixed in glutaraldehyde, and processed for examination in the transmission electron microscope.

Histological classification of the gastric and oesophageal mucosal lesions was done according to Rothery and Day (23) and Wyatt and Dixon (24). Endoscopy only is unreliable as a means of detecting gastritis or oesophagitis in dyspeptic

patients; therefore, our diagnoses were based on histological and histochemical methods only. Colonisation of gastric mucosa by H. pylori was graded as follows: 0 - absence of the spiral organisms; 1 - mild: sparsely distributed organisms in the superficial part of mucosa of isolated mucosal samples; 2 - moderate: more numerous bacteria on mucosal surface and a few in crypts in more than 2 mucosal samples and; 3 - severe: abundant organisms on the surface and in crypts present in all gastric mucosal samples.

RESULTS

Within the group of 178 patients, 176 had a complete upper gastrointestinal examination, and representative mucosal samples taken from the stomach. Samples were properly orientated and contained the whole thickness of mucosa, and, in many cases, muscularis mucosae. The average free mucosal surface length was 4 mm.

All 225 patients of the second group had satisfactory biopsies of antral mucosa.

There was no difficulty in detecting microorganisms by Warthin-Starry or Giemsa stains. Haematoxylin and eosin stains were sufficient to diagnose a severe degree of H.

pylori infestation, but they were less sensitive with low grades of colonisation.

In the first group of patients, in 40% of H. pylori-positive cases, organisms were detected in all gastric specimens, including those taken from the fundus; in the remaining 60%, organisms were detectable in the antral specimens only. Gastric colonisation by H. pylori was found to be positive in 75% of males, and in 68.4% of females. Sixty-one percent of patients younger than 35 years were affected, and more than two-thirds of them had a mild or moderate degree of colonisation.

A 71,6% incidence of H. pylori was noted in rural patients with dyspepsia without microscopical evidence of peptic ulcer or cancer. Two-thirds of these cases had a moderate to severe degree of colonisation (Fig. 2.1). Ninety percent of patients with duodenal or gastric ulcers, showed a severe degree of mucosal colonisation, especially in specimens taken from ulcers. Only sites showing intestinal metaplasia did not contain spiral organisms. Results given in Table 2.1 show an association of H. pylori colonisation with microscopical evidence of chronic atrophic type B gastritis (88,5%), and with other types of gastritis (79.1%). Only 34.2% of patients with gastric mucosa described as normal, showed the presence of H. pylori, (in most instances grade 1 colonisation). Seven patients with chronic gastritis,

present mainly or exclusively in the body mucosa (type A gastritis), were free from H. pylori. Among the H. pylori-positive patients with microscopic evidence of gastritis, 93% had active gastritis, in contrast to 70.3% who had quiescent gastritis. Three cases with lymphocytic gastritis (23), and two with reflux gastritis, were negative or mildly positive for H. pylori.

Histological examination of antral mucosa of urban patients revealed normal, or nearly normal gastric mucosa in 28.8%. The remainder showed various types of gastritis; active gastritis was the commonest (Table 2.3). Although selection of cases and different methods of sampling of gastric mucosa prevents direct comparison of groups of patients studied, there are more histological lesions seen in rural patients. Only 21.3% of the latter presented normal gastric mucosa. The prevalence of colonisation of antral mucosa in the urban patients (62.2%) was lower than in the rural (71.3%). Table 3 gives clear indication that H. pylori infestation is commonest in the active gastritis, and is directly associated with its activity. The prevalence and density of H. pylori organisms decreased with progression of mucosal atrophy and intestinal metaplasia. In a few of the cases with advanced intestinal metaplasia, H. pylori organisms were not present.

Gastric mucosa heavily colonised by H. pylori, showed, as a rule, active inflammatory changes in the lamina propria and/or subepithelial oedema. Depletion of intracellular mucin, and degeneration of superficial epithelium, were also detected in those cases (Fig 2.2).

Gastric mucosa of the patients with gastric cancer had strong H. pylori positivity. (The tumour itself was difficult to assess, due to the presence of superficial erosions, necrosis or intestinal type of glands).

The group of 159 patients shown in Table 2.2, with representative oesophageal biopsies, showed a close association between the presence of H. pylori in gastric mucosa, microscopic evidence of oesophagitis and glycogenic acanthosis.

Within this group, 63% of patients with normal oesophageal mucosa, and 82% of patients with oesophagitis or glycogenic acanthosis, also had gastric ulcer or gastritis. On oesophageal mucosa, only isolated organisms were present.

An ultrastructural examination of gastric mucosa shows single or loose groups of bow-shaped (Fig. 2.3), round (Fig. 2.4) or elongated (Fig. 2.5) organisms. The shape is a result of the orientation of the organism in the particular slide, and represents a part of a spiral form. The organisms

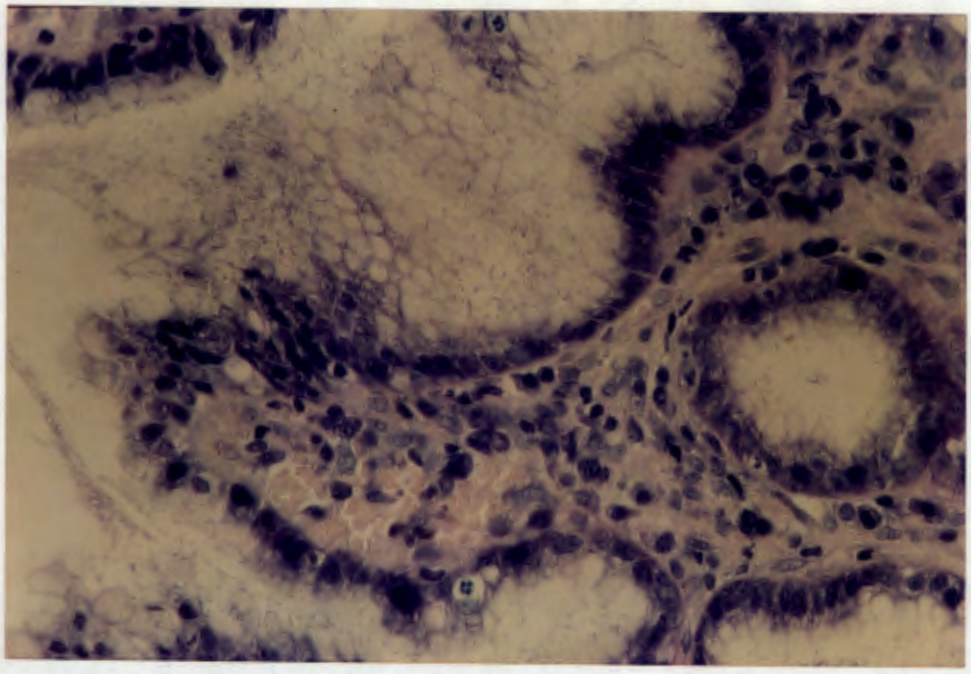

Fig. 2.1: Gastric antral mucosa is moderately colonised by H. pylori. Note numerous organisms in the mucin covering the superficial epithelium, and the irregularity of the lining cells. Giemsa stain X 450.

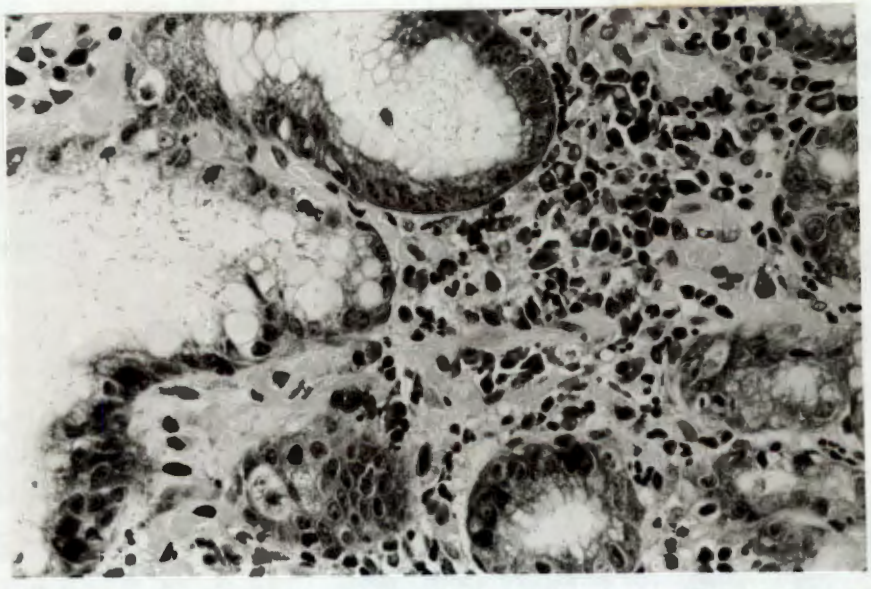

Fig. 2.2: Gastric mucosa is moderately colonised by H. pylori. The covering and crypt epithelium is invaded by inflammatory cells, partially distorted, and it shows abnormal mucin secretion. Polymorpho-cellular inflammatory infiltrate is present in the lamina propria. H & E X 420.

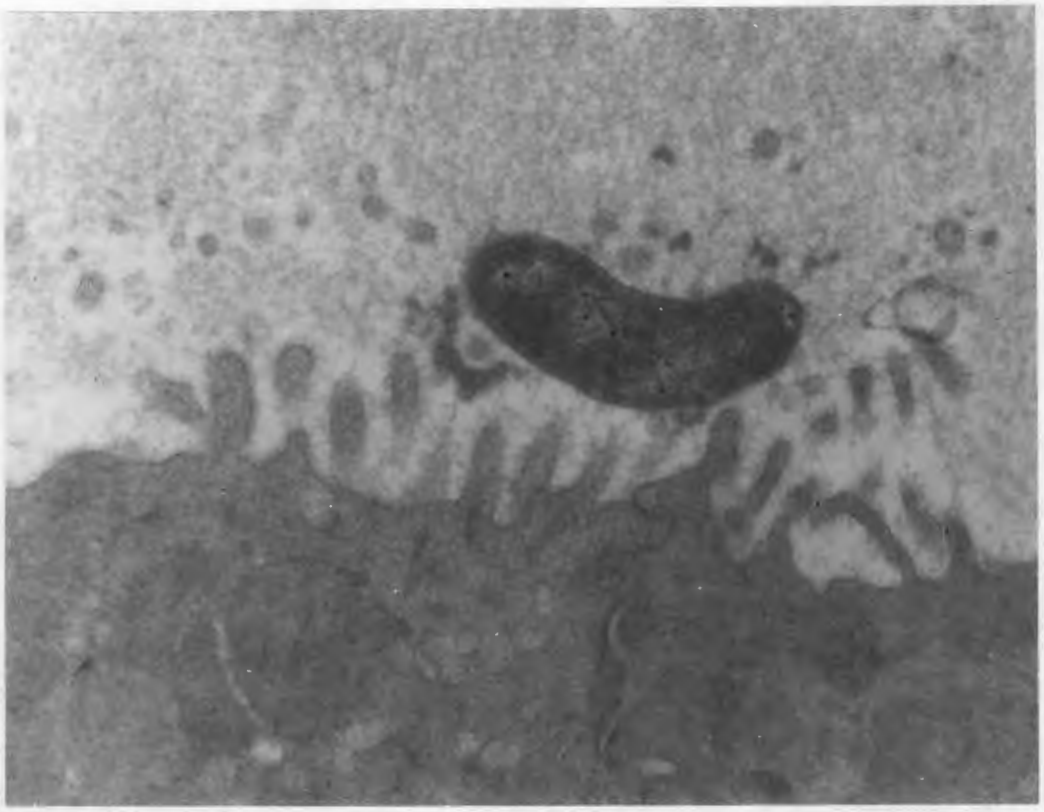

Fig. 2.3: H. pylori organism is in close opposition to the tight-junction complex. Partly resolved covering mucus and deformed microvilli. (Transmission electron microscope. Original magnification X 12 000).

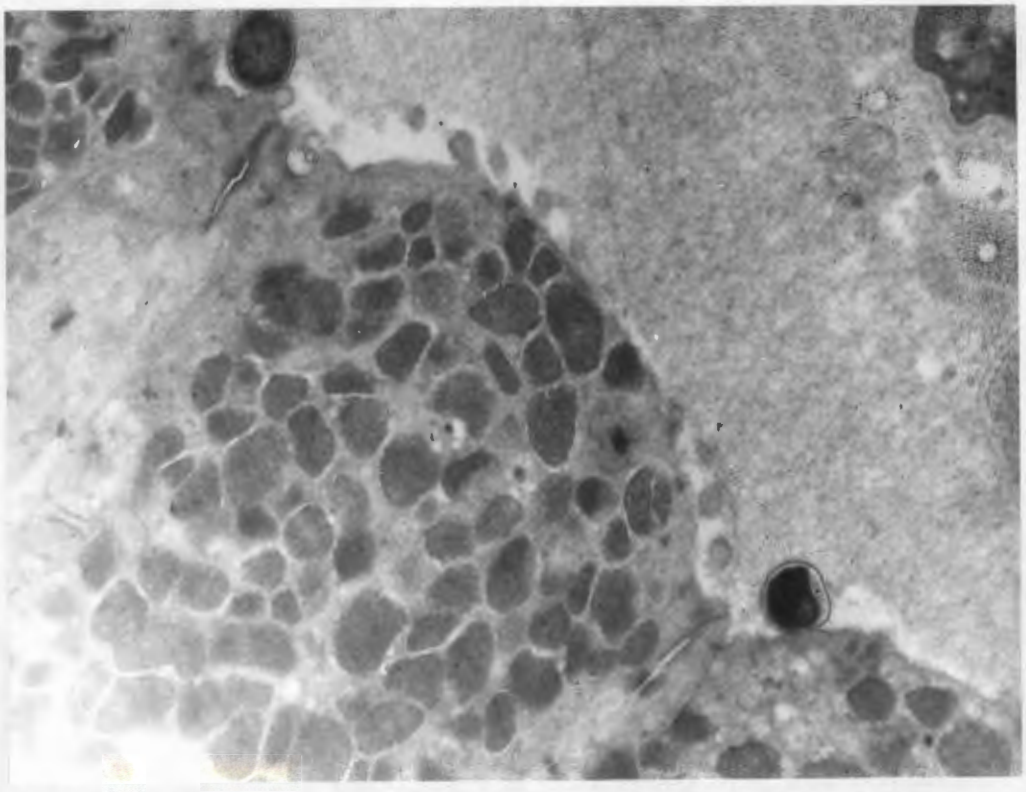

Fig. 2.4: Surface mucous cell with numerous granules and small microvilli. Some granules are partially resolved (translucent electron empty structures). Transversely sectioned H. pylori organisms lie close to the tight-junction complexes. (Transmission electron microscope. Original magnification X 8 000).

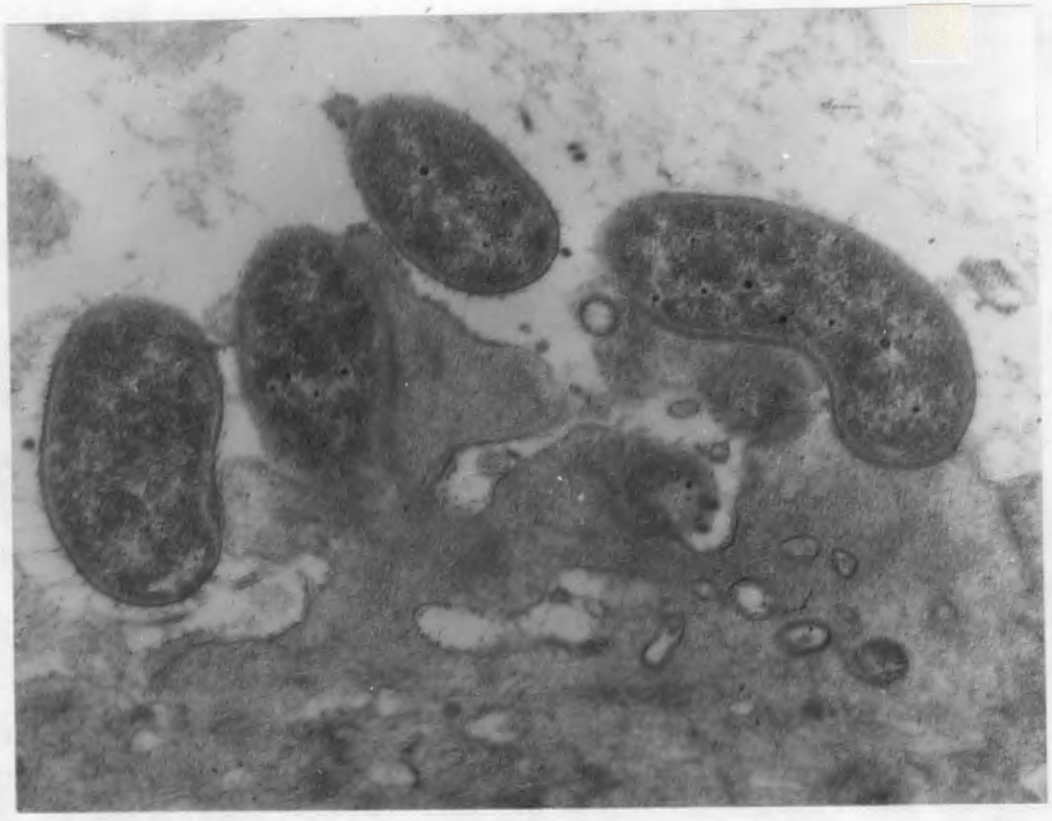

Fig. 2.5: The adhesion between the organisms and the epithelial cell is characterised by intimate contact between both cellular membranes. Note distorted microvilli and the tight-junction on the far right of the figure. (Transmission electron microscope. Original magnification X 17 000).

are loosely attached to the cellular surface, lying in covering mucus, and most often aggregate near an intercellular junction (Figs. 2.3 and 2.4). Mucus adjacent to the organisms shows a clear halo, microvilli are degenerated, shortened or kinky (Fig. 5). Adhesion of organisms to the cellular membrane is present (Fig. 2.5), but no intracellular invasion seen. Superficial mucous cells contain secretory granules of uneven density. Some of them are translucent or empty (Fig. 2.4).

DISCUSSION

In this study, as in recent publications (25), I have used histochemical methods only in the detection of H. pylori. The organisms can be considered to be H. pylori according to their microscopic characteristics. Due to the high sensitivity of histochemical methods, the average free mucosal surface length was 4 mm, and numerous biopsy specimens taken from each patient, suggested a high detection rate of H. pylori in this material. Easy to perform, inexpensive Giemsa stains can be advocated in similar studies of large population groups.

Ultrastructural studies confirm the superficial nature of the colonisation of H. pylori with secondary degeneration of the adjacent mucus, microvilli and secretory granules of mucous cells.

The results presented show a high H. pylori rate in non-ulcer and non-cancer dyspepsia, and are higher than reported by others (11,12,13), and similar to those noted in Natal (8). The association of H. pylori positivity and gastritis, as well as gastric or duodenal ulcers in this study, confirms reports of several others (4,8,11,12,14). Substantial differences between results obtained by some other authors (7,10,15) could be related to geographical variation, different methods used for H. pylori detection, sampling of gastric mucosa (e.g. small and/or few specimens), and to treatment prior to endoscopic biopsy. Some other possible causes of a false-negative finding are listed by Marshall et al (11).

The higher ratio, in material presented here, of H. pylori positive males as compared to females, supports the observations of others(13); and the lower H. pylori incidence in our younger population groups confirms the findings shown by others in symptomatic or asymptomatic individuals(26).

The high rates of H. pylori positivity detected in patients with gastric cancer, gastric ulcer and chronic atrophic type B gastritis, may indicate the possible pathological significance of H. pylori in the aetiology of gastric cancer. Chronic atrophic type B gastritis, an important precursor lesion for both gastric ulcer and cancer, is a common finding in our study population, and needs special attention in further studies. Sporadically noted body atrophic gastritis, antral reflux gastritis, lymphocytic gastritis and areas of intestinal metaplasia showed a negative association with H. pylori, as suggested by others (15,25).

Oesophagitis and glycogenic (clear cell) acanthosis, in patients with H. pylori, has not been studied before. The presence of isolated curved organisms in the oesophagus is most likely due to contamination from heavily colonised gastric mucosa. Although the raised incidence of H. pylori colonisation of gastric mucosa (including high grades) in patients with oesophagitis or glycogenic acanthosis suggests a direct relationship between these lesions, an indirect relationship is also possible, because of the high incidence of gastritis in the same patients.

To conclude, H. pylori infestation is common in a rural Cape population (at risk for gastric cancer) with dyspepsia, particularly in males older than 35 years. H. pylori is less

common in an urban population of higher socio-economic status, and is associated with chronic gastritis (particularly active B type), with gastric and duodenal ulcers, oesophagitis and glycogenic acanthosis. Association of H. pylori with gastric carcinoma, if any, is indirect, and is linked with other factors. A possible sequence of lesions in the gastric mucosa is as follows: active gastritis related to H. pylori infection - chronic atrophic gastritis - mucosal atrophy and intestinal metaplasia - dysplasia - intestinal type of gastric carcinoma.

TABLE 2.1

ASSOCIATION OF HELICOBACTER PYLORI AND MICROSCOPICAL EVIDENCE OF GASTRIC PATHOLOGY IN 176 PATIENTS WITH DYSPEPSIA.

Histological positive Findings	Number of Patients	H. pylori in gastric mucosa				
		Neg.	Mild	Moderate	Severe	Total (%)
Chronic atrophic type B gastritis	52	6	14	10	22	46(88.5)
Chronic atrophic type A gastritis	7	6	0	0	0	0 (0)
Other types of gastritis	48	10	6	14	18	38(79.1)
Duodenal and gastric ulcer	25	3	2	15	5	22(90.0)
Gastric carcinoma	6	0	1	0	5	6(100)
Normal gastric mucosa	38	25	7	4	2	13(34.2)

TABLE 2.2

ASSOCIATION OF HELICOBACTER PYLORI AND MICROSCOPICAL EVIDENCE OF OESOPHAGITIS AND GLYCOGENIC ACANTHOSIS IN 159 PATIENTS WITH DYSPEPSIA.

Oesophageal histol. findings	Number of patients	H. pylori in g. mucosa			
		Neg.	Mild to moderate	Severe	Total positive (%)
Oesophagitis	76	12	16	48	64 (84)
Glycogenic acanthosis	20	3	4	13	17 (80)
Normal oesophageal mucosa	63	30	10	23	33 (52)

TABLE 2.3

THE PREVALENCE STUDY OF H. PYLORI IN 225 DYSPEPTIC PATIENTS* IN CAPE TOWN AND ITS ASSOCIATION WITH GASTRITIS.

	no	%	H. pylori score (mean)
Normal mucosa	67	28.8	13 (0.19)
Chronic active gastritis	137	60.9	
mild	93		134 (1.44)
moderate	39		64 (1.64)
severe	5		5 (1.0)
Quiescent gastritis	21	9.3	7 (0.33)
Signs of mucosal atrophy	66	29.3	
mild	54		63 (1.17)
moderate	10		4 (0.4)
severe	2		2 (1.0)
Lymphocytic gastritis	2		0 (0)
Intestinal metaplasia	41	18.2	
mild	28		26 (0.93)
moderate	10		4 (0.4)
severe	3		0 (0)
*Total patients	225		
H. pylori positive	140	62.2	

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CHAPTER 3

LOCAL CELLULAR AND IMMUNE RESPONSE BY ANTRAL MUCOSA IN PATIENTS UNDERGOING TREATMENT FOR ERADICATION OF HELICOBACTER PYLORI.

ABSTRACT

Thirty-six patients with non-healing or recurrent duodenal ulcers (DU) were treated with omeprazole; 20 mg\day for one month and, in case of relapse of DU, triple therapy (metronidazole, 400mg 3 times daily, colloidal bismuth, 120mg 4 times daily and tetracyclin, 500mg 4 times daily), for 14 days was given.

At least 3 gastric mucosal samples were collected from the antral portion of the stomach and from duodenum at the beginning of the study, within 48 hrs after the omeprazole and 4 weeks after triple therapy. Samples were fixed in buffered formalaldehyde and glutaraldehyde and examined histologically and histochemically for inflammation, density of Helicobacter pylori colonisation and immunohisto-chemically for the density of gastrin-secreting cells, immunoglobulins (IgA, IgG, IgM), Kappa and Lambda light chains and T lymphocyte population. Ultrastructure of the surface of the antral mucosa was assessed in selected cases.

H. pylori colonisation of antral mucosa before treatment was present in 100%, and active gastritis was noted in 86% of patients. The clearance rate after omeprazole treatment was 47.3%, and after triple therapy, 69.5%. The prevalence of gastritis was observed in 63.9% and 33,3% respectively. Both therapies were associated with an accumulation of serous

fluid, increased population of lymphocytes and plasma cells, and secretion of immunoglobulins, particularly IgG and IgM in the upper part of lamina propria. These changes, together with increased numbers of T-lymphocytes within crypt epithelium and lamina propria, were associated with the presence of H. pylori organisms. Increased cellular response of lamina propria and accumulation of immunoglobulins in the upper part of mucosa may indicate lack of complete eradication of H. pylori, and predict relapse of DU.

Treated patients had hyperplasia of gastrin-secreting cells in the antrum, irrespective of the presence or absence of H pylori.

INTRODUCTION

Duodenal ulcer is a multifactorial disease (1). The aetiology is related to enhanced acid secretion, and influenced by many other factors including gender, genetic predisposition, alcohol consumption, drug intake, and smoking. There is substantial evidence that Helicobacter pylori (formerly Campylobacter pyloridis\pylori) is a major aetiological agent of chronic active type B gastritis, and that it may further predispose to peptic ulceration (2,3,4,5).

Colonisation of antral mucosa by H. pylori and antral gastritis has been regularly reported (6,7) in patients with duodenal ulcer (DU) disease. A strong association of H. pylori with DU, being present in over 90% of the samples of antral mucosa of patients with DU, was observed in our previous studies (8).

Numerous recent studies on eradication of H. pylori in patients with DU disease have been reported (2,3,9,10,11), with improved relapse rates following successful eradication of the organism. Recurrence of H. pylori infection and relapse of the disease was commonly noted after single therapy with bismuth salts, sucralfate, omeprazole, or H2-receptor antagonists; positive results are more stable after the currently advocated so-called triple therapy i.e. a bismuth salt combined with metronidazole and a second antibiotic, given for a 14 day period after ulcer healing.

There are some histological studies of gastric mucosa before and after treatment with cimetidine or bismuth preparations (3), as well as with omeprazole, or H2-receptor antagonists (10). Reports on the immune response of the gastric mucosa to H pylori are isolated (4, 12) and no works relating to the cellular and immunological response of the antral mucosa after eradication of H. pylori were found.

The purpose of this study is to investigate the possible changes in antral inflammatory and immunological responses following therapy with omeprasole and triple therapy.

MATERIAL AND METHODS

Forty-six patients (males 33, females 13) with non-healing or recurrent endoscopically documented duodenal ulcers were selected for this study. Patients were 18-65 years old.

At entry, active DU were confirmed endoscopically, and mucosal biopsies from the edges of ulceration were collected for histological examination. In addition, at least two good biopsies were taken from the gastric antrum within 5cm of the pylorus.

All patients received omeprazole, 20mg\day for one month. A second gastroscopy was performed within 48 hours of cessation of therapy, and 2 antral biopsies were collected as at entry. Subsequently, patients were randomised to receive triple therapy consisting of either a colloidal bismuth preparation, (120mg 4 times\day), plus metronidazole, 400mg 3 times\day) and tetracycline, (500mg 4 times\day). Patients were endoscoped a minimum of 4 weeks after cessation of triple therapy, and biopsies were taken as before.

Biopsies were fixed in buffered formaldehyde, embedded in paraffin blocks, and processed routinely. Tissue slides were stained with haemotoxylin, eosin and PAS\alcian blue for routine histology, and the Giemsa stain was used for the detection of H. pylori. Additional sections were stained by

the indirect immunoperoxidase technique for immunoglobulins A, G and M, for gastrin secreting cells, kappa and lambda markers, and for lymphocytes T using monoclonal Dako (Dakopatts) antibodies.

HISTOLOGICAL ANALYSIS

Classification of the histological activity of the gastritis was conducted as suggested by Whitehead (13): 0 - none or isolated polymorphonuclear cells, either associated or not with round cells in the lamina propria, and in the foveolar epithelium without any degenerative epithelial damage; 1 - mild but homogenous distribution of polymorphs, either associated or not with mononuclear infiltrate of the lamina propria, and the presence of polymorphs in the epithelium and epithelial degenerative changes; 2 - lesions as indicated above, but moderate; severe - markedly increased mononuclear cells and polymorphonuclear leucocytes, severe damage of the covering epithelium with numerous inflammatory cells in the crypt epithelium.

Quiescent gastritis was diagnosed in cases when the inflammatory infiltrate was predominantly mononuclear without cryptal epithelial lesions.

Grading of the colonisation of the gastric mucosa by H. pylori was performed as reported previously (8): 0 - absence of spiral micro-organisms; 1 - mild (sparsely distributed organisms on the superficial part of the mucosa in isolated mucosal samples); 2 - moderate (more numerous bacteria on the mucosal surface and a few in the crypts in two mucosal samples); 3 - severe (abundant organisms present on the surface and in crypts in all gastric mucosal samples).

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical analysis of the mucosal samples collected at the entry and after the therapies in each patient was performed in the same batch, using the indirect immunoperoxidase technique. This procedure was employed to minimise differences in stains which could be related to different laboratory conditions.

Plasma cell counts for each class, kappa, lambda markers, T-lymphocytes, and gastrin-secreting cell counts per unit area of mucosa were made using an eye-piece graticule, and values expressed per 0.25 mm2 of mucosa. Obtained figures included the amount of extracellular immunoglobulins, which were graded arbitrarily, divided into the 0-3 categories and collated into tables. Statistical significance of data was

assessed by Student's T-test and one-way analysis of variance.

RESULTS

A group of thirty-six patients (28 males and 8 females) completed the treatment trial, and from all of these patients satisfactory endoscopical biopsies were collected. The remainder completed only the first phase of the treatment, had incomplete triple therapy, or did not have satisfactory biopsy material, and were, therefore, excluded from further assessment.

At the initial examination, all 36 patients had endoscopically and histologically confirmed active DU. All patients had histologically diagnosed H. pylori colonisation of the mucosa of the gastric antrum, and 31 (86%) presented antral active gastritis of various degrees of severity (Table 3.1).

Half (eighteen) of the samples of the duodenal mucosa, collected at entry from the vicinity of the DU, showed a pyloric type of metaplasia and H. pylori colonisation.

Table 3.1 summarises H. pylori status and gastritis scores immediately after cessation of omeprazole and triple therapy. H. pylori microorganisms were found in 19 (52.8%) cases

after omeprazole, and in 11 (30.5%) cases after triple therapy. H pylori colonisation was significantly ($p<0.01$ and $p<0.001$ after omeprazole and triple therapy respectively) milder and gastritis significantly ($p<0.01$ and $p<0.001$ after omeprazole and triple therapy respectively) improved, when compared to the initial lesions. Relapse of DU, detailed assessment and long term results of therapy are outside the scope of this study.

Histological and immunohistochemical examination of gastrin-secreting cells in the antral mucosa showed hyperplasia of these in all treated patients. These cells were more numerous, distinct and larger as demonstrated by immuno-histochemical stains after omeprazole treatment, and remained numerous and prominent after triple therapy (Fig. 3.1). There was no influence of the grade of gastritis, or grade of H. pylori colonisation on the activity and population of gastrin secreting cells found.

Antral mucosa of patients after cessation of therapy showed a lack of H. pylori microorganisms, or their marked numerical decrease, the presence of coccoid microorganisms in the covering mucus, and sporadically degenerated fragmented mucus in the cryptal lumina (Fig. 3.2).

The healing effect of omeprazole and particularly triple therapy on gastritis was observed in the great majority of

the cases (Table 3.1). The activity of inflammation became less severe, quiescent, or the microscopical appearance of the mucosa became nearly normal. The population of polymorphonuclear leucocytes in the lamina propria was replaced by lymphocytes and plasma cells.

Lymphoid follicles in the lamina propria noted sporadically in few cases at the entry were present in the same cases after both therapies and were related to some degree of crypt atrophy.

Antral mucosa in treated patients after omeprazole and as well as triple therapy still colonised by H. pylori and those which cleared, but demonstrated incompletely healed gastritis, showed an oedematous and eosinophilic upper part of the lamina propria. Accumulation of IgG, IgM, and to a lesser extent IgA was found in this part of the lamina propria (Table 3.2 and Fig. 3.4). The kappa-positive mononuclear cells were predominant in the lamina propria, and superficial and foveolar epithelium showed increase of T-lymphocytes in H. pylori-positive samples (Fig. 3.5), but several cases from both therapies with histologically negative H. pylori and absent active gastritis showed similar changes (Table 3.2).

The differences in immune response of gastric mucosa between omeprazole and triple therapy were small. The rates of increased values of immunoglobulins and T cells in mucosal samples colonised by H. pylori were higher after triple therapy, than after omeprazole. In samples cleared from H.

pylori increased values of IgA, IgM and kappa light chain were more common (29%, 47%, 23% vs 16%, 40% and 8% respectively) in omeprazole-treated cases.

DISCUSSION

The present study confirms the previously reported high prevalence of antral gastritis in duodenal ulcer disease (2-8, 10,14), demonstrating gastritis of grade 2 and 3 in 76% of a group of consecutive cases (3). The study also confirms that the gastritis is closely associated with the presence of H. pylori.

The histologically assessed disappearance of H. pylori in ± 50% of patients treated with omeprazole is an interesting observation, which confirms the observations of others (9).

It should be noted, however, that this merely reflects clearance (i.e. absence of the organism immediately after cessation of therapy) and not eradication (i.e. absence of the organism a minimum of one month after cessation of therapy). The clearance rate of H. pylori was lower after omeprazole alone, than it was with the combination of metronidazole, bismuth and tetracycline. These results are similar to those reported by authors who used bismuth alone and triple therapy (2); omeprazole and H2-receptor antagonists (10).

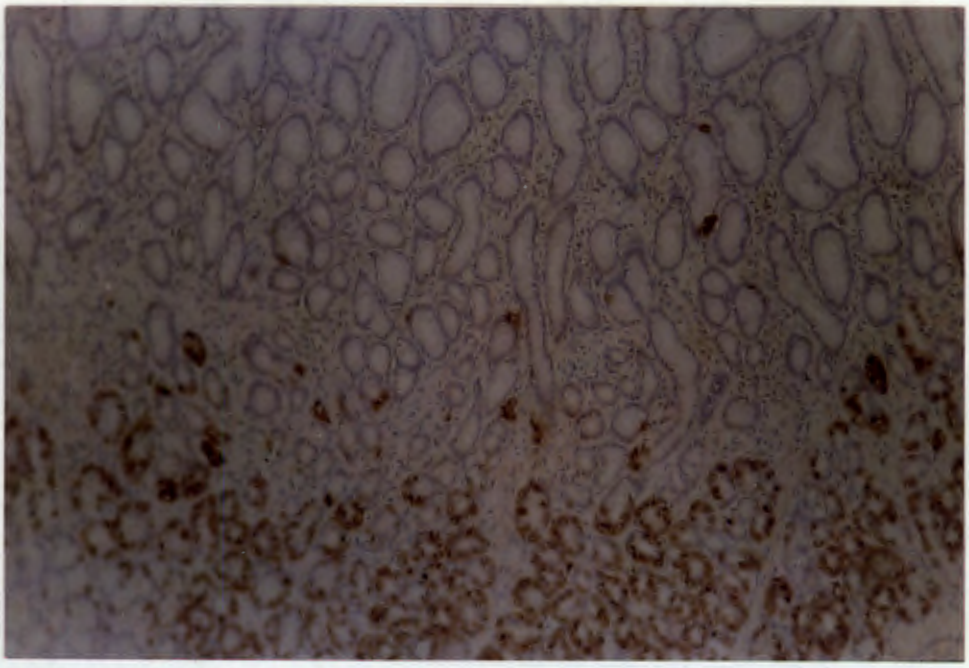

Fig. 3.1: Mucosa of the gastric antrum after omeprazole therapy. Gastrin-secreting cells are prominent. No evidence of active gastritis. Indirect immunoperoxidase stain with anti-gastrin monoclonal antibody X 420.

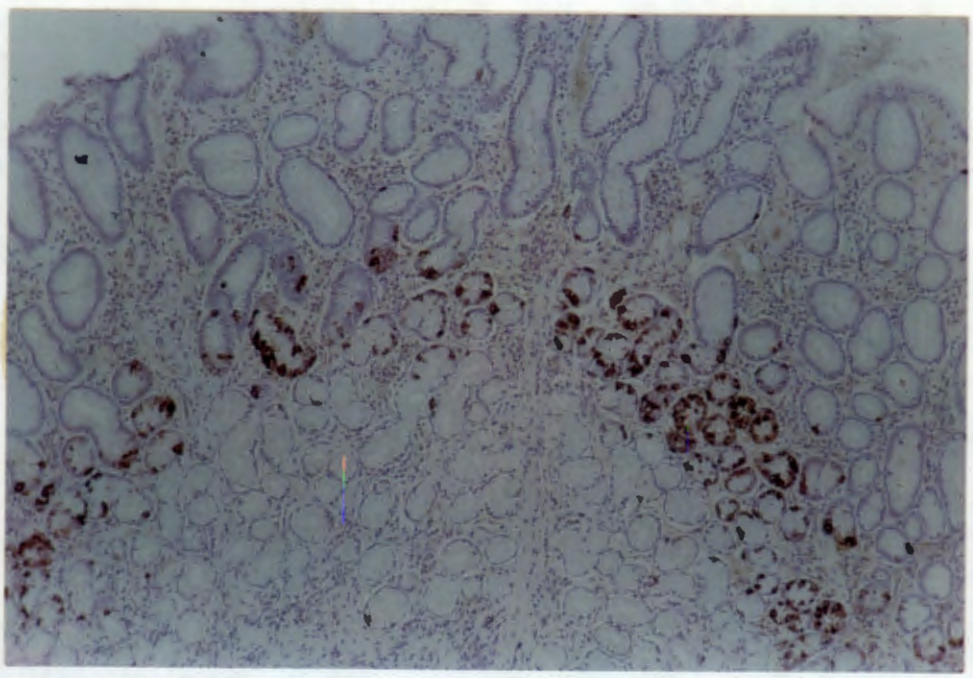

Fig. 3.1a: Antral mucosa of the same patient after triple therapy. Note intense stain of gastrin-secreting cells, and the absence of active gastritis. Indirect immunoperoxidase stain with anti-gastrin monoclonal antibody X 280.

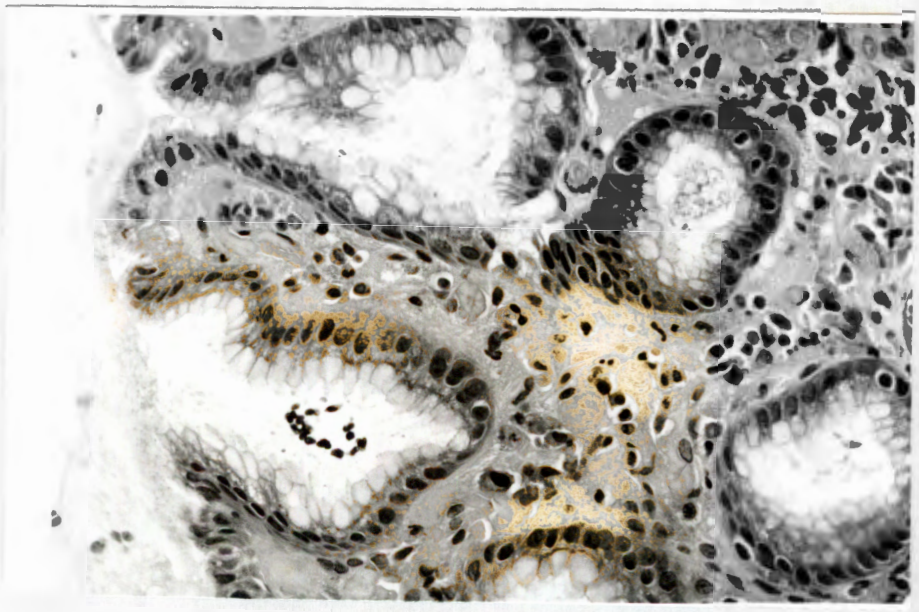

Fig. 3.2: The superficial part of the patient's gastric mucosa, after omeprazole treatment. Crypts contain degenerated mucus and several inflammatory cells. The lamina propria is slightly oedematous. H & E x 420.

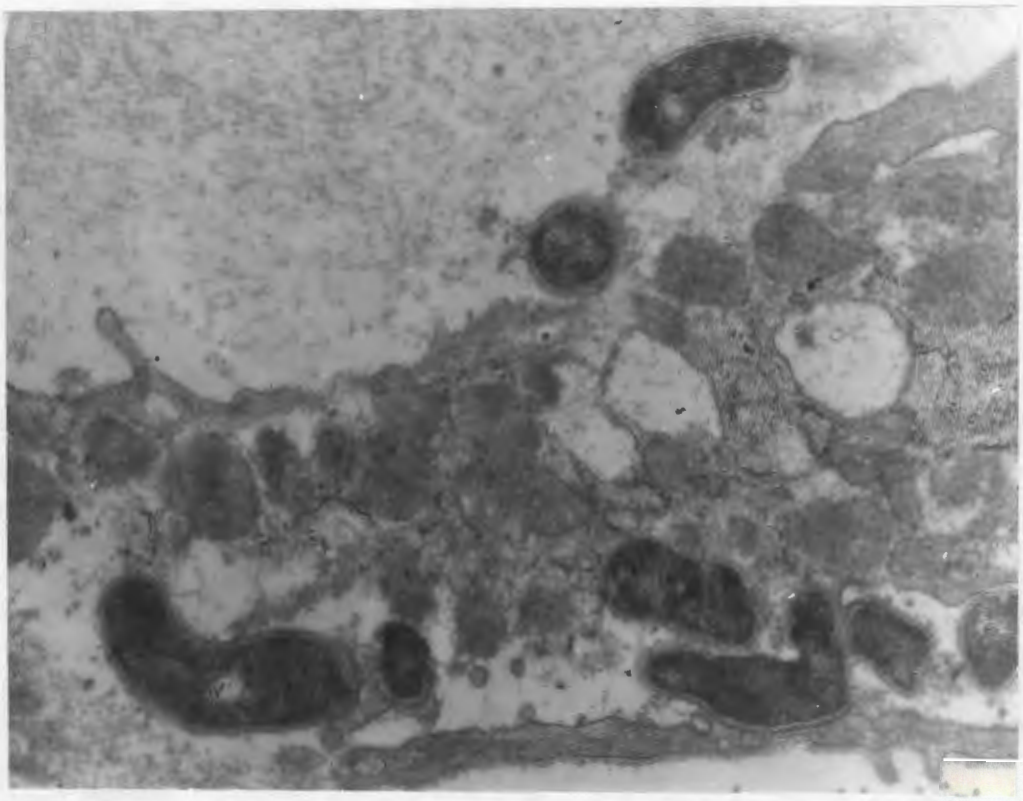

Fig. 3.3: Part of the crypt epithelial cell with distorted and vacuolated H. pylori organisms within the degenerated mucus. Patient after omeprazole treatment. (Transmission electron microscope. Original magnification X 12 000).

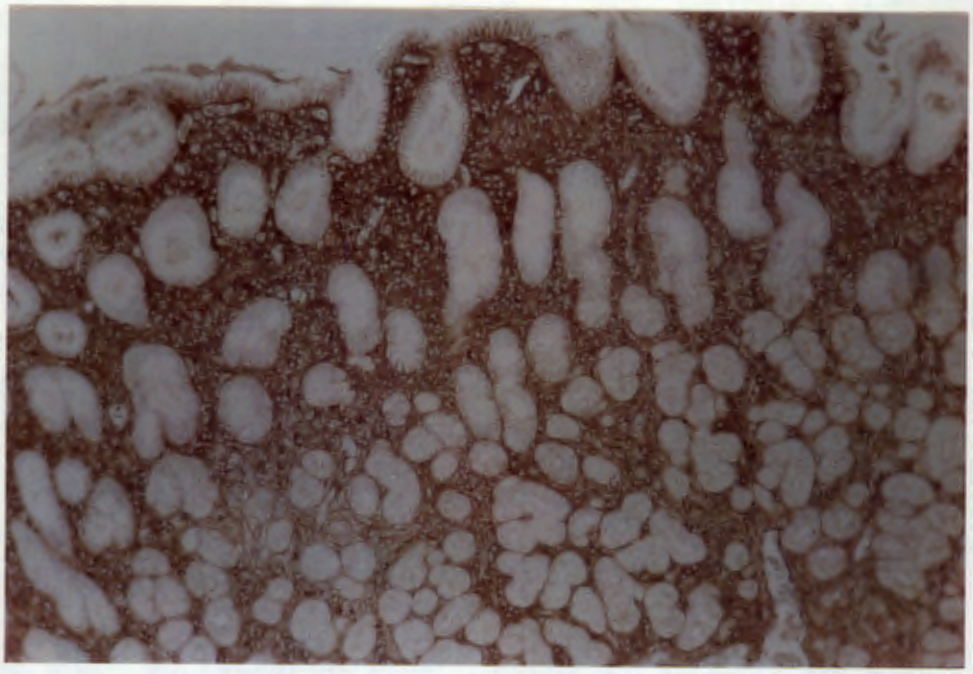

Fig. 3.4: Antral gastric mucosa after treatment with the omeprazole. Note the high amount of IgG at the upper part of the lamina propria. Patient after omeprazole, still infected with H. pylori. Indirect immunoperoxidasde stain for IgG X 280.

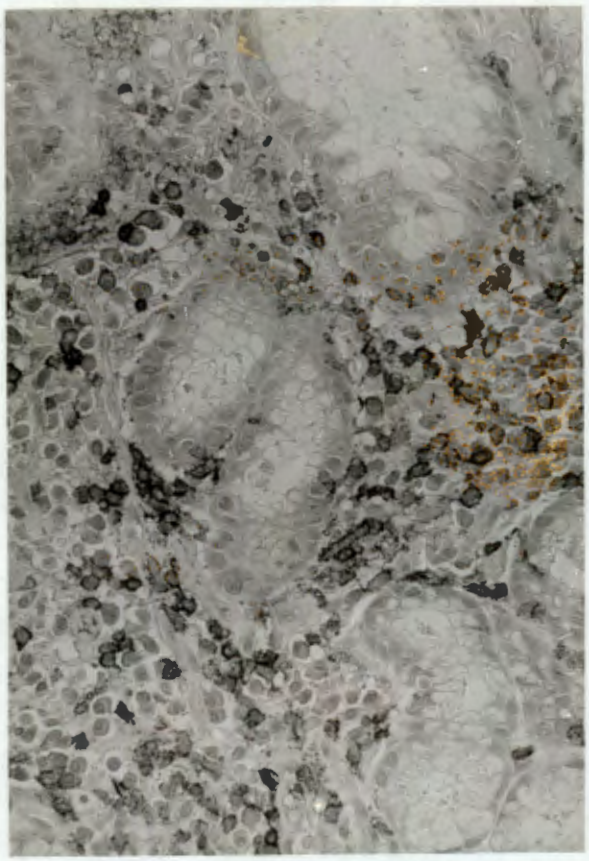

Fig. 3.5: Numerous T-lymphocytes within the lamina propria and crypt epithelium. Patient after triple therapy. Indirect immunoperoxidase UCHL-1 stain X 420.

Glupczynski and Burette (9) in a recent review suggest that combination therapy with antibiotics is the most effective (clearance rates ranging between 70 and 90%), and monotherapy with other agents has been almost totally ineffective (clearance rates 0-30%). Although acquired resistance of H. pylori to antibiotics has been reported, its precise role in treatment failures has not been thoroughly analysed. Development of infection in the histologically cleared but not eradicated from H. pylori gastric mucosa may be related to the immunological state of the host.

In studies related to the local humoral response and cell-mediated immunity in H. pylori-associated gastritis
Wyatt and Rathbone suggest that the morphological features of H. pylori-associated gastritis represent the immune response of the mucosa to the micro-organism (12). Their work, based on 7 cases of H. pylori-associated gastritis, and 8 with normal mucosa, showed that antibody production correlates with the plasma cell infiltrate. They conclude that the increase of lymphoid follicles in gastric mucosa implies antigenic stimulation and this is a specific feature of H. pylori-associated gastritis.

In this study increases of all classes of immunoglobulins in the superficial lamina propria in H. pylori-positive and several negative cases after both therapies indicate increased humoral immunological response of gastric mucosa

on persistent or recurrent infection with H. pylori or infection with other microorganisms which found to be present in H. pylori-cleared cases. Particularly high activity of plasma cells and T-cells in the absence of active gastritis, or histologically negative H. pylori, may indicate that infection has not cleared and that imminent recurrence of H. pylori-associated gastritis may follow.

Increased numbers of intra-epithelial T cells in cases with persistent infection indicates a local cell-mediated response to the luminal antigen. This response is probably non-specific. Most previous works on gastric cell-mediated immunity have concentrated on autoimmune (type A) chronic gastritis, but there are suggestions that T cells are increased in both the epithelial compartment and lamina propria in type B gastritis (15). Studies on T cell subsets (suppressor\cytotoxic or helper phenotype), and functional studies have given conflicting results. In lymphocytic gastritis, where T cells densely infiltrate the superficial epithelium, H. pylori was demonstrable in isolated cases in our previous study (16), and in less than half of their cases by Dixon et al (17). Nevertheless, the latter authors suggest H. pylori as the aetiology of lymphocytic gastritis. When organisms are absent on histological examination, the authors suggest two possibilities: the organism was present, but not detectable histologically, or the infection had cleared. The first suggestion is more acceptable to present

studies, where some cases were histologically cleared but still preserved an excess of the local humoral and cellular immune response.

The presence of lymphoid follicles in gastric mucosa as a specific feature of H. pylori-associated gastritis (12) is not confirmed in this study. It is more likely that lymphoid follicles are a result of a non-specific chronic response of the gastric mucosa to various factors antigenic included and signifying mucosal atrophy.

Gastrin-secreting cell hyperplasia in the antral mucosa, as observed in this study, is related to reduced acid secretion in omeprazole and triple therapy. By inhibiting acid secretion, omeprazole causes hypergastrinaemia. Trophic effects in the oxyntic and the antral mucosa differ. The expected trophic effects in the stomach such as an increased weight and thickness of the oxyntic mucosa and hyperplasia of enterochromaffin-like cells is observed in rats (18), but not in human studies (10,19). Extensive gastric biopsy data from patients enrolled in long-term studies indicates that omeprazole administration is not associated with clinically significant changes in the oxyntic endocrine cell population.

The present study of gastrin-secreting cells in the gastric antrum supports the clinical observations. No correlation between the population of gastrin-secreting cells and H. pylori clearance was found in our treated patients, reflecting the lack of correlation between serum gastrin concentrations and H. pylori status, as was also observed by others (20). Moreover, gastric acid outputs in patients with duodenal, or combined gastric and duodenal ulcers, are similar irrespective of the presence or absence of H. pylori (21, 22).

TABLE 3.1

THE INFLUENCE OF OMEPRAZOLE AND TRIPLE THERAPY ON THE ACTIVITY OF GASTRITIS AND H. PYLORI COLONISATION OF ANTRAL MUCOSA.

	Gastritis score					H. pylori score				
Grade	0	1	2	3	mean*	0	1	2	3	mean
Biopsy at entry	5	16	13	2	(1.33)	0	12	15	9	(1.92)
After omeprazole therapy	13	13	10	0	(0.92)	17	14	2	3	(0.76)
After triple therapy	24	10	2	0	(0.39)	25	6	4	1	(0.47)

*= the total score\number of cases (36).

TABLE 3.2

THE INFLUENCE* OF OMEPRAZOLE AND TRIPLE THERAPY ON THE MUCOSAL IMMUNOGLOBULINS AND T-LYMPHOCYTES.

Antral mucosa after	n	cases with increased values					
omeprazole treatment	36	IgA	IgG	IgM	K	L	T-cl
colonised by H. pylori	19	6	16	17	12	3	12
	%	31	84	89	63	15	63
cleared from H. pylori	17	5	5	8	4	1	2
	%	29	29	47	23	6	12
Antral mucosa after triple therapy	36						
colonised by H. pylori	11	5	11	10	8	5	8
	%	45	100	91	73	45	73
cleared from H. pylori	25	4	9	10	2	4	5
	%	16	36	40	8	16	20

* compared to the results at entry in each case.

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CHAPTER 4

LYMPHOCYTIC GASTRITIS IN NON-ULCER DYSPEPSIA

ABSTRACT

The prevalence of lymphocytic gastritis, a specific form of chronic gastritis, characterised by infiltration of gastric superficial epithelium with T-lymphocytes, has been established in non-ulcer dyspepsia.

Amongst a population sample of 586 patients at risk for gastric carcinoma, 0.8% of patients with non-ulcer dyspepsia, and 1.6% of patients with chronic active gastritis, showed lymphocytic gastritis. Amongst routine gastric biopsies from 5130 patients, only five cases met histological and immunohistochemical criteria of lymphocytic gastritis.

INTRODUCTION

Lymphocytic gastritis (LG) is a recently described histopathological entity, characterised by the infiltration of superficial and foveolar gastric epithelium by mature T-lymphocytes. The density of lymphocytic infiltration was patchy and varied between biopsies from the same patient, but was established as in excess of 30 mature T-lymphocytes per 100 epithelial cells (2,4,5) in the most densely infiltrated areas. Haot et al, in a retrospective review of 11,064 unselected endoscopic biopsies , found that 192 had been labelled as either varioliform gastritis (3), erosive gastritis or aphthoid gastritis (1,2). He considered that 92 of these 192 had microscopic features of lymphocytic gastritis. From the few published works, it appears that this lesion is not common, but incidence rates vary from 0.8 to 4-5% (3,4,5).

The aetiology, relationship to other parts of the digestive tract, clinical relevance and treatment of this unusual form of chronic gastritis are largely unknown.

MATERIALS AND METHODS

In order to determine the prevalence of LG in patients with non-ulcer dyspepsia (patients who presented with dyspepsia and subsequently found to be ulcer free), biopsies from an endoscopical screening study for gastric carcinoma taken in a local rural population, were used. The biopsies were from 586 rural Coloured (mixed origin) patients, known to be at high risk for gastric carcinoma (6).

The mean age was 45.3 $\pm$ 13 years, and the male/female ratio was 1.5/1. All patients were permanent inhabitants of the Cape Province, and had dyspeptic symptoms (heartburn, nausea and/or vomiting, epigastric pain, regurgitation etc), and were referred for endoscopy by local general practitioners.

Oesophagogastroduodenoscopies were performed, and gastric biopsies were collected for diagnostic purposes. At least 2 good mucosal samples were taken from each of the following sites: the angulus, 5 cm from the pylorus on the greater curvature and from the fundus.

The biopsies were fixed in neutral formalin, processed by the routine method, orientated under a dissecting microscope and embedded in paraffin blocks. Semiserial microtome sections were stained with haematoxylin and eosin, alcian blue/periodic acid Schiff and high-iron diamine stain for mucins, and Giemsa stain was used for the detection of Helicobacter pylori and other microorganisms.

Selected samples of gastric mucosa with a high number of intraepithelial lymphocytes (about 30 or more lymphocytes per 100 epithelial cells), were stained, using the monoclonal antibodies L-26, MT-1, UCHL-1, Kappa, Lambda, IgA, IgG, and IgM in an immunoperoxidase PAP technique, in order to clarify the nature of the intraepithelial lymphocytes.

The second part of the study involved a retrospective review of gastric biopsies taken from 5130 patients referred to the Gastro-Intestinal Clinic for upper GI endoscopy over a 20 month period. Samples suspected of LG were examined immunohistochemically as described above.

RESULTS

A total number of 9 cases of LG was found (Table 4.1). Within the endoscopically screened Coloured population, 4 cases fulfilled the required criteria for the diagnosis of LG: mature T-cell lymphocytic infitrates of superficial and foveolar gastric epithelium (Fig. 4.1 and 4.2).

Most of the lymphocytes observed in H & E stained preparations were surrounded by a clear rim (halo). The lamina propria contained an increased number of plasma cells and lymphocytes. Occasional epithelial erosions were detected in three cases. Identification of LG was found to be difficult in cases in which an active inflammatory infiltrate affected the superficial epithelium. Six such cases, primarily selected as LG, were ultimately dismissed, due to

an inadequate number of intraepithelial lymphocytes, and/or a lack of immuno-histochemical confirmation of their origin. Some cells, having a clear rim around the nuclei, were, in fact, degenerate epithelial or endocrine (gastrin-secreting) cells. In one instance, the lymphocytic infiltrate was found to be monoclonal, and further investigation confirmed a primary gastric B-cell lymphoma.

From the initial samples of the 586 endoscopically screened patients, a chronic ulcer was diagnosed in 88 cases, 157 patients were classified as chronic atrophic gastritis (CAG), 180 had superficial gastritis (SG) and 16 had gastric carcinoma (Table 4.1). CAG in this study is equivalent to idiopathic chronic pangastritis - uniform and SG is equivalent to Helicobacter pylori-induced antral gastritis when classified according to the Sydney System (7). Active gastritis was present in 245 patients with either CAG or SG, and non-ulcer dyspepsia was present in 498 patients. Therefore, it was established in this study that 0.8% of the patients with non-ulcer dyspepsia, and 1.6% with chronic active gastritis, had LG.

In the routine group of 5130 biopsies, 8 had features that were suggestive of LG, but only 5 met the pre-established criteria for LG. The remainder were excluded, due to a low ratio of intraepithelial lymphocytes (mean number 23 per 100 epithelial cells) and other intraepithelial lesions. Monoclonal antibodies L-26, MT-1 and UCHL-1 were sufficient for

the differentiation of B and T lymphocytes, clonality of which were assessed with antibodies kappa and lambda. IgA and IgG were predominant in mucosal plasma cells, and did not differ from normal distribution. One case with heavy lymphocytic infiltrate of lamina propria and epithelium, was diagnosed in later endoscopical and immunohistochemical examination as a lymphoma of the mucosa associated lymphoid tissue. Details of all cases of LG are collated in Table 4.2. The patients ranged in age from 39-59 years old (median - 47, mean - 46.9), with 5 females and 4 males. The lesions were present in gastric mucosa, both in body and antrum, in 8 patients, and confined to the antrum in only one.

One patient (G.C.) had 4 repeated endoscopical examinations during the 2 years of observation, and showed LG resistant to treatment with "tagamet", "motilium", "asilone" and "amoxil". At the initial examination, lesions were found throughout the stomach (Figs. 4.1, 4.2), but the duodenum was not involved. In the last histological examination, duodenal mucosa showed villous atrophy and numerous intra-epithelial lymphocytes. The patient was wasted and losing weight, but no other features of protein losing gastropathy, coeliac disease or dermatitis herpetiformis were detected. At each endoscopy, this patient presented with prominent mu-cosal folds in the fundus and body, inflammatory sessile polypoid lesions with erosions and finally polypoid lesions in the post-bulbar region of the duodenum. In other patients gastric mucosa at endoscopy was described as cobble-stoned, polypoid, with

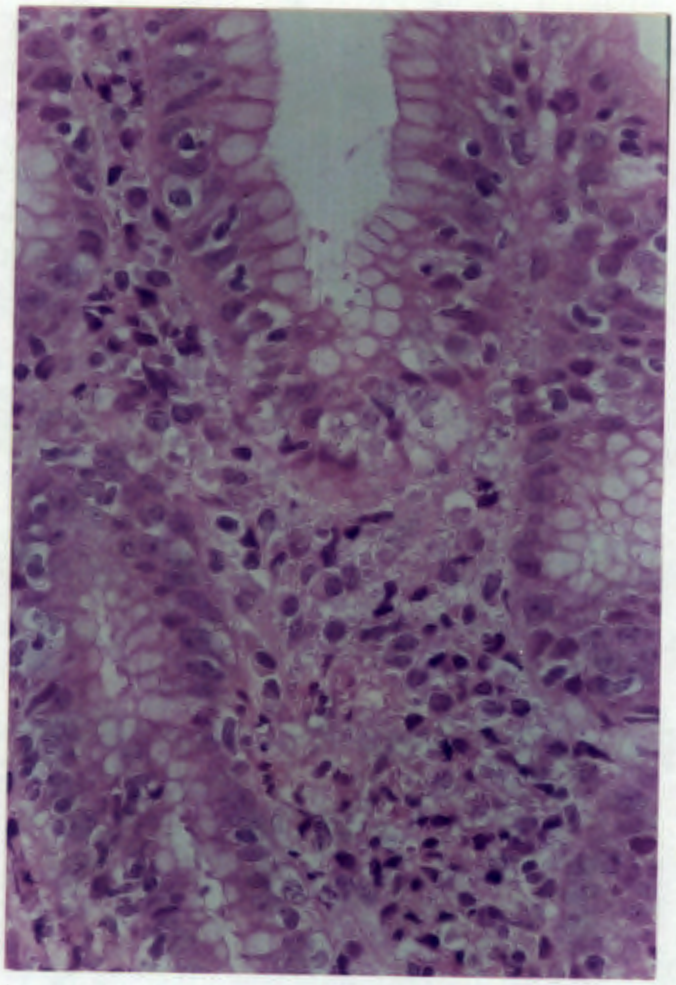

Fig. 4.1: Numerous inflammatory cells are present in the lamina propria and within the superficial and crypt epithelium. H & E X 450.

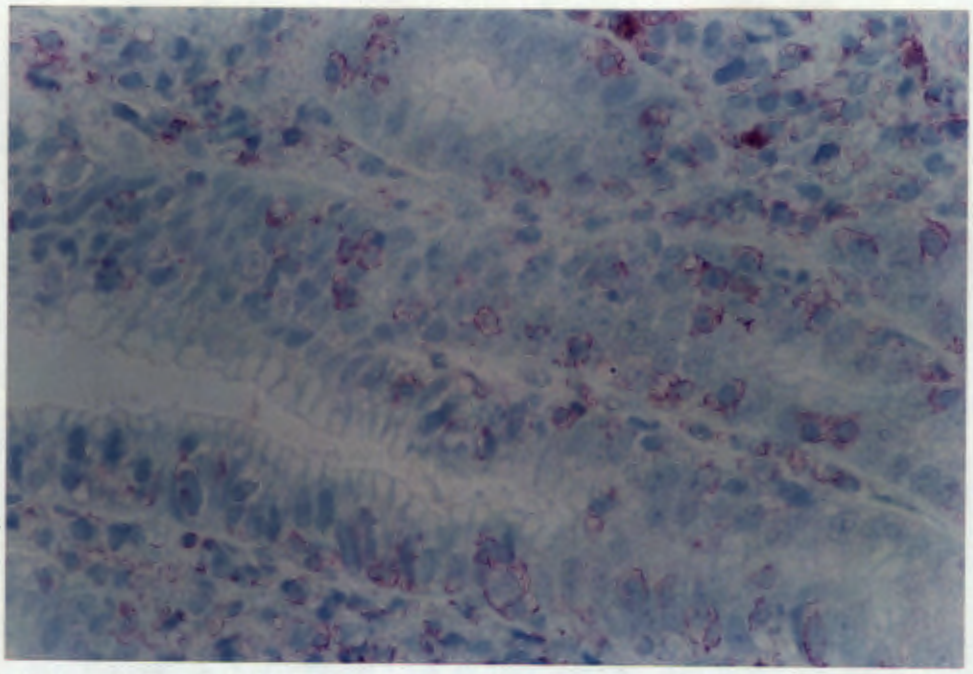

Fig. 4.2: Staining with antibody UCHL-1 reveals the positivity of numerous T-cells within the foveolar epithelium X 450.

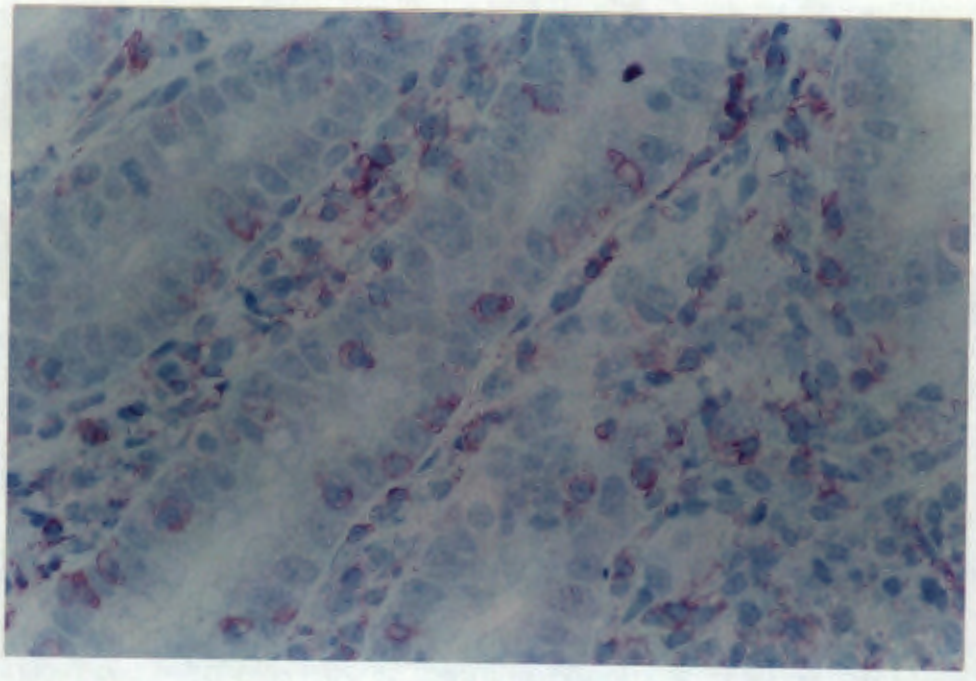

Fig. 4.3: Staining with antibody MT-1 confirms the presence of the numerous mature T-cells in the crypt epithelium X 450.

prominent or hyperaemic folds (Table 4.2). In one instance, the macroscopical appearance was described as normal, but histological section revealed LG with mucosal atrophy. Helicobacter pylori infestation of gastric mucosa was present in five patients out of nine.

Three patients related their dyspepsia to anti-tuberculous therapy (ethionamide, ethambucil and rifampicin). Associations with tobacco smoking and drinking of alcohol were found to be negative. A history of tuberculosis and related treatment in all of the studied patients was present in two percent.

DISCUSSION

The endoscopical study of the population with dyspeptic symptoms showed that the prevalence of LG is 0.8% in patients with non-ulcer dyspepsia, and 1.6% of patients with chronic active gastritis. The prevalence was very much lower among the patients attending the GI clinic (0.1%), but this may be related to the relative lack of adequate biopies away from the site of the lesion.

There are no other published reports based on uniform population samples available for comparison. All published works, including the most recent (9), are based on the histological material available for review at various departments of pathology, and suggest a prevalence of between 3.2 - 4.5% amongst patients with active gastritis, and

1.1-2.5% in non-ulcer dyspepsia (4,8,9). Haot et al (1) found that 92 cases corresponded to lymphocytic gastritis (0.8%) from unselected endoscopical material of 11064 endoscopies. In the most recent study of endoscopical biopsies from four gastroenterology departments, Haot et al.(9) reported 1.4% of varioliform gastritis corresponded histologically to LG in 82%. Differences in rates obtained from available reports are probably related to a variation in the preselection of patients for endoscopy, and a lack of uniformity in the quality of endoscopical biopsies, etc. in various centres. Other factors specific for our studied population, such as racial, high risk for gastric carcinoma, high prevalence of idiopathic atrophic and active (associated with H. pylori) gastritis, may play a role in the relatively low prevalence of LG.

A similar mean age of patients with LG, a female preponderance and diffuse involvement of gastric mucosa confirm the findings of others (4). Macroscopical diagnosis of varioliform gastritis is not a precondition for the diagnosis of LG in our observations. LG is based on histological/immunohistochemical features (Fig.4.3), because endoscopical descriptions are very much related to terminological traditions in different centres. [Varioliform gastritis was not used in our macroscopical assessment, although on most occasions, it was applicable].

These studies indicate the importance of monoclonal antibodies in the diagnosis of LG, particularly those cases with cellular infiltration of the lamina propria, to exclude a possibility of early stages of lymphoproliferative diseases.

The aetiology of LG is obscure. One can speculate as to whether it represents an allergic reaction to antigens present in food, or an atypical reaction to H. pylori (4). A very high prevalence of H. pylori was found in the first group (81%), the relatively low prevalence of LG and low number (5\9) of H. pylori positive LG cases does not support the last hypothesis. Another possibility is the relationship with tuberculosis or with anti-tuberculous treatment, present in 3 out of 9 of our patients.

The association between LG and protein-losing gastropathy has been reported (10), and a morphological resemblance between LG and coeliac disease has also been noted (11). This was present in one of the reported cases, but protein-losing gastropathy was not encountered. The role of tuberculosis or H. pylori has not been established, but histological similarity between LG, coeliac disease and recently described lymphocytic colitis (12), hints at the possibility of altered immune function following viral or other antigenic insult.

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TABLE 4.1

HISTOLOGICAL DIAGNOSES IN 586 PATIENTS UNDERGOING OESOPHAGO-GASTRODUODENOSCOPY FOR DYSPEPTIC SYMPTOMS.

Histological diagnosis	number of cases	%
Chronic atrophic gastritis	157	27
Superficial gastritis	180	31
Gastric ulcer	58	9.9
Gastric carcinoma	16	3
Reflux gastritis	16	3
Lymphocytic gastritis	4	0.8
Normal gastric mucosa	125	21

TABLE 4.2

DETAILS OF CASES OF LYMPHOCYTIC GASTRITIS STUDIED

No.	Patient	Age	Sex	Localisation of gastritis	Macroscopical appearance	H.pylori infestation*
1	EK	41	F	body, antrum	normal	-
2	KS	43	F	body, antrum	cobblestoned	++
3	MS	53	F	body, antrum	irregular, thick	+
4	SO	49	F	antrum	cobblestoned	++
5	GC	56	M	body, antrum duodenum	polypoid, erosions	+++
6	JD	39	M	body, antrum	hyperaemic	-
7	LT	47	M	body, antrum	erosions,polypoid	+++
8	VT	40	M	body, antrum	erosions,polypoid	-
9	MK	59	F	body, antrum	polypoid	-

*

- absent
+ mild
++ moderate
+++ severe

CHAPTER 5

CHRONIC ATROPHIC GASTRITIS AND MICRONUTRIENTS IN A POPULATION AT RISK FOR GASTRIC CARCINOMA

ABSTRACT

A micronutrient study in chronic atrophic gastritis, and in controls selected from a gastroscoped population at high risk for gastric carcinoma in Namaqualand, was undertaken.

Seventy-three patients, comprising 33 with histologically confirmed chronic atrophic gastritis and hypochlorhydria, and 40 controls with histologically normal gastric mucosa, were studied for the blood levels of mineral and trace elements, vitamin E and lipoproteins.

Nineteen cases with chronic atrophic gastritis, and 24 controls, were examined for a wide range of vitamins in venous blood, and nitrites and vitamin C in gastric juice.

Statistically, significant differences were found between iron, vitamin C (plasma and gastric juice), pyridoxine and folate levels in cases when compared to controls, which may suggest implications for the aetiology of atrophic gatritis.

One of the biggest problems in trying to relate nutritional factors to gastric cancer, is that it is difficult to determine the food consumed, and blood micronutrient levels at the time critical for the development of the cancer, e.g. in the initial stages of promotion.

Epidemiological studies (1,2,3) have shown an inverse relationship between risk of development of gastric cancer and consumption of foods rich in vitamin C. In addition, a relationship between vitamin C in plasma and levels of gastric vitamin C, pH and nitrites with gastro-intestinal disease has been demonstrated (5,6). However, the value of gastric vitamin C levels and hypochlorhydria in gastric carcinogenesis was questioned recently, due to the presence of hypochlorhydria and low vitamin C in gastric pathology not related to cancer risk (1,4,7). Moreover, low vitamin C status in gastric juice is related to the instability of this vitamin at elevated pH (6). It is, therefore, possible that factors other than, or in addition to, vitamin C are a function of the increased risk for gastric cancer. In this regard, since chronic atrophic gastritis (CAG) is thought to predispose to gastric carcinoma (8,9), it may represent the best model for quantifying the relationship, if any, between micronutrient status and malignancy. This paper reports on the mineral and vitamin status in patients with CAG.

Our previous study (10) showed a high incidence of gastric carcinoma in the screened populations, and confirmed a strong relationship of type B chronic atrophic gastritis (CAG) with nitrites, hypochlorhydria and low intragastric levels of gastric and plasma vitamin C.

MATERIAL AND METHODS

This work comprises the results of two studies carried out in a Namaqualand rural population which was at risk for gastric carcinoma. Participants of the studies were selected from about 600 patients suffering from dyspepsia. Preliminary endoscopical and histological studies of this homogenous rural population showed that 2.6% of the population had gastric cancers, and 30% had CAG and hypochlorhydria. Detailed dietary studies showed a strong association of CAG with low intake of red meat, fruit and green vegetables (11).

Patients who had dyspeptic complaints such as epigastric pain, heartburn, post prandial pain and bloating, vomiting or nausea, for a duration of at least 3 months, were preselected for endoscopy by local general practitioners.

Initial selection of cases and controls was done by age and sex, endoscopical assessment of gastric mucosa and pH of gastric juice. Patients with endoscopically identified

focal lesions, such as tumours or ulcers, were excluded, but patients with gastric mucosa suggestive of chronic active gastritis and gastric juice pH > 4, qualified as cases. Others with normal pH and a normal appearance of gastric mucosa, qualified as controls. Final classification of cases and controls was determined by histological assessment of gastric mucosa.

This study consisted of 116 patients, selected from a large population group in Namaqualand. Fifty-two patients presented histologically confirmed CAG/intestinal metaplasia and hypochlorhydria; 64 patients with histologically normal gastric mucosa and pH <4, served as controls (Table 5.1).

Approval from the the local hospital's ethical committee was given for all aspects of this study. All participants were asked about drinking of alcohol, smoking and dietary habits. Prior to endoscopy, informed consent was obtained. The endoscopical procedure, collection of gastric mucosal and juice samples have been previously described (10).

The pH of the gastric juice was measured, and the fluid was immediately transferred to tubes containing 1 ml of 1N HCl, in order to stabilise vitamin C(4).

Venous blood from 73 patients was drawn into EDTA for vitamin analysis and plain trace element-free tubes for

mineral analysis. In the blood collected in EDTA tubes, haematocrit was measured, and the blood was stored at 4°C. It was then separated and preserved accordingly for vitamin estimations, frozen, kept in the dark and transported within 4 days to the laboratory for processing.

The whole blood and plasma in the trace element-free tubes were frozen, transported to the laboratory and assessed for vitamin E, mineral elements: K, Na, Ca, Fe, Mg, Cu, and trace elements Zn and Se. Selenium was analysed in whole blood samples by the Grant method (12). Concentrations of sodium, calcium, potassium, iron, magnesium, copper and zinc in blood plasma were determined according to standard and atomic absorption and spectrophotometric methods (12). In addition, plasma cholesterol, triglycerides and low density lipoproteins (LDL) were estimated by routine methods.

In the group of 43 patients, a wide range of micronutrients were estimated in venous blood: vitamin C, A, E (tocopherol), riboflavin, nicotinic acid, vitamins B_6 (pyridoxine), B_1 (transketalase and TPP), B_{12}, serum and red blood cell folate.

Gastric juice samples were examined for nitrites (14) and vitamin C levels (6,15).

The biopsy specimens taken from all scoped patients were collected in 10% buffered formaldehyde. Biopsies were processed routinely and embedded in paraffin wax under a dissecting microscope. Sections were cut and stained with haematoxylin and eosin, periodic acid-Schiff (PAS) and alcian blue for neutral and acid mucins, and with high-iron diamine/alcian blue for sialo- and sulphomucins. The mucin stains were used for the accurate assessment of intestinal metaplasia, the type of gastritis, the amount and type of mucin produced by the gastric epithelium and the quantity of carbohydrates present. The Giemsa stain was used to detect spiral organisms of Helicobacter pylori.

For histological classification of the mucosal changes, we used the system devised by Correa (16), Rothery and Day (17), Wyatt and Dixon (18) and Kekki et al (19). Patients showing signs of antral only, or antral and body crypt atrophy (Fig. 5.1), and/or intestinal metaplasia (Fig. 5.2), and absence of these lesions in the fundus, were classified under CAG (synonyms: multifocal, environmental, type B, type AB chronic gastritis (16).

Patients presenting normal antral and body mucosa, with or without mild or moderate round cell infiltrates in the lamina propria, were classified as controls. Patients presenting other pathological changes, such as ulcers or

tumours, were subjected to further investigations and treatment, but were not included in this study.

RESULTS

Mineral and trace element study

Iron, calcium, zinc and selenium showed depressed values in the cases (as defined above), but a statistically significant difference was confirmed between levels of iron only in CAG when compared to controls (Table 5.2).

The frequency of the lowest readings of calcium and zinc in different study groups were similar. Although no significant differences were found in the Se levels between cases and controls, 40% of cases had Se (whole blood) levels below 100 ng/ml, whereas only 19% of the control subjects had markedly depressed Se levels, but this was not significant statistically.

Blood Se levels were not related to whether a participant of this study was a tobacco smoker or to levels of cholesterol, triglycerides or LDL in the plasma. Levels of plasma cholesterol, triglycerides and LDL did not show substantial differences within the groups.

Magnesium and zinc concentrations were below that expected in all groups studied, although no substantial differences between groups were noted.

Vitamin study

Results of biochemical values of micronutrients in CAG and subjects with normal gastric mucosa are collated in Table 5.3. No clinically detectable signs of vitamin deficiency were seen either in the cases or controls of this study. However, significantly low levels of vitamin C in plasma and in gastric juice were detected in CAG, accompanied by hypochlorhydria and the presence of nitrites in gastric juice. Increased nitrite levels in gastric juice were detected in 15/19 cases with hypochlorhydria (gastric pH>4) and CAG, whereas only 2 controls showed similar conditions. Median lipotrope levels were also lower in CAG than in controls. However, statistically significant values were detected in serum and red blood cell folate.

Vitamin B_6 levels were significantly lower ($p<0.05$) and vitamins A and E not significantly lower in CAG than in controls. Other vitamin levels did not show differences in different groups of patients.

Histological evidence for H. pylori was found in 81% of scoped patients, whereas only three patients with CAG from the first study had no evidence of H. pylori. Similarly, in the second study, five patients with normal histological mucosa and only three patients with CAG, were free from H. pylori infestation. A much lower infestation with H. pylori is expected due to the CAG-associated hypochlorhydria.

Therefore, a high rate of H. pylori positive biopsies showed such a close association with CAG, that no separate effect for the presence of H. pylori could be determined.

DISCUSSION

The strong association of CAG with hypochlorhydria, low levels of vitamin C in plasma and gastric juice, as well as nitrite in gastric juice, was confirmed by the present study and is in agreement with results reported by others (4-7).

In a recent review of the role of dietary minerals in colon carcinogenesis, Nelson (20) suggests that variations in dietary exposure to several minerals, such as iron, sodium, potassium, calcium, zinc and selenium, may alter the risk of acquiring colorectal cancer. There is also epidemiological and experimental evidence for zinc, copper, selenium and magnesium being involved in oesophageal carcinogenesis (21). However, very little information concerning minerals and gastric carcinoma was found.

Iron deficiency in gastric carcinoma was discussed in a high-risk Colombian population study (22). Several other epidemiological (23,24,25) and case-control analyses (26,27) pointed out that there was a depressed selenium status in a population at risk, or in patients with diagnosed gastric carcinoma. Blood selenium levels are usually lower in patients with any advanced cancer, but these low levels may

be a consequence rather than a cause of the disease. Moreover, selenium status depends on some other factors such as vitamin E, cholesterol and other lipoprotein levels in blood, and is also related to smoking (28). Significantly lower levels of iron and substantially lower selenium levels in patients with histologically proven CAG, compared to controls, suggests a long-term deficit in these micro-elements, and their possible role in gastric carcinogenesis.

CAG and achlorhydria have been described in association with chronic iron deficiency and/or associated nutritional defects per se (22), but the low status of selenium in the blood of patients with CAG is reported here for the first time. Lower levels of selenium interacting with vitamin E in cases seems to confirm the importance of micronutrients with antioxidant properties in CAG aetiology.

The variation of vitamin A and E levels in relation to gastric pathology reported by others (29), showed that these vitamins were lower in subjects with dysplasia than in subjects with normal mucosa and CAG. This study did not confirm our previous findings (10), although non-significant differences between cases and controls were present.

Low serum vitamin B_{12} levels, intrinsic factor antibody and anaemia, were observed in fundic CAG (30), which differs aetiologically from environmentally induced multifocal CAG

in our population group. The authors of a referred study (30) recorded no difference in serum levels of iron and vitamins A and E between patients with CAG and normal subjects. Surprisingly higher folate levels were reported in atrophic gastritis (type A), and an attempt was made to explain that an accumulation of S-methyl tetrahydrofolate in serum is due to vitamin B_{12} deficiency or greater folate synthesis by the intestinal flora, resulting from bacterial overgrowth secondary to hypochlorhydria.

In spite of hypochlorhydria and the presence of intragastric nitrites, patients with CAG in this study had lower serum and RBC folate, suggesting a dietary deficiency of these and other nutrients. A detailed case-control study of this population showed a significant association of CAG with deficient intake of animal protein, fresh vegetables and fruit (11).

Results of this micronutrient study in CAG indicate that altered dietary supply or altered absorption of minerals, particularly iron and selenium, and vitamins C, E, B_6 and folates, can play a role in gastric carcinogenesis. The association of similar micronutrients with malignant and premalignant oesophageal lesions shown in our previous report (31), suggests a common aetiopathogenesis of both gastric and oesophageal carcinoma, and that nutritional factors are important in CAG aetiology.

TABLE 5.1

DETAILS OF PATIENTS STUDIED

		Age (yr)		Sex
	n	mean	range	M : F
Chronic atrophic gastritis	57	57.1	37-65	32 : 23
Normal gastric mucosa	64	48.3	30-68	45 : 18

TABLE 5.2

VALUES OF MINERAL ELEMENTS IN BLOOD OF PATIENTS WITH CHRONIC ATROPHIC GASTRITIS AND IN CONTROLS

Mineral gastric element	Normal range	chronic atrophic gastritis (N=33) median	range	normal mucosa (N=40) median	range
Ca	2.1-26 mmol/L	2.16	1.83-2.33	2.27	1.89-2.37
Na	135-143 mmol/L	140.0	132-143	139.0	133-145
K	3.5-5.3 mmol/L	4.15	4.0-6.1	4.20	3.5-4.9
Mg	0.74-1.48 mmol/L	0.68	0.64-0.70	0.67	0.66-0.73
Fe	0.79-1.96 µg/ml	1.30	0.6-1.7**	1.46	09-2.1
Cu	0.68-1.82 µg/ml	1.16	0.9-1.4	1.16	1.0-1.5
Zn	1.0 ± 0.18 µg/ml	0.65	0.6-0.8	0.71	0.6-0.9
Se	112-200 µg/ml*	111.7	63-138	121.7	94-193

* normal value for the population group

** statistically significant ($p<0.05$), when compared to controls

TABLE 5.3

BLOOD AND PLASMA VITAMINS AND VITAMIN C IN GASTRIC JUICE IN PATIENTS WITH CHRONIC ATROPHIC GASTRITIS AND CONTROLS

Micronutrient	normal range	chronic atrophic gastritis (n=19) median	chronic atrophic gastritis (n=19) range	normal gastric mucosa (n=24) median	normal gastric mucosa (n=24) range
Vit C in plasma	0.25-1.2 mg/dl	0.24	0.10-0.42**	0.52	0.19-0.54
Vit C in gastric juice	0.23-0.58 mg/dl	0.23	0.12-0.40**	0.40	0.23-0.58
Vit A	20-70 µg/dl	45.1	37.5-44.5	46.6	37.1-51.5
Vit E	>6µg/dl	10.6	9.3-11.4	12.7	10.1-15.7
Vit B_6 (pyridoxine)	6-20 ng/mol	4.25	2.4-7.1***	5.36	2.6-7.1
Vit B_{12}	220-750 pg/ml	468.3	215-715	487.5	350-730
Serum folate	2.6-15.5µg/ml	3.24	2.1-5.0***	3.8	2.0-6.1
RBC folate	210-980 µg/ml	352.6	179-443***	409.9	302-559

** $p<0.005$ }
} when compared to controls
*** $p<0.05$ }

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CHAPTER 6

CHRONIC ATROPHIC GASTRITIS, GASTRIC pH, NITRITES AND MICRONUTRIENT LEVELS IN A POPULATION AT RISK FOR GASTRIC CARCINOMA

ABSTRACT

One hundred and seventy-eight patients at risk for gastric carcinoma had upper gastrointestinal endoscopy. Twenty-seven selected patients with the type B of chronic atrophic gastritis, 32 patients with normal mucosa, and 47 non-scoped healthy controls were tested for plasma vitamin C, retinol and tocopherol. The total vitamin C level was also assessed in the gastric juice of scoped patients. Micronutrient levels were related to gastric pH, nitrites and gastric mucosal pathology. The study showed a higher level of pH (> 4), and high nitrites in gastric juice in patients with chronic atrophic gastritis, gastric malignant and dysplastic lesions. Neither the hypochlorhydria nor the gastric nitrites affected the prevalence of C. pylori in gastric mucosa. Low gastric and plasma concentrations of vitamin C, observed in patients with chronic atrophic gastritis, showed an inverted relationship with pH level, and an inter-relationship of other vitamins with antioxidant properties (vitamins A and E).

The incidence of gastric cancer (GC) in some Western countries, including the USA, has shown a steady decline during the last several years. This declining tendency is not observed in Japan.

Gastric cancer in the South African population has been the most common cause of cancer death amongst Caucasians, Coloureds (mixed ancestry) and Asians, in both sexes(1). At present the incidence of GC for Coloureds is four times higher than that observed in other population groups(2).

Recent studies(3,4) of geographically distinct population groups, showed differences in gastric mucosal lesions, particularly intestinal metaplasia. An association between intestinal metaplasia and the intestinal type of GC, and a possible association with nutrition has been noted in countries with high GC incidence rates(5-8). For example, a relationship of gastric pathology with gastric pH, nitrites and vitamin C has been demonstrated recently in various gastric diseases(6-11). However, limited information is available on nitrites in gastric juice, and micronutrients in plasma of patients with type B chronic atrophic gastritis (CAG)(9).

This study formed a part of a larger endoscopical and nutritional survey aimed at early detection, aetiology and prevention of GC in populations at risk.

MATERIALS AND METHODS

The study population included 225 participants, of which 178 were pre-treatment patients with dyspeptic complaints such as epigastric pain, heartburn, post prandial pain and bloating, vomiting or nausea with a duration of at least 3 months. Patients were from the rural homogenous Coloured communities of the Western Cape, and were preselected by local practitioners. The patients were offered endoscopy, and informed consent was obtained. The remaining 47 participants were non-scoped, sex and age matched asymptomatic controls from the same area. Approval from the local hospital ethical committee was given for all aspects of this study.

Patients fasted overnight, before endoscopy was carried out, using an Olympus GIF-XQ-10 instrument. No sedation was used, but the pharynx was sprayed with xylocaine. Between each endoscopy, the suction channel of the endoscope was washed, and rinsed with Cidex and distilled water. On entering the stomach, approximately 10 ml of juice was aspirated using a sterile cannula passed through the biopsy channel. At the end of the endoscopy procedure, two mucosal biopsies were taken from each of the following sites: angulus; 5 cm from the pylorus, on the greater curvature and fundus.

The pH of the gastric juice was measured, following which, the juice was immediately transferred to tubes and frozen. The biopsy specimens were collected in 10% buffered formaldehyde. Biopsies were processed routinely, and embedded in paraffin under the dissecting microscope. Sections were cut and stained with haematoxylin and eosin, periodic acid-Schiff (PAS) and Alcian blue for neutral and acid mucins, and with high-iron diamine-Alcian blue for sialo- and sulphomucins. The mucin stains were used for the accurate assessment of intestinal metaplasia, the type of gastritis, the amount and type of mucin produced by the gastric epithelium and the quantity of carbohydrates present. Giemsa stain for spiral organisms was also used.

For histological classification of the mucosal changes, we used the systems devised by Correa(12), Rothery and Day(13), Wyatt and Dixon(14) and Kekki et al(15). Patients presenting with signs of antral or antral and body crypt atrophy, and/or intestinal metaplasia were classified under CAG (type B).

Round cell infiltration of the lamina propria without loss of crypts and intestinal metaplasia, was classified as superficial gastritis.

Patients presenting with pathological changes, such as ulcers or tumours, were subjected to further investigation and treatment. Patients with histologically diagnosed CAG/ intestinal metaplasia, and patients with normal gastric mucosa, were recalled for further studies as soon as possible. Venous blood was collected for vitamin assessment from 32 patients with normal gastric mucosa, 27 with CAG and 47 non-scoped control patients. Grading of the colonisation of gastric mucosa by Helicobacter pylori was performed as described previously (16). In H. pylori-infected gastric mucosa, the bacteria were seldom seen in the vicinity of the intestinal metaplasia, therefore, the colonisation assessment was done in non-metaplastic mucosal fields.

Venous blood was collected in plastic tubes, separated plasma was kept in the dark, and all samples, including gastric juice, were transported on ice to the laboratory as soon as possible .

Gastric juice nitrites were assessed, using Griess's reagent. Measurement of total vitamin C in gastric juice and plasma was done according to the method of Roe and Keuther (17). Plasma vitamin A (retinol) and vitamin E (tocopherol) were estimated as published before (18).

Most patients were middle-aged, and consequently, there were no substantial differences in mean age between groups.

RESULTS

Table 6.1 shows that, of 178 scoped patients, only 26.4% had histologically normal gastric mucosa. Thirty-six percent had superficial gastritis, and 26.4% were classified as CAG. Less common lesions were seen in the remainder of the patients.

Less frequent lesions were chronic gastric ulcers, cancer and dysplasia. There were 10 ulcers, three malignancies and seven dysplasias, both mild and moderate. Early intramucosal malignancy was confirmed by follow-up examination of post-surgical specimens.

Table 6.2 shows the relationships between gastric mucosal pathology, pH and nitrites in gastric juice. Patients with CAG, malignant and dysplastic gastric lesions, evidenced very high concentrations of nitrites and high pH. Patients with normal gastric mucosa differed little from those with chronic ulcers and superficial gastritis in nitrite concentration or gastric pH.

Patients with CAG (Table 6.3) may be classified into a number of classes and according to grade the mean pH and nitrite concentrations varied within these groups. However, significantly higher pH and nitrite concentrations were detected in type III (sulphomucin-positive) intestinal meta-

plasia and were related to the activity and severity of inflammation.

Plasma vitamin concentrations in endoscopically normal patients were very similar to the concentrations in asymptomatic, non-scoped control patients (Table 6.4). Mean values for all three vitamins were significantly higher in patients with normal gastric mucosa, compared to patients with CAG. Non-scoped control patients also showed significantly higher mean concentrations of vitamins C and A. However, the mean vitamin E concentration was higher in non-scoped control patients, but not significantly, compared to patients with CAG.

Plasma concentrations and gastric vitamin C levels were significantly lower in patients with hypochlorhydria ($p<0.001$). Only four patients out of 18 (27%), with pH lower than 4, had a substantially low plasma vitamin C level, whereas 25% of patients with hypochlorhydria had plasma vitamin concentrations comparable to the mean vitamin C level in controls. There was no such clear association of other vitamin levels with pH readings.

H. pylori colonisation was present in 81% of all patients examined and in 89% of patients with gastritis. Table 6.5 shows the association of the grade of H. pylori colonisation and values of pH and nitrite concentration in gastric juice.

The severity of mucosal colonisation by H. pylori is not related to gastric hypochlorhydria, and there is a lack of significant association of H. pylori colonisation and the presence of nitrites in gastric juice. Surprisingly, more severe H. pylori colonisation showed some depressing influence on gastric nitrites.

DISCUSSION

This study shows a high incidence of precursor lesions for gastric carcinoma in our local population, particularly CAG. Patients and controls discussed in this chapter come from a rural community in the Western Cape, close to the Namibian border. Other concurrent studies showed that CAG patients have a diet relatively rich in dried fish, but poor in red meat, fresh vegetables and fruits, when compared to controls. The finding of three gastric malignancies and seven dysplasias within this group of 178 patients, confirmed that they constitute a high-risk population group. Correlated with the gastric mucosal pathology, were hypochlorhydria, a high concentration of nitrites in gastric juice, low gastric vitamin C and low plasma concentrations of vitamins C, A and E.

Hypochlorydria and the formation of nitrite in gastric juice is known and has been reported in various gastric disorders

(6,9-11). Eisenbrand et al (9) noted a lack of significant nitrite changes in patients with gastric and duodenal ulcers or after proximal gastral vagotomy, but significant increases of gastric nitrites were noted in patients with CAG, and in those who had undergone Billroth I or Billroth II gastric resection. High pH values and high nitrite concentrations were observed by Watt et al (10) in patients subjected to various types of operations for duodenal ulcer, conditions not classified as precancerous lesions.

Recent studies (O'Connor et al (11)) of patients with various diseases showed a correlation between gastric juice and plasma concentration of vitamin C, where significantly lower vitamin C concentrations were observed in patients with hypochlorhydria. Low intragastric vitamin C was also observed in patients with gastric cancer, gastric or duodenal ulcers, pernicious anaemia, and after gastric surgery. A tendency towards a lower intragastric concentration of vitamin C was also found in patients with CAG, but tested non-significantly (11).

The study of a population at risk for gastric carcinoma showed a significant association of pH > 4 and high nitrites in gastric juice, with CAG and gastric malignant and dysplastic lesions. Within the patients with CAG, significantly higher pH and nitrite values were found in type III (sulphomucin-positive incomplete) intestinal metaplasia and

in active and more severe types of atrophic gastritis. The association of type III intestinal metaplasia with hypochlorhydria and nitrites, is a potentially important finding, due to the role that CAG and this type III of metaplasia plays in gastric carcinogenesis (19-22).

A non-significant trend in increasing nitrite concentration was associated with increasing severity of gastric lesions in Colombians, but not in Louisiana high-risk population groups in hospital-based, case-control studies (6). This study supports the Colombian study, and showed a similar prevalence of CAG to the recent report in the New Orleans population with increased risk of gastric carcinoma (23). In this report, the authors also pointed out an association of H. pylori and CAG, and suggested the possible role of H. pylori in the development of the precursor lesions of gastric cancer. Similarly, other authors (15,24,25) found a close association of H. pylori with CAG. Moreover, Blaser (25) suggests that hypochlorhydria with concomitant gastritis has been attributed to H. pylori infection. H. pylori may suppress gastric acid secretion through the production of nondialysable protein.(27) Siurala et al (26) showed a significantly decreased prevalence of H. pylori with increasing hypochlorhydria. In this study, neither the hypochlorhydria nor the amount of gastric nitrites affected the prevalence of H. pylori in the gastric mucosa. This is probably related to the exceptionally high prevalence of H.

pylori in the tested population (81%), but the question is important and remains open.

The vitamin studies support previous reports, concerning vitamin C in CAG and its inverted relationship with pH level(6,11,26). Very little is known about other anti-oxidants in relation to gastric pathology. Haenszel et al (28) found significantly lower plasma carotene, and less obvious vitamin E levels in gastric dysplasia. The presented study showed a significantly low plasma vitamin A and vitamin E concentration in patients with CAG, and indicates the importance of other micronutrients in the pathogenesis of the precursor lesions.

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TABLE 6.1I

DETAILS OF 178 PATIENTS SUBJECTED TO STUDY

Histological Diagnosis	n	Age (yrs) Mean Range	Sex M	F
Normal	47	48(19-82)	28	19
Superficial Gastritis	64	49.5(20-82)	30	34
Chronic atrophic Gastritis	47	53.7(37-65)	26	21
Chronic gastric Ulcer	10	46.1(24-65)	4	6
Gastric carcinoma & dysplasia	10	53.2(38-64)	6	4

TABLE 6.2

GASTRIC JUICE PH AND NITRITE VALUES AND MUCOSAL HISTOLOGY IN 178 PATIENT WITH DYSPEPSIA

Gastric Mucosal Histology	pH mean > 4.0		p-Value*	Nitrite ug/ml mean > 0.020		p-Value
No histological abnormalities	1.77	8.5%	=0,001	0.015	6.4%	<0.005
Superficial gastritis	2.65	26.4%	<0.005	0.098	15.8%	<0.05
Chronic atrophic gastritis	4.94	70.2%	-	0.274	51.1%	-
Gastric ulcer	1.70	11.1%	<0.001	0.003	0%	=0.0001
Gastric dysplasia or carcinoma	4.73	70.0%	NS	0.364	40.0%	NS

* Compared to chronic atrophic gastritis-group

TABLE 6.3

GASTRIC JUICE PH AND NITRITE VALUES AND CHRONIC ATROPHIC GASTRITIS TYPE, GRADE AND ACTIVITY

	INTESTINAL METAPLASIA		GASTRITIS ACTIVITY	
	Type I and II	Type III	Active, Moderate and severe	Quiescent, mild
	n = 25	n = 22	n = 29	n = 18
pH mean	4.48	6.26*	5.93	2.54**
Nitrite mean (ug/ml)	0.133	0.299*	0.328	0.075**

* $p < 0.05$

** $p < 0.005$

TABLE 6.4

VITAMIN C IN PLASMA AND GASTRIC JUICE AND PLASMA VITAMIN A AND E: MEAN VALUES IN PATIENTS WITH CHRONIC ATROPHIC GASTRITIS & IN CONTROLS

Gastric Mucosal Histology	Vit. C mg/100 ml		Vit. A ug/100 ml	Vit. E mg/Litre
	plasma	gastric		
Normal gastric mucosa n = 32	0.306**	0.337*	58.90*	19.77*
Chronic atrophic gastritis n = 27	0.109	0.230	43.02	14.88
Non-scoped asymptomatic patients n = 47	0.299**	-	58.76*	16.62

* $p < 0.05$]
] Compared to chronic, atrophic gastritis
** $p < 0.01$]

TABLE 6.5

RELATIONSHIP OF PH AND NITRITES IN GASTRIC JUICE AND H. PYLORI COLONISATION OF GASTRIC MUCOSA

H. Pylori Colonisation	pH>4 %	pH<4 %	NO^2>0.020 %	NO^2<0.020 %
Severe (+++)	34.8	36.0	31.4	43.0
Moderate (++)	30.4	32.0	28.6	28.4
Mild or absent	34.8	32.0	37.0	28.6

CHAPTER 7

SUMMARY OF RESULTS AND CONCLUSION

This study was performed in the rural communities of the Western Cape (Paarl, Malmesbury, Worcester, Vredenburg, Saldanha Bay and several areas in Namaqualand) at risk for gastric carcinoma, and outpatients from Cape Town who came from various socio-economic groups.

Endoscopical screening of patients at risk for gastric carcinoma and presenting unspecified dyspeptic symptoms (586 patients) revealed gastric carcinoma in 2.7%, being most common in males over 40 years of age. Of 4 affected females, two had diffuse histological variants. These figures confirm a high prevalence and characteristic age and sex distribution of gastric carcinoma. Only 3 out of 16 malignancies were early, confirmed in surgical specimens and potentially curable, therefore, the cost-benefit ratio for the diagnostic and therapeutic programme has to be carefully evaluated.

The study showed a high prevalence of cancer precursor lesions: chronic atrophic gastritis/intestinal metaplasia. Autoimmunological (type A) and pangastritis (type AB) were noted sporadically. Chronic atrophic antral (type B) gastritis was predominant, more common in males over 40 years and tobacco smokers. No relationship between the mucosal precursor lesions, and sulpho- and sialomucins was detected; therefore, this relationship which was reported by other authors, has not been confirmed in this study. High rates of chronic atrophic gastritis in males, more frequent extensive

distribution of intestinal metaplasia, and the frequency of its incomplete variant in males, are suggestive for their causal relationship with gastric carcinoma in the presence of a particularly high rate of malignant lesions in this particular group.

Other types of gastritis were found to be of a lesser frequency in the studied patients. The newly described entity - lymphocytic gastritis - is found in 0.83% of patients with non-ulcer dyspepsia, and 1.63% of patients with chronic active gastritis. Contrary to others, among our routine gastric biopsies from 5130 outpatients, only five cases met the historical and immunohistological criteria of lymphocytic gastritis. Presented results indicate an importance of immunological tests in differentiation of this unusual form of gastritis, gastrin-secreting cell hyperplasia, other mucosal infiltrates and early stages of lymphoproliferative diseases.

The study of Helicobacter pylori infestation of gastric mucosa in the rural population at risk showed the presence of H. pylori in 82% of all studied. A less common prevalence (61%) has been detected in the urban patients from higher socio-economic groups. There is a strong association of H. pylori and duodenal or gastric ulcer diseases and type B chronic active gastritis. The association with gastric carcinoma is indirect and is linked with other causal factors.

A possible sequence of lesions in the gastric mucosa is suggested: chronic active gastritis related to H. pylori infection - chronic atrophic gastritis - mucosal atrophy and intestinal metaplasia - dysplasia - intestinal type of gastric carcinoma. Other co-factors are probably involved in various stages of gastritis and transformation of gastric mucosa into metaplastic, dysplastic and malignant lesions.

Local cellular and immune response by antral mucosa was studied in patients undergoing treatment for eradication of H. pylori. Here again, H. pylori infection of the antral mucosa was present in all patients with duodenal ulcer disease, and strongly associated with chronic active gastritis. Treatment with omeprazole and triple therapy cleared infection and active gastritis, but not completely. The better and more permanent effects were after triple therapy. Both therapies were associated with an accumulation of serous fluid, increased population of mononuclear cells and secretion of immunoglobulins IgG and IgM in the upper part of the lamina propria. These changes, together with increased numbers of T-lymphocytes within the crypt epithelium and lamina propria, were associated with the presence of H. pylori organisms. Increased cellular response of the lamina propria and accumulation of immunoglobulins in the upper part of mucosa - even with histologically assessed clearance - may indicate a lack of complete era-dication of H. pylori, and predict relapse of duodenal ulcer.

Omeprazole treated patients had hyperplasia of gastrin-secreting cells in the antrum, irrespective of the presence or absence of H. pylori.

A micronutrient study in chronic atrophic gastritis and in controls selected from a fiberscoped population in Namaqualand showed statistically significant lower values of iron, vitamin C (plasma and gastric juice), pyridoxine and folate, in cases, when compared to controls.

Micronutrient levels were related to gastric pH, nitrites and gastric mucosal pathology. The study showed a higher level of pH and nitrites in the gastric juice of patients with chronic atrophic gastritis, gastric malignant and dysplastic lesions. Neither the hypochlorhydria nor gastric nitrites affected the prevalence of H. pylori in gastric mucosa. Low gastric and plasma concentrations of vitamin C observed in patients with chronic atrophic gastritis showed an inverted relationship with pH level, and an interrelationship of other vitamins with antioxidant properties (vitamin A and E). Concurrent dietary studies showed a lower intake of fruit and vegetables in cases, and support the implication of antioxidants and other vitamin deficiency in the aetiology of chronic atrophic gastritis. Hypochlorhydria and high nitrite concentration in the gastric juice of patients with chronic atrophic gastritis possibly act as promoting factors for gastric carcinogenesis.