UPPER GASTROINTESTINAL ENDOSCOPIC FINDINGS AND HELICOBACTER PYLORI STATUS IN DIABETIC OUTPATIENTS WITH DYSPEPSIA AT KENYATTA NATIONAL HOSPITAL

A DISSERTATION PRESENTED IN PART FULFILMENT FOR THE DEGREE OF MASTERS OF MEDICINE IN INTERNAL MEDICINE OF THE UNIVERSITY OF NAIROBI

BY

Dr. Johnson Masika Wafula

M.B., Ch.B (Nairobi)

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DECLARATION

This dissertation is original work and has not been presented for a degree in any other university.

Signed _____

Dr. Johnson Masika Wafula, M.B., Ch.B

This dissertation has been submitted for examination with our approval as university supervisors.

Signed _____

Prof. G.N. Lule

Associate Professor of Medicine,

Consultant Gastroenterologist,

Department of Medicine,

University of Nairobi.

Signed _____

Dr. C.F. Otieno,

M.B.,Ch.B, M.Med.

Lecturer, Department of Medicine,

University of Nairobi.

Signed _____

Prof. A. Nyong`o,

Associate Professor of Pathology,

Department of Human Pathology,

University of Nairobi.

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LIST OF ABBREVIATIONS

DM	-Diabetes mellitus
IDDM	-Non-insulin dependent diabetes mellitus
GIT	-Gastrointestinal tract
H. pylori	-*Helicobacter pylori*
KNH	-Kenyatta National Hospital
MALT	-Mucosal associated lympoid tissue
PMN	-Polymorphonuclear cells
Cag-A	-Cytotoxin associated protein
Vac-C	-Vacuolating associated cytotoxin
H2RA	-Histamine-2 receptor antagonists
PPI	-Proton pump inhibitors
NSAID	-Non steroidal antiinflammatory drugs
HIV	-Human immunodeficiency virus
BMI	-Body mass index
HbA1c%	-Percent glycosylated hemoglobin
ZN-stain	-Ziehl-Neelsen stain
ELISA	-Enzyme-linked immunosorbent assay
IgG	-Immunoglobulin-G
IgA	-Immunoglobulin-A
EDTA	-Ethylene diamine tetraacetate

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DEDICATION

To Nicole, Christina and Francois Kropf who have sacrificed so much for my well being.

ABSTRACT

OBJECTIVES

The aim of the study was to determine the prevalence of *Helicobacter pylori* and the associated upper gastrointestinal findings in diabetic outpatients with dyspepsia.

METHODS

This was a cross-sectional study in which diabetes mellitus outpatients aged 18 years and above were screened for dyspepsia. Demographic information (age, sex, residential area and employment status), and clinical information (type and duration of diabetes, weight, height, blood pressure, mode of glycemic control) was obtained from those who had dyspepsia and consented to an upper GIT endoscopy. HbA1c was determined and upper GIT endoscopy was performed. *H. pylori* was detected by the urease test and Cold ZN-stain while HE-stain was used for histological evaluation of the gastric biopsy specimens.

RESULTS

Of the 257 diabetics screened, 137 (53.3%) had dyspepsia. 71 of the dyspeptic diabetics underwent an upper GIT endoscopy, out of whom, 55 (77.5%) had *H. pylori* infection. The prevalence of *H. pylori* increased with HbA1c level but there was no statistically significant association with poor glycemic control (HbA1c >7.0%). Of the 71 patients, 48 (67.6%) had gastritis, 17 (25.7%) had duodenitis, 8 (11.3%) had oesophageal candidiasis, 7 (9.9%) had bile reflux and 6 (8.5%) had reflux oesophagitis. Six (8.4%) had ulcers (5 duonenal, 1 gastric) and 1 (1.4%) had gastric cancer. Endoscopically normal mucosa was found in 14 (19%) patients. The prevalence of *H. pylori* was 82.3% (32/38) and 82.4% (14/17) in patients with antral gastritis and duodenitis respectively. All ulcers and the cancer lesion (adenocarcinoma) were associated with *H. pylori*. Histological gastritis was found in 57 (81.8%) and was significantly associated with *H. pylori*.

CONCLUSION

Dyspeptic diabetics have upper GIT endoscopic findings and *H. pylori* prevalence which are comparable to those of non-diabetic dyspeptic historical controls.

LITERATURE REVIEW

INTRODUCTION

Gastrointestinal complications occur in up to 75 percent of diabetic outpatients **(1).** Most studies have implicated diabetic autonomic neuropathy as the major aetiology. However, abnormal release of gut peptides and other gastrointestinal regulatory substances have been implicated as well.

As a result of abnormal immunological responses, diabetic patients have increased susceptibility to infections. Abnormal motor function of the upper GIT in diabetes leading to reduced gastric emptying may further increase the risk of *H. pylori* infection **(2).** The role of *H. pylori* infection in causation of peptic ulcer disease and antral gastritis in non-diabetic patients is well established both internationally and locally **(3).** However, the contribution of *H. pylori* infection to the dyspepsia commonly seen in diabetes mellitus patients has not been established. The aim of this study was to determine the prevalence of *H. pylori* infection and the associated upper gastrointestinal findings in diabetic outpatients.

DIABETES MELLITUS AND DYSPEPSIA

Gastrointestinal complications are common in diabetes mellitus patients **(1, 4, 5).** In the upper GIT, these may manifest with symptoms of dyspepsia such as pain or discomfort in the upper abdomen, anorexia, nausea, vomiting, early satiety, postprandial fullness and flatulence.

In a study of 136 diabetic outpatients, Feldman and Schiller **(1)** found significant GIT manifestations in 75% of diabetic patients. They found abdominal pain in 34%, nausea and vomiting in 21%, dysphagia in 27%, and, diarrhea, faecal incontinence and constipation in 22%, 20%, and 60%, respectively.

In a similar study of 854 Sudanese diabetics, Awad et al **(6)** found 70% of the patients with significant GIT symptoms. In this study, 41% had nausea and vomiting, 28% had abdominal pain or discomfort, 6% had dysphagia, 3% had constipation and 7% had diarrhea. Other studies have shown that poor glycemic control is associated with increased GIT complications **(7)**. Severe GIT disorders in diabetes may lead to impaired metabolic control as may occur in persistent vomiting **(5)**.

Diabetic autonomic neuropathy is the main etiologic mechanism implicated. It is thought that oesophageal dysmotility arising as a result of autonomic motor dysfunction in diabetes may cause symptoms such as dysphagia and heartburn **(4).** Chronic nausea and vomiting may result from diabetic gastropathy, which is characterised by gastroparesis and antral hypomotility **(4, 8).**

An important observation however is that the symptoms tend to be intermittent such that abnormal GIT function tests may be demonstrable in many diabetics while only a fraction of these patients are symptomatic at any one time **(9).** Further, clinical improvement may also occur while objective measures of function still remain abnormal. Thus, there is lack of direct relationship between autonomic neuropathy, a slowly progressive

disorder, and the GIT symptom expression in diabetics. This implies that other factors play an important role in the exacerbation and remission of GIT manifestations in diabetics.

Other mechanisms postulated include abnormal release of gastrointestinal regulatory hormones and peptides, metabolic complications such as diabetic ketoacidosis and local GIT infections **(10, 11).** The role of *H. pylori*, a known gastric infection, has not been adequately investigated as a possible cause of dyspepsia in diabetes mellitus patients. In the general population, *H. pylori* is associated with 100% type B (antral) gastritis, and 95% of duodenal and 85% of gastric ulcers, which are major causes of dyspepsia **(12).**

These lesions, independent of diabetes, are associated with similar dyspeptic symptoms as seen in diabetes. Except for duodenal ulcers, which are relatively uncommon in diabetes mellitus, gastritis and gastric ulcers occur to almost the same extent as in non-diabetic patients with dyspepsia **(12, 13).** Other upper GIT mucosal lesions seen in diabetes at endoscopy include erosive oesophagitis, duodenitis, candida oesophagitis, atrophic gastritis, bile reflux gastritis and gastric malignancy as well as normal looking mucosa **(11, 13, 14).**

In Kenyan non-diabetic dyspeptic patients referred to KNH for endoscopy, Ogutu et al **(15)** found peptic ulcer disease (duodenal ulcer in 29.2% and gastric ulcer in 9.2%) as the commonest upper GIT endoscopic lesion occurring in 38.4% of the patients. Normal mucosa was the next commonest finding (34%). Other lesions found in this study included gastritis (31.7%),

duodenitis (17.5%), gastric cancer, oesophagitis and hiatus hernia. Lule et al found normal mucosa as the commonest lesion followed by peptic ulceration and then gastritis among other lesions **(3).**

Despite a high prevalence of GIT manifestations in diabetes, there is scarce and controversial data on the prevalence of *H. pylori* in diabetic patients. In 1993, Kojecky et al **(16)** found *H. pylori* in 61.1% of hospitalized type II diabetics with non-ulcer dyspepsia as compared to 56% in the non-diabetic controls.

In 1996, Persico et al reported a prevalence of 79% out of 29 Italian type II diabetics with non-ulcer dyspepsia **(17).** In 1998, de Luis et al found a significant association between the sero-prevalence of *H. pylori* and the duration of IDDM **(18).** In another study, Ma-lecki et al **(19)** found a low prevalence of *H. pylori* in 39 diabetics with symptoms of autonomic neuropathy (30%) as compared to the diabetic controls (68%). However, in 1998 Gentile et al **(2)** found a significantly high prevalence of *H. pylori* among 164 NIDDM patient (74%) as compared to controls (50%). In this study, they found no significant difference in the occurrence of peptic ulcers (21%) as compared to the controls (29.3%). From these studies, no firm conclusions can therefore be made on the prevalence of *H. pylori* in diabetes.

Diabetics as a group are at a considerable risk of bacterial infections **(20).** The increased susceptibility has been attributed to both ischaemic and neuropathic changes associated with diabetes. Earlier evidence arose from

studies which showed increased pharyngeal colonisation by gram negative bacilli in DM **(21, 22).**

Further evidence has been shown by the poor immunological defense against microbial invasion in DM due to poor mobilisation, chemotatic, adherence and phagocytic functions of PMN cells **(23)**. Modifiers of inflammatory response such as leukotrienes, prostaglandin-E and thromboxane B2 levels are also reduced in diabetes **(24)**. Likewise, functional abnormalities in the cell-mediated immune system may impair defense against mucosal infections.

Above abnormalities contribute to the poor mucosal immunity in diabetes mellitus. *H. pylori* is a virulent organism and is more likely to cause infection in states of reduced mucosal immunity **(25).** Reduced gastric emptying in diabetic neuropathy may further increase this risk **(2).** Since the immune response to *H. pylori* plays a significant role in the epithelial cell damage and thus determining the type of mucosal lesions **(25),** altered immune-competence in diabetes mellitus may modify the mucosal outcome.

From the foregoing, it is presumed that the prevalence of *H. pylori* may be high in diabetes. The prevalence of associated lesions may also be different from those found in the mostly immune dyspeptic non-diabetic patients.

As already alluded to, there is only scarce and controversial information on this subject internationally and none available locally. Western European studies of different subgroups of diabetic patients have reported a wide range of *H. pylori* prevalence (30%-88.2%) with some indicating clearly increased risk while others reporting low risk **(16-19, 26-28).**

HELICOBACTER PYLORI

Historical background

Spiral shaped organisms were observed in the stomachs of animals and man since the late 19th century but no pathogenetic link was made to gastroduodenal disease **(29-31).** This however changed after the pivotal 'discovery' by Warren and Marshall in 1983, who reported the presence of curved bacilli in the mucosa of patients with gastritis **(32).**

Subsequent investigation confirmed the presence of gram negative, micro aerophilic curved bacilli in 95% of patients with chronic active gastritis **(33).** The *campylobacter* like organisms described as motile, gram negative and curved rods that were oxidase, catalase and urease positive were first called *campylobacter pyloridis* and later as *campylobacter pylori* **(34).** In 1989 these organisms were placed in a new genus *Helicobacter* due to their unique morphologic, biochemical and genomic features **(35).**

Epidemiology

Approximately 30% of people in United States of America and Western Europe as opposed to 80% in the developing countries are infected with *H. pylori* **(36).** Locally, Lule et al **(3)** in 1991 found a prevalence of 57% in dyspeptic patients at KNH. In 1998, Ogutu et al **(15)** found a prevalence of 81.7% in 125 patients with dyspepsia. The prevalence increases with age, with most infections occurring during childhood **(37).** This effect has been observed in all other geographical situations and disease states including diabetes **(28)**. Locally, this observation has been demonstrated in a recent study by Ogutu et al (In press) who studied the seroprevalence of *H. pylori* in primary and secondary school going children in Nairobi. In this study, at

6-7 years age group, the seroprevalence was 42.5% and it increased with age such that it was 82.97% at 19-20 year age group.

Some of the risk factors associated include age, low social economic status, poor hygiene and over-crowding **(38).** Available evidence is supportive of faecal-oral mode of transmission **(39).** Oral to oral mode of transmission is suggested by the fact that spouses of patients with *H. pylori* infection and gastroenterologists are at an increased risk of infection as well **(40).**

Natural History and pathogenetic factors

H. pylori infection may cause transient gastritis or cause, over weeks to months, chronic superficial gastritis. It may then persist as chronic superficial gastritis over a period of years to decades, or lead to peptic ulcer disease. It may result in chronic atrophic gastritis which may then lead to gastric intestinal metaplasia and eventually gastric adenocarcinoma or B-cell lymphoma of the stomach **(41).**

Production of the urease enzyme by *H. pylori* confers to it the ability to survive the gastric acid by creating an alkaline microenvironment around itself **(42).** Other virulence factors include flageller motility **(43)** and production of the cytotoxins **(44),** cytokines **(45),** and enzymes such as proteases and lipases **(46).**

The outcome of *H. pylori* infection is determined by a complex interaction between bacterial, environmental and host modifying factors **(47).** Bacterial factors include *H. pylori* strain specific variations in their Cag-A gene and Vac-C gene expression, lipopolysaccharide structure and neutrophil activation. Environmental factors include low vitamin C, nitrates, early age

of *H. pylori* acquisition, and salt intake. Host factors include smoking, age, and host immunity.

Ultimately, mucosal changes giving rise to different outcomes occur as a result of two main mechanisms, direct bacterial cytotoxic effects and aggression by the mucosal immune inflammatory cells in response to *H. pylori* **(2, 48).** Due to abnormal immune function in diabetes, there may be a modification in the clinical outcome of *H. pylori* infection **(25).** In addition, presence of acid is necessary for the development of duodenal ulcers, in keeping with the new dictum 'no acid and no *H. pylori*, no ulcer'.

***Helicobacter pylori* and gastroduodenal disease**

H. pylori is strongly associated with type B (antral) gastritis **(12, 49, 50).** Inoculation by ingestion of *H. pylori* by two human subjects led to type B gastritis and then subsequently spread to the body **(51).** One of the two went on to develop chronic gastritis. Eradication of *H. pylori* is associated with resolution of gastritis **(51, 52).** Type A gastritis on the other hand is not associated with *H. pylori* **(53).**

Duodenal ulceration is strongly linked to *H. pylori* with many studies isolating *H. pylori* in 95 to 100% cases of duodenal ulcer patients **(12).** This may be due to increased basal and stimulated gastric output in *H. pylori* infected patients **(54, 55)** and direct spread of *H. pylori* infection into the duodenum which act together to induce duodenitis and eventual ulceration. *H. pylori* eradication leads to high cure rates and very low duodenal ulcer recurrence rates **(56 - 58).** Over 80% of gastric ulcers are associated with *H.*

pylori **(12, 59)** which on eradication leads to cure of the gastric ulcers **(60)**. Most of the remaining 20% of gastric ulcers are NSAID related.

Non ulcer dyspepsia especially when associated with antral gastritis has been linked to *H. pylori* infection in upto 70% of cases **(61, 62)**. Patients present with symptoms of peptic ulcer disease but no definite ulcer crater is demonstrable. Exclusion of pancreatic and gall bladder disease is necessary for diagnosis. Some clinical trials have reported improvement on *H. pylori* eradication while others have reported contradictory results.

Evidence through long term studies have shown progression from *H. pylori* associated gastritis to atrophic gastritis and eventually to intestinal metaplasia and dysplasia **(63)**. Earlier observations had taken note of the association between *H. pylori* and gastric carcinoma. Four of five gastric biopsies of gastric cancer patients were reported to have *H. pylori* by Marshall and associates **(64).** Studies have shown that *H. pylori* causes increased proliferation of gastric mucosal cells **(65)** and this process is significantly reduced when it is eradicated.

Potential mechanisms of carcinogenesis include its direct initiation of carcinogenesis, action as a co-carcinogen or indirectly by the immune inflammatory response to *H. pylori* infection. While normal gastric mucosa is free of inflammatory cells, *H. pylori* infection causes development of mucosal associated lymphoid tissue (MALT), a strong precursor of MALT B-cell lymphoma **(66, 67)**. Recently, *H. pylori* has been serologically linked to ischaemic heart disease **(68)**.

In a pioneering local study, Lule et al used culture method alone and isolated *H. pylori* in 87.5% of patients with antral gastritis and 57% with duodenal ulceration **(3)**. Subsequently, Ogutu et al while using rapid urease test and histological staining methods of *H. pylori* identification reported a prevalence of 100% and 84.6% in patients with peptic ulcers and gastritis respectively **(15)**. Maende **(69)** found a prevalence of 70.5% in sickle cell patients and 78% in dyspeptic controls. Karari et al in 1999 found a prevalence of 53.2% in patients with chronic renal failure **(70)**.

Diagnosis

H. pylori may be diagnosed invasively by endoscopic or non invasively by non-endoscopic methods **(71)**. Endoscopic methods involve, direct visualization of the upper GIT, thus allowing for diagnosis of macroscopic lesions such as peptic ulceration. Histology, urease test or culture is then performed on the biopsy tissues. **(72, 73)**

Histopathology is considered the gold standard for *H. pylori* diagnosis. Stains such as Cold Ziel-Neelsen (cold-ZN) described in appendix I or Giemsa stain are then used for *H. pylori* detection. Hematoxylin-Eosin (HE) stain (appendix II) may be used for histopathological diagnosis of the associated lesions.

Culture has high specificity approaching 100% but low sensitivity (50%-95%) thus giving a low predictive value if used alone and is time consuming as it takes upto 2 weeks for growth to occur. Culture may be achieved using a non-selective medium such as Chocolate agar, or a selective medium such as the Wilkins-Chalgren agar. *H. pylori* is identified on resultant colonies

histologically as above and by use of biochemical tests (urease, catalase and oxidase). Antibiotic sensitivity tests may then be performed. However, in-vitro determination of antibiotic sensitivity does not correlate well with clinical outcome of *H. pylori* therapy.

Urease test is based on the ability of *H. pylori* to produce the urease enzyme that breaks urea into carbon dioxide and ammonia. Gastric biopsy specimen is introduced into the test kit which contains urea and a pH indicator (phenol red) buffered at a pH of 6.0. Ammonia production causes an alkaline pH which results in colour change to magenta-red. One commercially available urease test kit is the CLO (Campylobacter Like Organism) test. A simpler, cheap and reliable alternative is the Hazell microtitre biopsy urease test **(74)** summarised in appendix III. Urease test is quick, sensitive (approximately 90%) and highly specific (approaching 100%). False negative test may occur in patients who have received PPI or high dose H2RA within 2 or 4 weeks respectively **(75)**

Non endoscopic methods of *H. pylori* diagnosis are indicated in situations where endoscopy is not indicated. They include urea breath test, ELISA and stool antigen testing. Urea breath test is a non-invasive, highly sensitive (84-100%) and specific test with a high predictive value **(76).** It is a measure of current *H. pylori* infection relying on *H. pylori* urease to hydrolyse urea labeled with radioactive carbon to produce isotopically labeled carbon dioxide in breath. It is however expensive due to the need to use mass spectrometry when Carbon C-13 is used or nuclear medicine when Carbon C-14 label is used. It may be used for confirmation of *H. pylori* eradication since a positive test indicates active infection.

ELISA is cheap, quick and non-invasive serological test based on the increase in serum levels of IgG and IgA antibodies to *H. pylori*. Cross-reaction with antigens of other enterobactericiae may occur thus reducing the specificity. One of the newer kits based on IgG has a sensitivity and specificity of 89% and 88% respectively. Serology is most useful during epidemiological studies of *H. pylori* prevalence. Since it takes months for the antibody titres to begin to decrease following successful treatment and may remain positive for years, blood antibody testing is not useful for monitoring post treatment *H. pylori* status **(77).**

Stool antigen testing is recently introduced enzyme immunoassay method of *H. pylori* detection in stool samples. It is rapid, highly sensitive (80-100%) and specific test **(78).** When positive, unlike serology, it denotes active *H. pylori* test. It can be used for confirmation of *H. pylori* eradication, in this case at least 4 weeks after eradication to avoid false positive result **(76).**

***Helicobacter pylori* eradication in treatment of dyspepsia**

The fact that dyspeptic patients with *H. pylori*–related peptic ulcer usually have a potentially curable disease means active search for such diseases should be sought and treated appropriately. This may also be true for some *H. pylori* associated non-ulcer dyspepsia, which occurs in upto 70% of non-ulcer dyspepsia **(62).** Since there is no symptom or symptom profile linked to specific gastric lesions or to *H. pylori* infection, upper GIT endoscopy is often necessary for evaluation of dyspepsia before appropriate therapy **(79).**

While this approach is expensive, the empiric therapy of dyspepsia with H2RA or PPI for 4-8 weeks, has been criticised for several reasons. It may promote prolonged use of inappropriate medications, weaken the value of

subsequent investigations, mask the symptoms of malignant ulcers and result, albeit rarely, in serious side effects **(80).**

The sequencing of the *H. pylori* genome has led into the possibility of developing a *H. pylori* vaccine **(81).** Future strategies may thus shift from treatment to vaccination against *H. pylori* infection in childhood as studies have shown that most infections occur in childhood. Such approach should target early childhood as shown by Ogutu et al (In press) in a recently concluded study showing a *H. pylori* seroprevalence as high as 42.5% in 6-7 year old school going children in Nairobi, Kenya.

JUSTIFICATION

Due to poor immunological function, diabetes mellitus patients are at increased risk of infections. *H. pylori* is a gastric pathogen which causes antral gastritis and eventual peptic ulceration which commonly causes dyspepsia in the general population. It is also associated with increased risk of gastric malignancy and has recently been serologically linked to ischaemic heart disease. Since a diagnosis of dyspepsia due to *H. pylori* associated duodenal and gastric ulcers and some *H. pylori* associated non-ulcer dyspepsia are potentially curable, these lesions should be actively sought. Despite this knowledge, the contribution of *H. pylori* associated lesions to the dyspepsia commonly seen in diabetics remains unknown. This study therefore sought to determine the prevalence of *H. pylori* in diabetic patients with dyspepsia, and the associated upper GIT mucosal findings. Further, this study may serve as a basis for other local studies of *H. pylori* in diabetes mellitus.

AIMS AND OBJECTIVES

Main Objective

To determine the prevalence of *H. pylori* and the associated upper GIT endoscopic findings in diabetic outpatients with dyspepsia.

Specific Objectives

1). To determine the prevalence of dyspepsia in diabetic outpatients.

2). To determine the upper GIT endoscopic findings in diabetic outpatients with dyspepsia.

3). To determine the prevalence of *H. pylori* in diabetic outpatients with dyspepsia

PATIENTS AND METHODS

Study design and Site

This was a Cross-sectional study carried out at KNH between June 2000 and March 2001. Recruitment, eligibility screening and upper gastrointestinal endoscopy were done at KNH. Histological and laboratory tests on the specimens were performed at Nairobi Hospital histology and Immunodiagnostic laboratories respectively.

Study population

Male and female patients aged 18 years and above previously diagnosed to have diabetes mellitus and attending the KNH diabetic outpatient clinic.

Ethical considerations

Approval was sought from the Department of Medicine of the Faculty of medicine of the University of Nairobi, and the KNH Ethical and Scientific Review Committee before commencement of the study. The investigator explained to every patient the nature and purpose of the study before consent was obtained (appendix IV). Patients were informed of their rights to withdraw from the study without prejudice to their future treatment.

Sample size

The minimum sample size required for this study was 64 endoscopically examined diabetic outpatients with dyspepsia as derived from the formula shown in appendix V.

Inclusion criteria

Inclusion criteria comprised of:

1) Male and female diabetes mellitus patients aged 18 years and above, who were attending the KNH diabetic outpatient clinic.
2) Those who were found to have dyspepsia according to the definition below.
3) Those who consented to undergo an upper GIT endoscopy.

Exclusion criteria

Patients were excluded if they had severe respiratory, renal, cardiac, liver, pancreatic or portal hypertensive disease; or if they had previous *H. pylori* eradication therapy, or treatment with PPI, H2RAs and bismuth compounds 4 weeks prior to interview, or antibiotics and NSAIDs 2 weeks prior to the interview. Those who were pregnant and those found to have jaundice or gallstones were also excluded.

Recruitment

Between June 2000 and March 2001, diabetes mellitus patients aged 18 years and above, were assigned random numbers before the commencement of the weekly diabetic clinic every friday morning. A table of random numbers was then used to select approximately a tenth of the patients attending the clinic on that particular day. This exercise continued until the target minimum sample size of 64 endoscopically examined diabetic dyspeptic patients was achieved. In total, 293 patients were randomly selected over the entire period; out of whom 36 were excluded. 12 were excluded due to history of use of H2RA or PPI within 4 weeks, 10 due to use of NSAIDS including low dose aspirin within 2 weeks and 7 due to use of antibiotics within 2 weeks of interview. Two were excluded due to previous *H. pylori* eradication therapy. Two patients with congestive cardiac failure and one with hepatitis were also excluded.

Screening for dyspepsia

257 patients who qualified for recruitment had a medical history taken to elicit symptoms of dyspepsia. Dyspepsia was defined as any combinations of the following symptoms:, upper abdominal pain or discomfort, anorexia, nausea, vomiting, early satiety, post-prandial fullness and flatulence (bloating); and such symptoms could be intermittent or persistent and should have been present for at least one month prior to the interview **(82).**

Informed consent (appendix IV) for an upper GIT endoscopy was then sought from patients with dyspepsia. Consenting patients were recruited into the study and allocated study numbers and details of their age, sex, weight, height, type of diabetes, duration since diagnosis, and the type of glycaemic

control were taken. History of other GIT symptoms, inter-current disease and use of medications was taken as well. Blood pressure and pulse rate was taken and a physical examination with emphasis on oral and abdominal findings was performed. The patients were then given appointment dates for the upper GIT endoscopy after fasting for at least 6 hours.

HbA1c

On the morning of the endoscopy, 2 milliliters of blood was collected in bottles with EDTA and send to the Immunodiagnostic laboratory where it was kept at a temperature of 2 to 8 degree centigrade. Total glycated haemoglobin and HbA1c were then determined within 7 days of collecting the blood samples using the Abbott IMx Glycated Haemoglobin test kit.

Endoscopy

Standard upper gastrointestinal endoscopy procedure **(83)** was performed at the KNH endoscopy room by the investigator in presence of two experienced endoscopists who confirmed all findings.

Inflammatory lesions such as gastritis, duodenitis, and oesophagitis were diagnosed if there was mucosal hyperemia, petichial hemorrhages or erosions or combinations of these lesions. Mucosal defects greater than 0.5 cm in diameter were diagnosed as gastric or duodenal ulcers. Bile reflux was diagnosed if bile was visualised in the oesophagus or stomach on initial entry prior to intubation of the duodenum. Esophageal candidiasis was diagnosed if there were white patches adherent on the mucosa.

Two biopsy specimens from the antrum, the incisura angularis and the body were taken for histological evaluation and *H. pylori* diagnosis. Biopsies of any lesions suggestive of malignancy were also taken for histopathological evaluation.

Histopathology and *H. pylori* detection

Biopsies for histology were preserved in formalin solution for at least 6hrs prior to processing and staining with Cold Ziehl-Neelsen staining method (appendix I) for *H. pylori* detection. Giemsa stain was used for quality control. Processed specimens stained with Hematoxylin-Eosin stain (appendix II) were used for histological evaluation. All the specimens were then examined by an experienced pathologist blinded to the urease test results.

Urease test was immediately performed after endoscopy on one of the biopsy specimens from each site following the procedure shown in appendix III. A positive test was indicated by a colour change from yellow to red. Negative test was recorded if there was no colour change at 24 hours.

H. pylori was declared positive if both histology and urease test were positive for *H. pylori* and negative if both or either test was negative.

STATISTICAL ANALYSIS

The data was collected using a standard questionnaire as shown in appendix VI. It was then coded, cleaned, verified and then entered and analyzed using the SPSS 10.0 software. The frequencies, percentages, means and standard deviations were calculated. The results were presented in tables and charts. Quantitative data was presented as percentages and where appropriate, associations determined. Kappa and Phi statistics were used to determine the levels of agreement. Chi-square test (x^2) was used to test the level of significance of association. Statistical significance was defined as p-value of less than 0.05.

RESULTS

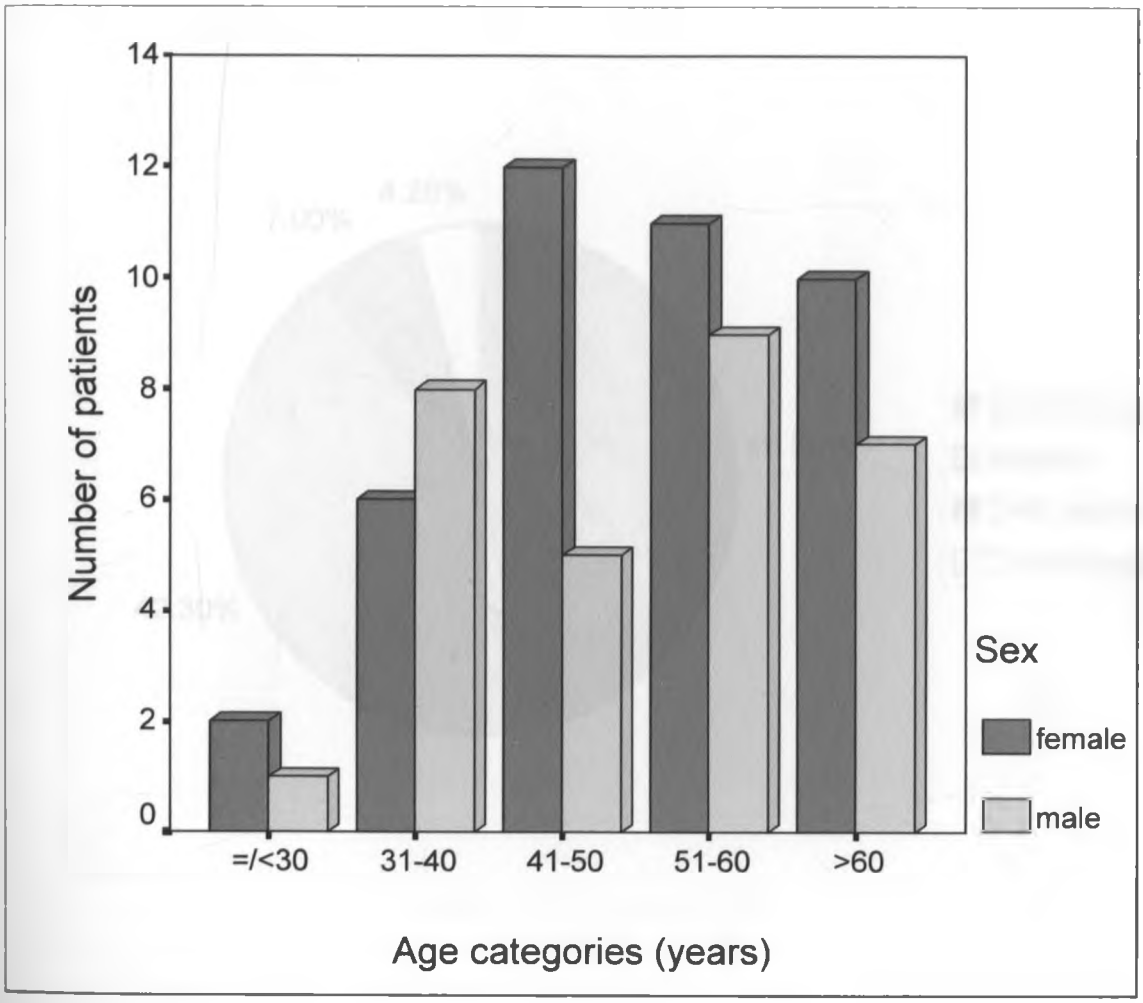
Between June 2000 and March 2001, 257 diabetic outpatients were screened, out of whom, 137 (53.3%) were found to have dyspepsia. Of the 137 dyspeptic diabetics, 103 consented to an upper GIT endoscopy but only 78 presented for endoscopy appointments. Three patients declined endoscopy at presentation while two did not undergo the procedure for having taken meals within 6 hours of the procedure. Upper GIT endoscopy was performed on 73 patients. Two patients were excluded from analysis when they were found to have esophageal varices at endoscopy. Thus, data of 71 patients was analysed.

Of the 71 patients, 30 (42.3%) were males and 41 (57.7%) females. 60 (84.5%) were type II while 11 (15.5%) were type I diabetics. The mean duration since diagnosis of diabetes mellitus was 7.31 years (SD +/- 7.18 years).

Thirty-three (46.5%) were known to have hypertension. Supine blood pressure above 140/90mmHg was recorded in another nine (12.3%) patients. Two (2.8%) patients were cigarette smokers and 11 (15.5%) took alcohol. The mean BMI was 25.92 kg/m^2 with 95% CI of 24.71-27.09kg/m^2. The mean BMI for females was significantly more than for males (27.34 vs. 23.91 kg/m2), (P=0.010).

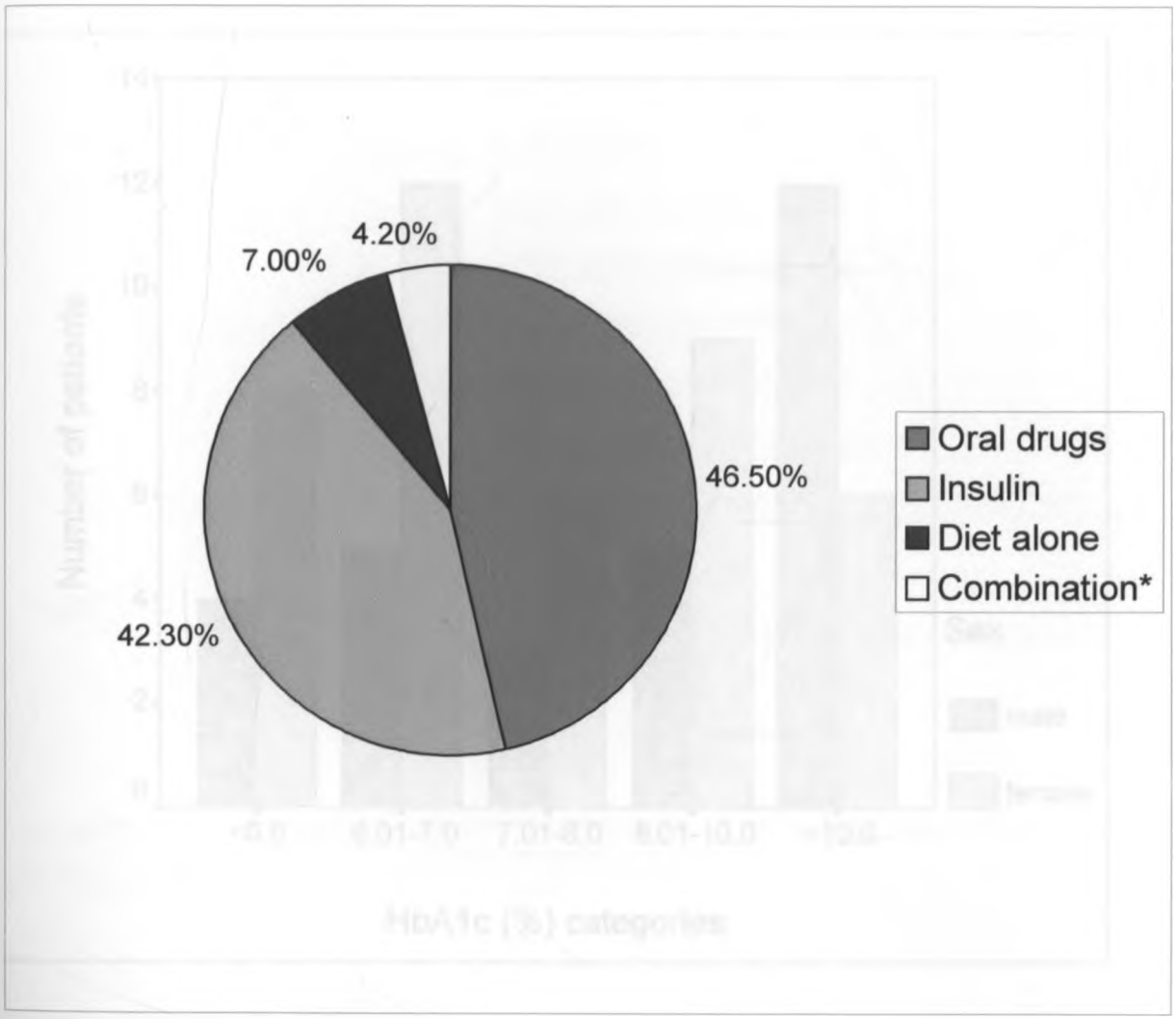
Twenty nine (40.8%) resided in the rural areas, 27 (36.6%) in urban high-density areas and 16 (22.5%) in the urban low density areas.

Figure 1: The age and sex distribution of the 71 diabetic outpatients with dyspepsia studied at KNH.



Most of the patients studied were aged over 30 years. The mean age was 53.13 years with a range of 23 to 102 years.

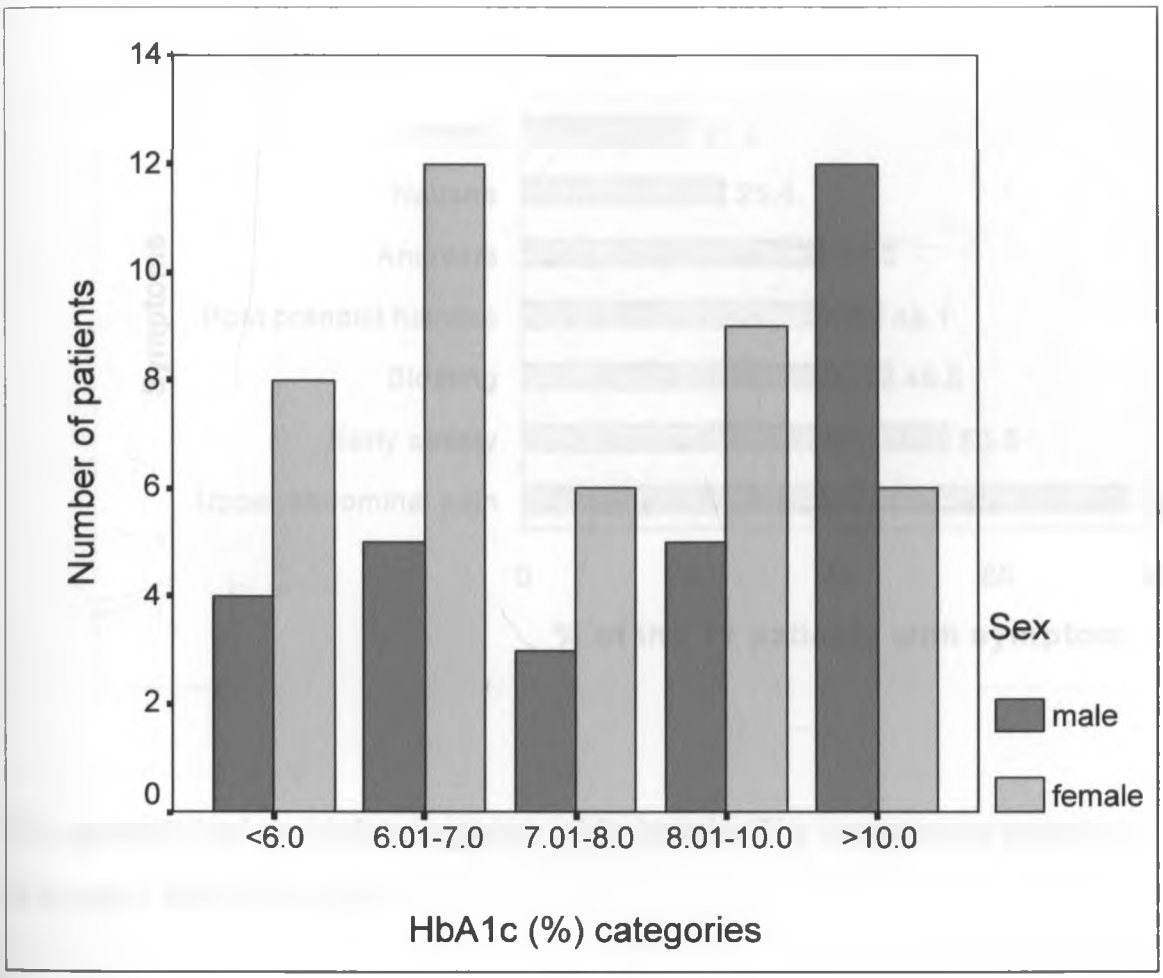
Chart 1: The modes of glycemic control of the 71 diabetic outpatients with dyspepsia studied at KNH.



Combination*= Insulin and oral drugs

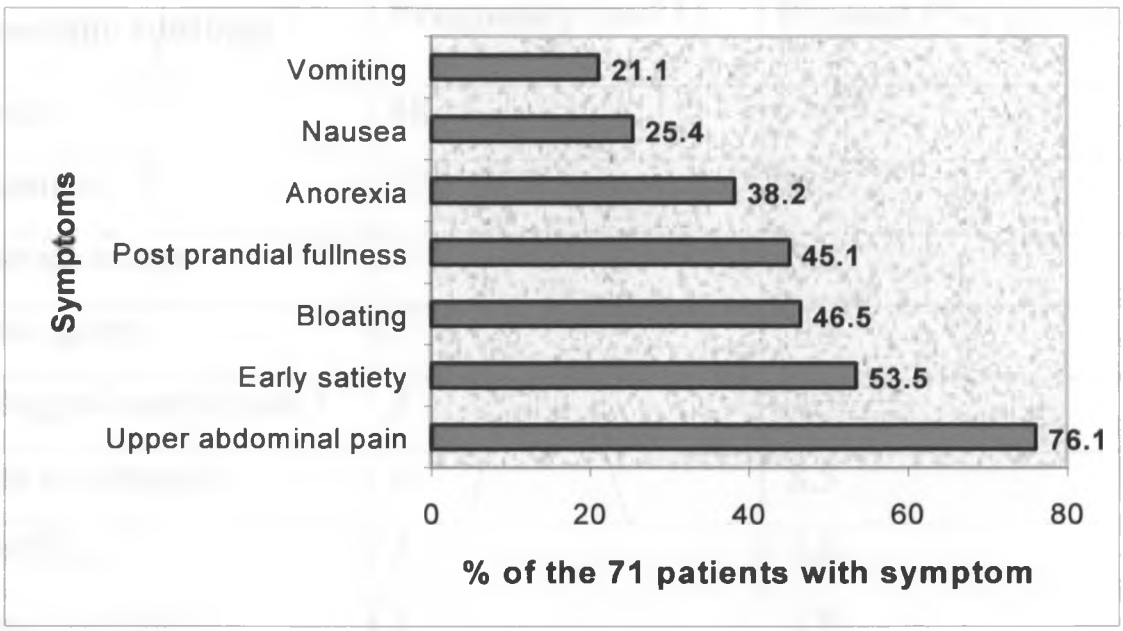
Most patients were controlled on oral agents and insulin. The mean HbA1c was 7.21% for dietary mode of control, 8.06% for oral agents, 8.57% for insulin and 7.90% for combination use of insulin and oral drugs together.

Figure 2: The level of glycemic control (HbA1c) according to the sex of the 71 diabetic outpatients with dyspepsia studied at KNH.



Most of the patients had poor glycemic control with 43 (58.6 %) having HbA1c of over 7.00 %. The males had a higher mean of HbA1c (8.81%) as compared to females (7.81%). This difference was however not statistically significant (P=0.131).

Figure 3: The prevalence of symptoms of dyspepsia in the 71 diabetic outpatients with dyspepsia studied at KNH.



The patients had multiple symptoms of dyspepsia. The commonest symptom was upper abdominal pain.

Table 1: Upper GIT endoscopic findings of the 71 diabetic outpatients with dyspepsia studied at KNH.

Endoscopic findings[π]	Frequency (n=71)	Percent (%) (n=71)
Gastritis	48	67.6
Duodenitis	17	25.7
Duodenal ulcers	5	5.7
Gastric ulcers	1	1.4
Esophageal candidiasis [μ]	8	11.3
Reflux esophagitis	6	8.5
Bile reflux	7	9.9
Gastric nodules [α]	2	2.8
Deformed antrum [γ]	2	2.8
Normal	14	19.7

n = Number of patients studied

π = Some patients had more than one endoscopic lesion. 14 of the 17 patients with duodenitis also had endoscopic finding of gastritis. 3 patients with esophageal candidiasis also had gastritis. Bile reflux occurred together with refux oesophagitis in 4 patients. There were multiple other combinations of findings occuring together.

μ = Only one had oral thrush but all had HbA1c above 7.00%.

α = All were benign at histology.

γ = At histology, one patient had gastric cancer (adenocarcinoma)

Antral gastritis was the commonest form of gastritis (38 out of 48) while predominantly fundal gastritis was present in 10 of the 48 patients with gastritis. None of the patients with history of alcohol intake and smoking had duodenal or gastric ulcers. However, 8 of the 11 patients with history of alcohol intake had histological gastritis.

Endoscopic diagnosis of gastritis correlated poorly with histological diagnosis of gastritis. (Kappa statistic= 0.285, P=0.015). The most common histological finding was gastritis which was found in 81.8% of the 71 studied patients. The commonest form of gastritis was chronic (61.6%), followed by active chronic gastritis (11.2%) and then atrophic gastritis (9.0%). Normal histology was found in 16.8%. Gastric malignancy (poorly differentiated adenocarcinoma) was present in one (1.4%) patient, which at endoscopy had appeared as deformed antrum in a 60 year old female.

Only one of the patients with oesophageal candidiasis had oral thrush. The candidiasis was significantly associated with poor glycemic control (HbA1c >8.00%, P=0.009).

The prevalence of *H. pylori*

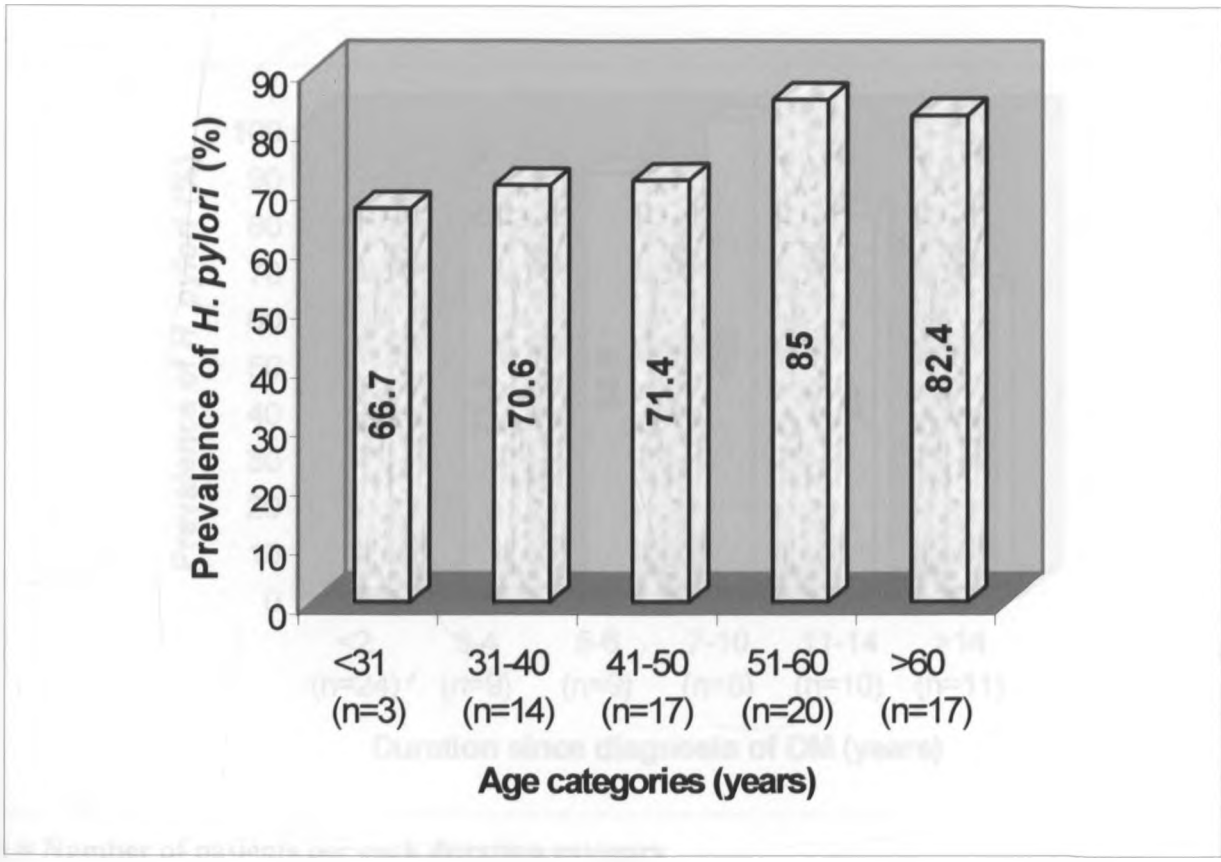
H. pylori was detected in 55 (77.5%) of the 71 patients by both urease test and histology. The biopsy urease test correlated well with the histological detection of *H. pylori* (Phi statistic =0.779, P= 0.000). Four patients with a positive urease test but with negative histology and one with positive histology alone were considered negative for *H. pylori.*

Table 2: Significance of associations between *H. pylori* prevalence and some demographic and clinical characteristics of the 71 diabetic outpatients with dyspepsia studied at KNH.

H. pylori Status		**Positive**	**Negative**	**P value**
SEX	**Male**	21	8	0.428
	Female	33	8	
Type of DM	**Type I**	9	2	0.707
	Type II	46	14	
Alcohol	**Yes**	9	2	0.707
	No	46	14	
Smoking	**Yes**	1	1	0.346
	No	54	15	
BMI (kg/m2)	**<25.0**	21	4	0.309
	>25.01	33	12	

H. pylori was not significantly associated with the sex, type of diabetes, BMI, cigarette smoking or alcohol intake.

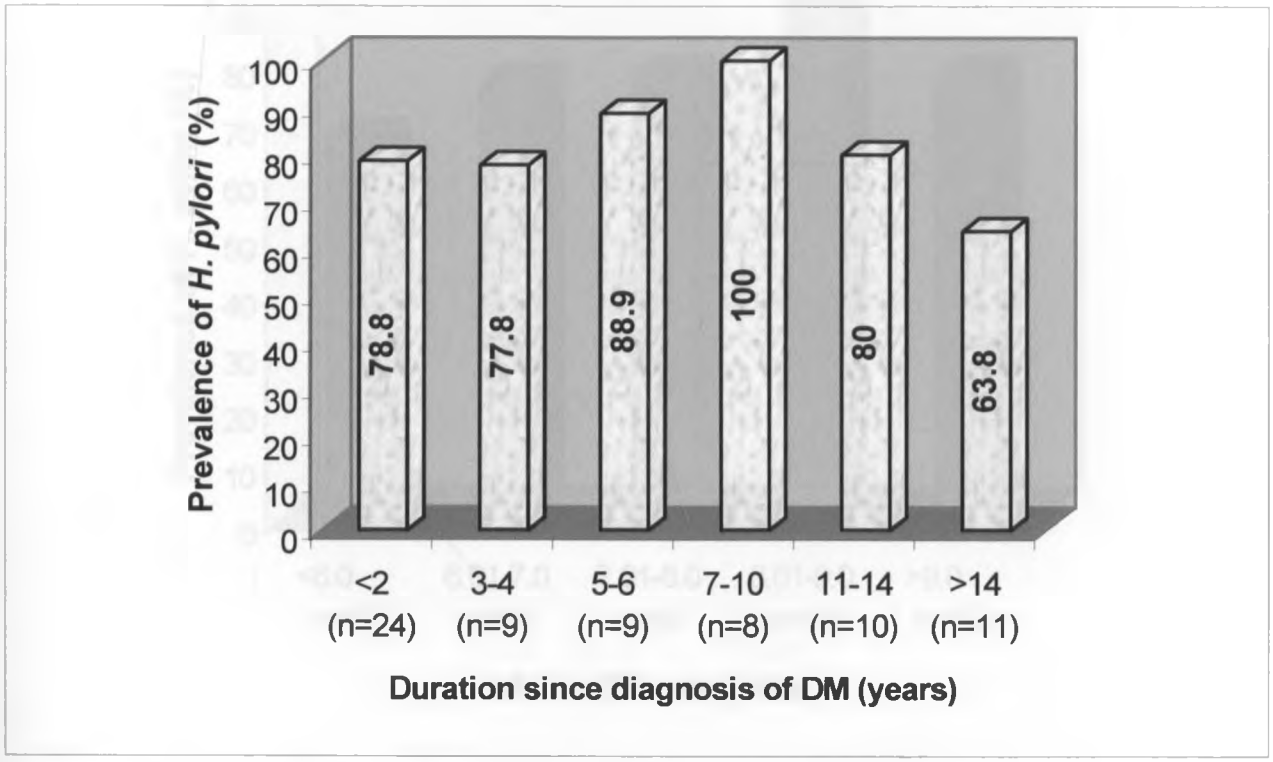
Figure 4 :The variation of *H. pylori* prevalence with age in the 71 diabetic outpatients with dyspepsia studied at KNH.



n=number of patients in each age category

The prevalence of *H. pylori* increased with age with a tendency to level off after the sixth decade.

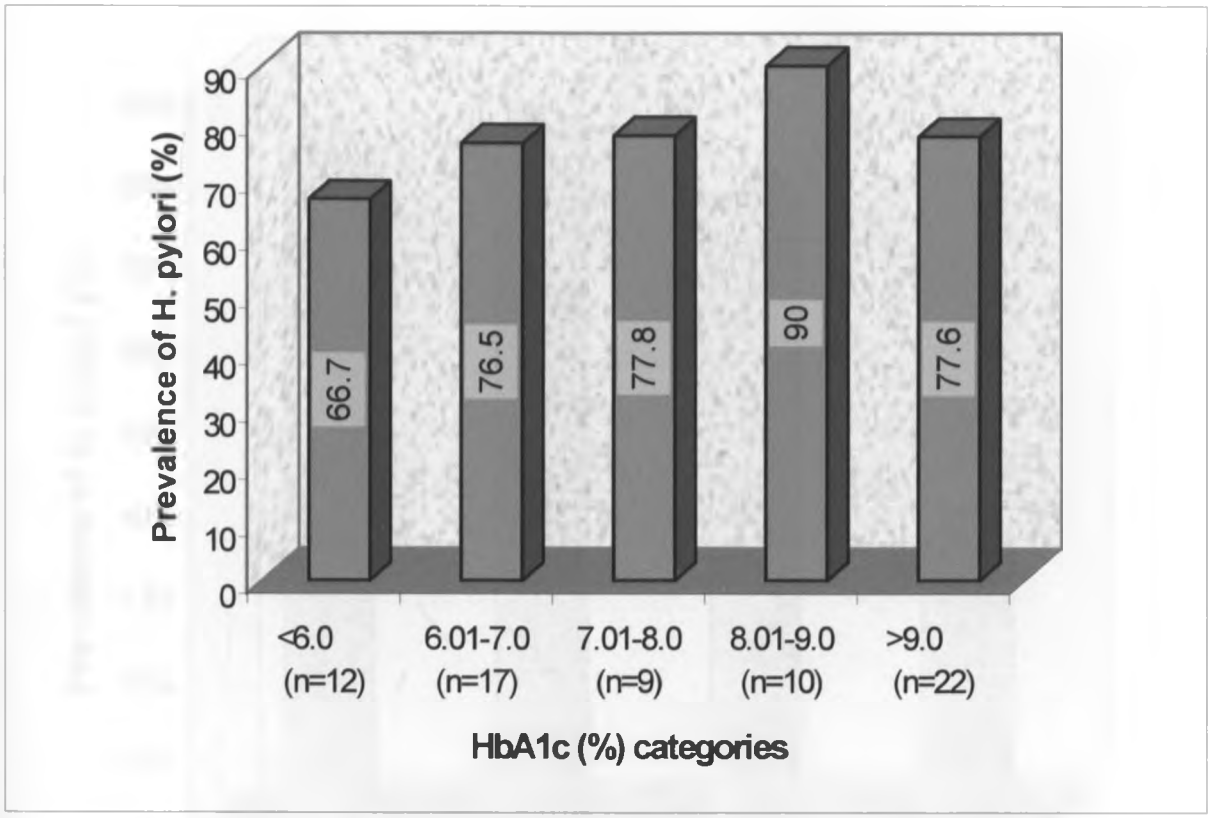
Figure 5: The variation of *H. pylori* prevalence with duration (since diagnosis) of DM of the 71 diabetic outpatients with dyspepsia studied at KNH.



n = Number of patients per each duration category

The prevalence of *H. pylori* increased with the duration of diabetes peaking at 6 to 10 years (since diagnosis) of diabetes.

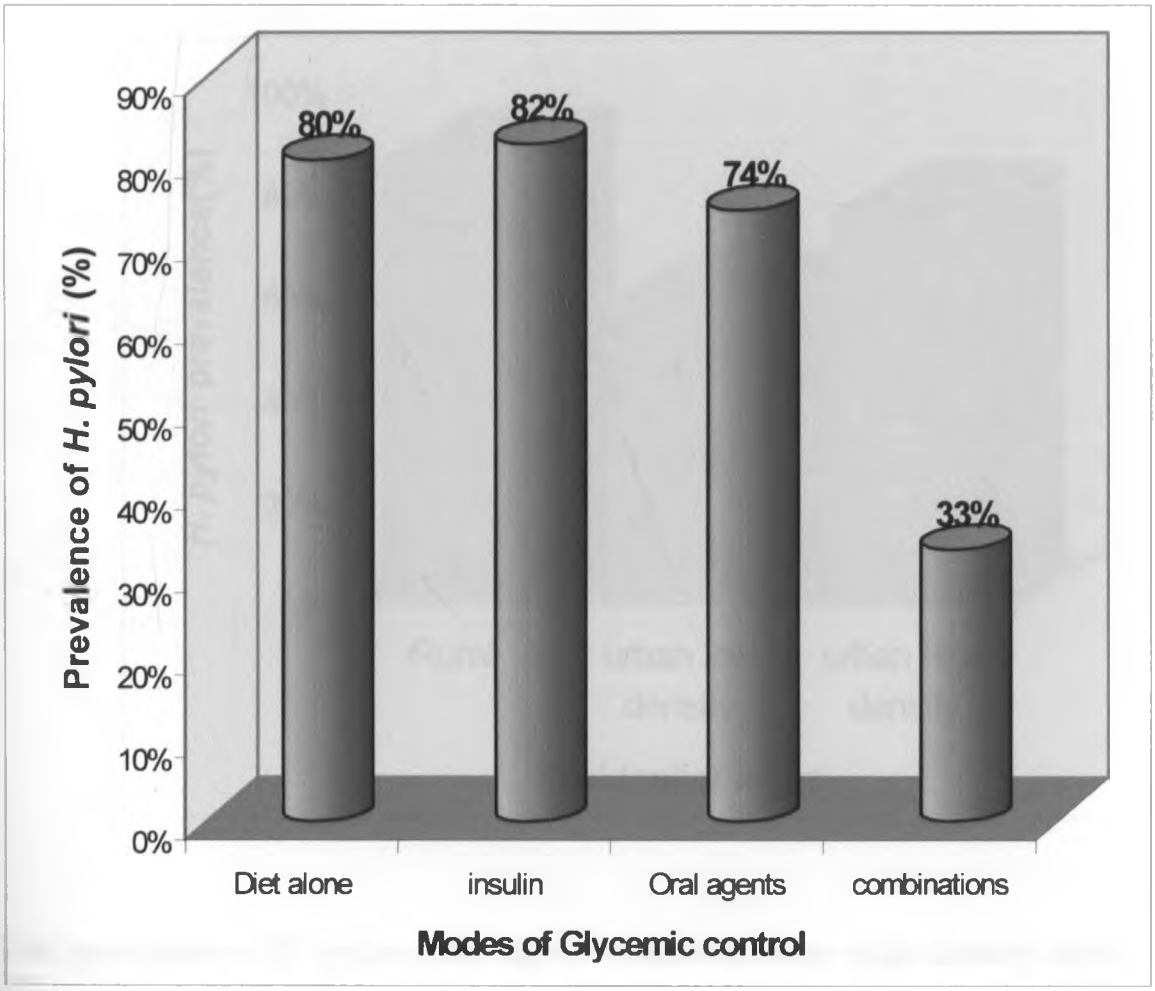
Figure 6: The variation of *H. pylori* prevalence with HbA1c of the 71 diabetic outpatients with dyspepsia studied at KNH.



n = Number of patients per each duration category

There was a general trend of increase in the prevalence of *H. pylori* with HbA1c. However, there was no statistically significant difference in the prevalence between patients who were well controlled (HbA1c <7.0%) and those who were poorly controlled (HbA1c >7.0%), (P= 0.428).

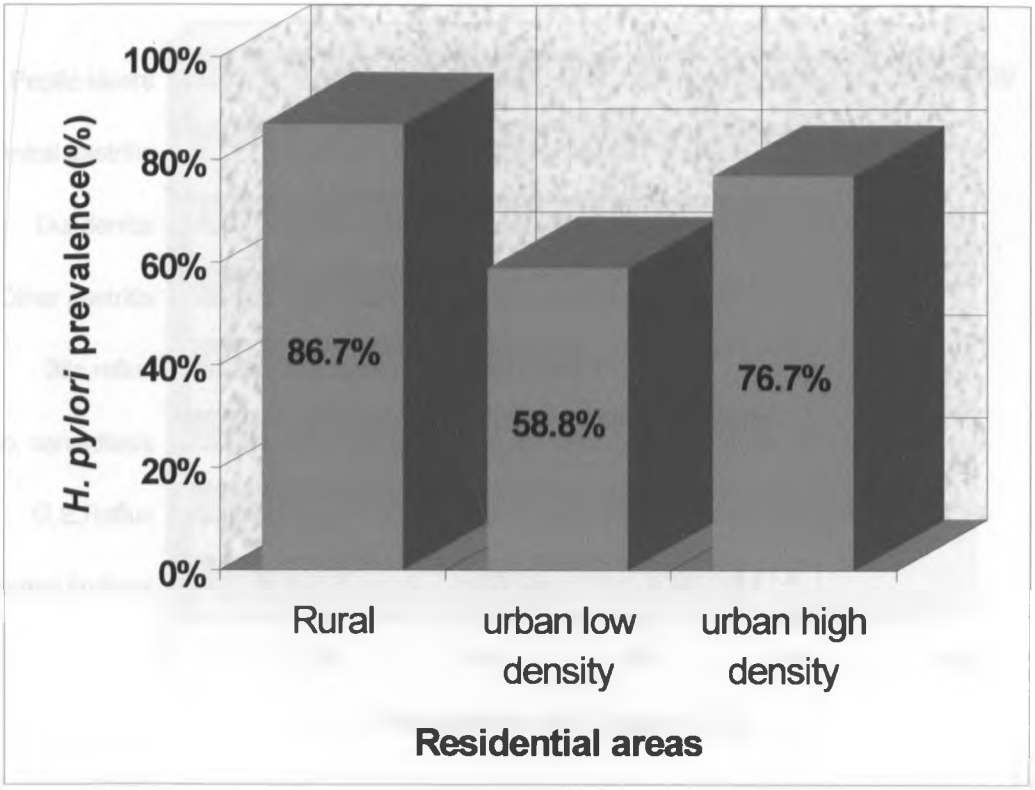
Figure 7: The prevalence of *H. pylori* within the modes of glycemic control of the 71 dyspeptic diabetic outpatients studied at KNH.



Combinations= Insulin and oral agents

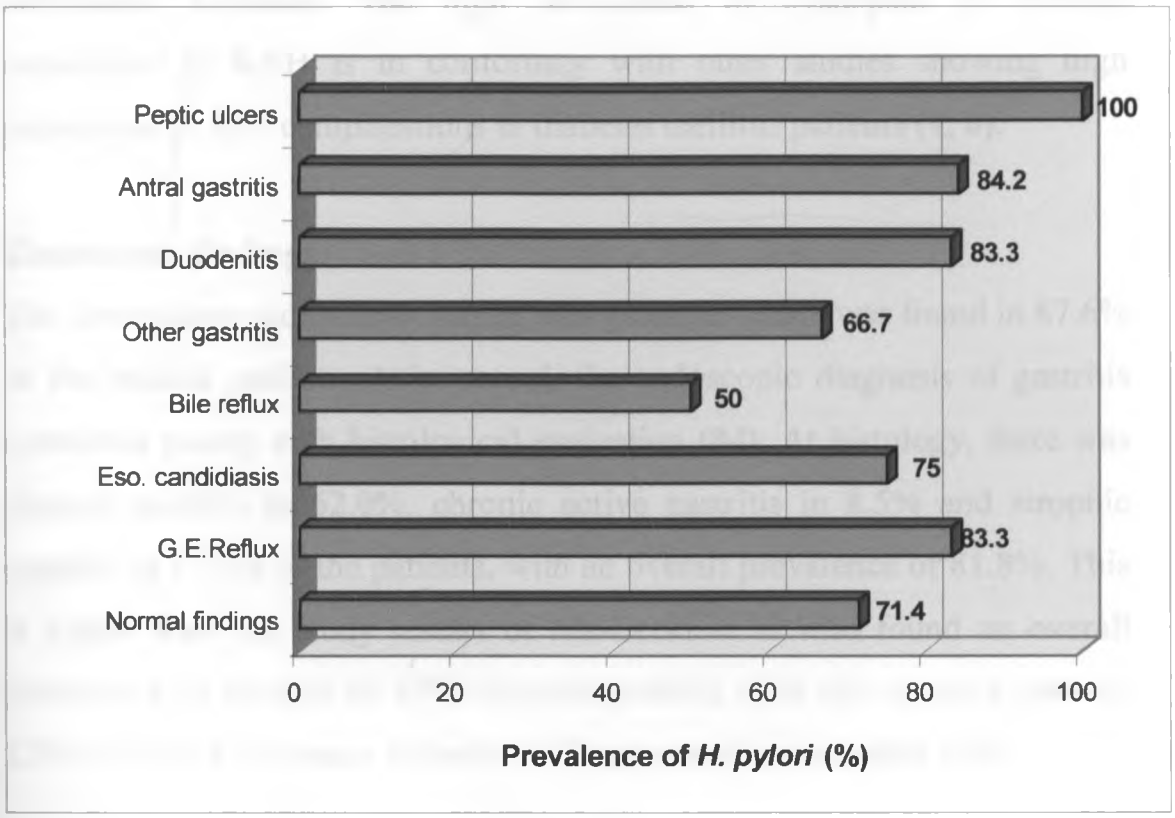
H. pylori prevalence did not differ significantly between the three main modes of glycemic control. The very small number of patients on combination mode of glycemic control could explain the low prevalence observed.

Figure 8: The *H. Pylori* prevalence within the residential areas of the 71 diabetic outpatients with dyspepsia studied at KNH.



The prevalence of H. pylori was high in rural and urban high-density areas than in the urban low-density areas. However, the association was not statistically significant, (P= 0.190), even after post hoc Bonferroni and Sidak tests of multiple variance.

Figure 9: The prevalence of *H. pylori* in association with endoscopic findings of the 71 diabetic outpatients with dyspepsia studied at KNH.



G.E. Reflux = Gastroesophageal reflux
Eso. Candidiasis = Esophageal candidiasis

H. pylori prevalence was high and was significantly associated with peptic ulcers (P= 0.021), histologically confirmed antral gastritis (P = 0.024), and duodenitis (P=0.050). In contrast, other endoscopic findings had lower prevalence of *H. pylori*.

DISCUSSION

Dyspepsia was found in 137 (53.3%) of the 257 randomly selected diabetic outpatients screened. The high prevalence of dyspepsia in diabetic outpatients at KNH is in conformity with other studies showing high prevalence of GIT complications in diabetes mellitus patients **(1, 6)**.

Endoscopic findings

The commonest endoscopic finding was gastritis, which was found in 67.6% of the studied patients. As expected, the endoscopic diagnosis of gastritis correlated poorly with histological evaluation **(84).** At histology, there was chronic gastritis in 62.0%, chronic active gastritis in 8.5% and atrophic gastritis in 11.3% of the patients, with an overall prevalence of 81.8%. This is higher than the study results of Ma-Lecki et al who found an overall prevalence of gastritis of 63% (chronic gastritis 41% and reactive gastritis 22%) of the 39 European diabetics with autonomic neuropathy **(19).**

This contrasts with most studies done in predominantly non-diabetic patients with dyspepsia. Lule et al **(3)** reported 16 out of 66 (24%) patients with gastritis while Ogutu et al **(15)** found a prevalence of 31.7%. In these two studies, the commonest finding was normal mucosa (39.4% and 34.2% respectively). It comes out strongly from this study that gastritis is a far more common finding in diabetes mellitus, with endoscopically normal mucosa being found in only 19%. However, this is higher than 11 % of chronic renal failure patients with normal mucosa reported by Karari et al **(70)**.

Peptic ulcer disease was only found in 8.2% as compared to other local studies that reported higher rates **(3, 15, 69,70).** The lower prevalence of ulcers observed in this study may have been influenced by three possible confounding factors. Previous researchers, for example Lule et al **(3)** and Ogutu et al **(15),** studied highly selected patients (dyspeptic patients referred for upper GIT endoscopy) and thus could have had more severity of dyspepsia. Peptic ulceration may be less symptomatic in diabetics due to associated autonomic neuropathy, and thus could have been missed out at the screening stage. Another possibility is `crowding out` of peptic ulcers as a cause of dyspepsia by the high prevalence of other causes of dyspepsia in diabetic subjects.

Future comparative studies between diabetics and non-diabetics with dyspepsia, and between dyspeptic diabetics and non-dyspeptic diabetics may help determine the contribution of these confounding factors. However, our results concur with previous studies that have shown very low peptic ulcer prevalence rates in diabetics **(11, 13, 16).** From these studies it can be said that dyspeptic diabetics have a lower prevalence of peptic ulcers. Thus, most dyspepsia seen in diabetes mellitus is non-ulcer dyspepsia associated with gastritis. This observation may be explained by the fact that mucosal damage is partly mediated by the hosts` immune response to *H. pylori* infection **(25),** and reduced immunity in diabetes mellitus may alter the mucosal outcome of *H. pylori* infection.

Oesophageal candidiasis was found in 11.3% of the studied patients and was significantly associated with poor glycemic control. The fact that only one of these patients had oral thrush means that oesophageal candidiasis should

always be considered as a contributing factor to dyspepsia especially if the patient has poor glycemic control with HbA1c above 8.00%. However, since HIV status was not assessed in this study due to lack of ethical justification, some cases of candidiasis may be attributable to HIV infection. Reflux oesophagitis and bile reflux found in this study may reflect the state of GIT dysmototility associated with diabetic autonomic neuropathy **(4)**.

Malignancy was found in one patient (1.4%) as compared to previous local studies that have found prevalence range of 2.7% to 9.1% in non-diabetics **(3, 15, 70)**. The different study populations and selection criteria of the studied patients may explain this wide range. The ratio of duodenal to gastric ulcer in this study was 5:1, which is within the range of 7.5:1 and 3:1 observed from previous local studies in non-diabetics **(3, 15, 70)**.

The prevalence of *H. pylori*

We found *H. pylori* prevalence in the diabetic dyspeptics to be 77.5% which is comparable to 81.7% found in non diabetic dyspeptic patients by Ogutu et al **(15)**. Maende **(69)** found a prevalence of 70.5% and 78% in adult sickle cell patients and dyspeptic controls respectively, which is also comparable to the findings in this study. This was above 60.6% found by Lule et al **(3)** who used only culture method for *H. pylori* identification. Gentile et al **(2)** found a prevalence of 74% in 29 Italian type II diabetics, which is comparable to our finding. Other western European studies looking at different subgroups of diabetics have found prevalence rates between 30% and 88% **(16, 17, 18, 19, 26, 36)**. This wide range may also be partly explained by the different selection criteria used by the different researchers and the different geographical settings of the studies.

The *H. pylori* prevalence increased with age, with a tendency to level off after the 6th decade. This compares with findings by Lule et al in a study of non-diabetics with dyspepsia **(3)** and Maende in a study of adults with sickle cell anaemia **(69)** who reported a peak in *H. pylori* prevalence with subsequent decline in later decades. On the contrary, most other studies have demonstrated a cohort effect whereby the prevalence of *H. pylori* increases with age regardless of the geographical location or type of patients including diabetes **(28, 70)**. A possible reason is the fact that most epidemiological studies are serologically based such that once infected, the persistence of antibodies gives positive tests regardless of the true *H. pylori* status **(77)**. Another possible reason is the fact that the older Kenyan age groups (above 60 years) experienced better socio-economic conditions and lived in sparse populations during their childhood and thus accounting for lower *H. pylori* infections and lower current prevalence.

The prevalence of *H. pylori* increased with duration of diabetes mellitus, with a peak at 6-10 years after diagnosis where a prevalence of 100% was found and then lower rates thereafter. A possible explanation for this observation comes from a study by Ma Lecki et al who reported a lower prevalence of *H. pylori* in patients with diabetic gastropathy (a feature of autonomic neuropathy) as compared to diabetic controls without dyspepsia **(19)**. We postulate that the initial increase in the *H. pylori* prevalence is due to low immunity in diabetes but later reduces as autonomic neuropathy sets in. This drop may be due to increased use of antibiotics as a result of increased rate of infections as autonomic neuropathy develops. However, the sample size of this study could not allow for controlling for the effect of age

and other factors and therefore a study large enough to allow statistical validation of this pattern is recommended.

The prevalence of *H. pylori* in the diabetics from rural (86.7%) and urban high-density (76.7%) areas was higher than those from the urban low-density (58.8%) areas. Though categorization by residential areas alone is not a good indicator of the socio-economic status, this pattern may reflect the influence of this factor on the prevalence of *H. pylori.*

No significant association was found between *H. pylori* and sex, current mode of glycemic control, and level of glycemic control. Although there was a tendency for the prevalence to increase with HbA1c, no statistically significant difference was found between the poorly controlled and the well-controlled diabetics. This is expected since a single HbA1c reading is only a measure of the glycemic control in the recent 3 months, and does not reflect the quality of control over the entire duration of diabetes. On the other hand, it is possible that there is no association between HbA1c level and *H. pylori* infection. Large subsequent studies may help validate this observation.

H. pylori was significantly associated with histologically confirmed diagnosis of gastritis (81.8%), P= 0.024. This is in conformity with both local studies in non diabetics and studies from the developed countries in diabetic dyspeptics **(2, 3, 15, 17).** This is a finding that is not unexpected as previous studies have consistently demonstrated *H. pylori* association with type B (antral) gastritis **(13, 49, 50, 51)**. In contrast to antral gastritis, body gastritis and pangastritis had lower prevalence of *H. pylori,* as expected **(53).**

All the diabetics with peptic ulcers in this study had *H. pylori* infection, which is similar to reports from studies in non-diabetics in which 100% of duodenal ulcers are found to be associated with *H. pylori* **(12, 15).** Similarly, *H. pylori* was identified in the gastric cancer lesion, not a surprise finding since *H. pylori* is an established carcinogen that is implicated in causation of gastric cancer **(63, 64, 65).**

From the foregoing discussion, it can be summarised that at KNH, dyspepsia occurs in upto 53% of diabetic outpatients with *H. pylori* infection occurring in upto 77.5% of these dyspeptic diabetics. The *H. pylori* prevalence lies within the range observed from studies of non-diabetic local historical controls. Further, histologically confirmed gastritis was found, as in non-diabetics, closely associated with *H. pylori* infection. However, there are two outstanding differences arising from this study, the relatively fewer ulcers and more oesophageal candidiasis found in dyspeptic diabetics.

CONCLUSION

Dyspeptic diabetics have upper GIT endoscopic findings and *H. pylori* prevalence which are comparable to those of non-diabetic dyspeptic historical controls.

LIMITATIONS

1). A comparative study with diabetic patients without dyspepsia or with non-diabetic matched controls would have provided more power to the study.

2). Diabetic autonomic dysfunction of the GIT, renal failure and causes of dyspepsia-like symptoms such as pancreatitis and cholelithiasis were not studied or excluded by laboratory investigations in this study.

3). HIV infection, a possible cause of oesophageal candidiasis, was not ruled out in this study.

RECOMMENDATIONS

1). Diabetic patients with dyspepsia should be treated no differently from non-diabetic dyspeptics.

2). *H. pylor*i prevalence studies comparing dyspeptic diabetics with non-dyspeptic diabetics, and also dyspeptic non-diabetics with dyspeptic diabetics are recommended.

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Appendix I

Cold Ziehl-Neelsen Stain

Procedure:

- dewax with xylene twice each time for 5 minutes
- hydrate sequentially using 100%, 90%, 80%, 70% alcohol, then with water each for 2 minutes
- stain in Carbol fuschin at room temperature for 1 minute
- rinse in water to clear slide of excess stain
- stain in Loefflers methylene blue at room temperature for 1 minute
- rinse in water
- dehydrate sequentially in 70%, 80%, 90%, then 100% alcohol each for 1 minute
- Clear in Xylene for 3 minutes twice
- Mount in synthetic mountant (DPX)

Results-Helicobacter	-blue
Background	-pale
Mucosa	-Lilac
Nuclei	-Magenta

Carbol Fuschin Preparation:

-Basic Fuschin	1gm
-Phenol crystals	5gm
-Isopropyl alcohol	10ml
-Water	100ml

Appendix II

Haematoxylin and Eosin staining method

Procedure

-Bring sections to water.

-Stain with haematoxylin for 10 minutes.

-Rinse in tap water.

-Differentiate with 1% acid-alcohol.

-Blue in Scotts tap water subsitute.

-Rinse in tap water.

-Stain with 0.5% eosin for 5 minutes.

-Rinse in tap water.

-Dehydrate, clear and mount.

Appendix III

Biopsy urease (The Hazell microtitre biopsy urease) test

Procedure

-Place 2 drops of the urease reagent into two wells of microtitre plate

-Place the gastric biopsy into one well, the other serves as a control

-Seal the well with a transparent plastic tape

-Record the name of the patient, date, site of biopsy and start time of test

-To improve sensitivity, incubate at 37°C to for the next 3 hrs, then at room temperature thereafter.

-A positive test is indicated by a colour change from yellow to red.

-Negative test is recorded if there is no colour change at 24 hours.

Reagent:	Urea	10g
	Phenol red (0.5%)	50ml
	$NaH_2PO_4.H_20$	0.218g
	Na_2HPO_4	0.51g
	NaN_3	100g

Appendix IV

INFORMED CONSENT

This is to confirm that I have agreed to participate in the research " Upper gastrointestinal endoscopic findings and *Helicobacter pylori* status in diabetic outpatients with dyspepsia at Kenyatta National Hospital."

As explained to me, this will involve undergoing an upper gastrointestinal endoscopy and biopsy. I still have the right to decline participation before or after commencement of the procedure, and in so doing my treatment will not be prejudiced. While the results remain a confidential property of the investigator, significant findings that may influence further management of my condition may be made available to me.

Patient's Name .....

Signed..... Patient / Guardian

..... Investigator

Appendix V

Sample size derived from the equation

$$n = \frac{p\,(100 - p)}{e^2}$$

n = minimum sample size

p = estimated prevalence

e = degree of precision equivalent to 5%

Assumption: p = 80 %, the estimated prevalence of *H. pylori* based on the prevalence of *H. pylori* of 81.7% obtained by Ogutu et al **(15)** in a study of dyspeptic patients at KNH.

Appendix VI

Questionaire

Study No.....

Age (years).....

Year of diagnosis of diabetes.....

Duration of diabetes (Years).....

Weight (kilograms):.....

Height (Metres).....

Sex	1: Male 2: Female	☐
Smoking status	1:Yes 2: No	☐
Alcohol intake status	1:Yes 2: No	☐
Education level	1: None 2: Primary 3: Secondary 4: College 5: University	☐
Employment status	1: Yes 2: No	☐
Residential area	1: Rural 2: Urban low density 3: High density (Slum areas)	☐
Type of Diabetics	1: Type I 2: Type II	☐

Current mode of glycemic control ☐

1: Diet alone
2: Oral Hypoglycemics
3: Insulin
4: Combination 2 and 3

Specify.....

Symptoms of dyspepsia	**Duration**	**1= Present, 2 =Absent**
Anorexia	----------	☐
Early satiety	----------	☐
Abdominal pain	----------	☐
Nausea	----------	☐
Vomiting	----------	☐
Postprandial fullness	----------	☐
Upper abdominal discomfort	------	☐
Flatulence (Bloating)	----------	☐

Other GIT symptoms

Heart burn	----------	☐
Weight loss	----------	☐
Constipation	----------	☐
Diarhoea	----------	☐
Others, Specify	----------	☐

Other symptoms

Numbness	---------	☐
Sweating	---------	☐
Diziness	---------	☐
Erectile dysfunction	---------	☐

Intercurrent illness **1=Yes, 2=No**

Hypertension	☐
Renal disease	☐
Cardiac disease	☐
Others,	☐

specify.....

Physical Examination findings

Blood Pressure (mmHg): Supine----------, Erect----------

Pulse Rate (/minute): Supine----------, Erect----------

Abnormalities noted on general examination.....

Oral mucosa examination findings .....

Abdominal examination .....

Laboratory test HbA1c (%).....

Endoscopy (macroscopic) findings

Esophagus.....

Stomach.....

Duodenum.....

Sphincters.....

Helicobacter pylori detection

Urease test	1: Positive 2: Negative	☐
Cold ZN (or Giemsa stain)	1: Positive 2: Negative	☐

Histopathology findings

Haematoxylin-Eosin stain: .....