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DECLARATION

I, **Dr Alick Bwanga**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature_____ Date _____

SUPERVISORS

This dissertation has been submitted for examination with my approval.

Signature_____ Date_____

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ABSTRACT

Gastroduodenal perforation contributes a significant proportion of surgical patients undergoing emergency laparotomy worldwide and is associated with high morbidity and mortality. It is well established that *Helicobacter pylori (H. pylori)* is the main cause of peptic ulcer disease. The prevalence of *H. pylori* infection in gastroduodenal perforation varies with geography, age, race, ethnicity and socioeconomic status. The correlation between *H. pylori* infection and gastroduodenal perforation is not well established. This study was set out to explore the role of *H. pylori* infection in adults presenting with spontaneous gastroduodenal perforations at the University Teaching Hospitals (UTH), Lusaka, Zambia.

This was a cross-sectional study carried out in patients aged 18 years or above who presented to the surgical department of UTH with gastroduodenal perforation between June 2018 and March 2019. A total of 60 patients who had an acute perforation in the stomach and/or duodenum identifiable at laparotomy were enrolled. The perforation site was biopsied with 3 millimetre margins and sent for *H. pylori* histopathological examination. Simple closure of the perforation, omentopexy and peritoneal lavage were done. Post-operatively, stool was obtained for a rapid stool antigen test for *H. pylori*. The sociodemographic characteristics alongside relevant past medical and drug history were obtained using the questionnaire. Data was analysed using STATA version 13.

Out of 60 patients, 48 were males, and 12 were females. The median age was 40 years (IQR, 31 – 52). Only 7 (12%) patients were positive for *H. pylori* and 53 (88%) patients were negative on histological examination of biopsies. There were 50 gastric perforations and 10 duodenal perforations. *H. pylori* infection had no significant statistical association with patient's demographic characteristics, site and size of the perforation. Histological examination with Warthin-Starry silver stain was found to have superior sensitivity of 65% to stool antigen test (55%) in the detection of *H. pylori* infection.

The prevalence of *H. pylori* infection in adults presenting with gastroduodenal perforation at UTH was found to be 12% and the histological examination was more sensitive than stool antigen test in the detection of the infection.

Keywords: Gastroduodenal perforation, *Helicobacter pylori*, Peptic Ulcer Disease

DEDICATION

To my loving wife Chifundo and my beautiful daughters Chengelo and Chabu

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

BP	Blood Pressure
ERES	Educational Records Evaluation Services
GDU	Gastroduodenal ulcer
IRB	Institutional Review Board
MALT	Mucosa Associated Lymphoid Tissue
NSAIDs	Nonsteroidal anti-inflammatory drugs
PCR	Polymerase Chain Reaction
PUD	Peptic ulcer disease
SD	Standard deviation
RVD	Retroviral disease
STATA	Statistics and data
UTH	University Teaching Hospitals

DEFINITIONS

Brier Score: a proper score function that measures the accuracy of probabilistic prediction.

Gastroduodenal perforation: a perforation in the stomach and/or duodenum.

Goodness of fit: the extent to which observed data matches the values expected by theory.

Helicobacter pylori: a Gram-negative, microaerophilic bacterium of the family Spirillaceae that causes gastritis and peptic ulcers and has been implicated in gastric carcinogenesis.

Omentopexy: a surgical procedure in which a part of the greater omentum is used to cover or fill a defect, augment arterial or portal venous drainage, absorb effusions, or increase lymphatic drainage.

Sanders resolution: the average across groups of such “expected” errors.

CHAPTER ONE: INTRODUCTION

1.1.1 Background

Gastroduodenal perforation contributes a significant proportion of surgical patients undergoing emergency laparotomy worldwide and is associated with high morbidity and mortality. In Europe, gastroduodenal perforation is one of the most deadly gastrointestinal complications accounting for approximately 23.5% of mortality (Lau *et al.*, 2011). At the University Teaching Hospital (UTH) in Lusaka, Zambia, gastroduodenal perforation is associated with mortality rate of almost 40% (Sondashi *et al.*, 2011). While *Helicobacter pylori (H. pylori)* is among the frequent causes of gastroduodenal ulcer disease, there is no formal data on the prevalence of *H. pylori* infection among patients with perforated stomach and duodenum at the UTH.

1.1.2 Helicobacter *pylori* infection in gastroduodenal ulcers

Epidemiological studies revealed a very strong association between *Helicobacter pylori* infection and duodenal and gastric ulcers. The ultimate proof of *H. pylori* as the main cause of ulcer disease was the permanent cure of peptic ulcers by eradication of the infection (Rangaswamy & Rubby, 2016; Malfertheiner *et al.*, 2000; Rauws & Tytgat, 1990). More than 50% of the world's population have a chronic *H. pylori* infection of the gastric mucosa, in spite of that only 5–10% of those infected develop ulcers. Factors determining whether the infection will produce disease are the pattern of histological gastritis induced; changes in homeostasis of gastric hormones and acid secretion; gastric metaplasia in the duodenum; interaction of *H. pylori* with the mucosal barrier and immunopathogenesis; ulcerogenic strains; and genetic

factors (Malfertheiner *et al.*, 2009). *H. pylori* colonises the entire gastric epithelium, from the prepyloric antrum to the cardia. Clinical outcomes are dependent on the pattern of chronic mucosal inflammation induced (Sipponen, 1992).

Prevalence of *H. pylori* varies with geography, age, race, ethnicity, socioeconomic status and is seen to decrease with improved hygiene (Dogra *et al.*, 2014). In developing countries, *H. pylori* infection is usually acquired during childhood, with infection rates ranging from 13.4 - 24% (Brown, 2000; Okuda et al., 2019).

1.1.3 Magnitude of the problem

Perforated peptic ulcer is one of the most common surgical emergencies worldwide with associated mortality up to 30-50% (Møller *et al.*, 2011; Sondashi *et al.,*2011). As many as 60% to 90% (gastric and duodenal) of ulcers are associated with *H. pylori* (John *et al.*, 2017).

Global prevalence of peptic ulcer disease has decreased in recent decades, but this has not been followed by a similar reduction in complications from peptic ulcers (Lassen *et al.*, 2006). Complications are encountered in 10% to 20% of these patients and 2% to 14% of the ulcers will perforate (Bertleff & Lange, 2010; Svanes, 2000).

Gastroduodenal perforation is a potentially fatal surgical emergency that remains a formidable health burden worldwide (Svanes, 2000). Furthermore, it represents the leading cause of gastrointestinal tract perforations (Behrman, 2005).

1.1.4 Detection of *H. pylori* infection

Several diagnostic methods can be employed for the detection of *H. pylori* such as non-invasive serological tests which measures specific anti *H. pylori* immunoglobulins IgG and/or IgA and invasive tests such as bacterial culture, histopathological examination of biopsy specimen with different stains and assays for urease activity (Goossens *et al.*, 1992; Wang et al., 2015).

1.1.5 Local Perspective

In 1991, Bem indicated that the incidence of gastroduodenal perforation at UTH was 16%. About 20 years later, Sondashi *et al* noted a rise to 29.7% in the rate of perforation of gastroduodenal ulcers. Sondashi *et al* also found that the gastric perforations (82.8%) were more common than duodenal ones, and cigarette smoking and alcohol were the top associated risk factors for perforated peptic ulcer (Sondashi *et al.*, 2011). However, the prevalence of *H. pylori* infection in these patients was not studied.

As far as literature was searched, there are no formal data on the prevalence of *H. pylori* infection in adults presenting with spontaneous gastroduodenal perforation at UTH. This study aimed to find an association between *H. pylori* infection and gastroduodenal perforation at UTH.

1.1.6 Overall purpose of the study

This study was mainly intended to explore the role of *H. pylori* infection in spontaneous gastroduodenal perforation cases by gastric or duodenal biopsy histopathological examination and rapid antigen stool test. Along with this, other factors like type and location of the perforation, and patient sociodemographic characteristics were studied. This would help the surgeon in deciding whether *H. pylori* eradication therapy should be offered to all patients with gastroduodenal perforation or it should be applied selectively.

1.2 Statement of the problem

A large number of patients are admitted to the surgical department due to acute peritonitis. Upon emergency laparotomy, a significant number are found to be due to gastroduodenal perforation. These perforations are associated with high morbidity and mortality at UTH.

In a period of 12 months (April 2014 to April 2015), 50 out of 189 (26.4%) of all emergency laparotomies for generalised peritonitis were found to have perforated gastroduodenal ulcers at UTH (retrieved from Emergency Theatre Record Books). Sondashi *et al* found about 30% incidence and almost 40% mortality associated with perforated peptic ulcers among operated cases for peritonitis (Sondashi *et al.*, 2011). However, the prevalence of *H. pylori* infection in the patients with gastroduodenal perforation at UTH has not been studied.

Gastroduodenal perforation contributes a significant proportion of surgical patients undergoing emergency laparotomy and is associated with high morbidity and mortality at UTH. There are

no formal data on the prevalence and role of *H. pylori* infection among patients with perforated gastroduodenal ulcers at UTH.

1.3 Study justification

Emergency operations for gastroduodenal perforation result in a mortality of 6–30% (Blomgren, 1997; Hermansson *et al.*, 1997; Svanes *et al.*, 1994; Wakayama *et al.*, 1994). Perforation accounts for more than 70% of deaths associated with peptic ulcer disease, mortality rates for perforated duodenal and gastric ulcers being 0–10% and 10–40%, respectively (Branicki, 1996).

Although the factors associated with perforated gastroduodenal ulcers (mainly smoking, alcohol and NSAIDs use) have been definitively established at UTH, the role of *H. pylori* infection in gastroduodenal perforation is not known (Sondashi *et al.*, 2011). Therefore, this study aimed to address this knowledge gap.

CHAPTER TWO: LITERATURE REVIEW

2.1.1 Prevalence of *H. pylori* infection in perforated gastroduodenal ulcers

A study in Madrid showed a mean prevalence of *H. pylori* infection in patients with perforated peptic ulcer to be about 60%, which contrasted with the 90-100% figure usually reported in non-complicated ulcer disease (Gisbert *et al.*, 2004). In India a comparable prevalence rate of 61% for *H. pylori* infection was noted in patients with perforated peptic ulcer (Sharma et al., 2015). A recent study in India found the prevalence rate of *H. pylori* infection in patients with perforated peptic ulcer to be approximately 61% (Rehmani & Pathak, 2018). In a series in Hong Kong, Ng *et al* found 70% prevalence of *H. pylori* infection in patients with duodenal perforation (Ng *et al.*, 1996). Mihmanli obtained similar results in which the prevalence of *H. pylori* infection in 16 patients operated for perforated duodenal ulcer was 88.8% (Mihmanli *et al.*, 1998). *H. pylori* infection in peptic ulcer perforation, in a study by Dogra, was 92% (Dogra *et al.*, 2014). On the contrary, Chowdhary studied a series of 45 patients: 15 had a perforated duodenal ulcer and none had evidence of *H. pylori* infection (Chowdhary *et al.*, 1998). In another study by Reinbach it was concluded that there was no clear association between *H. pylori* infection and duodenal ulcer perforation (Reinbach *et al.*, 1993).

Africa has the highest pooled prevalence of *H. pylori* infection (70.1%; 95% CI, 62.6 - 77.7), whereas Oceania has the lowest prevalence (24.4%; 95% CI, 18.5 - 30.4). Among individual countries, the prevalence of *H. pylori* infection varies from as low as 18.9% in Switzerland (95% CI, 13.1 - 24.7) to 87.7% in Nigeria (95% CI, 83.1 - 92.2). Based on regional prevalence estimates, there were approximately 4.4 billion individuals with *H. pylori* infection worldwide in 2015 (Hooi *et al.*, 2017).

2.1.2 Relationship between sociodemographic characteristics and *H. pylori* infection in gastroduodenal perforation

H. pylori is very common in the developing world. High seropositivity rates are related to overcrowded and unsanitary living conditions, and poor hygiene and domestic services are associated with an enhanced risk of infection (Mendall *et al.*, 1992; Smith et al., 2018). Dakubo *et al* studied gastroduodenal perforation at the University of Ghana and reported that the mean age was 40.9 years, with a male to female ratio of 4.5:1 (Dakubo *et al.*, 2009).

Bin-Taleb found an overall mean age of 39.08 years, with a male to female ratio of 8:1 (Bin-Taleb, *et al.*, 2008). This is comparable with the studies reported later by Dogra (Dogra *et al.*, 2014). In another study, the general incidence of perforation of peptic ulcer did not show changes; however, the percentage of women with perforated duodenal ulcer markedly increased. Patients with perforated gastric ulcer (regardless of gender) and females suffering from perforated duodenal ulcer were, on the average, about 10 years older than males with perforated duodenal ulcers. The mean age of male and female patients with perforated duodenal ulcer over the last 45 years showed an insignificant upward trend (Wysocki *et al.*, 2011). At UTH, the mean age for peptic ulcer perforation was found to be 39.9 years with a male to female ratio at 5.9:1 (Sondashi *et al.*, 2011).

2.1.3 Complications of gastroduodenal ulcers

Mortality increases with age and comorbidities and is higher in patients without previous history of PUD (Fashner & Gitu, 2015; Lau *et al.*, 2011). Kuremu found that perforations were the commonest complications at 56.6% followed by gastric outlet obstruction at 34.0%

(Kuremu, 2002). In Tanzania, Ngerageza found that perforation (51.0%) was the commonest complication followed by bleeding (16.0%) (Ngerageza, 2011). Sondashi *et al* noted that in 2009, 29.7% of emergency surgery accounted for benign perforated peptic ulcer in all patients operated for generalised peritontis (Sondashi *et al.*, 2011).

2.1.4 Ratio of gastric to duodenal perforations

In the study by Dakubo *et al*, it was noted that there were 88% duodenal, 7.1% prepyloric, and 4.9% type 1 gastric ulcer perforations (Dakubo *et al.*, 2009).

Bin-Taleb reported that the perforated duodenal ulcer and perforated gastric ulcer ratio was 4.38:1 (Bin-Taleb *et al.*, 2008). Out of 50 cases studied, 42 patients (84%) were found to be having duodenal ulcer perforation and only eight patients (16%) had gastric ulcer perforation. Duodenal perforation was found on the anterior wall of the first part of duodenum in all the cases. Of the cases having involvement of stomach, five had perforation over the anterior wall in a prepyloric region, two had posterior wall perforation, and one patient had perforation over the lesser curvature (Dogra et al., 2014). In Zambia, Tuakli observed that out of the 60 patients who presented with upper gastrointestinal bleeding, 55% had chronic doudenal ulcers and only 1.7% had gastric ulcers (Tuakli & Wosornu, 1977). Furthermore, Kelly *et al* found duodenal ulcer (17%) and gastric ulcer (12%) in a review of endoscopy and pathology reports (Kelly *et al.*, 2008). Paradoxically, Sondashi noted that gastric perforations (82.8%) are commoner than duodenal perforations (14.3%) (Sondashi *et al.*, 2011). Similarly, a study in Tanzania by Ngerageza showed the ratio of gastric to duodenal perforations to be 2.5:1 (Ngerageza, 2011). Additionallly, Ohene-Yeboah reported the ratio of gastric to duodenal perforations at 2.8:1 (Ohene-Yeboah & Togbe, 2006).

2.1.5 Detection of *H. pylori* infection in gastroduodenal perforation

H. pylori infection can be diagnosed by mucosal biopsy, but noninvasive tests offer an effective screening tool and do not require an endoscopic procedure. It is now accepted that histologically confirmed chronic gastritis and duodenal ulceration is caused by *H. pylori* infection in over 90% of cases and *H. pylori* infection is responsible for 50% of gastric ulcer (Warren & Marshall, 1983). A study in Netherland showed that Giemsa stain has high validity for detecting *H. pylori*. Lofelld *et al* conducted a study on 302 patients with perforated peptic ulcers and found 78% accuracy in detecting *H. pylori* by Giemsa stain from biopsy sample (Loffeld *et al.*, 1991). In a study in London, *H. pylori* was identified in 14 sectioned stained with haematoxylin and eosin (10/16 biopsy specimen with chronic gastritis, 4/7 biopsy specimen with MALT lymphoma, 0/5 sections gastrectomy specimens). *H. pylori* could be detected at a still greater frequency in Warthin-Starry stained sections; 23 were positive (14/26 biopsy specimen with chronic gastritis, 6/7 with MALT lymphoma, and 3/5 gastrectomy sections). In all cases the bacteria were more prominent and easier to detect in the immunostained than in sections stained tinctorially (Ashton-Key *et al.*, 1996). In a study by Rehmani and Pathak, rapid urease test for *H. pylori* detection had more positive result compared to histological examination (Rehmani & Pathak, 2018). Similarly, Kumar *et al* evaluated 86 patients with perforated peptic ulcer disease and 43 (50%) were found positive on rapid urease test and 29 (33.72%) patients on histology (Kumar & Sinha, 2002).

Despite high histology sensitivity, the site, number, and size of biopsy specimen affect diagnostic accuracy. Although a single biopsy taken from the body and/or antrum of the stomach, in untreated *H. pylori* positive patients, can detect *H. pylori* presence in more than 90% of cases, diagnostic accuracy can be increased with multiple biopsies from the greater curvature and the body (Lee & Kim, 2015).

When atrophic changes occur in the gastric mucosa, a high percentage of endoscopic biopsy samples become negative for a bacterial histology (Kokkola *et al.*, 2000). Moreover, in metaplastic areas, *H. pylori* is undetectable by either conventional or special staining techniques in the majority of cases, despite serologic evidence of infection (Loffeld et al., 1991). Atrophic gastritis (AG) and intestinal metaplasia(IM) are considered premalignant lesions of gastric cancer. Thus, the appropriate biopsy site for detecting *H. pylori* infection in gastric cancer patients is similar to that of AG or IM patients. Kim *et al* reported that the antrum showed 55% sensitivity and the body on lesser curvature side showed 80% sensitivity in detecting *H. pylori*, whereas the body on greater curvature side showed 95% sensitivity (Kim et al., 2009). Enomoto *et al* also reported that the *H. pylori* detection rate varied from 30% at the antrum lesser curvature to 100% at the body greater curvature in surgically resected gastric specimens (Enomoto *et al.*, 1998). Their results suggest that the adequate biopsy site for detecting *H. pylori* in gastric cancer patients is the body, especially the body greater curvature side.

Non-invasive testing for *H. pylori* can be done by measuring exhaled ^{13}C-labelled CO_2 (known as the urea breath test, UBT), by serology and by analysing body materials such as faeces, saliva and urine (Kelly *et al.*, 1994; Logan et al., 1991; Sobala *et al.*, 1991; Syam *et al.*, 2015; Yamamoto *et al.*, 2000). However, positive results obtained by serology do not necessarily indicate current infection by *H. pylori*. The UBT requires an expensive instrument such as a mass spectrometer, which is not always available in clinical laboratories.

The *H. pylori* stool tests represent a non-invasive concept for diagnosis of infection.The tests include culture, PCR, and enzyme immunoassay (Kabir, 2001). In a study in India, the sensitivity and specificity of stool PCR in untreated patients were 72.5% and 100% respectively (Mishra et al., 2008). The sensitivity of stool antigen test in simple ulcer as reported in literature is in range of 88- 100% (Makristathis *et al.*, 2004).

2.1.6 Role of *H. pylori* in gastroduodenal perforations

Although the role of *H. pylori* in causation of duodenal and gastric ulcer is beyond doubt according to various studies, the role of *H. pylori* in various complications of PUD is not well understood. Various authors have reported an association of *H. pylori* infection with perforated peptic ulcer disease and opinion is still divided on this issue (Rehmani & Pathak, 2018).

2.2 Research question

What is the role of *H. pylori* infection in adults presenting with spontaneous gastroduodenal perforation at the University Teaching Hospital (UTH), Lusaka, Zambia?

2.3 Study objectives

2.3.1 Main Objective

To explore the role of *H. pylori* infection in adults presenting with spontaneous gastroduodenal perforation at UTH, Lusaka, Zambia.

2.3.2 Specific Objectives

i. To determine the prevalence of *H. pylori* infection in adults with spontaneous gastroduodenal perforation at UTH.

ii. To establish the factors associated with *H. pylori* infection in adults with spontaneous gastroduodenal perforation.

iii. To compare the sensitivity of the histological examination and stool antigen test for *H. pylori* detection in adults with gastroduodenal perforation.

CHAPTER THREE: METHODOLOGY

3.1 Study design

This was a Cross-sectional study that explored the role of *H. pylori* infection in adults with spontaneous gastroduodenal perforation presenting at the Department of Surgery, Adult Hospital, University Teaching Hospitals, Lusaka, Zambia. The study took 10 months, running from June 2018 to March 2019. The study and target populations were all patients presenting with peritonitis secondary to spontaneous gastroduodenal perforation at Adult Hospital, UTH, and the those who satisfied the inclusion criteria, respectively.

3.2 Inclusion criteria

i. Patients 18 years or above. At 18 years, somebody is an adult and can severally sign an informed consent for laparotomy.
ii. Patients with a clinical diagnosis of peritonitis, and identifiable perforation in the stomach or duodenum at laparotomy (biopsies taken from the perforation).

3.3 Exclusion criteria

i. Patients with established diagnosis of gastroduodenal malignancy preoperatively because perforation is a known sequela (not spontaneous) for this disease.
ii. Refusal to give consent. This is in-keeping with the principles of research involving human subject as prescribed by the Helsinki Declaration.

3.4 Sampling strategy

Consecutive sampling of all consented patients who met the inclusion criteria was used throughout the study.

Sample size: 59

$$N = \frac{Z^2 \times P(1-P)}{(D)^2}$$

Where:

N = Sample size, Z = 1.96 **(**when using a 95% Confidence interval),

P = 4% (Bertleff & Lange, 2010; Svanes, 2000), D = +/-5% the accepted accuracy range.

$$N = \frac{1.96^2 \times 0.04\ (1 - 0.04)}{0.05^2}$$

$$N = 59$$

Variables that were investigated:

Independent variables:

Sex, age, ethnicity, socioeconomic status, smoking and alcohol, NSAID and steroid use.

Dependent variable:

Site and size of perforation, *H. pylori* infection detection in biopsy and stool, histological pattern of the perforation.

3.5 Procedure

Patients satisfying the inclusion criteria were enrolled.

After resuscitation and baseline investigations, all the patients were managed by surgical exploration through midline abdominal incision and sucking out or mopping off the peritoneal fluid. Stomach and duodenum were inspected in all the cases to locate the perforation in the anterior or posterior wall. The exact site and size of perforation were noted. With a toothed dissecting forceps gently placed on one end and using a scalpel, the sample was taken from the perforation with intact mucosa and 3 millimetre clear margins from the lesion and put in a 10% formalin medium for histopathological examination. The specimens were prepared and stained with Haematoxylin and Eosin (H & E) for *H. pylori* identification. Silver stain (Warthin-Starry) was used on the specimen which did not show *H. pylori* on H & E and re-examined and reported accordingly. On the other hand, specimens were evaluated for acute and chronic inflammation, lymphoid aggregates, proliferation, mucosal atrophy, intestinal metaplasia, dysplasia and neoplasm. Simple closure of refashioned perforation was carried out by using absorbable suture followed by omentopexy - using a pedicle of the omentum to augment and cover the suture line. Peritoneal lavage and drainage were carried out. Abdominal wall mass closure was done. Nasogastric tube was inserted for gastric decompression.

Stool sample was taken and stool antigen test for *H. pylori* done by using OnSite^{TM} *H. pylori* Ag Rapid Test (CTK Biotech Inc., San Diego, USA). OnSite^{TM} *H. pylori* Ag Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of *H. pylori* antigen in human faecal specimen. The sample diluent vial was labelled with sample ID and the cap was removed. Using a wooden stick applicator, a pea-sized stool sample (approximately 0.1g) was added to vial of sample diluent. For liquid or semi-solid stool samples 100µL of the sample

was added to the vial using a disposable pipette. 100µL was the approximate volume to the first notch on the pipette. Cap was replaced. Homogenizing for 15 seconds on a vortex mixer or manually shaking vigorously to mix. Using a disposable plastic pipette 350µL of the stool suspension was added to a test tube. A test strip was placed in the tube with the uncovered end down, so that the base of the strip is immersed in the sample. The test strip stood vertically at room temperature for 15 minutes. Test results were read visually within 5 minutes of the end of the 15 minutes incubation period. Results were interpreted as positive, negative or invalid (in which case were repeated).

3.6 Ethical Considerations

The study was conducted according to the principles of research involving human subjects as prescribed by the Declaration of Helsinki.

i. Risks:

Apart from the risks associated with the standard surgical management of gastroduodenal perforation (operation), there was minimal risk or injury associated with this study. There may have been psychological trauma due to some sensitive questions asked during the patient interview.

ii. Benefits:

No financial remuneration was provided to the patients recruited in the study as all procedures, investigations and follow up was confined to routine procedures that are part of standard care and management. Nonetheless, the investigator covered the cost of the *H. pylori* investigations involved.

iii. Voluntarism:

Participation in this study was purely voluntary. Patients participated of their own accord; no coercion was used and if the patient felt injured or inconvenienced, they were at liberty to withdraw from the study at any time without any implication to their management.

iv. Written informed consent:

Written informed consent was obtained from every patient participating in the study prior to their enrolment.

v. Confidentiality

vi. The data collected was kept confidential and available only to the researcher. It was kept in a locker with keys kept by the researcher. Once transferred to a personal computer; the data was kept securely under password protection accessible only by the researcher.

Ethical clearance and approval were sought from the Institutional Review Board, the ERES Converge IRB (IRB No. 00005948). Permission was obtained from UTH Management, and the Department of Surgery.

3.7 Data Analysis

All the data collected was entered in excel spread-sheet and exported into STATA version 13 (STATA Corp. College Station, Texas, USA) for analysis. All categorical variables were tabulated with the outcome variable in order to determine an association using Fisher's exact test because in some cells the expected frequency was less than 5. All continuous variables were tested for normality using Shapiro-Wilk test. Skewed continuous variables, such as age, were reported using median and interquartile range. A correlation matrix was run to understand how independent variables relate to each other. To determine possible predictors/risk factors and to rule out confounders for *H. pylori*

infection in gastroduodenal perforation, multiple logistic regression was used. To predict the probability of *H. pylori* infection in gastroduodenal perforation, marginal commands and marginal plots were used. For all statistical tests, a p value of <0.05 was considered significant.

3.8 Study limitations

Tests which have been shown to be more sensitive in *H. pylori* detection such as Polymerase Chain Reaction (PCR) (Bakhtiari *et al.*, 2019), Urea Breath Test (UBT) (Molina-Molina *et al.,* 2019), immunohistochemistry (Ginestet *et al.*, 2017) and in-situ hybridization (Fontenete *et al.*, 2017) were not used in this study. This could explain the low prevalence of *H. pylori* infection observed in this study.

The study only involved one centre and ran for a duration of under 12 months with a sample size of 60.

The histological examination of *H. pylori* is operator dependent and may therefore vary from one operator to another.

CHAPTER FOUR: RESULTS

4.1 Background

In this study, a total of 280 patients underwent emergency laparotomy for acute peritonitis between June 2018 and March 2019.

Table 4.1: Baseline demographic characteristics of study participants with gastroduodenal perforation

Characteristic	n=60	
Age (years)	40 (IQR, 30.5 - 52)	
	Category	Proportion (%)
Gender	Female	12(20)
	Male	48(80)
Education level	Primary	17(28.33)
	Secondary	27(45)
	Tertiary	16(26.67)
Marital status	Married	36(60)
	Not married	24(40)
Occupation	Employed	27(45)
	Not employed	33(55)
Residence	Low density	15(25)
	High density	45(75)
Smoking status	Smokers	25(41.67)
	Ex-smokers	8(13.33)
	Non-smokers	27(45)
Alcohol use	Drinkers	42(70)
	Ex-drinkers	7(11.67)
	Non-drinkers	11(18.33)

IQR: Interquartile range

Sixty (21.43%) had gastroduodenal perforations. Out of the 60, 48 were males and 12 were females, in the ratio of 4:1. The median age was 40 years (IQR, 30.5 – 52). All the participants were black-skinned Zambians and had abdominal pain as the main symptom. In this study, 71.67% of the participants had at least secondary education, 60% were married, 45% were employed, 75% came from high density areas, and 41.67% gave the history of active smoking and as many as 42% had history of alcohol consumption. These demographic characteristics are shown in Table 4.1 above.

The duration of symptoms for participants with gastroduodenal perforation was studied and the median was 3 days (IQR, 2 – 6) for *H. pylori* negative compared to *H. pylori* positive 6 days (IQR, 4 – 8); p=0.06, as shown in Figure 4.1 below.

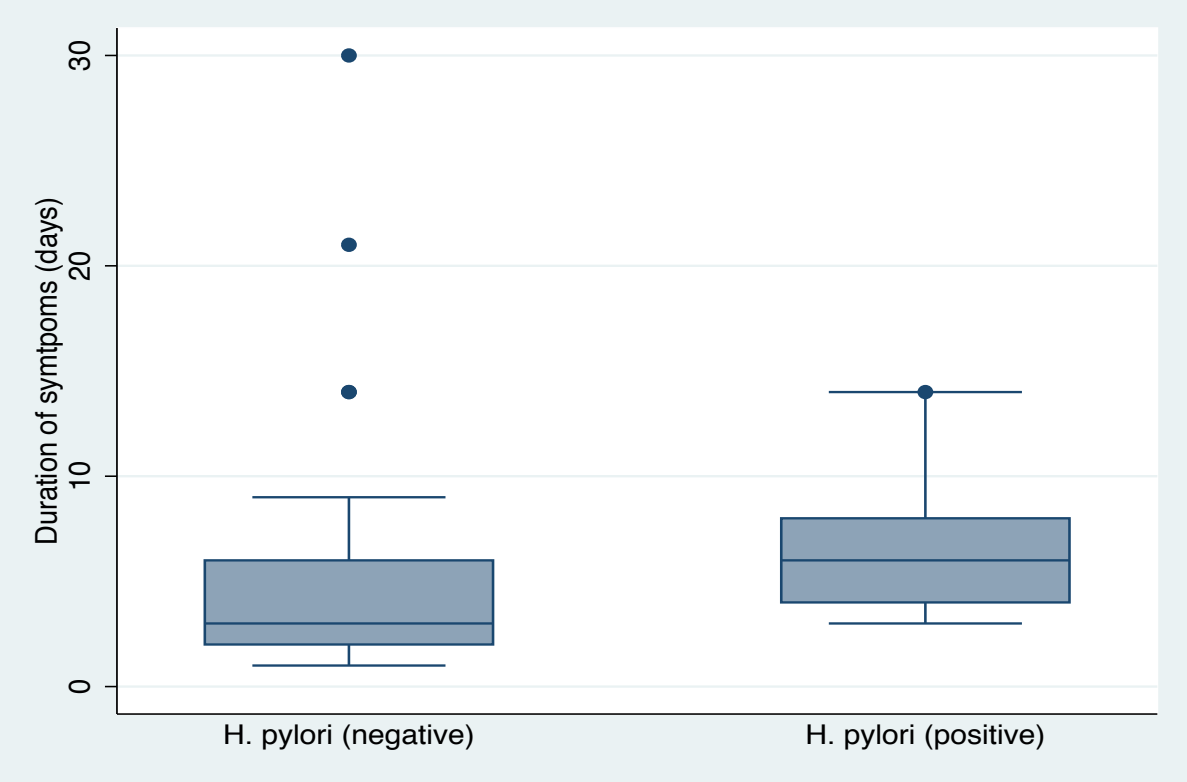

Figure 4.1: Duration of symptoms in *H. pylori* negative and positive individuals in gastroduodenal perforation

Preoperative data of patients were: median pulse 102.5/min (IQR, 87.5 – 126), median respiratory 22/min (IQR, 20 – 27.5), mean temperature 36.3° C (SD, 1.02), mean haemoglobin 13.9g/dL (SD, 2.8), BP: 38 were normotensive, 16 were hypertensive and 5 were hypotensive. Out of the 60 participants studied, only 7 (11.67%) had a positive result for *H. pylori* in the tissue biopsy. The association between *H. pylori* status and demographic characteristics of these participants was performed using Fisher's exact test as shown in Table 4.2.

Table 4.2: Association between *H. pylori* status and demographic characteristics in gastroduodenal perforation

Characteristic	H. pylori positive	H. pylori negative	p-value
Gender			
Female	1	11	0.688
Male	6	42	
Education level			
Primary	3	14	0.584
Secondary	2	25	
Tertiary	2	14	
Marital status			
Married	6	30	0.140
Not married	1	23	
Occupation			
Employed	6	27	0.082
Not employed	1	26	
Residence			
Low density	2	13	0.816
High density	5	40	
Smoking status			
Smokers	4	21	0.626
Ex-smokers	1	7	
Non-smokers	2	25	
Alcohol use			
Drinkers	6	36	0.411
Ex-drinkers	1	6	
Non-drinkers	0	11	

In this study, there were 48 [83.33% (95% CI, 71.3 – 91.0] participants with gastric perforations and 12 [16.67% (95% CI, 9.0 – 28.7) with duodenal perforations. This ratio of gastric to duodenal perforations was 5:1. Intra-operatively, 26 patients (43%) has fibrinopurulent peritoneal contents while 34 patients (57%) had gastric content in the peritoneal cavity. The histology of the 60 perforations were as follows: 57 (95%) chronic non-specific ulcers and 3 (5%) adenocarcinoma from the gastric region. The ratio of perforations is as shown in Figure 4.2.

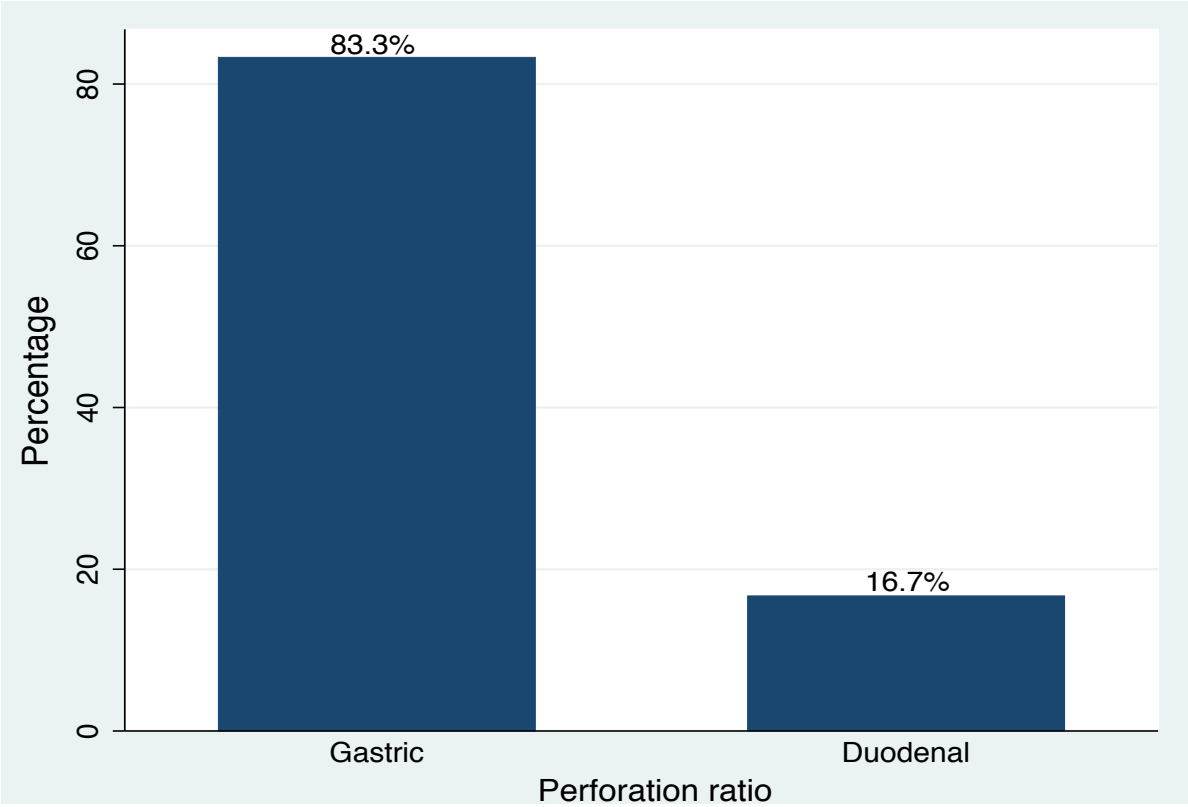

Figure 4.2: Ratio of gastric to duodenal perforation

Furthermore, the correlation between the independent variables of the study participants was examined. There was positive and significant correlation between occupation with sex (r=0.30), marital status (r=0.40), and residence (r=0.48); education with residence (r=0.61) and occupation (r=0.39), smoking with sex (r=0.52), and alcohol with occupation (r=0.38) and smoking (r=0.43). Negative and significant correlation was observed between residence with

age (r= -0.42), education with age (r= -0.36), and smoking with level of education (r= -0.27). These results are as indicated in Table 4.3.

Table 4.3: Correlation matrix showing the relationship among independent variables in study participants with gastroduodenal perforation

Variable	Gender	Age	Marital status	Residence	Occupation	Education	Smoking
Gender	1.0000						
Age	-0.1232	1.0000					
Marital status	0.1817	0.2278	1.0000				
Residence	0.0000	-0.4203*	-0.0000	1.0000			
Occupation	0.3015*	-0.1597	0.4042*	0.4835*	1.0000		
Education	-0.0112	-0.3572*	0.0275	0.6100*	0.3863*	1.0000	
Smoking	0.5195*	-0.2061	0.0439	-.0.0621	0.1836	-0.2666*	1.0000
Alcohol use	0.2229	-0.1187	0.1907	0.0858	0.3819*	0.0721	0.4343*

Two-tailed correlation * $p<0.05$

To determine the association between *H. pylori* infection and clinical predictor variables, multivariable logistic regression model was constructed. When univariate regression model was constructed, history of PUD, RVD, Type 2 (duodenal) perforation and perforation size of greater than 2 cm were all associated with increased infection with *H. pylori* but were not significant. Other major illness (diabetes, hypertension) and use of NSAID were protective but not significant as shown in Table 4.4. In multivariable logistic regression analysis after

adjusting for all baseline characteristics (Table 4.1), there were no major changes in the estimates and the 95% CI and all were still not significant (Table 4.4).

Table 4.4: Multiple logistic regression analysis of the association between *H. pylori* infection and predictor variables

	Univariate			Multivariable		
Variable	**OR**	**95% CI**	**p-value**	**aOR**	**95% CI**	**p-value**
History of PUD						
No	Ref.					
Yes	4.91	0.71 – 33.71	0.11	6.45	0.62 -66.65	0.08
Major illness						
No	Ref.					
RVD	1.06	0.10 – 10.48	0.96	1.52	0.13 – 17.27	0.73
Others	0.822	0.08 – 7.94	0.87	0.47	0.03 – 7.41	0.59
NSAID use						
No	Ref.					
Yes	0.94	0.19 - 4.65	0.94	1.18	0.21 – 6.5	0.21
Perforation site						
Duodenal	Ref.					
Gastric	0.44	0.07 – 2.69	0.37	0.93	0.11 – 7.74	0.95
Type of perforation						
Type 1	Ref.					
Type 2	1.8	0.29 – 10.99	0.52	1	1	1
Type 3	1	1	1	1	1	1
Size of perforation						
<2 cm	Ref.					
>2 cm	2.86	0.55 – 14.7	0.20	2.33	0.44 – 13.6	0.34

PUD: peptic ulcer disease; OR: odds ratio; CI: confidence interval; aOR adjusted odds ratio, NSAID: Non-steroidal anti-inflammatory drugs. The analysis was adjusted for all baseline characteristics in Table 4.1.

The Area Under Curve (AUC) for histology and stool for prediction of *H. pylori* infection were 0.65 (95% CI: 0.60 - 0.72) and 0.55 (95% CI: 0.49-0.58) respectively. Histology significantly predicted *H. pylori* but not stool p-values (0.04 vs 0.07) respectively and the diagonal line (45° line) is the reference line as shown in Figure 4.3.

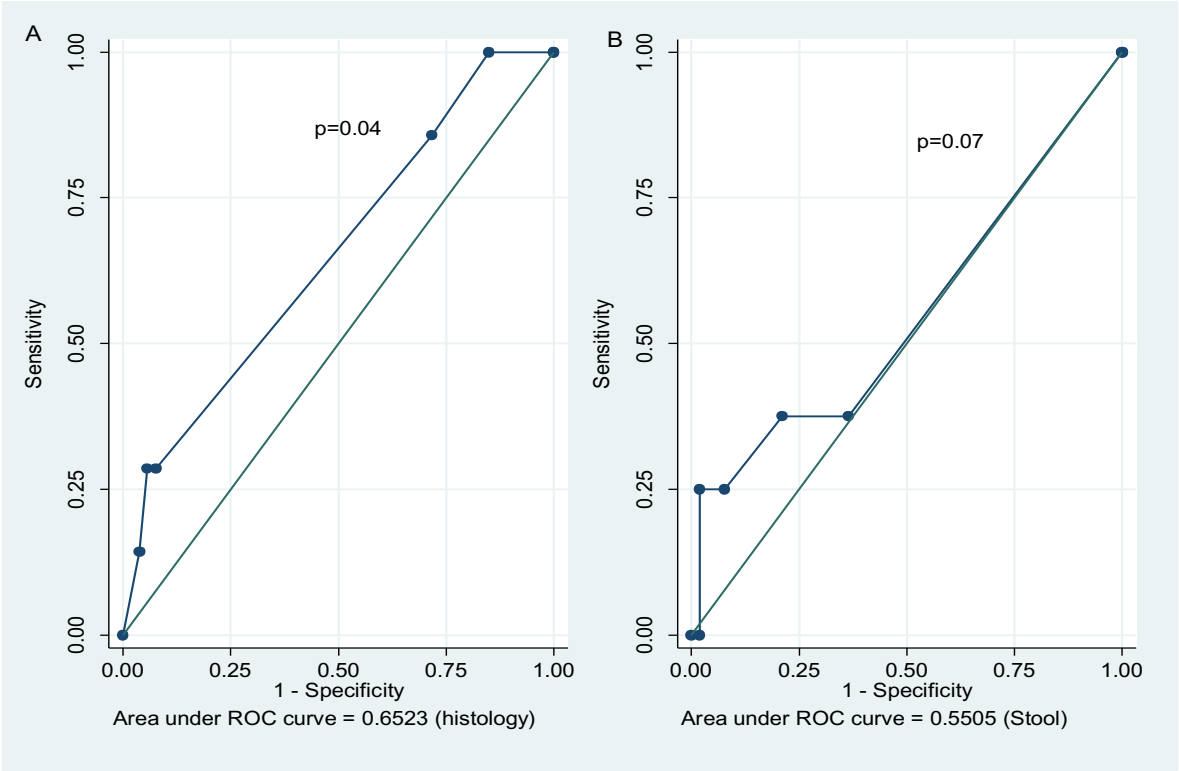

Figure 4.3: Histology (A) and Stool (B) prediction of *H. pylori* in patients with gastroduodenal perforation

The overall performances of histology and stool are shown in Table 4.5. Brier score of histology and stool were 0.15 and 0.16, respectively. Reliability of 0.05 and 0.07 for histology and stool respectively. The goodness of fit in the prediction of *H. pylori* infection in gastroduodenal perforation for histology was 74.8% and 69.3% for stool.

Table 4.5: Overall performance of histology and stool in diagnosis of *H. pylori*

Model	Brier score	Sanders resolution	Reliability	Goodness of fit (%)
Histology	0.15	0.09	0.05	74.8(%)
Stool	0.16	0.11	0.07	69.3(%)

After constructing a logistic regression model, the predicted probability of getting a positive result for *H. pylori* in a biopsy using histology was studied. The probability of a positive sample was 10.7% (95% CI, 1.91 – 19.64). Percentage points when a patient has no history of PUD versus history of PUD (41.8% 95% CI, -5.14 - 88.85). Holding all other variables at their means as shown in below for patients with no major illness, the predicted probability was 14.52% (95% CI, 3.41 - 25.51); p-value 0.01; patients with RVD was 19.72%, (95% CI, -10.77 - 50.22); p-value 0.21 and patients with other major illness was 7.98% (95% CI, -8.31 - 24.26); p-value = 0.33 holding all other variables at their means as shown in Figure 4.4.

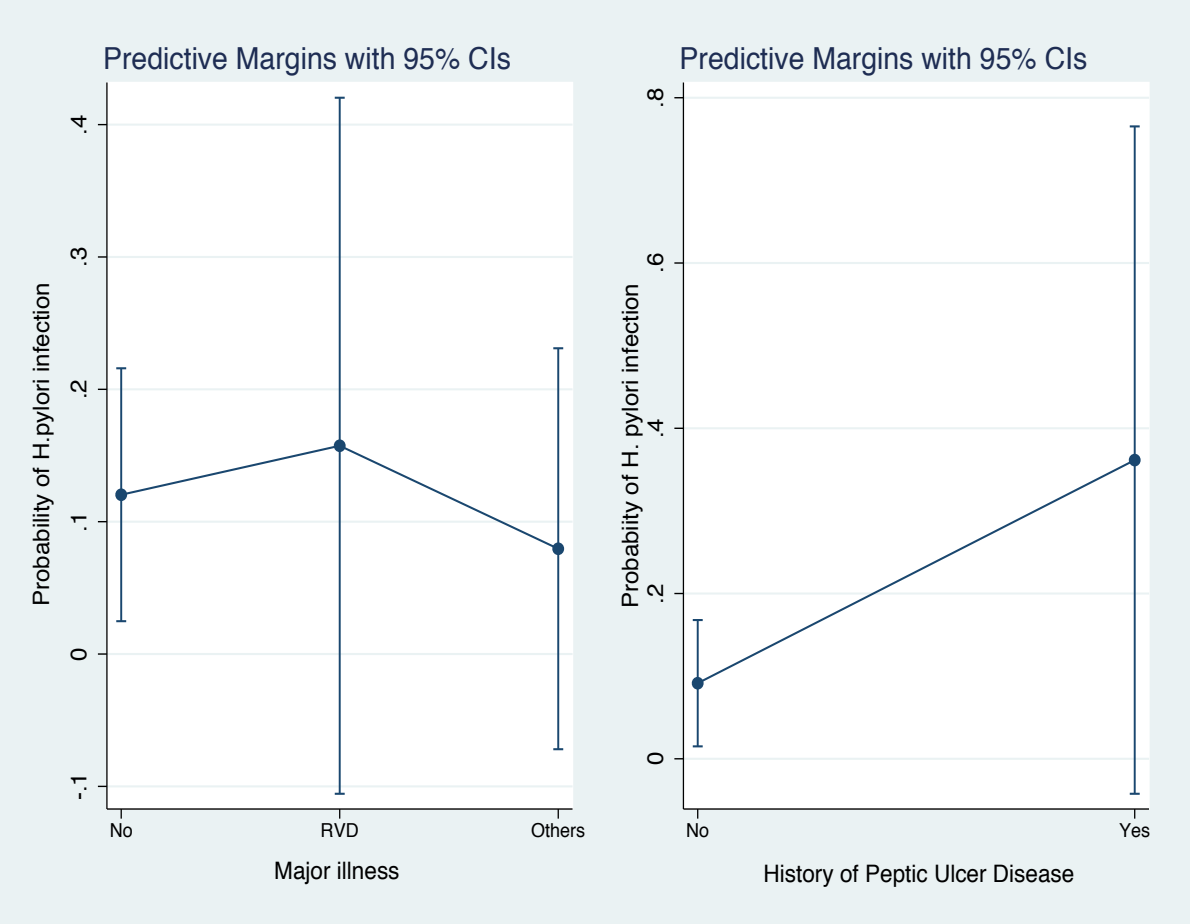

Figure 4.4: Predictive margins of *H. pylori* infection in major illnesses and Peptic Ulcer Disease

The predicted probability for non- NSAID users was 12.74% (95% CI, -0.40773 - 25.89), p=0.05 and for NSAID users was 14.51% (95% CI, 2.11 - 26.9); p=0.02 holding all other variables at their means as shown in (Figure 4.3A) below. For perforation sites, the predicted probability was not estimated due to collinearity (Figure 4.5).

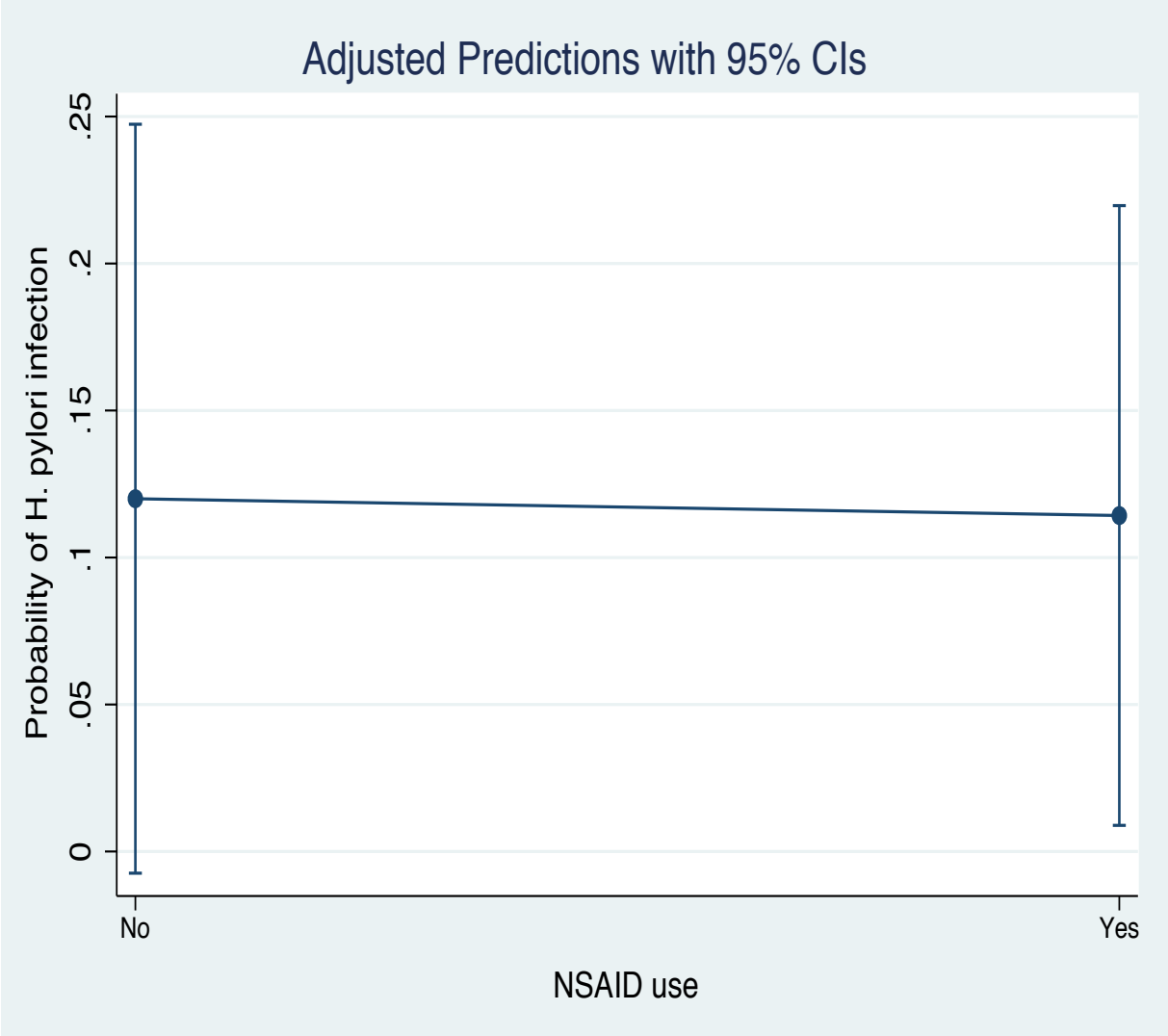

Figure 4.5: Predictive margins of *H. pylori* infection in NSAID use

The predicted probability for size of perforation less than 2 cm, was 10.91% (95% CI, 10.99 - 20.73369; p=0.02 and size of perforation more than 2 cm was 21.26% (95% CI, -0.12 – 4266); p=0.053 (Figure 4.6). The probability for the type of perforation was not estimated because of collinearity.

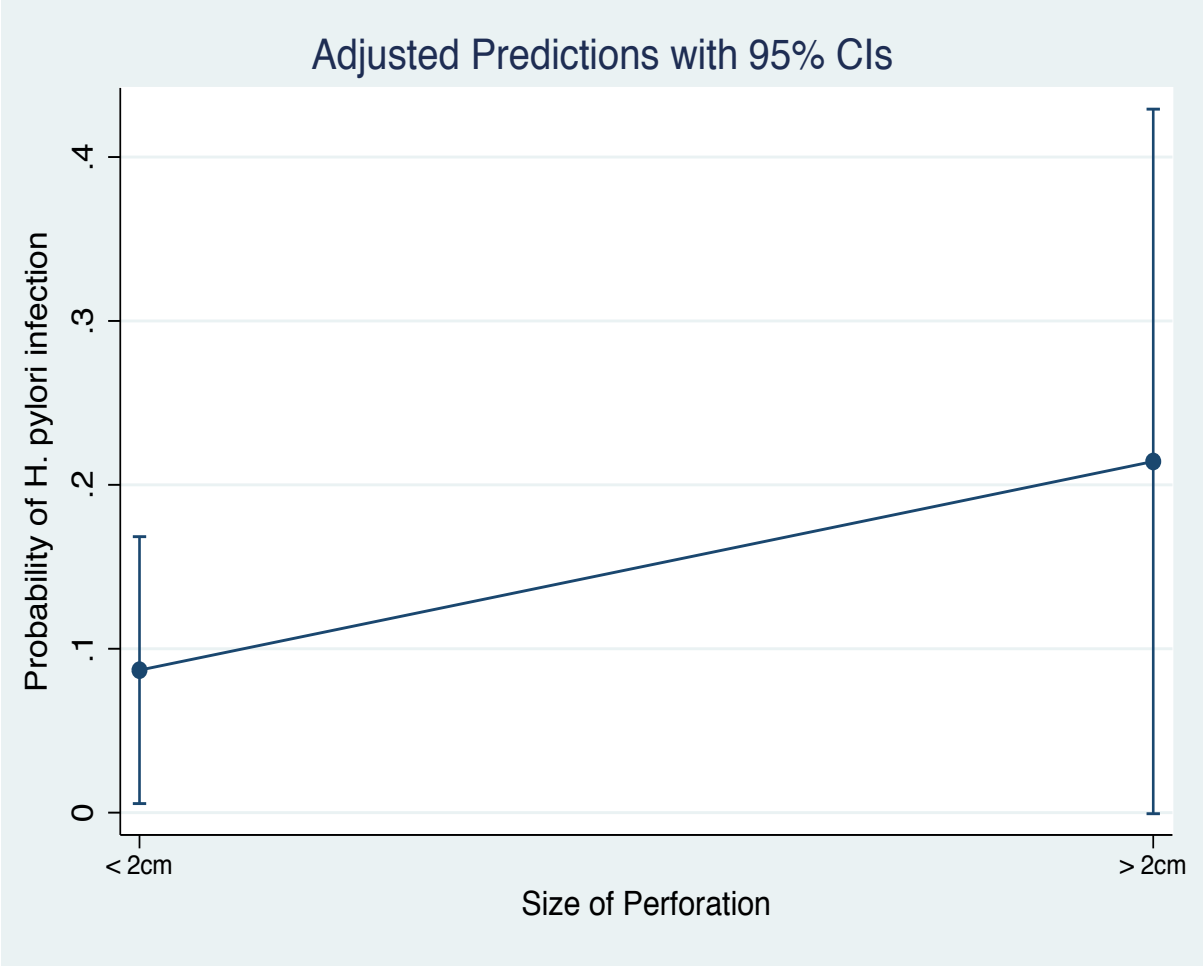

Figure 4.6: The predicted probability for size of perforation in *H. pylori* infection

CHAPTER FIVE: DISCUSSION

5.1. Background

This study was set out to ascertain the role of *H. pylori* infection in adults presenting with spontaneous gastroduodenal perforation at the University Teaching Hospital in Lusaka, Zambia. There is contradictory data regarding the infection rate of *H. pylori* in gastroduodenal perforation. Studies report *H. pylori* infection rates ranging from 0 – 92 percent (Sharma *et al.*, 2015).

5.2 Baseline Demographic Characteristics

Sixty participants who had spontaneous gastroduodenal perforation were enrolled. This represented one-fifth of emergency laparotomies for peritonitis carried out between June 2018 and March 2019 at UTH. In this study, most of the patient were middle-aged with a male to female ratio of 4:1. This is similar to the findings obtained by Ng *et al*, and Aman *et al* (Ng *et al.*, 1996; Aman *et al.*, 2008). Three-quarters of participants resided in high density areas, two-thirds had at least secondary education and 45% were in employment. However, there was no statistical significance in the relationship between *H. pylori* infection status and demographic characteristics of study participants (Table 4.2). This finding is in defiance of what Rosenstock *et al* and Mendall *et al* reported in their studies where *H. pylori* infection was associated with low economic status (Rosenstock *et al.*, 1996; Mendall *et al.*, 1992).

This could probably be explained by the fact that the studies were done in different geographical locations. Additionally, the sample size was too small to demonstrate the

statistical relationship between *H. pylori* infection in gastroduodenal perforation with demographic characteristics.

In this study, there was positive and significant association between independent variables of study participants. These include, occupation with sex, marital status and residence; education with residence and occupation; smoking with sex; and alcohol use with occupation and smoking. Statistically significant negative association between smoking and education could be that people who are educated are more likely to know the harm of smoking and subsequently abstain from it.

Logistic regression analysis module could not depict any associated factors *H. pylori* infection. This could be due to the small sample size. Other studies have shown that the use of NSAID increases the chance of *H. pylori* infection (John *et al.*, 2017; David 2002).

5.3 *H. pylori* Infection in Gastroduodenal Perforation

Gastric to duodenal perforation ratio was found to be 5:1 with the *H. pylori* infection rate of 11.76% (7/60) on histological examination of the perforation biopsies. This is incomparable to the *H. pylori* infection rates of 92% reported by Dogra et al and 61% by Sharma et al in perforated peptic ulcers (Dogra *et al.*, 2014; Sharma *et al.*, 2015). In his study, Ng *et al* asserted that *H. pylori* play an important role in the aetiology of non-NSAID related ulcers (Ng *et al.*, 1996). However, Chowdhary *et al* observed that there was evidence of *H. pylori* infection in patients who presented with perforated duodenal ulcers (Chowdhary *et al.*, 1998). Parallel results were obtained by Reinbach where there was no clear association of duodenal perforation with *H. pylori* infection (Reinbach *et al.*, 1993). This could imply that in this study, patients may have been exposed to a lot of antibiotics before presenting to the hospital.

In this study, the infection rate of *H. pylori* was less (by 56%) in the gastric perforations than in duodenal perforations, but not significantly so (p=0.36 and p=0.95). This is in keeping with other studies by Dogra *et al* and Tsuji *et al* who reported higher duodenal infection rates (Dogra *et al.*, 2014, Tsuji *et al.*, 1999). Hamlet too reported a higher infection rate of the duodenum when compared to the stomach (Hamlet *et al.*, 1999). The lower infection rate of stomach by *H. pylori* could be due to the highly acidic gastric environment.

5..4 Performance Characteristics of Histology and Stool Antigen Test in *H. pylori* Detection

In this study, the prediction of *H. pylori* infection by histological examination by silver stain (Warthin-Starry) was quite significant. A comparable finding was obtained when Loffeld *et al* studied 302 patients with perforated peptic ulcers and observed 78% accuracy in detecting *H. pylori* by histological examination using Giemsa stain (Loffeld *et al.,* 1991). The study by Lee and Kim observed histological examination for *H. pylori* to have a sensitivity 95% and specificity of 99%. Histological examination has a number of limitations, including high cost, high turnover time, dependence on the skill of the operator. More so, the density of *H. pylori* can vary at different sites and this may lead to sampling error. Furthermore, the sensitivity of *H. pylori* may decrease in patients taking proton pump inhibitors (Lee & Kim, 2015). Fashner and Gitu reported sensitivity of 69 – 87% and specificity of 87 – 93% for stool *H. pylori* antigen test in their study (Fashner & Gitu, 2015). The disadvantage of the stool antigen test is the possibility of false negative as a result of recent use of antibiotics or proton pump inhibitor or bismuth (Mishra *et al.*, 2008).

5.5 Predictors for *H. pylori* infection in gastroduodenal perforation

Participants who had a history of PUD were about five times more likely to have *H. pylori* detection on histology. For major perforations (size > 2 cm), *H. pylori* infection rate was 2.86 times more than those with perforation size < 2 cm. However, there was no statistical significance. This could be because of a smaller number of patients. For predicted probability of *H. pylori* infection in NSAID users was 14.51% (p=0.02) and 12.74% (p=0.05) for non-users. *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drugs (NSAIDs) are independently associated with PUD, but the potential interaction between these factors is arguable. Large population-based studies have not succeeded to identify a synergistic effect, while results from cohort studies suggest both an additive risk and a protective effect associated with *H. pylori* infection and NSAID use (David, 2002).

As depicted in Figure 4.1, it is interesting to note that patients who had *H. pylori* infection detected also had longer duration of symptoms. This is significant in our setting so as not to ignore patients with abdominal symptoms. We have to emphasise the use of gastroscopy which is readily available. This will reduce the number of patients coming with perforations since they will be identified in good time and treated adequately for PUD.

CHAPTER SIX: CONCLUSSION AND RECOMMENDATIONS

6.1 Conclusion

There was a low prevalence of *H. pylori* infection in adults presenting with gastroduodenal perforation at the University Teaching Hospital in Lusaka, Zambia. There was no correlation between *H. pylori* infection and participants demographic characteristics; site, size and histology of the perforation. Ninety-five percent of the perforations were chronic nonspecific ulcers.

The detection of *H. pylori* by histological examination is observed to have a higher sensitivity of 65% than stool antigen test (55%).

6.2. Recommendations

i. In adults admitted with spontaneous gastroduodenal perforation at the UTH, *H. pylori* eradication therapy should be given only to those who have a positive histology result for *H. pylori*.

ii. Further studies to be done to identify the causes of spontaneous gastroduodenal perforation apart from *H. pylori* infection and malignancy.

iii. Superior tests for detection of *H. pylori*, such as immunohistochemistry, polymerase chain reaction (PCR) to be used in subsequent studies.

iv. All patients with gastroduodenal perforation to have histological examination of biopsies for detection of *H. pylori.*

v. Longitudinal study be done to evaluate the outcome of patients with gastroduodenal perforation.

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APPENDICES

Appendix I: Participant Information Sheet

Code No _____

Consent to participate in the study of the role *Helicobacter pylori* infection in adults presenting with spontaneous gastroduodenal perforation at the University Teaching Hospitals, Lusaka, Zambia.

Introduction:

I am Dr Alick Bwanga, a postgraduate student at The University of Zambia, School of Medicine, in the Department of Surgery.

The purpose of the study:

To study the role of Helicobacter pylori infection in adults presenting with spontaneous gastroduodenal perforation at UTH. This will help in understanding how prevalent H. pylori infection is in spontaneous gastroduodenal perforation and manage patients accordingly.

What participation involves?

Once you consent to participate in this study, and the operation has been done and biopsy of the perforation taken, you will need to answer a questionnaire (when stable and fit enough to do so). You will be requested to submit a stool sample for investigation. All the results will be interpreted and made available to you and the management will be instituted accordingly.

Confidentiality:

All data collected on questionnaires will be entered into a personal computer with a code number. The questionnaires will be handled with great privacy in order to maintain confidentiality throughout the study.

Risks:

All the procedures done are part of the standard surgical care for patients with this condition. Complications may arise because of the magnitude of the condition and the major operation. However, there is no additional risk associated with this study.

Right to withdraw:

Participating in this study is entirely voluntary. If you choose not to participate in the study, you will continue to receive all services that you would normally get from the hospital.

Benefits:

The study will cover the cost of the investigations. The findings of the study will help to improve the health care delivery and follow up of patient with spontaneous gastroduodenal perforation.

Who to contact?

A committee that works to protect your rights and welfare reviews all research. If you have questions or concerns about your rights as a research participant or comments regarding the conduct of this research, you may contact:

Dr. Alick Bwanga Registrar Surgeon Principal Investigator Department of Surgery University Teaching Hospital Lusaka Zambia +260977262288 drbwanga28@gmail.com	Prof. Etienne Odimba Professor of Surgery Department of Surgery University of Zambia School of Medicine Lusaka Zambia +260978206335 etienne.odimba@yahoo.com	The Chairperson ERES Converge IRB 33 Joseph Mwilwa Road Rhodes Park Lusaka Zambia +260955155633/4 eresconverge@yahoo.co.uk

Appendix II: Consent Form

I …… have read the content of this form. All my questions have been answered; I have been told I can ask at any time during the course of the study and that I am free to opt out of the study at any stage. I, therefore, agree to participate in this study.

Signature or thumb print of participant ……...

Signature or thumb print of witness ……

Date of signed consent …….....

Participant agrees / Participant does NOT agree

Appendix III: Clinical Assessment Tool

1. Preoperative data:

   BP …../….. Pulse ……./min Resp. ……./min Temp. …….^{o}C

   Hb …… g/dL

2. Intraoperative (peritoneal) findings:

   2.1. Serous fluid 2.2. Fibrinopurulent fluid 2.3. Gastric content 2.4. Blood-stained

   Perforation site: ……

   Perforation size: ……...

3. Biopsy technique:

   - Peritoneal fluid suctioning
   - Inspection of stomach and duodenum to identify the perforation site
   - Estimation of the size of perforation
   - With a toothed dissecting gently placed on one end of the perforation, intact mucosa with 3mm clear margins is cut using a scalpel
   - Placement of the sample in 10% formalin medium and labelling for histological examination
   - Histological examination includes ulcer characteristics (acute or chronic), cellular architecture (atrophy, metaplasia, dysplasia, neoplasia) and staining with haematoxylin and eosin (H & E) and silver stain.

4. Rapid *H. pylori* Antigen Stool Test

   Stool sample will be collected as soon as the patient starts to open bowels. The stool sample will be tested using the OnSite™ *H. pylori* Ag Rapid Test. Using a wooden stick the sample will be placed in a diluent vial. The sample will be homogenised for 15 seconds. The test strip will be placed into the vial and put to stand vertically at room temperature for 15 minutes and the result will be read thereafter.

Appendix IV: Questionnaire

Code Number : ……

1. SOCIODEMOGRAPHICS

1.1. Gender: 1.1.1. Male 1.1.2. Female

1.2. Age: …….

1.3. Race: 1.3.1. Negroid 1.3.2. Caucasian 1.3.3. Mongoloid 1.3.4. Other

1.4. Marital Status: 1.4.1. Single 1.4.2. Married 1.4.3. Divorced 1.4.4. Widowed

1.5. Residence: 1.5.1. High density 1.5.2. Low density

1.6. Occupation: 1.6.1 Employed 1.6.2. Self-employed 1.6.3. Other

1.7. Education: 1.7.1. None 1.7.2. Primary 1.7.3. Secondary 1.7.4. Tertiary

1.8. Smoking: 1.8.1. Current smoker 1.8.2. Ex-smoker 1.8.3. Non-smoker

1.9. Alcohol use: 1.9.1. Never 1.9.2. Yes 1.9.3. Quit

2. CLINICAL DATA

2.1. Chief complaint: 2.1.1. Abdominal pain 2.1.2. Abdominal distension
2.1.3. Vomiting 2.1.4. Other

2.2. Duration of symptoms (hours): 2.2.1. <2 2.2.2. 2 to <6
2.2.3. 6 to 12 2.2.4. >12

2.2. History of PUD: 2.2.1. Yes 2.2.2. No

2.3. PUD diagnosis: 2.3.1. Clinical 2.3.2. Endoscopy 2.3.3. Serology 2.3.4. Other

2.4. Major illness: 2.4.1 DM 2.4.2. HTN 2.4.3. Arthritis 2.4.4. TB

2.5. NSAID use: 2.5.1. Yes 2.5.2. No

2.6. Steroid use: 2.6.1. Yes 2.6.2. No

3. HISTOPATHOLOGY

3.1. Perforation site: 3.1.1. Gastric 3.1.2. Duodenal 3.2.1. Both

3.2. Size of perforation: 3.2.1. Up to 2 cm 3.2.2. More than 2 cm

3.3. Type of perforation: 3.3.1. I 3.3.2. II 3.3.3. III 3.3.4. IV 3.3.5. V

3.4. Biopsy report: 3.4.1. Benign 3.4.2. Malignant 3.4.3. Indeterminate

3.5. *H. pylori* in tissue biopsy: 3.5.1. Positive 3.5.2. Negative

4. MICROBIOLOGY

4.1. Stool antigen test for *H. pylori*: 4.1.1. Positive 4.1.2. Negative