PATTERN OF HELICOBACTER PYLORI ASSOCIATED GASTRIC LESIONS AT MUHIMBILI NATIONAL HOSPITAL USING IMMUNOHISTOCHEMISTRY

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By

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A Dissertation Proposal Submitted in Partial Fulfilment of the Requirements for the Degree of Masters of Medicine in Anatomical Pathology of the Muhimbili University of Health and Allied Sciences

July, 2018

CERTIFICATION

The undersigned certify that they have read and hereby recommend for the acceptance of dissertation entitled Pattern of Helicobacter pylori Associated Gastric lesions at Muhimbili National Hospital using Immunohistochemistry in fulfilment of the requirements for the degree of Master of Medicine (Anatomical Pathology) of the Muhimbili University of Health and Allied Sciences.

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(Supervisor)

Date: -----------------------------------

DECLARATION AND COPYRIGHT

I, Advera Isaac Ngaiza, declare that this dissertation is my own original work and that has not been presented and will not be presented to any other university for similar or any other degree award.

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DEDICATION

To Almighty God

To my beloved mother

To my husband

To my beloved son

To all my teachers

To all my friends and colleagues

I dedicate this work.

ABSTRACT

Background: *H. pylori* is a bacterium which affects the majority of population worldwide, and accounts for more than 50% of the population. The *H. pylori* infection is common in developing countries like Tanzania where more than 50% of the population are infected. *H. pylori* infection is a communicable disease which is transmitted through the faecal-oral route in contaminated water and food. It is associated with acute, chronic and atrophic gastritis, peptic ulcers and malignancies such as adenocarcinoma and mucosal associated lymphoid tissue lymphoma. The cost of management of patients to the country is high and may result into a vicious cycle of poverty. Immunohistochemistry is one of the superior methods and is regarded as a gold standard for detection of *H. pylori,* hence it will help in the establishment of the relationship between the bacteria and the associated lesions as it has been reported in this study. Despite its worldwide distribution, few studies have been done in Tanzania to detect the *H. pylori* in gastric biopsies in relation to the gastric pathologies it causes.

Objective: To determine the pattern of *H. pylori* associated gastric lesions at MNH using immunohistochemistry.

Methodology: A descriptive cross-sectional study, retrospective laboratory based study was conducted at Muhimbili National Hospital Histopathology Laboratory in which 50320 specimens were retrieved and 743 were gastric specimens. 170 FFPE blocks and slides were retrieved from the archive of the year 2012- 2016, and were then stained with Hematoxylin and Eosin. All cases were immunohistochemically stained using a polyclonal antibody (DAKO) to detect *H.pylori* antigens and reviewed by two senior surgical pathologists (supervisors). The protocols for the procedures were adopted from MNH SOPs.

Results: 170 gastric biopsies were identified (patient mean age 50, 109 males, 61 females). Morphologies included 61% (103/170) inflammatory lesions, 4% (7/170) benign-neoplastic lesions and 35% (65/170) malignant lesions. *H. pylori* was detected in 11.2% of cases (19/170) by H&E and 37% (63/170) by immunohistochemistry, and was most frequently seen in cases with chronic inflammation 55.6% (35).

Conclusion: *H. pylori* is a common bacterial infection most frequently associated with chronic inflammation and was identified at a prevalence lower than described in the literature. Immunohistochemistry was more sensitive than H&E alone in identifying *H. pylori*. Additional studies are needed to determine the prevalence in the general population.

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LIST OF ABBREVIATIONS

AhpC: Alkyl hydroperoxidase C

BabA2: Blood group Antigen Binding Adhesions 2

CLO Test: Campylobacter Like Organism test

MDGs: Millennium Development Goals

DNA PCR: Deoxyribose Nucleic Acid Polymerase Chain Reaction

DPX: Dibutyl Phthalate Xylene

FFPE: Formalin Fixed Paraffin Embedded

GIST: Gastrointestinal stromal tumour

H&E: Hematoxylin and Eosin

HCL: Hydrochloric acid

H_2O_2: Hydrogen peroxide

IceA: Induced by Contact with Epithelium Allele

IHC: Immunohistochemistry

KatA: Catalase activity

MALT: Mucosal Associated Lymphoid Tissue

MNH: Muhimbili National Hospital

MUHAS: Muhimbili University of Health and Allied Sciences

SOP: Standard Operation Procedures

SPSS: Statistical Package for Social Science

VacA: Vacoulating Cytotoxin A

DEFINITION OF TERMS

Helicobacter pylori: Is a gram negative bacterium associated with various gastrointestinal lesions.

Peptic ulceration: Is a defect in the gastric and duodenal mucosa with at least the diameter of 0.5 centimetres.

Gastritis: Is an infiltration of neutrophils and mononuclear cells in the mucosa of the stomach which has acute or chronic course.

Metaplasia: Is the reversible transformation of one differentiated cell type to another differentiated cell type.

Adenocarcinoma: Is a type of cancer of epithelial tissue that has glandular origin, glandular characteristics or both.

Immunohistochemistry: It is a process of detection and localization of antigens (e.g., proteins) in cells of a tissue section based on the principle of antibody-antigen binding reaction and visualized on microscope.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

H. pylori is a bacterium which was first isolated in the gastric contents of humans in nineteenth century (1,2). The bacteria are widely distributed in the stomach particularly in the parietal cells of the gastric glands, and was commonly seen in patients with different gastric complaints (3). *H. pylori* is associated with various gastric conditions ranging from benign conditions to malignancies. *H. pylori* is often contracted in childhood and the bacteria may remain harboured to adulthood (4).

H. pylori infection is common in all age groups along with many other infections. The pathologies result from *H. pylori* infections are associated with high morbidity and mortality (5). Although the incidence of *H. pylori* in developed world has been decreasing, it is still a major problem in developing countries (3,6–10). The trend of infection has been decreasing in the younger population globally and it is expected to decrease even more in the next few years with improvement of living conditions and implementation of the Millennium Development Goals (11–13).

Studies have shown that *H. pylori* infection begins in childhood and manifests in adulthood. In developing countries the prevalence is reported to be high in young people. However, there are some risk factors which are reported in the literatures as contributory to *H. pylori* infection and are related to living conditions during childhood such as living in overcrowded places, areas with unreliable supply of clean water and poor sanitation (14). Living in a developing country in areas with poor sanitation and where people are overcrowded is common and has a higher risk of *H. pylori* infection (15). It has been shown in a number of studies that *H. pylori* infection is more commonly diagnosed in patients with low socio-economic status. Other studies have shown that males are two times more affected than females, however, some studies have shown no gender predilection in *H. pylori* infection (16,17).

H. pylori is a gram negative bacterium which measures about 2-4 micrometre length with two to six unipolar flagella of about 3 micrometre length each (17,18). It is spiral shaped, but can appear coccoid after administration of antibiotics or when dead (6). The flagella help the bacteria to move faster towards the neutral environment (19). The growth of *H. pylori* requires optimal conditions such 2.5% of oxygen, 5-10% of carbon dioxide, high humidity and neutral pH ranging from 6-8. Helicobacter pylori is urease, catalase and oxidase positive (6). The urease activity helps the bacterium to survive in the high acidic medium of the stomach.

The pathological conditions associated with *H. pylori* infection include inflammatory conditions such as acute, chronic and atrophic gastritis, peptic ulcers and malignancies such as gastric adenocarcinoma and mucosal associated lymphoid tissue lymphoma (12).

Acute and chronic gastritis: The colonization of gastric mucosa by *H. pylori* leads to neutrophils and mononuclear inflammatory cells infiltration in the antrum and corpus of the stomach (5,7,18,19). However, other factors which may lead to gastritis include atrophic mucosa, vagotomy inhibition and the use of acid suppressing drugs.

Peptic ulcer disease: Peptic ulceration, either gastric or duodenal, is defined as a mucosal defect with a diameter of at 0.5 centimetre penetrating the muscularis mucosa (6). Most of the gastric ulcers occur at the lesser curvature near the transition zone from corpus to the antrum and is associated with long standing chronic inflammation (20).

Gastric cancer (gastric adenocarcinoma and mucosal associated lymphoid tissue lymphoma): Gastric cancer is among the most common leading causes of death worldwide. *H. pylori* is a class I (definite) human carcinogen as deemed by the World Health Organization and the International Agency for Research on Cancers (8,20). The occurrence of gastric cancer varies among populations depending on prevalence of *H. pylori* infection in the area, *H. pylori*-associated gastritis, bacterial virulence, host genetic factors and the types of foods consumed (5,22–24). A study done in Tanzania showed that gastric cancer develops following chronic inflammation and metaplastic changes (25).

Clinical presentation: Most of the patients with *H. pylori* infection present with dyspeptic symptoms or epigastric discomfort especially in early stage of the disease. Such symptoms include heartburn, nausea, epigastric pain and early satiety (28,29).

Detection of Helicobacter pylori: The diagnosis of *H. pylori* is very crucial towards proper eradication of the bacteria. The diagnostic tests can be invasive and non-invasive, and include histological evaluation, culture, polymerase chain reaction (PCR), the rapid urease test (RUT), the urea breath test (UBT), serology and stool antigen test (SAT) (1). Histological evaluation of *H. pylori* includes histochemical and immunohistochemical staining.

There is a number of histochemical stains which include Hematoxylin and Eosin (H&E) stain, Giemsa stain and Warthin-Starry stain.

Hematoxylin and Eosin stain: The bacterium can be seen on the H&E stain but with difficulties, however, a careful scrutiny using a high power objective is often necessary, however, it is likely to fail if bacteria are few in number (27). The H&E stain is quite less expensive but with the lowest specificity and sensitivity and therefore remains to be unreliable screening test (28).

Giemsa stain: The stain is scientifically silver based stain. It is relatively inexpensive compared to immunohistochemical method. Its ability to stain properly is entirely based on the competence of the laboratory to provide the best results as it requires optimal conditions. It has a high sensitivity and specificity compared to H&E stain (15).

Warthin-Starry stain: It is also a silver based stain which stains the glycogen content of the cell wall. This is more sensitive than the Giemsa stain(17).

Rapid Urea test: This is among the best histological evaluations of *H. pylori* as it has a high sensitivity and specificity compared to the above mentioned ones. It detects the urease produced by the *H. pylori* on and in the gastric mucosa (3). It has a relatively high sensitivity and specificity compared to H&E and Giemsa stains (29). It is subjected to false negative results as urease production in a patient tends to be impaired as the patient takes proton pump inhibitors or antibiotics.

Culture: It is not routinely done, but it involves culturing of biopsy tissue containing the *H. pylori*. It has the highest specificity but less sensitive hence it is not commonly done (17).

Polymerase chain reaction (PCR): It is a molecular study for detecting *H. pylori* in the biopsy specimen or in the stool. This is highly sensitive than any other histological methods but is very expensive and use advanced technology (30–32). However, culture remains to be more specific than PCR (3).

Immunohistochemistry: Immunohistochemical (IHC) stains have become more commonly used. This type of stain relies on antibody-antigen reaction. IHC for *H. pylori* is a very sensitive (>90%) and specific test. It is easier for a pathologist to read and therefore to detect the organisms, it is more accurate but relatively expensive (33).

Non-invasive methods:

These methods aim at detecting bacteria themselves or their products and most of them have low specificity and high sensitivity (33).

Urea breath test: It uses Carbon 13 and 14 isotope labelled urea where it detects the urease produced by the bacteria in the stomach (26,32,33). This test is subjected to false positive results as there are other bacteria which produce urease apart from *H. pylori* such as Helicobacter helmannii.

Faecal antigen test: This method aims at detecting the *H. pylori* in the stool. It's sensitivity and specificity are as for urea breathe test (33,34).

Serological test: This method uses detection of antibodies against the H. pylori antigens which include IgG and IgA (35,36).

1.2 Literature Review

1.2.1 Helicobacter pylori infection

It is estimated that about 50% and 90% of the population globally is infected by *H. pylori* (12,20). The pathological conditions related to H. pylori infection are associated with significant morbidity and mortality in both developed and developing countries. This is

even more pronounced in developing countries where there are socioeconomic challenges on hygienic statuses of individuals. Also in most sub-Saharan countries particularly Tanzania where more than 80% of the population live in the rural areas with no access to electricity hence inability to refrigerate their remained foods and lack of clean water supply to prevent the spread of waterborne diseases, *H. pylori* infection was found to be very high at 91.1% after adjusting for the age-standardized rate of the world population. In the tropics where the temperature is too high and humid, villagers tend to store their left overs locally since they can't afford to buy refrigerators to store their foods appropriately (37), thus, most of their foods tend to get decomposed easily (37,38). Moreover, if the struggle towards increasing life expectancy is to be achieved in developing countries the proper diagnostic approach and treatment of *H. pylori* infections and its associated pathologies should be spearheaded. Despite the improved diagnostic approaches and treatment, *H. pylori* infection remains to be the most common among individuals who develop benign to malignant gastric conditions. Such conditions include inflammatory conditions, peptic ulceration and neoplastic conditions as a whole (39,32,40). A study done in Iran revealed that more than 80% of cases tested for *H. pylori* infection were positive. This shows the high burden of the infection globally (20).

The prevalence of *H. pylori* infection has been decreasing in the developed world in contrast to the developing countries since many individuals are still living in unhygienic conditions, overcrowded populations which lack clean water supply (37). In Tanzania, *H. pylori* infection is not considered a major public health problem despite the existence of the relationship of *H. pylori* with various gastric conditions that include malignancies (12,30,41,42,37).

1.2.2 Histomorphological changes of Helicobacter pylori infection in relation to age and sex

Studies have consistently shown that *H. pylori* infection has a chronic course of which most patients tend to harbour infection during childhood and manifest in adulthood (43,37). The study done in Australia also showed 82% of children tested positive for *H. pylori* (4). Most of the studies done in Tanzania involving adult patients used other techniques to detect *H. pylori* and no study was done utilizing immunohistochemical techniques which are among the superior methods. However, several studies conducted on

gastric biopsy tissues revealed *H. pylori* infection to be more common in males as compared to females (45,38). In contrast, the study carried out in 2014 using CLO test found female to male ratio of 1.1:1. On the other hand, most of the studies involving the detection of *H. pylori* in adults showed that the mean age of patients was between the third and fourth decades (42,46,20,38), contrasted with the study conducted in Tennessee which showed that most patients were in their fifth decade and above (4). The study also showed that females were more infected than their male counterparts. This study was concordant with another study conducted in Iran which showed that females were slightly more infected than male by the ratio of 1.1:1 (35).

Another study conducted in Germany showed no gender predilection among patients who had *H. pylori* infection, and this was concordant with the study conducted in Italy. Also among patients who had gastric lesions, *H. pylori* was found more in patients who had severe gastritis (48,49). The results of this study was similar to another study done elsewhere which showed that the patients who tested *H. pylori* positive were those who had greater curvature corpus gastritis (14,5).

1.2.3 Association between Helicobacter pylori and gastric lesions

H. pylori infection is among the common bacterial infections worldwide affecting the majority of people. It takes a chronic course of the infectious period. *H. pylori* is associated with various gastric lesions including acute and chronic gastritis, atrophic gastritis, peptic ulcerations, metaplasia, mucosal associated lymphoid tissue lymphoma and gastric adenocarcinoma. Several studies showed a direct association between gastric lesions and *H. pylori* infection (10,11,16,31,34,50,51,52). The study conducted in Tanzania involving patients who presented with dyspeptic symptoms showed that 67% of cases tested positive for *H. pylori* using CLO-test. Also 63% of patients who had *H. pylori* infection presented with gastritis (25). Another comparative study was carried out in Tanzania, Japan and China, and of the three countries, Tanzania showed highest prevalence of *H. pylori* infection especially in patients who had chronic atrophic gastritis (37). Also among patients who had MALT lymphoma, 85% were found to be *H. pylori* positive using DNA-PCR, which shows a direct causal association, and was concordant with the study conducted in the United States (50). The same comparative study was also found to be concordant with another study which was done in Japan and showed that patients who had

two different subtypes of lymphoma had a prevalence of 86% *H. pylori* infection (51). Another important finding in this study was that upon withdrawal of the intriguing agent such as *H. pylori* from the mucosal associated lymphoid tissue lymphoma, there was degeneration of the neoplasm and the inflammatory process (34,50,54). The occurrence of gastric cancer varies among different group populations. This depends on prevalence of *H. pylori* infection, *H. pylori*-associated gastritis, bacterial virulence, host genetic factors, and the type of foods consumed (5,22–24). The study done in Tanzania showed that gastric cancer develops following chronic inflammatory and metaplastic changes (25). It also showed that metaplastic changes in the stomach were statistically significant associated with *H. pylori* infection.

1.2.4 Detection of Helicobacter pylori

i. Immunohistochemical method

Immunohistochemical detection of *H. pylori* is regarded as the gold standard in diagnosis of *H. pylori* infection. However, there are other detection methods which can be used such as Hematoxylin and Eosin stain, Silver stain, CLO-test and others with basically lower sensitivity and specificity compared to immunohistochemical method. This is because immunohistochemical technique can detect non-viable and non cultureable state of *H. pylori* (10). Immunohistochemical method is relatively more expensive than other staining methods (10). The study done in South Korea showed that immunohistochemical method was highly sensitive and specific by more than 95% (26). This was similar to another study done in different countries in Asia which also showed that immunohistochemical technique was more sensitive as compared to other staining methods (53). Also among patients who had MALT lymphoma, 85% were found to be *H. pylori* positive using immunohistochemical technique, this showed a direct causal association, and was also similar to two studies, one carried out in the United States (50) and the other one done in Japan. These studies showed that patients who had two different subtypes of lymphoma had a prevalence of 86% *H. pylori* infection (51).

ii. Other detection methods

Apart from immunohistochemistry, there are other methods of detecting *H. pylori*. They include both invasive and non-invasive methods (3).

- **Non-invasive methods:** They include urea breath test and faecal antigen test, and aim at detecting the products produced by the bacterium itself, and these methods have high sensitivity but low specificity (33).
- **Urea breath test:** This method uses Carbon 13 and 14 isotopes labelled urea where it detects the urease produced by the bacteria in the stomach (26,32,33). This test is subjected to false positive results as there are other bacteria such as Helicobacter helmannii which produce urease.
- **Faecal antigen test:** This method aims at detecting the *H. pylori* in the stool. Its sensitivity and specificity is similar to that of Urea breath test (33,34).
- **Invasive methods:** These methods involve different invasive procedures and they include;
- **Serological test**: this method uses detection of antibodies against the *H. pylori* antigens. The antibodies detected include IgG and IgA (35,36).
- **Rapid Urea test:** This is one of the reliable histological evaluations of *H. pylori* as it has both high sensitivity and specificity compared to other methods described above (17,27,38).
- **Hematoxylin and Eosin staining:** The bacteria can be seen on the H&E stain but with difficulties, however, a careful scrutiny using a high power objective is often necessary, and is likely to fail if organisms are few in number (27).
- **Giemsa staining:** The stain is scientifically silver stain-based. The method is relatively inexpensive compared to immunohistochemical method. Its ability to stain properly is entirely based on the competence of the laboratory to provide the best results as it requires optimal conditions (1).
- **Warthin-Starry staining:** This is also a silver based stain, which stains the glycogen content of the cell wall. This method is more sensitive than Giemsa stain (1).

1.3 Conceptual Framework

This is a scheme of concepts (or variables) which the researcher used to operationalize in order to achieve set objectives. It is a schematic (or a diagrammatic) presentation of the theory. The theory was presented as a model where research variables and the relationship between them was translated into a visual picture to illustrate the interconnections between the independent, extraneous and dependent variables.

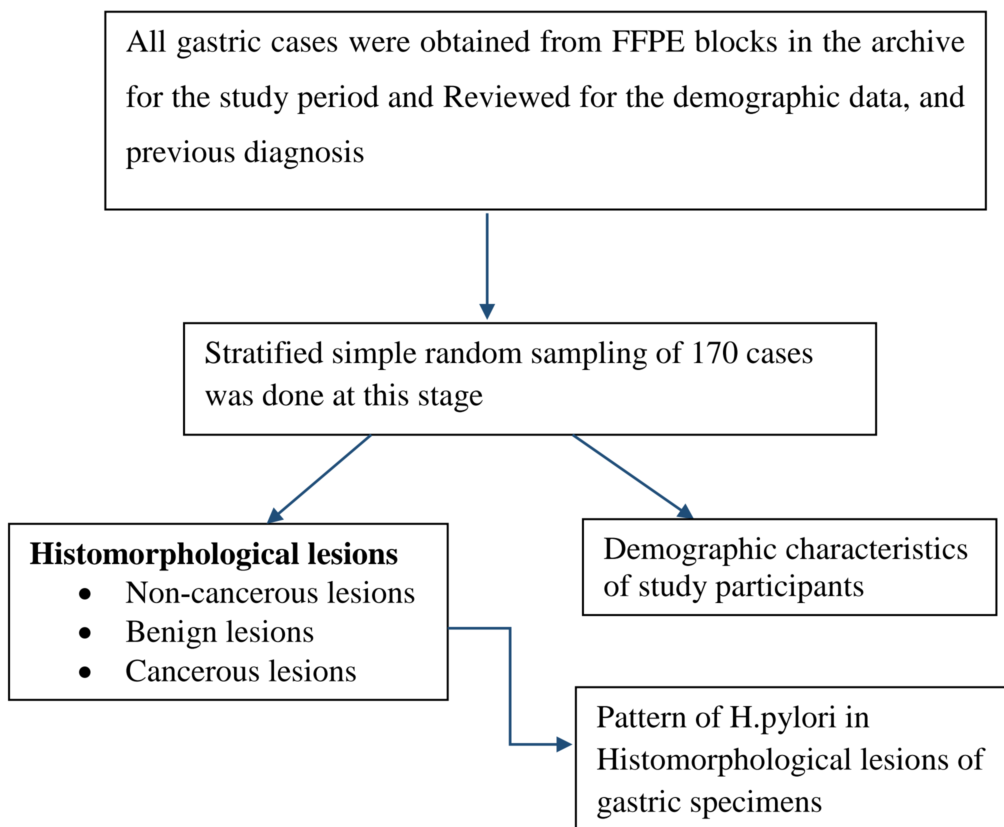

Figure 1: The conceptual framework of this study

1.4 Problem Statement

H. pylori infection is the most common gastric condition worldwide, and in Tanzania more than 50% of population is habited with the bacteria (25). The pathologies associated with *H. pylori* infection may have fatal outcomes but few studies have been carried out in Tanzania to find out the magnitude of *H. pylori* infection in the gastric lesions. The studies

conducted previously used less sensitive and specific methods compare to Immunohistochemistry. Furthermore, no study has been done in Tanzania to ascertain the gastric mucosa changes associated with *H. pylori* infection using immunohistochemistry (8,25).

1.5 Study Rationale

This study will not only help the hospital and the patients in particular in reaching early and accurate diagnosis of *H. pylori* infection, but also it will lay grounds for the future scientists to use the data available for more studies. Nevertheless, it will help in improving the registry and the survival rate of patients through proper management of inflammatory and precancerous conditions.

1.6 Research Questions

i. What is the pattern of histopathological lesions of gastric biopsy specimens by age and sex at MNH?
ii. What is the pattern of H. pylori in the gastric biopsy specimens by histomorphological features using polyclonal antibody at MNH?

1.7 Objectives

1.7.1 Broad Objective

To determine the pattern of H. pylori associated gastric lesions at MNH using immunohistochemistry.

1.7.2 Specific objectives

i. To describe the histopathological lesions of gastric biopsy specimens by age and sex in the FFPE blocks of patients presenting with gastric lesions.
ii. To determine the pattern of H. pylori by histopathological lesions in gastric biopsy specimens

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study Design

This was a descriptive cross-sectional study, laboratory based whereby FFPE tissue blocks of the registered gastric cases between January 2012 and December 2016 were retrieved, and sampled cases were reviewed to confirm the diagnosis and immunostained for H. pylori.

2.2 Study Duration

The study was conducted for a period of 6 months from June to December 2017.

2.3 Study Area

This study was conducted at Muhimbili National Hospital (MNH) for both inpatients and outpatients in the department of Histopathology. MNH is the national referral and teaching hospital with 1,500 bed capacity attending about 1,000-1,200 outpatients and inpatients per week (www.mnh.or.tz). Muhimbili National Hospital has the histopathology department capable of handling 13,000 tissue biopsies per year and some of them are special-stained and immunostained. The gastric cases ranges from 600 to 800 in a year.

2.4 Study Population

All FFPE tissue blocks from patients who underwent gastric endoscopy and gastrectomy from January 2012 to December 2016 at MNH.

2.4.1 Inclusion criteria

FFPE tissue blocks of all gastric specimens from patients whose necessary information is retrievable.

2.4.2 Exclusion criteria

i. All FFPE tissue blocks with crushed biopsy specimens.
ii. The registered FFPE tissue blocks which are nowhere to be found.
iii. The registered FFPE tissue blocks from patients whose request forms are not found.

2.6 Sample Size Estimation

The sample size was calculated from Fischer's formula:

n = [DEFF*Np(1-p)]/ [($d^2/Z^2_{1-\alpha/2}$*(N-1)+p*(1-p)] where:

n= Minimum required sample size

N=Population size

D=Confidence interval limits as % of 100 (absolute +/- %):5%

DEFF= Design effect: 1

$Z^2_{1-\alpha/2}$ = 1.96 at 95% Confidence Interval which was assumed for the study

P = Proportion of gastric cases with the characteristic of interest of which it's estimated proportion of patients with *H. pylori* in gastric specimens is 79.6% (Raziye et al,).

d^2 = Margin of error which is conventionally taken as the sampling error at 1.96 and is thus taken as 5% in this study.

The minimum estimated sample size (n) is thus taken to be 170 FFPE tissue blocks.

2.5 Sampling Procedure

A total of 743 gastric cases were obtained from January 2012 to December 2016. Of those 15 gastric cases were excluded; 8 had no request forms, 5 had no blocks and 2 were crushed. Of 728 gastric cases, 434 were inflammatory lesions, 258 were malignant lesions and 36 were benign lesions. Stratified sampling procedure was used and 170 gastric cases were selected in a ratio of inflammatory lesion: benign : malignant lesions by 12:1:7 respectively.

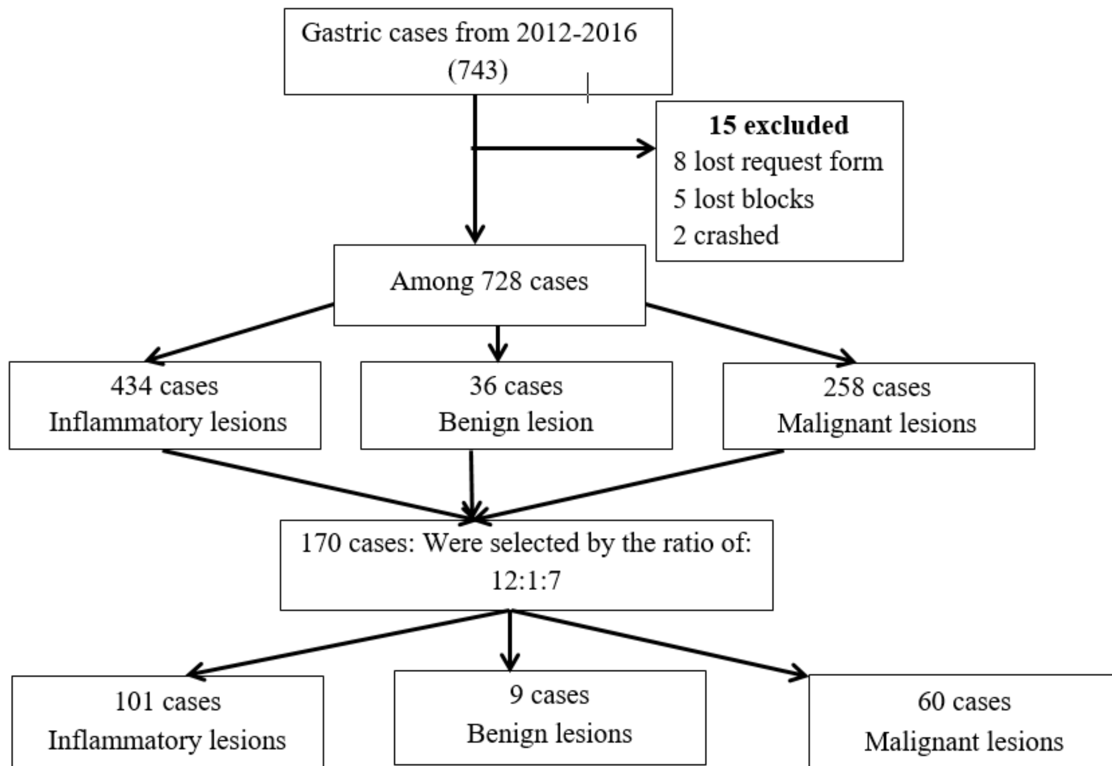

Figure 2: Sampling procedure

2.7 Laboratory Methods

All FFPE tissue blocks of gastric cases received from January 2012 to December 2016 were retrieved for clinical, histopathological and immunohistochemical analysis. Retrieved blocks were sectioned and stained using Haematoxylin and Eosin. The review and categorization of the histopathological diagnoses were performed according to World Health Organization criteria (WHO 2013). Thereafter, H. pylori was detected using immunohistochemical staining method specifically anti-*H.pylori* polyclonal antibody (DAKO) code No. BO 471 lot 076 edition 04.02.00.

2.7.1 Retrieval of the blocks

FFPE tissue blocks to be retrieved were identified by screening the specimens' register book and histology reports kept in the archive of the histopathology unit where they were arranged in order of their laboratory histopathological number (HP number). Tissue blocks from gastrectomy and gastric endoscopy biopsies of patients and which corresponded to

gastric conditions were retrieved for resection using a microtome. For the purpose of this study, new sections for H&E and immunohistochemical staining were prepared.

2.7.2 Microtome and Sectioning

The rotary microtome (SAKURA model SRM 200 CW) was used to cut the thin sections of three to five micrometres where by two sections were analysed per case. The thin section was allowed to float on water bath containing distilled water at 45^0C and mounted on standard frosted glass slides. Thereafter, slides were labelled with respective registration number of the retrieved cases. Then the slides were allowed to drain before de-waxing them on the hot plate at 60^0C for 50 minutes.

2.7.3 H&E staining (MNH protocol was used).

The sections were de-waxed by placing slides on the hot plate before bringing it down in three changes of Xylene for three minutes each and brought down to water through descending grades of ethyl alcohol (absolute, 95%, 80% and 70%), thereafter they were stained in Harris Haematoxylin for 10 minutes, washed in tap water until it disappeared, differentiated in 1% HCL in alcohol, then washed in running tap water and subsequently blued in running tap water for approximately five minutes. The sections were counter-stained in 1% aqueous eosin for five minutes, washed in tap water, then dehydrated in ascending grades of ethanol (70%, 80%, 95%) by making ten dips then cleared in changes of xylene and then were mounted in DPX and labelled accordingly.

2.7.4 Interpretation of H&E stained slides: The slides were read using microscope (Olympus Model CX31) at low, medium and high powers and the final diagnoses were confirmed by the senior pathologists (Dr. Edda Vuhahula and Dr. Henry Mwakyoma). This stage addressed the objective number one.

2.7.5 Immunohistochemical staining (MNH protocol was used).

The FFPE tissue blocks were sectioned at four to five micrometres thickness using a microtome and were left to float on the water bath containing distilled water at 45^0C. The slides were deparaffinized in Xylene then rehydrated in Ethanol 100%, 95% and 70%. They were rinsed with running tap water for one hour and a section on the slide was circled using DAKO pen to limit over spillage of the antibody solution. The slides were allowed to dry overnight and then stained. Blocking of endogenous peroxidase activity was done by

incubating sections in 3% H2O2 solution in methanol for 10 minutes and then rinsed in tap water twice for 5 minutes each time. Antigen retrieval was done by using microwave method where by a working solution of BD Pharmingen™ Retrievagen A was prepared by mixing 18mls of solution 1 and 82mls of solution 2. The final volume was mixed with 1 litre of distilled water. The slides were placed in a plastic coplin jar filled with Retrievagen A working solution and then heated in a microwave oven to 203°F (95°C). The Retrievagen A working solution in the coplin jar was mixed using a disposable pipet and then incubated with the slides at 203°F (95°C) for 10 minutes. Then the coplin jar with the slides was removed from the microwave oven and covered tightly, and then the solution was allowed to slowly cool to room temperature for 20 minutes to enable the protein molecules to fold properly. The slides were rinsed in PBS three times for 5 minutes each time. Thereafter, the slides were allowed to cool and cover slipped for interpretation. The reading was done using a microscope at low, medium and high powers.

2.7.6 Interpretation of immunostained slides:

This was done first by the investigator and then reviewed by two senior pathologists (supervisors). The results were considered positive if spiral to rod shaped bacteria appeared brownish stained in their cell membrane. This stage addressed the objective number two.

2.8 Description of Study Variables

The first objective is to describe the histomorphological features of gastric biopsy specimens from patients presented with gastric lesions in relation to age and sex at MNH. In this case the morphological features of gastric specimens presented at histopathology unit were determined to find out the frequency and the pattern of the gastric conditions in the specimens submitted. It was determined using H&E staining method.

i. **Dependent variables**: These were histomorphological features of gastric biopsy specimens from patients presented with gastric conditions at MNH.

ii. **Independent variables**: These were comprised of age and sex.

The second specific objective was to determine the distribution of *H. pylori* by histopathological features among gastric biopsy specimens from patients presented with gastric conditions at MNH. In this regard, *H. pylori* in the gastric tissue specimens were

stained using polyclonal antibody technique in order to look for its relationship to gastric lesions.

Dependent variable: This was comprised of histomorphological patterns of the gastric lesions in relation to presence of *H. pylori*.

Independent variable: This was the presence of *H. pylori*.

2.9 Data Collection Techniques

The preformed data collection sheet was used. It was comprised of;

1. Socio demographic characteristics: age and sex
2. Histological diagnosis:
    i. Inflammatory
    ii. Benign
    iii. malignant
3. *H. pylori* status (positivity)

2.10 Data Analysis

The raw data was captured in the SPSS computer software version 20 and the data was managed electronically in the computerized software program for analysis.

Variables were summarised as mean, median and percentages. A two-tailed P-value <0.05 was considered significant. SPSS computer software version 20 was used for data cleaning, analysis and drawing tables and graphs.

2.11 Validity and Reliability of the study

i. Specimen retrieval

The FFPE tissue blocks to be used in the study were obtained from the archive with the aid of the request forms and registered numbers (histopathological numbers). Thereafter each individual case was given a unique identification number to ensure confidentiality. The blocks were re sectioned and stained using Hematoxylin and Eosin. Afterwards the principle investigator reviewed the diagnosis against the previous one which was then confirmed by a senior pathologist.

ii. Examination and reporting

All slides were initially read by the principal investigator then confirmed by two senior pathologists (E. V and H. M). Each case was given a unique identification number to ensure confidentiality. The findings were recorded on the same collection data sheet as clinical data. The Helicobacter pylori in the immunostained tissue appeared brownish under the microscope.

iii. Data handling

Each case was identified by newly given identification number denoted as SN number. This enabled the cases not to mix up during subsequent processes and reporting. However, in order to ensure the reliability of the data, laboratory specific standard operating procedures were adopted in the entire period of this study and for case of polyclonal anti-*H.pylori* antibody, the procedures followed the MNH standard operating procedures and also adoption of the manufacturer standard operating procedures was taken into account to ensure proper results. Also control cases were included in order to validate the results.

2.12 Quality Assurance

The principle investigator was trained in tissue processing, histotechnology methods and immunohistochemistry. The retrieved tissue blocks were clearly labelled and processed while adhering to standard operating procedure (SOP).

The principle investigator reviewed the histopathological features and diagnosis. The two supervising pathologists independently reviewed the findings.

Data was carefully entered into respective data collection forms to avoid mix-ups.

2.13 Ethical considerations and Approval

The proposal was presented to the Department of Pathology of the Muhimbili University of Health and Allied Sciences for approval. Ethical clearance was granted from the Research and Publication Committee of the School of Medicine and from the Senate Research and Publications Committee of the Muhimbili University of Health and Allied Sciences. Administrative permission to conduct the study was obtained from Muhimbili National Hospital as per the hospital management protocols. The study cases were

identified by histopathological numbers recorded on the record sheets. Confidentiality was observed by provision of each individual case by a unique identification number and unauthorized people had no access to the data collected. The data collected were soon entered into the computer software which was encrypted with password which only the investigator had an access.

2.14 Study limitations

Financial constraints did not allow the use of multiple approaches to staining methods like Modified Giemsa, Silver stains and PCR which would have been very informative in diagnosing *H. pylori* in tissues since each of the method has a different detection rate. However, DNA polymerase chain reaction test would have been better compared to IHC. This would help to suggest which method would have been suitable for our set up. Most of the request forms had no detailed history including site of the biopsy.

CHAPTER THREE

RESULTS

A total of 743 gastric cases were obtained from January 2012 to December 2016. Among which 15 gastric cases were excluded; 8 had no request forms, 5 had no blocks and 2 were crushed. Of 728 gastric cases, 434 were inflammatory lesions, 258 were malignant lesions and 36 were benign lesions. Stratified sampling procedure was used and only 170 gastric cases were selected in a ratio of inflammatory lesion: benign: malignant lesions by 12:1:7 respectively. There were 109 (64%) males and 61 (36%) females, and the age ranged from 4 to 96 years. The mean ages for males and females were 49.11+/-19.58 and 52.13+/-18.05 respectively. There were various gastric pathologies found in this study which included inflammatory lesions 103 (59.5%), benign lesions 9 (5.3 %) and neoplastic conditions 60 (35.3%).

3.1 Demographic Characteristics.

Of the 170 gastric biopsies of patients were analyzed, the majority were males 109 (64 %) and the mean age at encounter was found to be 50 +/- 19.04. The majority of the study participants were more than 40 years of age 123 (72.4%).

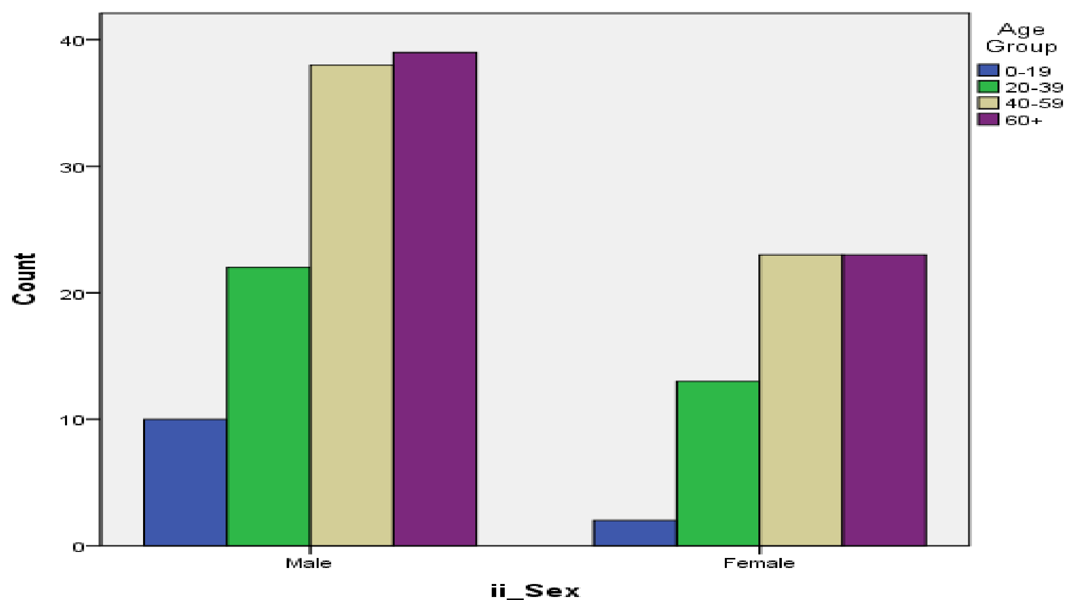

Figure 3: Demographic characteristics of 170 patients with various gastric lesions.

3.2 Histomorphological Features

3.2.1 Histomorphological features of gastric lesions by Age

Of 170 study participants whose gastric biopsies were studied, majority were over 40 years which accounted for 123 (72.3%) cases of which most had inflammatory lesions 71 (41.8%), followed by neoplastic conditions 45 (36.6%). Of the 12 cases aged less than 20 years, 9 (75%) had inflammatory lesions. The results showed that gastric lesions are more common with advancing age (p-value 0.667).

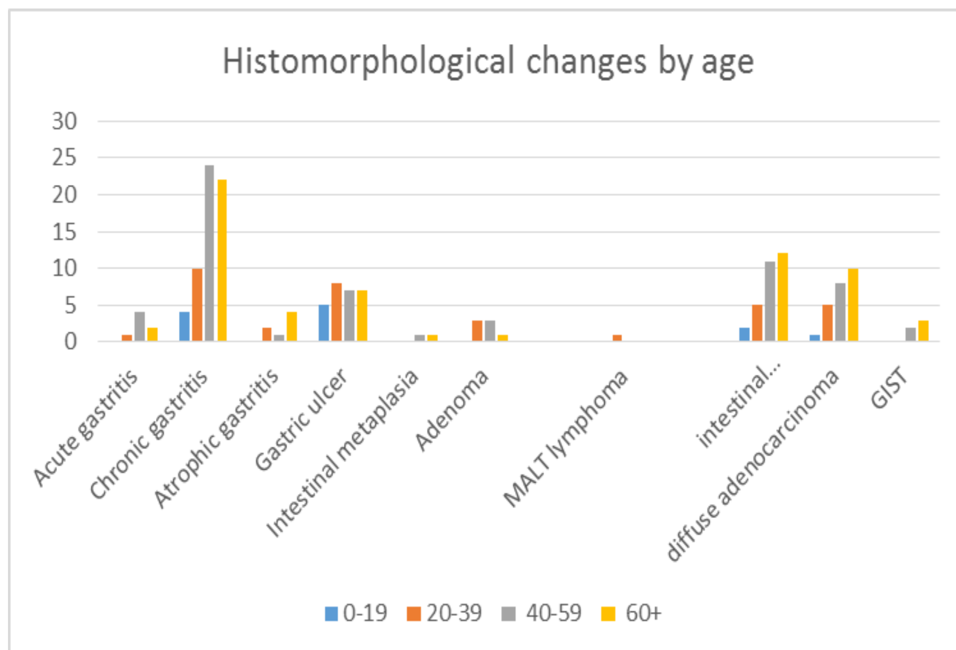

Figure 4: Histomorphological features by age

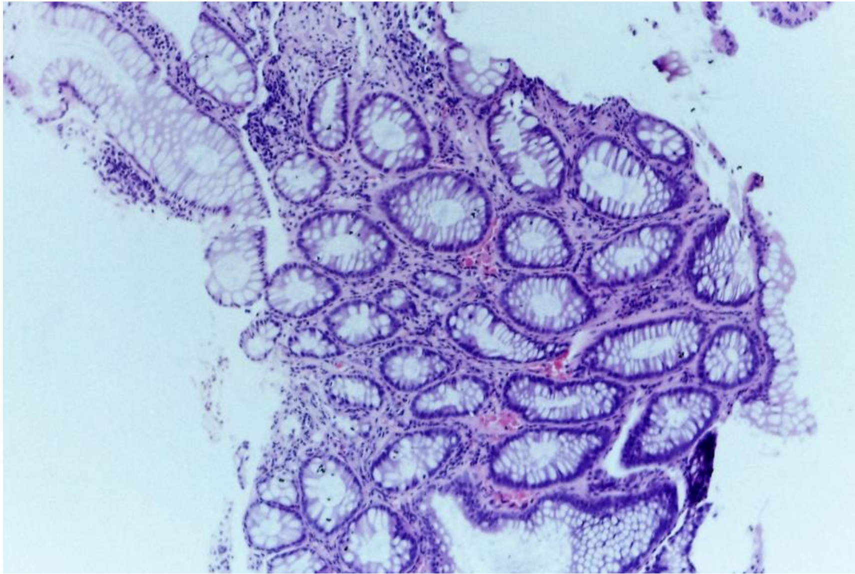

Figure 5: A photomicrograph showing acute gastritis x40 magnification

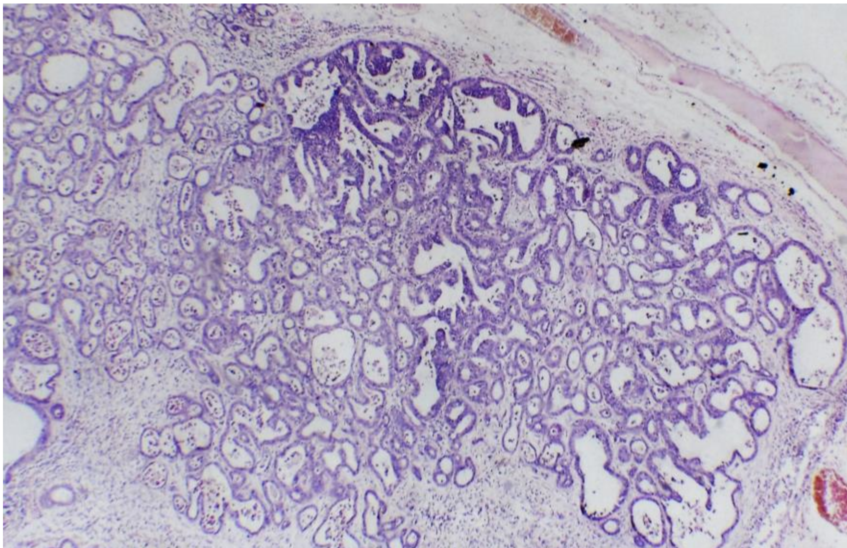

Figure 6: A photomicrograph showing a well differentiated adenocarcinoma x40 magnification

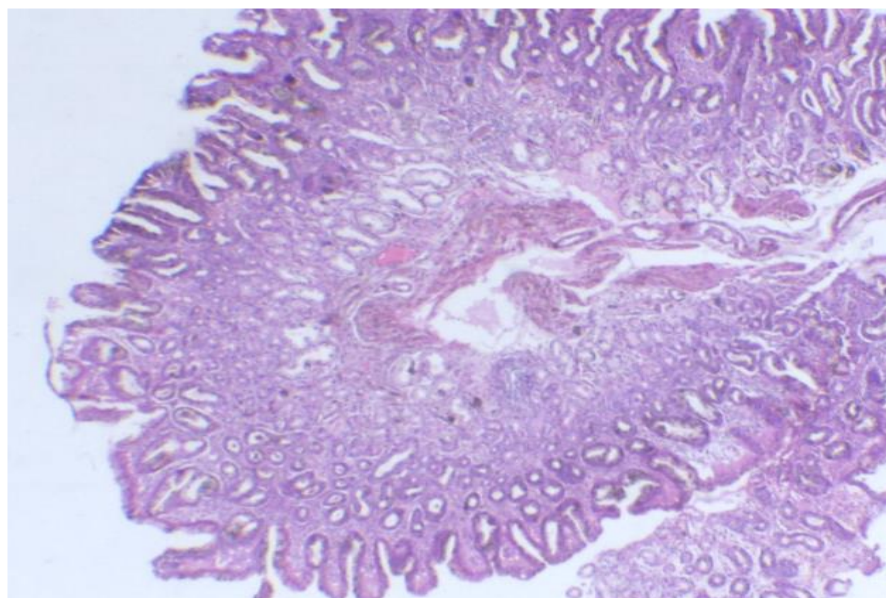

Figure 7: Gastric adenoma ×40 magnification

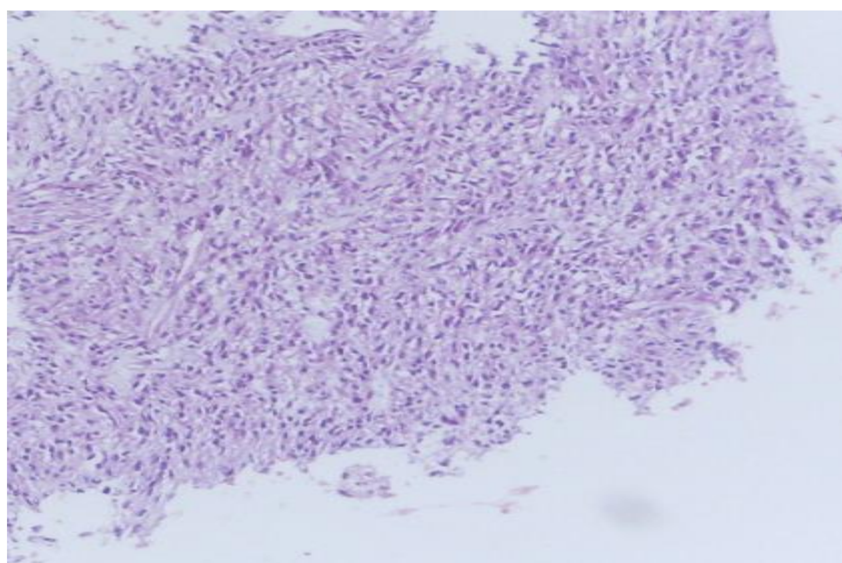

Figure 8: Gastrointestinal stromal tumour of the stomach x100 magnification

3.2.2 Histomorphological features of gastric lesions by sex

Of the 170 gastric cases, the majority were males 109/170 (64%) while females were 61/170 (36%). Of the 109 males with various gastric lesions majority had inflammatory lesions 70 (64.2%) especially chronic gastritis 39/109 (35.78 %) followed by malignant

neoplasms 34 (31.2%), and benign lesions were 4.6%. Gastric adenoma was seen in males which was only benign lesion seen. The majority of female were found to have inflammatory lesions 50.8% while benign lesions were 6.6% (p value was 0.148).

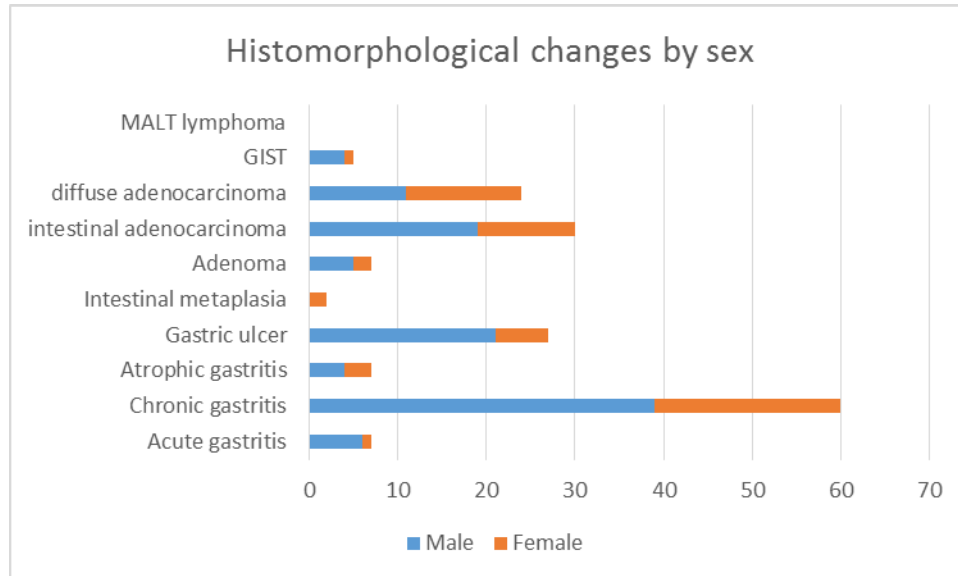

Figure 9: Histomorphological features by gender

3.3 Helicobacter pylori status by histomorphological features of gastric lesion type

A total of 170 gastric biopsies were stained for *H. pylori* and among these 63/170 (37.1%) were positive for *H. pylori*. Out of 63 biopsies which stained for *H. pylori*, 56/63 (88.9%) were inflammatory lesions. In the category of benign lesions which constituted of adenomas, only one adenoma was positive for *H. pylori*. Among all inflammatory lesions, chronic inflammation accounted for 34.7% which stained positive for *H. pylori* followed by gastric ulceration which accounted for 15.8% while the least was acute gastritis whereby only one case was positive for *H. pylori* of 6 cases retrieved. Of 60 cases of malignant lesions only 6 (10%) were stained positive and all of them were adenocarcinoma (p value was <0.01).

Table 1: Helicobacter pylori status by histomorphological features of gastric lesions

Disease category	**Histomorphological lesions**	**Helicobacter pylori status**		
		Positive	Negative	Total
Inflammatory lesion	Acute gastritis	1	6	7
	Chronic gastritis	35	25	60
	Atrophic gastritis	4	3	7
	Gastric ulceration	16	11	27
	Intestinal metaplasia	0	2	2
Benign lesions	Adenoma	1	6	7
Malignant lesions	Intestinal Adenocarcinoma	4	26	30
	Diffuse adenocarcinoma	2	22	24
	MALT lymphoma	0	1	1
	GIST	0	5	5
	Total	63	107	170

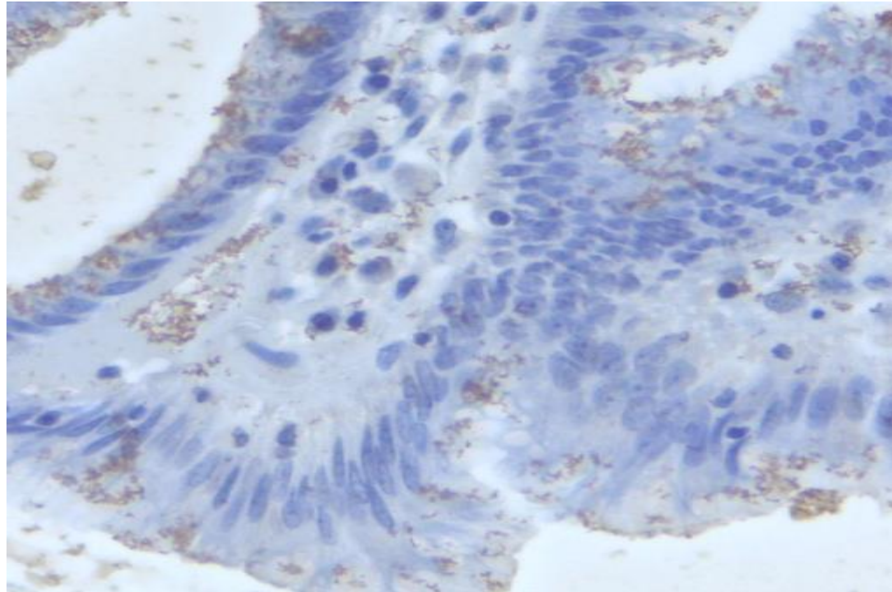

Figure 10: A photomicrograph showing positive staining of *H. pylori* using polyclonal antibody in acute gastritis x100 magnification

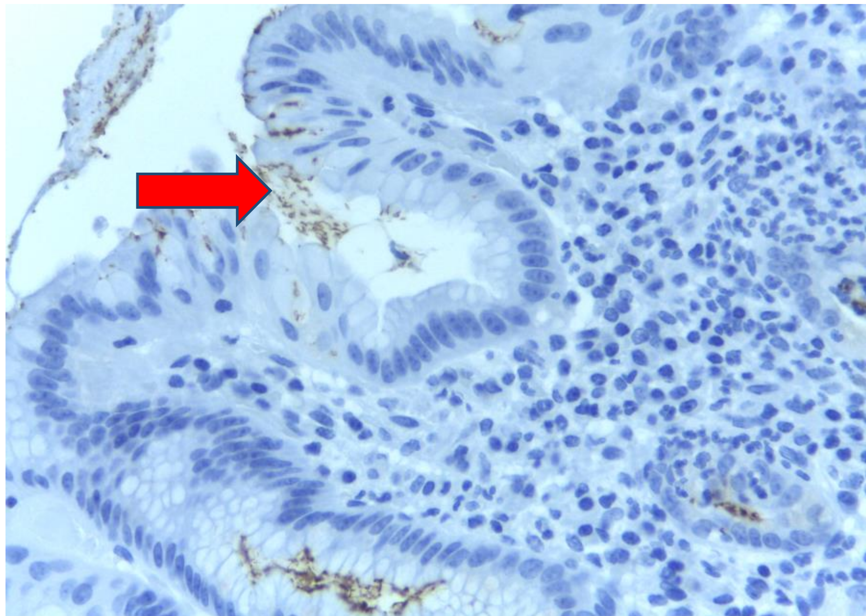

Figure 11: A photomicrograph showing *H. pylori* in a tissue section with gastric ulceration x100 magnification

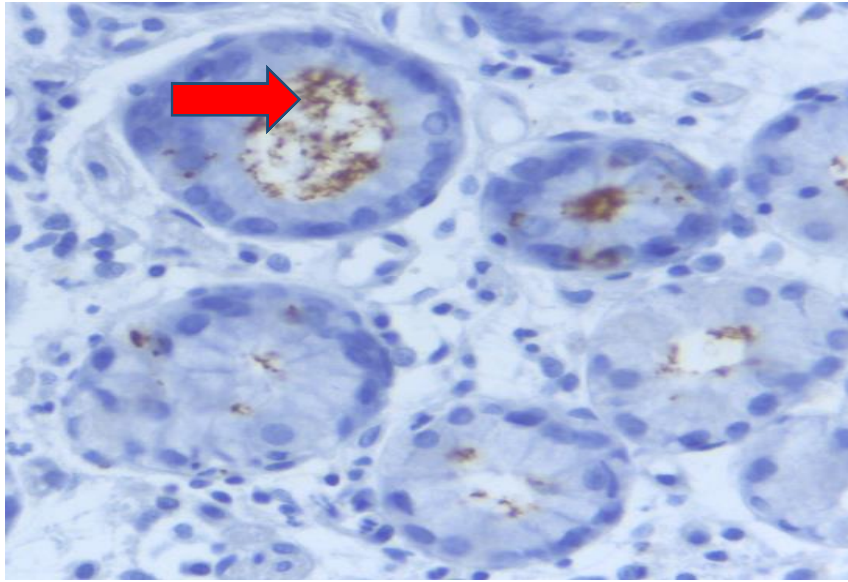

Figure 12: A photomicrograph showing *H. pylori* in a tissue section with chronic gastritis x100 magnification

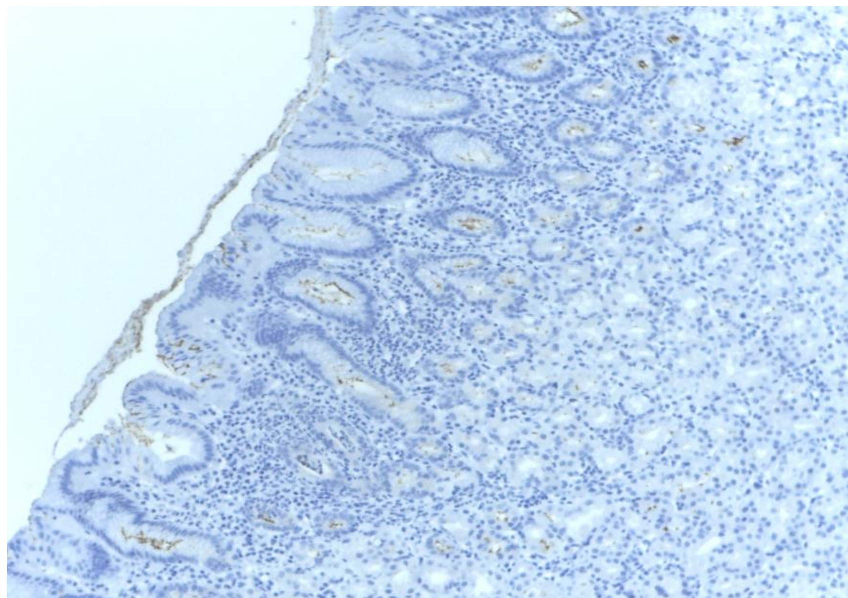

Figure 13: A photomicrograph showing *H. pylori* in a tissue section with chronic gastritis x40 magnification

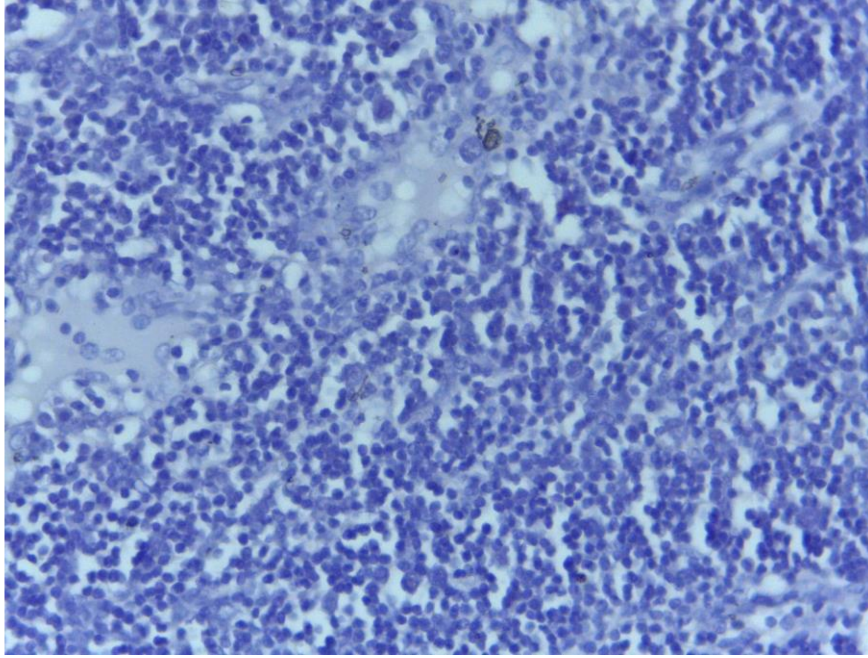

Figure 14: A photomicrograph showing a Mucosal Associated Lymphoid Tissue lymphoma x100 magnification.

CHAPTER FOUR

DISCUSSION

H. pylori infection is among the most common bacterial infection which is associated with various gastric lesions including benign and malignant conditions such as inflammatory conditions, ulceration, adenoma, adenocarcinoma and MALT lymphoma.

In this study, the age distribution of the study participants ranged from 4 to 96 years. This range was similar to that of other studies carried out elsewhere, however, majority of participants were old. About seventy percent of the study participants were more than 40 years, and this age distribution was also seen in other studies done elsewhere (25). In this study, participants aged less than 20 were the minority and most were males. This small number of young participants in this study may be explained by the fact that children get contacted with bacteria and remain asymptomatic through adulthood (6).

The mean age of this study population was 50+/-19.04. This was similar to other studies conducted in Tanzania, Japan and United States of America with mean ages 53.8, 57.9 and 50 respectively (21,37,39). The slight lower mean age of the patients seen in this study may be described by the fact that most of the patients present with late stages clinically. Tanzanian patients seek medical care at late stages of the disease, this may be due to lack of awareness and knowledge on the particular disease. The situation is slightly different from other developed countries where diagnostic equipment are readily available hence most patients tend to be diagnosed at less than 50 years (54).

In this study, all gastric lesions were involved. Inflammatory lesions accounted for the majority of the gastric lesions seen in the patients aged more than 40, and this is similar to what other studies have shown (37,39). This may be explained by the fact that majority of individuals harbour the infection from the childhood but clinical symptoms tend to manifest during the adulthood. This is because *H. pylori* has a chronic course in which clinical symptoms may show after a long period of time of acquiring the infection (6,8,41,55,56).

Gastric carcinomas affected older patients more than younger ones.

In this study, there were more males (64%) than females. The pronounced male predominance was observed among patients with inflammatory lesions. Similar findings were also seen in other studies (20,21,25,37,39,42,45,50), however, this was not possible to elucidate, but other studies have stated the reason may be due to differences in health seeking behaviours among males. As compared to females, more males were found to have inflammatory lesions more than other lesions.

In this study gastric lesions were seen more in males than in females. Similar findings were found in Japan where gastric lesions were more common in males, however, the methodology used was quite different where only patients with gastritis from the family with familial adenomatous polyposis were included (45).

This study showed that chronic inflammatory lesions accounted for the most of the inflammatory lesions diagnosed in both sexes. The study findings were similar to other studies conducted in other areas (3,4,16,37, 48), however, the study done in Croatia by Maliha et al showed female predominance.

More females were found to have malignant lesions especially adenocarcinoma than males in this study. To the limit of my knowledge, this may be explained by different levels of exposure to the risk factors based on different geographical locations (35), and most of African populations seek medical care at late stages where the disease is already advanced (25).

In this study, none of the male participants had intestinal metaplasia or MALT lymphoma. From this findings there is a probability that patients are not screened to find out the precancerous lesions and they seek medical care while have already transformed into malignancy. Out of all participants, only one female had MALT lymphoma, and this may probably be probably due to less number of the lesions or are misdiagnosed.

It has been shown that males and females are slightly equally affected with atrophic gastritis in this study, which will later lead to malignancy, and this may explain the slight difference observed in adenocarcinomas among males and female.

H. pylori is associated with various gastric lesions such inflammatory lesions, benign tumours such as adenoma and malignant tumours such as adenocarcinoma and MALT

lymphoma (6,41,55,56). The slightly low prevalence (37%) of gastric lesions were found to be *H. pylori* positive. This may be explained by the fact that, in this study all gastric biopsies were stained for *H. pylori* in which it was not expected to be in some lesions. However, high prevalence of this bacteria was found in Ugandan and Japanese patients where only patients with dyspepsia and those who were on medications were included and excluded respectively (10,16).

Malignant lesions (9.5%) accounted for the least of all the gastric lesions with *H.pylori* infection, however, normal section rather than the malignant sections of the gastric biopsies had *H. pylori* infection. This can be explained by the natural history of the bacteria where it can't survive in the inappropriate balanced pH environment following destruction of parietal cells by malignancy (6).

It has been documented that *H. pylori* causes intestinal metaplasia and adenoma, however, in this study no *H. pylori* were detected in the lesions using immunohistochemistry. This may be explained by a small number of those gastric biopsies. This finding is similar to the study done in Iran (20).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

H. pylori infection is a bacterial infection most frequently associated with chronic inflammation and was identified at a prevalence lower than described in the literature. Immunohistochemistry is a reliable technique for the detection of *H.pylori* and it was more sensitive than H&E alone. Immunohistochemistry is expensive to be done on each case of gastric lesion hence it should be performed on the inflammatory lesions especially the chronic inflammation.

Additional studies involving histochemical stains and DNA-PCR are needed to determine the prevalence of *H. pylori* infection in the general population.

5.2 Recommendations

The diagnosis of *H. pylori* in our set up is very crucial as 37% of patients were found to have harbour the bacteria. The gastric biopsies are routinely processed and reported in the histopathology unit at Muhimbili National Hospital. Some cases of gastric lesions in this study were found to have *H. pylori* infection on routine Hematoxylin and Eosin staining, which were not diagnosed previously. We recommend the pathologists at Muhimbili National Hospital to concentrate more on chronic inflammatory lesions in order to isolate *H. pylori* when it comes to gastric specimen submitted to histopathology unit especially those with chronic gastritis in patients with dyspeptic symptoms. We observed the late presentation of patients to hospital in this study, hence, other rapid and cheap methods such as giemsa staining, toluidine staining and other silver staining methods should be validated in our set up for early and accurate diagnosis.

REFERENCES

1. Garza-gonzález E, Perez-perez GI, Maldonado-garza HJ, Bosques-padilla FJ, Garza-gonzález E, Maldonado-garza HJ. A review of Helicobacter pylori diagnosis , treatment , and methods to detect eradication. WJG 20th Anniv Spec Issues(6). 2014;20(6):1438–49.

2. Genta RM, Hamner HW, Graham DY. Gastric lymphoid follicles in Helicobacter pylori infection: Frequency, distribution, and response to triple therapy. Hum Pathol. 1993;24(6):577–83.

3. Tu T, Lee C, Wu C, Chen T, Chan C, Huang S, et al. Comparison of invasive and noninvasive tests for detecting Helicobacter pylori infection in bleeding peptic ulcers. Gastrointest Endosc J. 1999;49(3):18–20.

4. Cherian S, Forbes D, Sanfilippo F, Cook A, Burgner D. The epidemiology of Helicobacter pylori infection in African refugee children resettled in Australia. MJA 2008. 2008;189(8):6–9.

5. Fock KM, Ang TL. Epidemiology of Helicobacter pylori infection and gastric cancer in Asia. J Gastroenterol Hepatol 25. 2010;25(2010):479–86.

6. Kusters JG, Vliet AHM Van, Kuipers EJ. Pathogenesis of Helicobacter pylori Infection. Clin Microbiol Soc. 2006;19(3):449–90.

7. Mitchell H, Mégraud F. Epidemiology and diagnosis of Helicobacter pylori infection. Helicobacter. 2002;7(Supplement 1):8–16.

8. Mbulaiteye SM, Gold BD, Pfeiffer RM, Brubaker GR, Shao J, Biggar RJ, et al. H . pylori -infection and antibody immune response in a rural Tanzanian population. Infect Agent Cancer. 2006;7(Sept 2006):1–7.

9. Palaniappan V, Janarthanam V, Swaminathan K. Histomorphological profile of Gastric antral mucosa in Helicobacter associated gastritis . Int J Currres Med Sci 2. 2016;2:22–8.

10. Roberts BCA. Immunohistochemistry Detection of Helicobacter Pylori. Lancet.

2004;64(2):1–6.

11. Shimizu T. lmmunohistochemical Detection. 1996;Number4.19(Blackwell Science, Inc. 1083-4389/96/$ IO.501 I97 197-206):197–206.

12. Wotherspoon AC, Diss TC, Pan L, Isaacson PG, Doglioni C, Moschini A, et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet. 1993;342(8871):575–7.

13. Y HC, Hartzen SH, Roshanisefat H, Percival L, Angeliki K. Molecular methods for typing of Helicobacter pylori and their applications. FEMS Immunol Med Microbiol. 1999;24.

14. Duynhoven YTHP Van, Jonge R De. Transmission of Helicobacter pylori : a role for food ? 2001;79(99):455–60.

15. Rugge M, Pennelli G, Pilozzi E, Fassan M, Ingravallo G, Russo VM, et al. Gastritis : The histology report on Helicobacter pylori among patients presented with gastritis. Dig Liver Dis. 2011;43S(2011):S373–84.

16. Lee JY, Kim N. Diagnosis of Helicobacter pylori by invasive test : histology. Ann Transl Med. 2015;3(1):1–8.

17. Wabinga HR. Comparison of immunohistochemical and modified Giemsa stains for demonstration of Helicobacter pylori infection in an African population. Afr Health Sci. 2002;2(2):52–5.

18. H. J. Epple, F. W. Kirstein, C. Bojarski, J. Frege, M. Fromm E 0. R&, Schulzke JD. Helicobacter pylori -induced epithelial cell signalling in gastric carcinogenesis. Brazilian J Microbiol. 2003;76(2990):23–34.

19. Tomb J, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, et al. The complete genome sequence of the gastric pathogen Helicobacter pylori. Nature. 1997;389(September):1211–2.

20. Tajalli R, Nobakht M, Mohammadi-Barzelighi H, Agah S, Rastegar-Lari A,

Sadeghipour A. The immunohistochemistry and toluidine blue roles for Helicobacter pylori detection in patients with gastritis. Iran Biomed J. 2013;17(1):36–41.

21. Fujiya K, Nagata N, Uchida T, Kobayakawa M, Asayama N, Akiyama J, et al. Different gastric mucosa and CagA status of patients in India and Japan infected with helicobacter pylori. Dig Dis Sci. 2014;59(3):631–7.

22. David Y.Graham CO. Pathology of Helicobcater pylori;virulence factors. Suppl Pathol 1. 2000;5(1083–4389):1–4.

23. Joossens J V, Hill MJ, Stamler R, Stamler J, Lesaffre E, Dyer A, et al. Dietary Salt , Nitrate and Stomach Cancer Mortality in 24 Countries. Int Journal Epidemiol. 1996;25(3).

24. Uemura N et al: H. pylori infection and the development of gastric cancer. Keio J Med. 2002;51:63–8.

25. `Ayana SM, Swai B, Maro VP, Kibiki GS. Upper gastrointestinal endoscopic findings and prevalence of Helicobacter pylori infection among adult patients with dyspepsia in northern Tanzania. Tanzania Journal Heal Res. 2014;65:87-92

26. Joo M, Ji EK, Sun HC, Kim H, Chi JG, Kim KA, et al. Helicobacter heilmannii-associated gastritis: Clinicopathologic findings and comparison with Helicobacter pylori-associated gastritis. J Korean Med Sci. 2007;22(1):63–9.

27. Laine L, Lewin DN, Naritoku W, Cohen H. Prospective comparison of H & E , Giemsa , and Genta stains for the diagnosis of Helicobacter pylori. Gastrointest Endosc J. 1997;45(6):463–7.

28. Wilcox MH, Dent THS, Wraight EP, Brown DFJ, Wight DGD. Accuracy of serology for the diagnosis of Helicobacter pylori infection- a comparison of eight kits. J Clin Pathol. 1996;49(2):373–6.

29. Tv P, Ravikumar V, Km U. Detection of Helicobacter Pylori By Invasive Methods and Evaluation of Rapid Urease Test in a Dyspeptic Patient'S in and Around

Hosakote, Bangalore Rural, South India. Int J Basic Appl Med Sci. 2014;4(1):374–7.

30. Khalifehgholi M, Shamsipour F, Ajhdarkosh H, Daryani NE. Comparison of five diagnostic methods for Helicobacter pylori. Iran J Microbiol. 2013;5(4):396–401.

31. Roberts CA. Immunohistochemistry Detection of Helicobacter Pylori. Nationslab. 2013;78:67-90

32. Lee JY, Kim N. Diagnosis of Helicobacter pylori by invasive test: histology. Ann Transl Med [Internet]. 2015;3(1):10. PMC4293485

33. Dogar T, Khan SA, Jaffer R, Majid S, Qureshy A. Identification of Helicobacter Pylori in Gastric Biopsies : a Comparison of Haematoxylin and Eosin Staining With Immunohistochemistry. Biomed J Sci. 2012;28(2012):121–5.

34. Calik Z, Karamese M, Acar O, Aksak S. Investigation of Helicobacter pylori antigen in stool samples of patients with upper gastrointestinal. Brazilian J Microbiol [Internet]. 2015;47(1):167–71.

35. Sheikhaman, Ataherian D. prevalence and risk factors for hpylori IRAN.pdf. Khorramabad: A. Sheikhian; 2008. p. 56–78.

36. Haapiainen R, Kokkola A, Rautelin H, Puolakkainen P, Sipponen P, Fa M, et al. Diagnosis of Helicobacter pylori Infection in Patients with Atrophic Gastritis: Comparison of Histology, 13 C-Urea Breath Test, and Serology ¨. Scand J Gastroenterol. 2000;2(18).

37. Aoki K, Kihaile PE, Castro M, Disla M, Nyambo TB, Misumi J. Seroprevalences of Helicobacter pylori Infection and Chronic Atrophic Gastritis in the United Republic of Tanzania and the Dominican Republic. Environ Health Prev Med. 2004;9(July):170–5.

38. Satoh K, Kimura K, Taniguchi Y, Kihira K, Takimoto T, Saifuku K, et al. Biopsy Sites Suitable for the Diagnosis of Helicobacter pylori Infection and the Assessment of the Extent of Atrophic Gastritis. Am J Gastroenterol. 1998;93(4):569–73.

39. Cutler AF, Havstad S, Ma CK, Blaser MJ, Perez-perez GI, Schubert TT. Accuracy of Invasive and Noninvasive Tests to Diagnose Helicobacter pylori Infection. J Gastroenterol. 1995;12(1995):36–141.

40. Demetter P. Classification of gastritis. Misiewicz JJ, J Gastroenterol Hepatol 1991. 1991;64(1995):1–3.

41. Frenck RW, Fathy HM, Sherif M, Mohran Z, Mohammedy H El. Sensitivity and Specificity of Various Tests for the Diagnosis of Helicobacter pylori in Egyptian Children. Paediactrics J. 2006;118(4):e1195-1201.

42. Huang JIAQ, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the Relationship Between Helicobacter pylori Seropositivity and Gastric Cancer. J Gastroenterol. 1998;114(1998):1169–79.

43. Meliha K. The Relationship of Helicobacter pylori Positivity with Age , Sex , and ABO / Rhesus Blood Groups in Patients with Gastrointestinal Complaints in Turkey. Helicobacter ISSN. 2007;2(2007):244–50.

44. Ame P, East F. Hpylori Antibiotic Resistance: Prevalence, Importance, and Advances in Testing. group.bmj.com. 2004;53(2004):4–64.

45. Clyne M, Drumm B. Impact of Helicobacter pylori infection and Mucosal atrophy on gastric lesions in patients with familial adenomatous polyposis. Microbiology. 2004;72(9):5464–9.

46. Miljk B. Otkrivanje Helicobactera pylori u uzorcima dobijenim biopsijom i resekcijom ž e luca. UDC J. 2005;62(9):39–43.

47. Faller G, Steininger H, Kranzlein J, Maul H, Kerkau T, Hensen J, et al. Antigastric autoantibodies in Helicobacter pylori infection: implications of histological and clinical parameters of gastritis. Gut. 1997;41(5):619–23.

48. Al-khatib AJ, Abu-zaiton AS. The Prevalence of Helicbacter Pylori Among Patients Complaining from Abdominal Pain. Eur Sci J. 2013;9(30):358–63.

49. Yong X, Tang B, Xiao Y, Xie R, Qin Y, Luo G, et al. Helicobacter pylori

upregulates Nanog and Oct4 via Wnt / β-catenin signaling pathway to promote cancer stem cell-like properties in human gastric cancer. Cancer Lett [Internet]. 2016;374(2):292–303.

50. Wang F, Sun GP, Zou YF, Zhong F, Li XQ, Wu D. Helicobacter pylori infection predicts favorable outcome in patients with gastric cancer. Curr Oncol. 2013;20(2013):388–95.

51. Ohnita K, Isomoto H, Mizuta Y, Maeda T, Haraguchi M, Miyazaki M, et al. Helicobacter pylori infection in patients with gastric involvement by adult T-cell leukemia/lymphoma. Am Cancer Soc. 2002;94(5):1507–16.

52. Genta RM, Hamner HW, Graham DY, Wotherspoon AC, Diss TC, Pan L, et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. Hum Pathol. 1993;342(8871):575–7.

53. Vilaichone RK, Mahachai V, Shiota S, Uchida T, Ratanachu-Ek T, Tshering L, et al. Extremely high prevalence of Helicobacter pylori infection in Bhutan. World J Gastroenterol [Internet]. 2013;19(18):2806–10.

54. Godkhindi VM, Meshram DP, Deshpande SA, Kadam PN, Chavan YH. The Histopathological Study Of Various Gastro-duodenal Lesions and Their Association with Helicobacter pylori Infection . 2013;4(3):51–5.

55. Naja F, Mph NK, Sullivan T, Naja F, Kreiger N, Helicobacter TS. Helicobacter pylori infection in Ontario : Prevalence and risk factors. 2007;21(8):501–6.

56. Antonia R. Sepulveda, MD, PhD; Madhavi Patil M. Practical Approach to the Pathologic Diagnosis of Gastritis. Arch Pathol Lab Med. 132:17–9.

APPENDICES

Appendix I: Checklist Guide

Serial no:- **-……………………………..**

H/P no:- **-……………………………..**

Date: **-……………………………..**

1. Age **-……………………………..**

2. Sex **-……………………………..**

3. Histomorphological gastric lesions using Hematoxylin and Eosin

   i. Acute gastritis…………………..

   ii. Chronic gastritis……………………..

   iii. Atrophic gastritis………………………..

   iv. Peptic ulceration…………………….

   v. Mucosal associated lymphoid tissue lymphoma…………….

   vi. Gastric adenocarcinoma………………………..

   vii. Others………….(Mention)

4. Helicobacter pylori status

   i. Positive

   ii. Negative

Appendix II: Standard operating procedure (SOP) guidelines

SOP for H&E histochemistry

Table 2: SOP for H&E histochemistry

1.0 Purpose	To provide instructions during hematoxylin and eosin staining.
2.0 Scope	This procedure is applicable during manual Hematoxylin and eosin staining at Muhimbili National Hospital, Central Pathology Laboratory.
3.0 Responsible staff	The head of Unit Histopathology is responsible for ensuring the effective implementation and maintenance of this procedure.
4.0 Principle	Tissue structures contain groups of cells that are made up of the nucleus and cytoplasm. The nuclei of the tissues which are acidic in nature (due to their nucleic acid content i.e. DNA and RNA) have the affinity for basic dyes. Haematin is the oxidation product of Hematoxylin. When used in conjunction with a mordant (e.g. Potassium alum which is included in Hematoxylin solution) it will provide a stable link called lake which binds to the acid phosphate groups of DNA and RNA and stain the nuclei into blue colour. The cytoplasm on the other end is basic in nature and will have an affinity for the acidic dyes. Eosin which is acidic in nature is the most suitable stain to combine with alum- Hematoxylin for demonstration of cytoplasm architecture by staining it red/shades of pink. It is vital to use the correct concentration of reagents such as 1% HCL acid in 70% ethyl alcohol for differentiating the stains to avoid undesired staining results.
5.0 Reagents and Supplies	• Xylene • Harris Hematoxylin • 1%Acid in 70%ethyl Alcohol • Scott’s Tap Water Substitute • 1% aqueous Eosin Staining Solution

	• Absolute ethyl alcohol • DPX. (Mountant). • Containers for Reagents and Solutions • Gloves • Laboratory coat. • Cover slips. • Frosted Slides
6.0 Reagent preparation	Refer to reagents preparation sop HISTO no 29
7.0 Equipment	• Light microscope • Timer
8.0 Sample and Container Types	Histological tissue sections
9.0 Special Safety Precautions	• All specimens should be considered as potentially infectious. • Xylene is carcinogenic; always wear gloves, mask and goggles when handling it.
10.0 Quality control	• Positive control - Well processed tissue which should be cut and stained each week and every time when the reagents are changed. • Negative control - poor processed tissue which should be cut and stained each week and every time when the reagents are changed.
11.0 Detailed procedures	1. Dewax sections and bring down to water. 2. Stain in Harris hematoxylin for 10 minutes. 3. Rinse in running tape water. 4. Differentiate in 1% acid alcohol for 5-10 seconds. 5. Rinse in tap water 6. Blue the sections in tap water for 10 minutes. 7. Counter stain in 1% aqueous eosin for 3 minutes. 8. Rinse in tap water.

	9. Dehydrate the sections through ascending grades of alcohol 10. Clear in two changes of xylene (3 minutes in each) 11. Mount with DPX or mounting machine.
13.0 Interferences and Limitations	• Poor preparation of reagents. • Poor storage of stains. • Poor quality of tissue sections
14.0 Results Interpretation	• Nuclei - Will stain blue color • Cytoplasm and intercellular substances - shades of pink and red • Cells with much RNA or acid mucopolysaccharide - Purplish

SOP for Immunoperoxidase stains

Table 3: SOP for Immunoperoxidase stains

1.0 Purpose	To provide instructions during manual immunohistochemistry staining.
2.0 Scope	This procedure is applicable during immunohistochemistry staining at Muhimbili National Hospital, Central Pathology Laboratory
3.0 Responsible staff	• Qualified and trained Medical Laboratory Technicians, Technologists and scientists are responsible for doing this test procedure. • The head of Unit Histopathology is responsible for ensuring the effective implementation and competency assessment for this procedure.
4.0 Principle	Immunohistochemistry (IHC) is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions. By the employment of a detection system and a chromogenic end product, these antigen-antibody complexes can be visualized under light microscopy.
5.0 Reagents and Supplies	• Antigen Retrieval solution • TBS (wash buffer) • Peroxidase blocking solution • Different types of primary antibodies • HRP • DAB • Harris hematoxylin • 1% HCl in 70% alcohol • Pressure cooker • Electrical hot plate. • Slide Humidity chamber • Containers for Reagents and Solutions • Gloves • Laboratory coat.

	• Cover slips. • Timer • Microtome • PAP pen
7.0 Reagent Preparation	**1. Antigen retrieval solution** Antigen retrieval solution …………. 150 ml Distilled water …………..1350 ml 2. **Hydrogen peroxide block of endogenous peroxide**. Distilled water ……………..388 ml Hydrogen peroxide ……………… 12 ml 3. **DAB**. Substrate buffer ……….. 1 ml DAB ………. 1 drop.
7.0 Equipment	• Microtome • Light microscope • Timer
8.0 Sample and Container Types	Histological tissue sections.
9.0 Special Safety Precautions	• All human samples are potentially infectious HANDLE WITH CARE. • Hydrogen peroxide causes burns, and is highly oxidizing, always wear gloves and face protection when handling. • DAB is known CARCINOGENIC handle with EXTREME CARE, always wear gloves, clean up spills immediately; neutralize and discard down sink. • Pressure cooker causes severe burns, wear gloves and face mask when handling boiling liquids.

10.0 Quality control	1. Quality control of Primary Antibodies. New primary antibodies must be worked up to establish optimum titre, antigen retrieval and optimum control tissue. Data relating to these issues and guidance for antibody work up is provided by the manufacturer in the form of a specification sheet. A 3-step titration of the diluted antibody is performed using the manufacturer's suggested optimum diluted range, antigen retrieval method if necessary and appropriate control tissue. These details are found on the specification sheet accompanying the antibody. Primary antibodies may be re-titred if results appear to be drifting from the expected staining quality. This drift may include weak staining, increased background staining and new control tissue not staining optimally. 2. Control tissue. The procedure is a quality controlled by staining known positive control sections for the test antigen under analysis. This control tissue is obtained from previous test tissue that has been assessed and shown to demonstrate the antigen to an optimum level. Note that some controls may be difficult to obtain and may not be ideal. This should be taken into account when assessing results. A negative control (no primary antibody applied to the test section) is also run to assess background staining from endogenous peroxidase or cross reaction from the detection kit. The control tissue sections are cut at the same time as the test sections and if possible placed on the same slide. The control is placed on the bottom half of the slide; this minimizes wash time disparities between test section and control.
11.0 Detailed procedures	• Cut on Dako slides and bake for 30 minutes on hot plate. • Dewax sections in xylene and rehydrate to tape water. • Ring sections with PAP pen and add 2-3 drops of peroxidase blocking solution, leave it for 15 minutes.

<table>
<tr><td></td><td>

- Place slides in tap water.
- Heat antigen retrieval solution or citrate buffer in pressure Cooker until it starts boiling.
- Place slides in antigen retrieval solution in pressure cooker and close the lid.
- Time for 2 minutes at full pressure
- Remove slides from pressure cooker and place in water. Do not allow slides to dry from this step onwards.
- Add Tris Buffer Saline (TBS) for 3 minutes.
- Drain off TBS and cover sections with primary antibody at appropriate dilution and incubate for 60 minutes.
- Wash gently TBS on slide for 5 minutes
- Drain off TBS from slides and cover sections with 2-3drops of Horse raddish Peroxidase (HRP) for 30 minutes, the reagent should be at room temperature before use.
- Wash gently for 5 minutes with TBS (make up DAB reagent by adding one drop of DAB to 1 ml of substrate buffer)
- Drain TBS from slides and apply working DAB reagent to the sections and incubate for 5 minutes.
- Wash slides well in water
- Counterstain with haematoxylin for 30 seconds, dip in 1% acid alcohol 2 dips.
- Blue in tap water for 2 minutes.
- Dehydrate, clear in xylene and mount with DPX.

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<tr><td>12.0 Interferences and Limitations</td><td>The limitations of this procedure relate to the specificity and sensitivity of the primary antibodies used and the quality of the tissue sections.</td></tr>
<tr><td>13.0 Results Interpretation</td><td>

- DAB - Brown (intensity reflects amount of antigen present)
- Nuclear counter stain - Blue

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