HISTOPATHOLOGICAL PATTERNS AND HELICOBACTER PYLORI STATUS OF GASTRIC LESIONS AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM, TANZANIA

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By

Leonard Mlemwa

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Medicine in Anatomical Pathology of

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for the examination of dissertation entitled "**Histopathological patterns and Helicobacter pylori status of gastric lesions at Muhimbili National Hospital,Dar es salaam, Tanzania"** in fulfilment of the requirements for the degree of Master of Medicine (Anatomical Pathology) of Muhimbili University of Health and Allied Sciences.

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Prof. E.E. Kaaya

(Supervisor)

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Date

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Dr. H.A. Mwakyoma

(CO-Supervisor)

_____

Date

DECLARATION AND COPYRIGHT

I, Leonard Mlemwa, 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.

Signature_____ Date_____

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DEDICATION

To Almighty God

To my beloved Parents

To my lovely wife

To my beloved children

To all my teachers

To all my friends and colleagues

I dedicate this work.

ABSTRACT

Background: Gastric cancer is the second leading cause of cancer-related deaths and the fourth most common cancer worldwide. Its frequency in different geographical areas is markedly variable. Etiologically, the majority (>90%) of malignant gastric lesions are the sporadic type mostly caused by long-standing inflammatory premalignant conditions. The more differentiated variant, the intestinal-type adenocarcinoma, is closely linked with Helicobacter pylori (H. pylori) infection and is associated with well-characterized dysplastic precursor premalignant lesions. There is a scarcity of data regarding gastric lesions at Muhimbili National Hospital and Tanzania in general. **This study was carried out to describe the histomorphological patterns of gastric lesions, their relationship with the age of patients, sex, anatomical tumour locations and to determine the frequency of H. pylori infection.**

Methods: This was a prospective hospital-based cross-sectional study of gastric lesions received at Histopathology unit of Muhimbili National hospital between July 2018 and February 2019. Gastric tissue biopsies were stained for routine H&E and Modified Giemsa stain for H. Pylori detection. A designed data collecting sheet collected data and analyzed by using SPSS computer software version 23.0.

Results: A total of 189 cases of gastric lesions were registered in the study with a male to female ratio of 1.5:1. **The mean age of patients was 53(SD±15.881) years, with age ranging from 17 to 85 years**and the majority were >40 years (76.2%). Majority of cases had inflammatory lesions (56.6%) with chronic gastritis dominating. Gastric adenocarcinoma was the most common gastric malignancy (86.6%) with its Intestinal type the most common in patients with < 40 years of age (p=0.019). The antrum was the most frequent anatomical site for both**inflammatory, benign and malignant**lesions (42.3%). The frequency of H. Pylori was 24.9% of all cases, and among the positive cases, 91.5% were inflammatory lesions.

Conclusion: Gastric lesions are common conditions in Tanzania, affecting persons from childhood to late adulthood. They are represented by chronic gastritis for inflammatory lesions and adenocarcinoma for malignant tumours, respectively. Males are moreaffected than females. Gastric cancer occurs mostly in adults of more than 40 years of age; however, intestinal-type adenocarcinoma shows a tendency towards relatively young age at diagnosis. H. pylori infection is more common in patients with chronic gastritis.A significant proportion of Tanzanian population with gastric cancer has precursor inflammatory and benign lesions, which can be discovered and treated earlier before giving rise to cancer.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABREVIATIONS

AG	Atrophic gastritis
CG	Chronic gastritis
DNA	Deoxyribonucleic acid
GC	Gastric cancer
GI	Gastrointestinal
H&E	Hematoxylin and Eosin stain
HRP	Horseradish Peroxidase
IARC	International Agency for Research on Cancer (IARC)
IM	Intestinal metaplasia
MNH	Muhimbili National Hospital
MUHAS	Muhimbili University of Health and Allied Sciences
OR	Odds Ratio
PAP	Papanicolaou
PPI	Proton Pump Inhibitors
SOP	Standard Operation Procedure.
USA	United States of America
WHO	World Health Organization

DEFINITION OF TERMS

Chronic gastritis: Is a long-term condition in which the mucus lined of the stomach (gastric mucosa) is infiltrated by mononuclear inflammatory cells and irritated for long time.

Atrophy: Is a decrease in size, wasting away or progressive decline of body part ortissue

Dysplasia: The presence of cells of an abnormal type within the tissue which signify a stage preceding the development of cancer

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

Gastric ulceration: Is an open sore that develop in epithelial mucosal lining of the stomach

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

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

CHAPTER ONE

1. 0INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Gastric cancer (GC) is the second leading cause of cancer-related deaths and the fourth most common cancer worldwide (1,2,3,4,5). Its prevalence in different geographical areas is markedly variable. Etiologically, gastric cancer is associated with Helicobacter pylori (H. pylori) infection, dietary, lifestyle factors, and genetics (2).

The majority (approximately 90%) of malignant gastric lesions are adenocarcinomas, but there are also other types, including Mucosa-Associated Lymphoid Tissue (MALT) lymphoma, and gastric stromal related leiomyosarcomas(3). Annually, around 990,000 people are diagnosed with GC worldwide, with a mortality of approximately 738,000, thus making GC the highest contributor of cancer burden, as indicated by disability-adjusted life years lost (1,4).

Approximately 10% of GC are hereditary, but genetic factors involved in familial malignancies remain mostly elusive, although apparent mutations have been well-characterized in a subgroup of patients (5). Thus, the majority of GCs are sporadic, most of them caused by long-standing inflammatory conditions primarily due to infections, chemical agents or autoimmune diseases causing unidentified interplay of molecular and phenotypic imbalances (7).

The Lauren classification, which histomorphologically categorizes gastric cancer into intestinal and diffuse types, is used in epidemiological studies revealing that the intestinal type of GC, is linked closely to H. pylori infection. Also, well-characterized dysplastic precursor lesions such as Chronic gastritis (CG),Gastric epithelial dysplasia (GED), Gastric atrophy (GA) and Intestinal metaplasia (IM) (1,2,3,6).

Recently, gastric cancer has shown a change in trend and presentation both clinically and histologically and is increasingly seen in different age groups in the black population, mostly in resource-limited countries, including Tanzania. In these countries, the affected patients present with high-grade cancer and at advanced stage hence poor prognosis (4, 9,10).

Helicobacter pylori bacteria infects over half the world's population, making it one of the most successful bacterial pathogens (14). Several studies have indicated that infection rates are highest in developing countries, and gastric colonization is usually lifelong. Unless the infection is treated, this ultimately leads to peptic ulcer disease, gastric mucosal atrophy, intestinal metaplasia, dysplasia, gastric adenocarcinoma and MALT Lymphoma (2,6,7,8,11,13,14).

H. pylori is found in all parts of the world, although the prevalence is higher in developing countries, and almost all infections occur before the age of 10 years (10). **In industrialized countries H. pylori seroprevalence in children younger than five years of age is 1–10%, whereas in developing countries rates of more than 50% are common in children of the same age group. (10).**

Although the incidence of H. pylori in the developed world has been decreasing, it is still a significant problem in developing countries (11–16). Therefore, reduction of mortality of GC demands the identification of high-risk group for disease development of management strategies to retard and prevent its progression to malignancy. Moreover, it is more cost-effective to detect gastric cancer in an early stage, by using easily available and cheap methods since it can be readily treated by endoscopic sub mucosal resection than is more advanced gastric cancer (17, 22).

Methods of detection of Helicobacter pylori

- **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 (17).**

- **Immunohistochemistry detection of Helicobacter pylori is regarded as a gold standard in diagnosis of Helicobacter pylori infection. This is because immunohistochemical technique can detect non-viable and non culturable state of**

Helicobacter pylori.(14)Immunohistochemical method seems to be relatively more expensive than other staining methods (14).

- **Urea breath test: This method uses Carbon 13 and 14 isotope labelled urea where it detects the urease produced by the bacteria in the stomach (18–20). 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 Helicobacter pylori in the stool. Its sensitivity and specificity is similar to that of Urea breath test (18,21).**

- **Invasive methods: These involve different invasive procedures and they include;**
- **Serological test: this method uses detection of antibodies against the Helicobacter antigens. The antibodies detected include IgG and IgA (22,23).**

- **Rapid Urea test: This is one of the reliable histological evaluations of Helicobacter pylori as it has both high sensitivity and specificity compared to other methods mentioned above (24–26).**

- **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 (24).**

- **Warthin-Starry: This is also a silver based stain, which stain the glycogen content of the cell wall. This method is more sensitive than Giemsa stain (27).**

1.2 Conceptual Framework

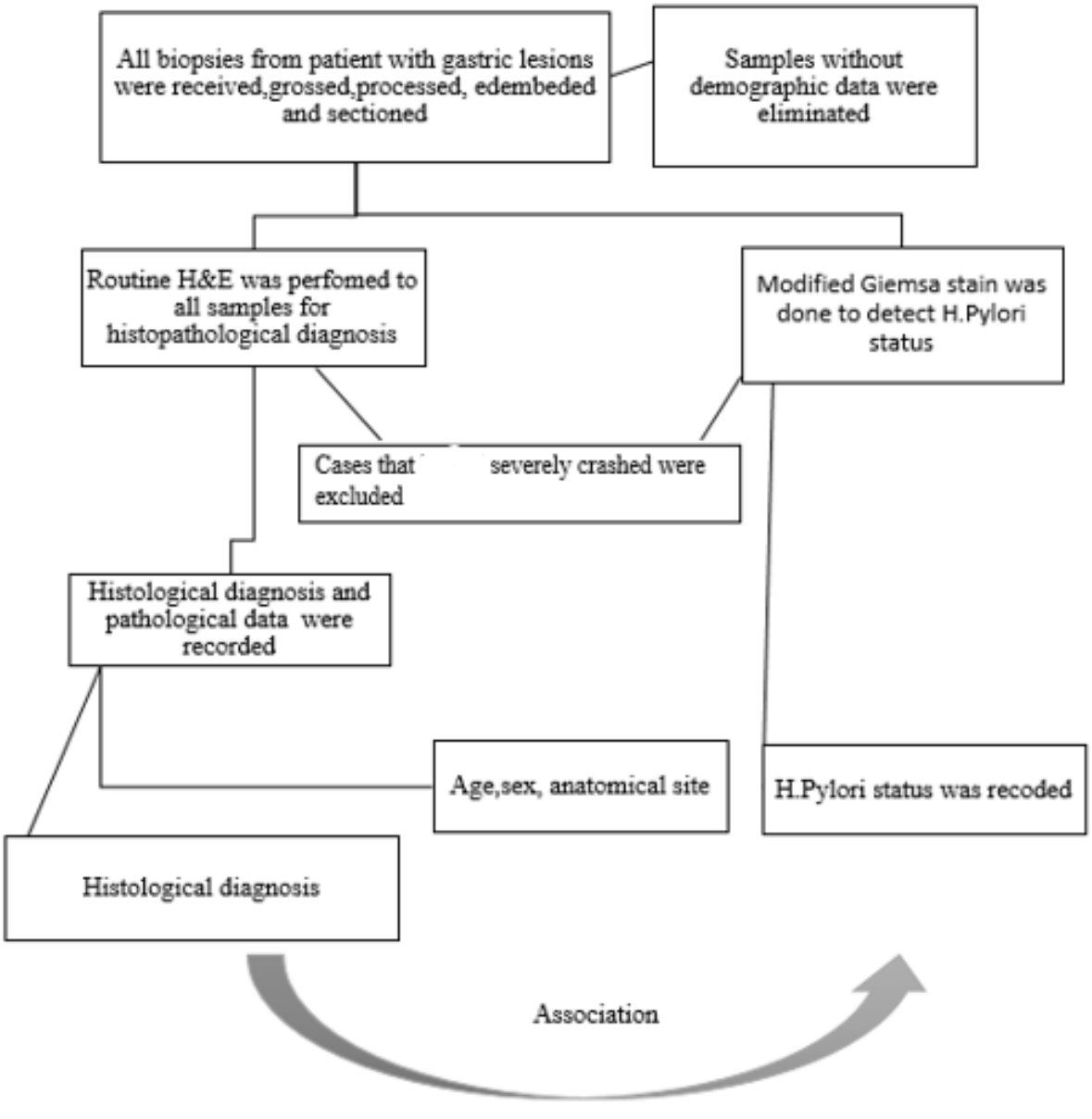


Figure 1: Diagram illustrating the conceptual framework of this study

1.3 Statement of the Problem

The WHO report (2014),indicates that in the United Republic of Tanzania, there were 19,900 deaths due to cancer among 403,000 total deaths (28). Gastric cancer contributed to 4.1% of deaths in males and was the third most frequent cause of death after cancer of prostate and oesophagus (38). This situation demands for the establishment of a baseline data on improving the survival rate and care of patients through proper management of pre-malignant, inflammatory and benign lesions before their transformation to malignancy through detection of H. pylori and early effective therapeutic interventions.**Very little information is available regarding the magnitude of gastric inflammatory, benign and malignant lesions and their associated risk factors, namely age, geographical location, diet and H. pylori infection in Tanzania.**

In addition to the above, there are no established protocols and techniques for detecting H. pylori in tissue biopsies at MNH, and other hospitals in Tanzania. The occasionally used Immunohistochemistry (IMHC) method is relatively expensive and requires a well-equipped laboratory.

Also, most patients with gastric cancer in the limited-resource countries like Tanzania come from the low socioeconomic status group and therefore the disease present at an advanced stageof high-grade cancer with poor prognosis (4,27). Modified Giemsa stain is a relatively cost-effective method of detecting H. pylori in gastric biopsies and have shown good sensitivity (85%) and specificity (89%) and can be used to improve the early management of patients infected with H. pylori(26). With the above background in mind this study, coupled with other studies parameters, modified Giemsa stain is used to detect H. pylori in gastric biopsies.

1.4 Rationale of the Study

The information obtained from this study will add to the already available data on the subject, especially for Tanzania and MNH. The knowledge generated will help pathologists in early diagnosis and clinicians to improve the care of patients by increasing survival ratethrough proper management of precancerous, inflammatory and benign conditionsthrough early therapeutic interventions or establishment of a screening program in high-risk groups.

At the laboratory level, the study will establish a routine diagnostic technique for detecting H. pylori in tissue biopsies at MNH and other hospitals in Tanzania. Furthermore, the results from this study will be beneficial for further research and scientific enquiry on pathogenesis and early detectionof gastric cancer and its variations, and to estimate the magnitude of the disease in various populationsin the future.

1.5 Hypothesis of the study

Gastric lesions are common in patients at MNH and are associated with H. pylori infection.

1.6 Research Questions

(i) What**are** the histopathological pattern**s** and frequency of different gastric lesions at MNH from July 2018 to February 2019?

(ii) What is the association between gastric lesions with patients' age groups, sex and anatomical site at MNH from July 2018 to February 2019?

(iii) What is the status of Helicobacter pylori infection among patients with gastric lesions at MNH July 2018 to February 2019?

1.7 Objectives

1.7.1 Broad Objective

To describe the histopathological pattern and frequency of gastric lesions, their relationship with age, sex, anatomical site and the status of Helicobacter pylori infection using modified Giemsa stain at MNH from July 2018 to February 2019.

1.7.2 Specific Objectives

(i) To determine the histopathological patterns and frequency of different gastric lesions at MNH from July 2018 to February 2019.

(ii) To determine the association between gastric lesions and age groups, sex and anatomical site of the patients at MNH from July 2018 to February 2019.

(iii) To determine the frequency of Helicobacter pylori infection among gastric lesions using a modified Giemsa stain at MNH from July 2018 to February 2019.

1.8 Review of Literature

1.8.1 General Pattern and Frequency of Gastric Lesions

The International Agency for Research on Cancer (IARC) (2008) estimates of the global incidence and mortality rates of Gastric Cancer (GC), showed that there were 989,000 new cases of gastric cancer (7.8% of all reported cancer cases) in 2008 (2),making GC the fourth most common malignancy globally, behind lung, breast and colorectal cancers (2, 3, 5).

Significantly more GC cases were noted in less developed regions compared to more developed regions. About half of the total gastric cancer burden was located in East Asia, especially China (2). East Asia is also the home of the most high-risk countries such as Korea, Mongolia, Japan and China, with the age-specific rates of 62.2, 48.2, 46.8 and 41.3 per 100,000, respectively and mostly in males (2).

The majority (approximately 90%) of GC are adenocarcinomas, which arise from the glands of the most superficial layer, or the mucosa, of the stomach (1, 3). There are, however, other types of cancer arising from the stomach, such as Mucosa Associated Lymphoid Tissue (MALT) lymphoma, and leiomyosarcomas. These arise from the muscles and lymphoid tissue in the mucosa (1,3). A. Lauren stratifies gastric adenocarcinomas into two major histological

types: diffuse and intestinal. These two types not only look different under the microscope but also differ in gender ratio, age at diagnosis, and other epidemiologic features (1, 3).

GC incidence rates have been on the decline in most parts of the world (2, 3). In the United States, for example, the incidence rates decreased by 1.7% for men and 0.8% for women annually from 1992 to 2010.Furthermore, the incidence is relatively low, particularly in Whites, with an estimated incidence rate per 100,000 of 7.8 and 3.5 in non-Hispanic White men and women, respectively (3). Also, rates of GC vary across races, for example, in the United States, rates are higher in Latinos (13.9 per 100,000 in men and 8.2 per 100,000 in women) than in non-Hispanic White populations(3). In The Netherlands, as in many Western countries, the incidence of GC is relatively low, with approximately 6.9 cases/100,000/year (29).

The geographic variations in GC incidence also exist within a country. For instance, in China, the risk of GC in Changle, Fujian, was 5-fold that of Hong Kong (2). Muping County in Shandong, China also had a significantly higher risk of gastric cancer when compared to Yanqing County in Beijing. These variations may be related to differences in risk factors exposure, such as chronic infection such as H. pylori, tobacco smoking, dietary factors, alcohol, environmental and occupational exposure.(2, 20).

A comprehensive review done by Annemarie C. de Vrie et al.,on premalignant inflammatory and benign lesions in different countries, reported that prevalence rates vary widely, from Australia (22%), Sweden (28%), Netherlands (34%), Hungary (37%), Norway (50.5%), Japan (52.9%) to 98.1% in China (27). AG and IM in these countries were more frequent and were considerably more frequently seeninH. pylori-positive subjects, compared to only 10% of uninfected individuals. Prevalence of gastric GED varies from 0.5 to 4% in Western populations and from 9 to 20% in high-risk areas for gastric carcinomas (31).

Studies have established that intestinal gastric cancer is frequently preceded by a sequence of histological activities starting with diffuse CG and ultimately leading to AG, IM, and GED (8). IM had a yearly incidence of gastric malignancy of 0.25% within five years after diagnosis (1). An epidemiological study in Italy showed that patients with IM have more than a 10-fold

increased risk of developing gastric malignancy (1). High-grade GED has been identified close to 40% to 100% of Early Gastric Cancers (EGC), and 5% to 80% of advanced adenocarcinomas. Furthermore, epithelial dysplasia was also an indicator of risk for cancer elsewhere in the gastric mucosa. GED often exists in the mucosa adjacent to foci of adenocarcinoma, and elimination of dysplastic lesions decreases the subsequent risk of cancer (6).

Compared with other risk factors, AG and IM increase the risk of gastric cancer exponentially. Therefore, individuals with AG and IM are a high-risk group for GC (1). Detection of GC at a curable stage substantially improves morbidity and survival. For instance, nationwide mass screening programs for gastric neoplasia in Japan have resulted in a higher detection rate of early gastric cancer(8). In a study conducted in a rural Chinese population at high risk of GC, when residents with precancerous lesions were followed-up for five years, odds ratios of gastric cancer in subjects with IM were 17.1 to 29.3(32). Another study,on topographic patterns of IM, showed that the presence of IM is significantly associated with increased cancer risk(1, 10).

The prevalence of GC has also been reported to increase in young age groups (28, 29). A study done by*Theuer CP et al.,* in California to determine unique features of GC in young age groups revealed that 5.5% of cases of gastric carcinoma in a population-based Southern California historical cohort occurred in patients who were less than 41 years of age. The age-adjusted incidence of gastric carcinoma during the 11- year study period was 10.2/100,000. The frequency of gastric carcinoma, and the proportion of cases of gastric adenocarcinoma occurring in young patients, however, remained constant throughout the 11-year study period(33).Also, in a study of 176 Jordanian patients with GC who were managed at the King Abdullah University Hospital, Irbid-northern Jordan and its affiliated facilities between 1991 and 2001, the frequency of early-onset GC was 9.7%. Similar to its counterpart in older patients, the incidence of gastric adenocarcinoma in young patients appears to decrease with time. The frequency of early-onset gastric adenocarcinoma was reduced from 4.3% in the early 1990s to 2.6% in late 1990s(34).

The predicted frequency and mortality rates of GC in Africa is 4/100,000 and 3.8/100,000, respectively (7). In Africa, GC is ranked twelfth most common cancer with the estimated incidence and mortality rates of gastric cancer in Africa are 4/100,000 and 3.8/100,000 of the population, respectively (26). There is, however, a substantial variation in reported incidence and mortality from GC within individual African countries. For instance, in Mali, GC is the most common malignancy in males with an incidence rate of 21.6/100,000 and mortality rate of 21.1/10,000 (7). In a study done in a teaching hospital in North-Western Tanzania,it was shownthat out of 5,134 patients who were diagnosed with malignancies, 232 with GC were enrolled in the study signifying the prevalence of 4.5% (4).

1.8.2 Association of Gastric Lesions with age groups and sex

GC is usually a disease of the aged, with a mean age of approximately 50 to 60 years; patients younger than 40 years represent 2%to 8%of all patients with gastric carcinoma (37). A study was done in Korea on the incidence and long-term outcome of young patients with GC according to sex. The total was 1299 patients, (Males 865 - 66.6% and females 434 - 33.4%), and the sex ratio was 1.99:1. Among them, 175 (13.5%) were classified as a young group and remaining 1124 patients (86.5%) classified as an older group, and the male to female ratio was 2.13 (37).

The incidence rate of GC rises progressively with age, as has been demonstrated in several studies. Worldwide (3, 18, 37).In a study done inthe United States, of the cases diagnosed between 2005 and 2009 approximately 1% of cases occurred between the ages of 20 and 34 years, whereas 29% occurred between 75 and 84 years with a Median age at diagnosis of GC of 70 years(3).

GC incidence rates vary wildly between men and women and across different countries (3, 20, and 23). Rates are 2- to 3-folds higher in men than women (3, 20). For example, the annual age-standardized GC incidence rates per 100,000 in men are 65.9 in Korea versus 3.3 in Egypt(3, 23).

A comprehensive review done byTiing Leong Ang et al., on the worldwide clinical epidemiology of GC, showed that male gender cases (466,900) were more frequently reported in less developed regions compared to developed areas (173,700. The corresponding case burden for the female gender was 247,000 and 102,000 cases, respectively.The age-standardized incidence rate (ASIR) for males was generally twice that for females (2, 23).

A study done in the Netherlands with a cohort of 92,250 patients with a first diagnosis of a premalignant gastric lesion showed a 1:1 male-to-female ratio. Median age at initial diagnosis was significantly higher with increasing severity of premalignant gastric lesions. Women were considerably older than men at the initial diagnosis of AG (median age 63.2 years vs 57.8 years for men), IM (68.7 vs 64.6 for men), mild-to-moderate dysplasia (70.9 vs 66.9 for men), and severe dysplasia (77.6 vs 72.1 for men) (8).

In another study done by *den Hoed C M. et al*. with total of 383 patients, and a male to female ratio of 1:1the mean age was 53.1 years (rangeof 17 to 86 years)(29). The cases with pre-malignant gastric lesions were significantly older than subjects with either normal gastric mucosa or CG with the mean age of 60 versus 52.5 years. Furthermore, 95% of premalignant gastric lesions occurred mostly in the subjects over 60. IM and GED were highly prevalent in the higher age groups. IM was present in 2.3% of the 30–40 years old subjects, increasing to 13.4% in the age group 60–70 years(29) this was different from the study done in Finland showed that prevalence of AG increased significantly with age, reaching 8% in the age group 70 years or over(37).

In a study done in northern India of 100 gastric mucosa biopsies of 10 from November 2012 to October 2013, the age of patients varied from 17 years to 80 years. The majority of the patients were in the 4th decade (33%), followed by 5th and 6th decades. Among them, 65% were males, and 35% were females with an M: F ratio of 1.86:1. On histopathological examination of biopsy specimens, CG was the most common lesion seen in 89 % cases; five cases had gastric carcinoma (5%), 1 case of MALT Lymphoma (1%)(38).

In a study, of 176 patients with GC at the King Abdullah University Hospital - Jordan, between 1991 and 2001, a total of 17 (9.7%) patients were 40 years or younger, and 159 patients were more than 40 years old. Their mean age was 36.3 years and ranged from 24 to 39 years, for the young group, and 63.8 years and range 41–91 years, for the older group. The proportion of females in the young group (59%) was significantly higher than the proportion (35%) in the older group.The male to female ratio usually increases with age (21).

Several studies, however, have described the clinical and pathologic features of gastric adenocarcinoma in young adults (20, 21). A population-based analysisofunique features of GC in young patients in California showed that young patients with gastric adenocarcinoma varied from 6% to 15% in a single institutional case studies. The study defined young patients as being less than 41 years of age. One hundred and sixty-seven patients (5.5%) were less than 41 years; the mean age was 33 years, while the mean age of 2,853 old patients was 69 years. Among young and old patients, there was no difference in the mean age between males and females. The male to female ratio of the two groups was similar (1.5:1) and no significant difference in male: femaleratios between young and old patients was noted between races(33).

In general, approximately 2–16.2% of GC occurs in patients younger than 41 years old, and only 1.1–3.3% of gastric cancer cases occur in a patient younger than 30 years of age (21). Evidence suggests that there are differences in the clinicopathological features and pathogenetic mechanisms of gastric adenocarcinoma between young and older patients. Several reports have indicated that younger patients are frequently diagnosed with advanced tumour stages and that GC has a poorer prognosis in young in comparison to older patients (34).

In Africa, the incidence of GC is higher in men (4.7/100,000) than in women (3.3/100,000). There is a great deal of variation in reported incidence and mortality within the individual African countries. For instance, in Mali, West Africa, gastric cancer is the most common cancer in men with an incidence rate 21.6/100000 and mortality rate 21.1/100000. (26, 30).

In a study done to find incident cases of gastric cancer in part of Kenya’s Eastern Province between 200 cases of GC were found giving an annual average crude incidence rate of 7.01

per 100,000 males and 3.7 for females. Previous incidence estimates for the same area of Kenya were reviewed, and a 10-fold increase in the recorded, indirectly standardized incidence rate between the periods (39).In another study done in the teaching referral hospital in Northern-western Tanzania, 232 patients were included in the study; 172 (74.1%) were males and 60 (25.9%) females giving a male to female ratio of 2.9:1. The modal age was found to be 51 to 60 years making 53.4% of cases (4).

1.8.3 Association of Gastric lesions with anatomical site location.

The stomach is divided into anatomic subsites, which includes the cardia (proximal), fundus, body, pylorus, and the antrum (distal). The sites are distinguished by anatomic demarcations, histological differences, or both (3).The proximal and distal GC have different epidemiological, clinical features and risk factors (3,18). Thus proximal gastric cancer may be epidemiologically distinct from the distal carcinomas (21-23). Risk factors for proximal GC include obesity, gastroesophageal reflux disease and Barrett's esophagitis, whereas distal GC risk is increased by the presence of H. pylori infection (3,18,21-25). Other risks are a family history of GC, low socioeconomic status, cigarette smoking and food rich in salt, smoked food and low consumption of fruits and vegetables (3,40).

The prevalence of malignant gastric lesions by tumour anatomical location has also been described to differ broadly on geographic location, race and socioeconomic status (4, 21). There is a decreasing trend in distal cancers in developed Western countries, whereas the trends in proximal GC rates have remained stable or increased(3,26,27). Such different patterns for proximal and distal GC may result from distinct etiologies (3).

Unlike for distal GC, H. pyloridoes not seem to be a risk factor for proximal GC in Western countries (43). Therefore its decline in prevalence would not be expected to affect proximal GC rates (3, 26-28). In contrast, obesity and gastroesophageal reflux seem to be risk factors for cardia but not non-cardia GC. Obesity has been increasing in prevalence in Western countries, which may contribute to the rates of cardia GC(3).

Proximal gastric malignant lesions are common among whites in higher social, economic status in developed countries, while distal gastric malignant lesions are more common in

blacks in developing countries, and in lower socioeconomic classes (4,22). However,a study done in a medical University in Iran showed no significant difference in the tumour anatomical subsite distribution by sex with proximal gastric involvement observed in 104 of 261 males and 38 of 91 females (44).

In another study of 176 Jordanian patients with GC between 1991 and 2001, the involvement of the middle and distal part of the stomach were most common sites of the tumour(84.7%) than the proximal third (15.3%) (21).

The study done to find the incidence and long-term outcome of a young patient with GC according to sex and tumour location in Korea showed that the upper third (11.7%), middle third (40%), lower third (57.1%) (34).

The study done in Bugando Medical Centre in Tanzania showed that the antrum was the most occupied subsite by GC (56.5%) followed by fundus (17.2%), body (12.1%), diffuse and cardia (5.2%) (4).

1.8.4 Association of Helicobacter pylori and Gastric lesions.

H. pylori is a gram-negative, spiral-shaped organism that colonizes the human gastric mucosa (7, 8, 11, 12, 13). H. pylori has a universal, worldwide distribution as it is claimed to be the most frequent bacterial infection worldwide (7, 8, 14). It can colonize the acid-secreting portion of the stomach where it remains for an extended period, possibly for life (12, 14).

The morphology of H. pylori is not constant because, under adverse conditions, it becomes coccoid (7, 8, 12), but there is controversy about the nature of the coccoid form (12). Some researchers have stated that this form is either a contaminant or a dead bacterium (12). Others consider it to be a metabolically active form that cannot be cultured in vitro (13, 14).H. pylori is microaerophilic; optimal growth occurring in the presence of 5–15% oxygen, 5% CO2, pH range of 4.5–9, temperatures of 30–37^{o}C. All the required growth conditions are met in the gastrointestinal tract of all warm-blooded animals (10). **Childhood is the critical period for infection, and transmission from person to person most significantly through oral–oral and faecal-oral routes (11).**

The bacteria are widely distributed in the stomach, particularly in the parietal cells of the gastric glands and is commonly seen in patients with different gastric complaints ranging from benign premalignant conditions to malignant ones (16). Colonization with H. pylori is a significant risk factor for GC. It is one of the most common infections in humans with a prevalence of up to 90% in many developing countries in young adults; the prevalence in the developed world varying between 30% and 50% (2, 18). H. pylori cause gastritis in virtually all infected patients, which can lead to AG, IM, GED and eventually invasive cancer (2,18,29,38,39,40).

H. pylori infection can be diagnosed by endoscopic followed by techniques which include biopsy and histological examination, culture, or polymerase chain reaction. Non-endoscopic methods (serology, urea breath test, urine or blood, detection of H. pylori antigen in a stool specimen) can also be used for H. pylori diagnosis (45).

The prevalence of premalignant gastric lesions shows considerable geographic differences and is associated with the regional incidence of H. pylori infection (8, 29, 38, 39). In the Western European population, H. pylori infection prevalence is approximately 30–40%. Atrophic gastritis is present in about 25–30% and intestinal metaplasia in about 45% of H. pylori-infected individuals, whereas only 5–10% of uninfected individuals have these lesions. In Asia, the overall prevalence of H. pylori infection is approximately 60% and is more often associated with pan-gastritis (9).

The prevalence of H. pylori infection is high in developing countries (90%), whereas in industrialized countries, the figure is lower (50%) and decreasing (11). The causal association between H. pylori infection and GC is established by many epidemiologic and clinical studies (33, 34, 35). More than 90% of GC patients have current or past H. pylori infection (1, 34, 35).

Globally, H. pylori infection affects 50% of the population (2, 36), and it is the most important causal factor for distal GC (2, 3, 21, 34). An analytical study of 12 prospective case-control studies by the H. pylori and Cancer Collaborative Group showed that for distal cancer OR was 2.97 (95% CI 2.34–3.77) for H. pylori infection, conversely, for proximal cancers, no statistically significant association was demonstrated. Furthermore, when the pooled analysis

was restricted to cases occurring at least ten years after the diagnosis of H. pylori, the OR for distal cancer increased to 5.93 (95% CI 3.41–10.3) (2).

Based on the average H. pylori prevalence of 35% and 85% in developed and developing countries, respectively, it was estimated that about 65% – 80% of distal gastric cancers attributable to H. pylori infection are potentially preventable (2). Regions with high GC incidence rate tend to have high seroprevalence rates for H. pylori infection (2, 3). A prospective, placebo-controlled, randomized study found that subjects with persistent H. pylori infection had a significantly higher risk of progression to intestinal metaplasia than those with successful H. pylori eradication (OR 2.13, 95% CI 1.41–3.24). Thus, mass eradication and chemoprevention of H.pylori were effective in significantly reducing the incidence of gastric atrophy (77.2%; 95% CI 72.3%–81.2%), although the reduction in the incidence of intestinal metaplasia was not significant (3).Two other meta-analyses, which addressed the controversial issue regarding the impact of H. pylori eradication on gastric atrophy and intestinal metaplasia, showed considerable improvement in gastric atrophy but not gastric intestinal metaplasia (2). Research duringthe two decades has established that H. pylori is an apparent cause of GC, with relative risks of approximately 6 for distal GC. Certain H. pylori types, mainly those positive for the virulence factor cytotoxin-associated gene A (CagA), are more likely to cause GC. H. pylori is estimated to cause 65% to 80% of all GC cases, or 660,000 new cases annually (3).

The H. pylori seroprevalence rates in less developed or developing countries are higher than in developed countries as it is reported in Bangladesh (92%), in India (79%), in Vietnam (74.6%). On the other hand, the seroprevalence rates in more developed countries were generally lower, like in Australia (15.1%). In Asian countries that became developed or industrialized in recently, the seroprevalence rates were higher than in Australia. Among East Asian countries, the overall seroprevalence rate was 58.07% in China, 39.3% in Japan, 59.6% in South Korea and 54.5% in Taiwan. Among Southeast Asian countries, the reported seroprevalence rate was 35.9% in Malaysia, 31% in Singapore and 57% in Thailand (46).A study done in Finland showed that H. pylori infection is still common in society. Among all the 4,256 volunteers (averageage 56 years, age range 18–92 years), 819 (19%) had an ongoing

H. pylori chronic gastritis. Altogether 150 (3.5%) of all 4,256 volunteers had advanced moderate or severe AG (37).

The study done in the Netherlands to study H. pylori infection in premalignant gastric lesions revealed that H. Pylori was demonstrated in 22% subjects with H. pylori gastritis. The prevalence of H. pylori gastritis increased with age. Eleven per cent of subjects under 40 years of age were colonized with H. pylori. This prevalence rose to a maximum of 33% in subjects 50–60 years of age. In the non-Caucasian patients, the prevalence of H. pylori was 44% overall, ranging from 33% in the patients under 40 years of age to 57% in those above 50 years. In 11% of the subjects with histopathologically diagnosed gastritis, no signs of H. pylori were identified (29).

Helicobacter pylori play a significant role in the pathogenesis of low-grade B cell gastric lymphoma (MALT lymphoma) and GC (40,47). The role of H. pylori infection in GC pathogenesis was further supported by a study that comprised 2,722 early GC patients and 13,976 controls. The study demonstrated a higher H. pylori prevalence in patients with early gastric cancer (87%) than in the control group (61%) (40,48).

In Korea, gastric cancer is the most common malignancy and a leading cause of deaths due to cancer. In 1998, a nationwide epidemiologic survey of H. pylori infection in Korea showed 17.2% seropositivity among children below age 16 and 66.9% among adults. This amounts to an overall rate of 46.6%, the transition state from a developing country to a developed country(49). Likewise, the study which included 176 participants in Jordan showed that H. pylori had been established as a significant risk factor for GC and the prevalence of H. pylori infection increases with age and reaches a plateau in those older than 40 years (21).

In another study done in Western Iran to assess the prevalence and risk factors of H. pylori infection in health centre referrals, the mean age was 28.5 years and showed an overall frequency of 43%. Seroprevalence of disease in men was 42.5% and women 43.6 %. There was an association between age and prevalence. The lowest age with prevalence rate group (22.9%) <20 years and 30-39years (52.3%)(22).

CHAPTER TWO

2. 0 MATERIALS AND METHODS

2.1 Study design

This was a cross-sectional hospital-based study. Tissue biopsies were collected from patients with gastric lesions attending both the Gastroenterology unit and Surgery Departments and sent to the Histopathology unit at Muhimbili National Hospital (MNH). The duration of the study was seven months from July 2018 to February 2019.

2.2 Study area/setting

The study was conducted at MNH in Tanzania. The mainstay of the study was the Histopathology unit in Central Pathology laboratory MNH. MNH is a National referral hospital which serves as the Teaching Hospital for MUHAS, receiving patients from different parts of the countrywith 1,500 bed facility, attending 1,000 to 1,200 outpatients per day, admitting 1,000 to 1,200 inpatients per week.

2.3 Study population

All biopsies from patients with gastric lesions obtained between July 2018 and February 2019 at the gastrointestinal unit and surgical department of MNH and submitted to Histopathology unit at MNH were included in the study.The histopathology unit receives 1000 to 1100 samples annually.

2.4 Eligibility criteria

2.4.1 Inclusion criteria

Gastric tissue biopsy specimens were taken and collected during the study period, with investigation forms containing adequate patients' demographic particulars and registration number.

2.4.2 Exclusion criteria

Tissue biopsies with missing important social-demographic details and those, which the anatomical subtype of the tumour in the stomach was not indicated clearly, as well as severely crushed specimens.

2.5. 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 a sample size

N=Population size, 278

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 Helicobacter pylori in gastric specimens using modified Giemsa stain is 44.4%(50)

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 **161** cases.

2.6. Sample collection and sampling procedure

Tissue biopsies were obtained through a surgical or endoscopic procedure from patients with gastric lesions and immediately fixed in histological specimen containers with 10% buffered formalin, and submitted as per MNH tissue collection protocol. The collected biopsies were fixed for 24 hours.

2.7 Laboratory procedure.

2.7.1 Grossing

The collected samples were given identification numbers and taken in by documenting important gross features. Grossing of large samples was correctly done by taking representative samples, and the tiny endoscopy biopsies were kept into cassettes.

2.7.2 Processing

Automatic processing was done for 12 hours overnight by using SAKURA Tissue-Tek VIP machine.

2.7.3 Embedding

Embedding was done by using SAKURA Tissue-Tek TEC embedding and cryomodule

2.7.2 Microtome

Four (4) micrometer thick sections were made from iced paraffin-embedded blocks using a rotary microtome, Sakura model, SRM200 CW. The sections were mounted on slides with ID numbers and allowed to drain before placing on a hot plate at 60 degrees centigrade for 15-30 minutes.

2.7.3 Hematoxylin and Eosin stain

All sections were stained with Hematoxylin and eosin (H&E) following the MNH SOPs (**Appendix I**).

2.7.4 Modified Giemsa stain (Diff quick stain)

Sections were then be stained for Helicobacter pylori using Modified Giemsa (Diff quick) stain as described under MNH SOPs. The positive controls were sections known to contain H. pylori and negative control. (**Appendix II**).

2.7.5 Microscopy

After the above procedures**,** histological studies were doneusing a LEICA DM 750 light microscope to identify pre-malignant and malignant lesions. H&E stained adenocarcinomas lesions were classified according to Lauren classification as intestinal and diffuse type. Other malignancies and premalignant lesions were diagnosed and reported based on their histomorphological characteristics. Under Diff quick stain Helicobacter pylori was interpreted as positive when a dark blue-coloured helical or curved rods were seen and comparable to similar rods in the positive control. For record-keeping, pictures were taken using a 12-megapixel Samsung Galaxy S7 rare camera, with the aid of the Vankey cell phone microscope adapter for H&E sections, and Motic Easy scanner for Diff quick stained sections. The photographed images were transferred to the Principal Investigators laptop, which is password secured.

2.8 Data collection sheet

A specially designed instrument with various sections as per the objectives of the study was used to collect data from the selected cases, which had met the inclusion criteria. Socio-demographic data, histological diagnosis, tumour anatomical sites, were filled in and also pretested at MNH before the actual commencement of the study, to ensure validity and reliability of the data collection instrument **(Appendix III).**

2.9 Study tools, validity and reliability

2.9.1 Sample collection and processing

The fixation, processing, embedding, microtome and staining of slides followed the hospital and manufacturer's SOP, and before the staining of study slides, there was pretest of staining to assure validity and reliability of the stains. Data collection instrument was used to gather the data.

2.9.2 Reporting

The investigator read both the H&E and Diff quick slides and confirmed by the pathologist. H&E stained slides were examined, and gastric lesions were classified into inflammatory, benign and malignant lesions. Gastric Adenocarcinoma was classified according to the Lauren classification of gastric cancers (51). Diff quick slides were examined and classified as positive (in the prevalence of H. pylori bacteria) and negative in the absence of H. pylori.

2.10 Data analysis plan

The data were transferred from the data collection sheet and recorded into software and analysed using SPSS version 23 computer software. The data were summarized into proportion and frequency tables. Chi-square test was used to test for significance of associationsbetween the predictor and outcome variables in the categorical variables. Categorical variables with data less than 5, Fischer exact test, was used. Significance was defined as a P-value of <0.05.

Specific Objective 1: To achieve specific objective one, descriptive statistical analysis for pattern and categories of gastric inflammatory, premalignant and malignant lesions was performed to determine the frequency of cases and was summarized in frequency tables and diagrams.

Specific Objective 2: To achieve specific objective two, data was summarized in the form of proportions and frequency tables and diagrams for categorical variables.

Specific Objective 3: To achieve specific objective three, descriptive statistical analysis for the categorical variable was performed, to determine the proportion of cases with a positive and negative result for Helicobacter Pylori infection.

2.11 Ethical clearance

Before the study started, the research proposal was presented to the Department of Pathology, MUHAS for approval. Ethical consent was sought from the MUHAS Institutional Review Board (IRB). Administrative permission to conduct the study was obtained from Muhimbili National Hospital as per the hospital management protocols after getting the ethical clearance.

CHAPTER THREE

3.0 RESULTS

3.1Study population and socio-demographic data.

A total of 196 gastric tissue biopsies were obtained at the histopathology unit of MNH from July 2018 to February 2019. Among those seven (7) gastric biopsies were excluded; four (4) had request forms without socio-demographic features or anatomical sub-site site of biopsy in the stomach, and three (3) were severely crushed biopsies.The study population, therefore, consisted of gastric biopsies from 189 patients. The median age of the patients was 54 years; the youngest patient was 17 years, while the oldest was 85 years, with a mean age of 53.06 (SD+15.881). The majority of the patients were more than 40 years 141 (76.2%), while 45 (23.8%) occurred below 40 years. The studied population had more men represented (114; 60.3%) than women (75; 39.7%) with a male to female ratio of 1.5:1

3.2 General histopathological pattern and frequency of gastric lesions.

In this study, the inflammatory lesions predominated with 107 cases (56.6%) followed by malignant lesions (67; 35.4%), and benign lesions with (15; 7.9%) were the least category. Chronic gastritis topped up among all lesions with 81 (42.9%) followed by intestinal-type adenocarcinoma 46 (24.3%) whereas MALT lymphoma (1.6%) was the least diagnosis. Among the malignant lesions, Adenocarcinoma with 58/67 (86.6%) patients was the most frequent malignant lesion **(Table 1**and**Figure 3-10).**

Table 1: General histopathological pattern and frequency of gastric lesions

Histological category	Histological diagnosis	Frequency (%)	Group percentage
Inflammatory lesions	Chronic gastritis	81(42.9)	
	Gastric ulceration	14(7.4)	**56.6%**
	Atrophic gastritis	12(6.3)	
Benign lesions	Intestinal metaplasia	8(4.2)	
	Gastric epithelial dysplasia	5(2.6)	**7.9%**
	Adenomatous polyp	2(1.1)	
Malignant lesion	Adenocarcinoma (Intestinal type)	46(24.3)	
	Adenocarcinoma (Diffuse type)	12(6.3)	**35.4%**
	Malignant GI stromal tumour	6(2.3)	
	MALT Lymphoma	3(1.6)	
Total		**189(100)**	

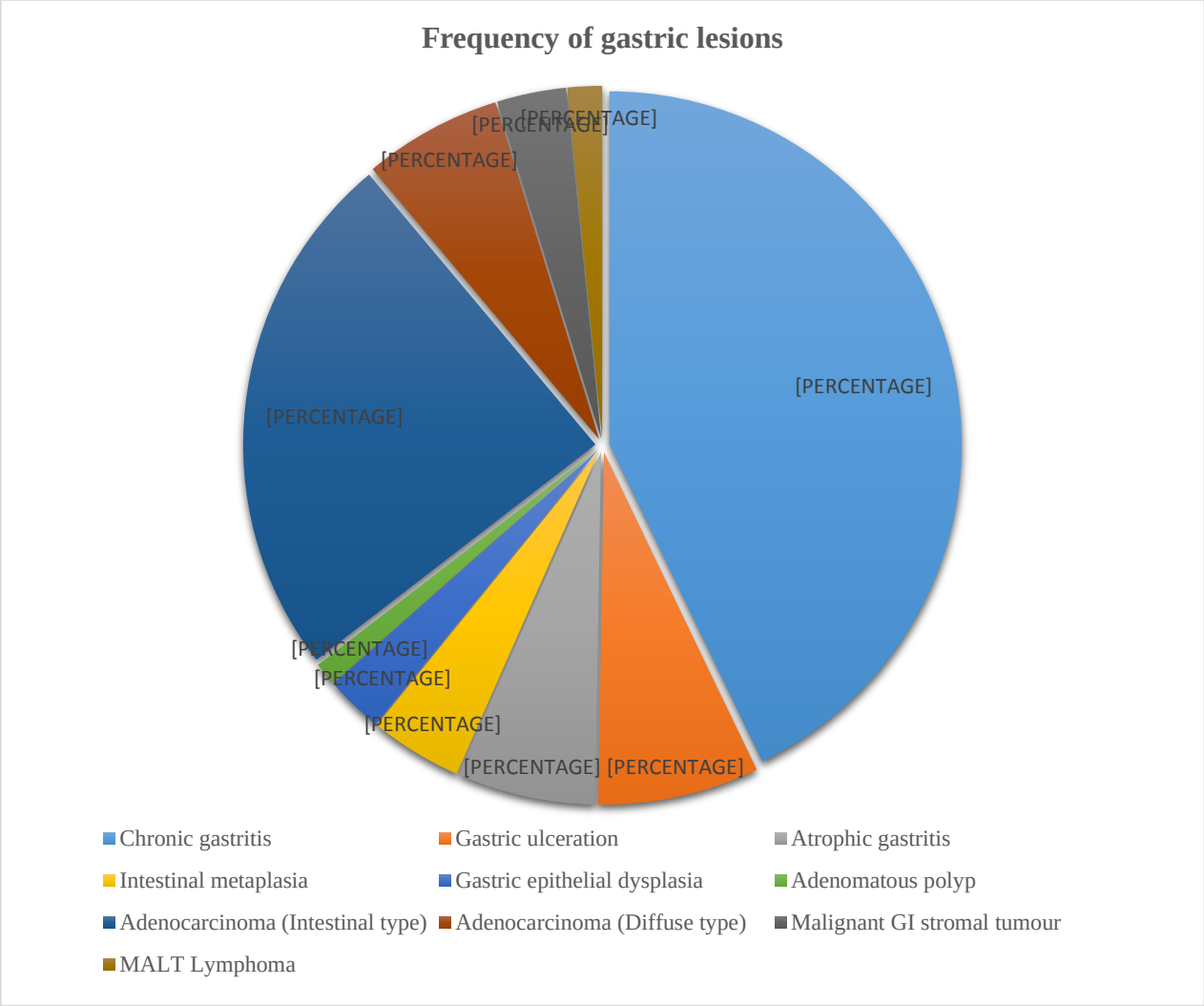


Figure 1: Frequency of gastric lesions.

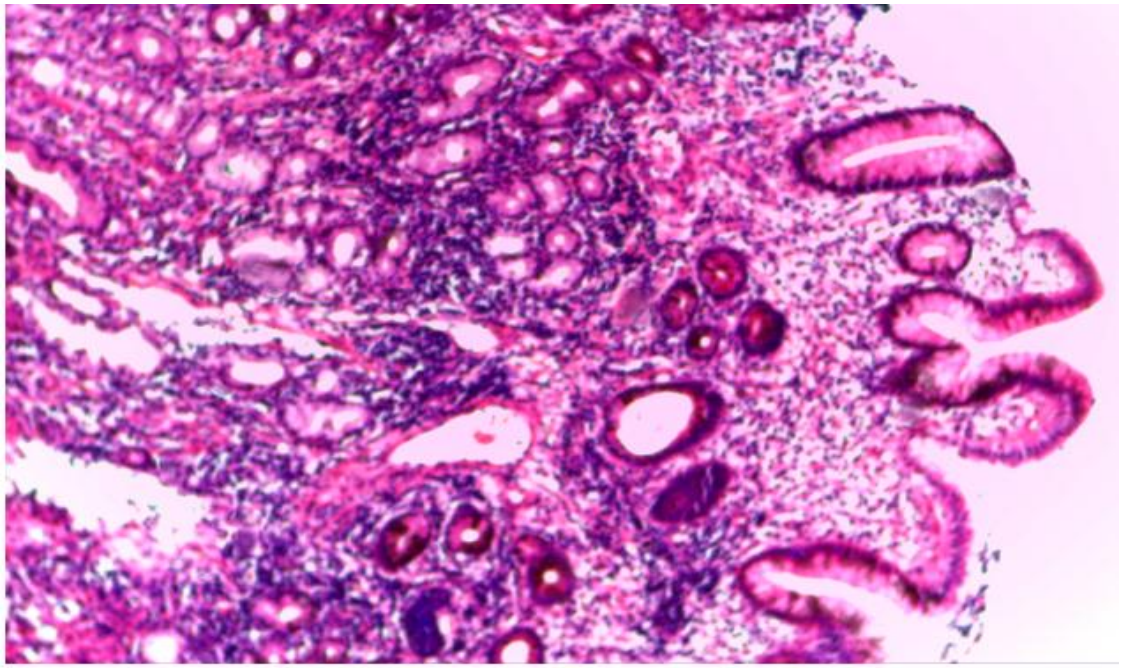


Figure 3: Atrophic gastritis: Glandular atrophy, compression and mononuclear cells infiltration (HE x40).

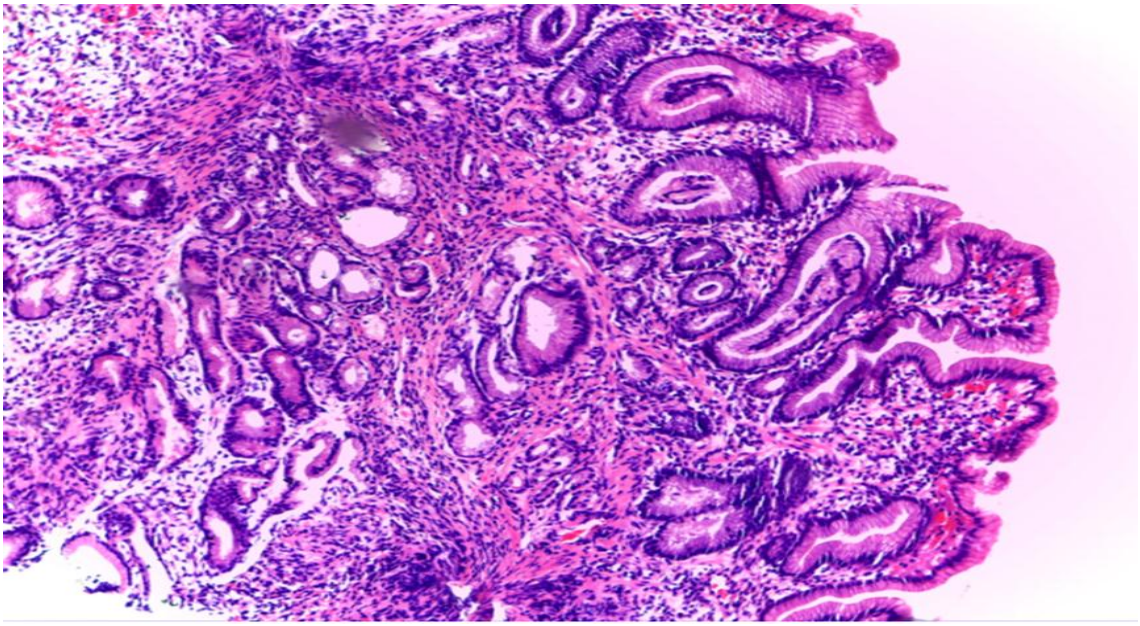


Figure 4: Chronic gastritis; Infiltration of chronic mononuclear inflammatory cells in gastric mucosa (HE x40).

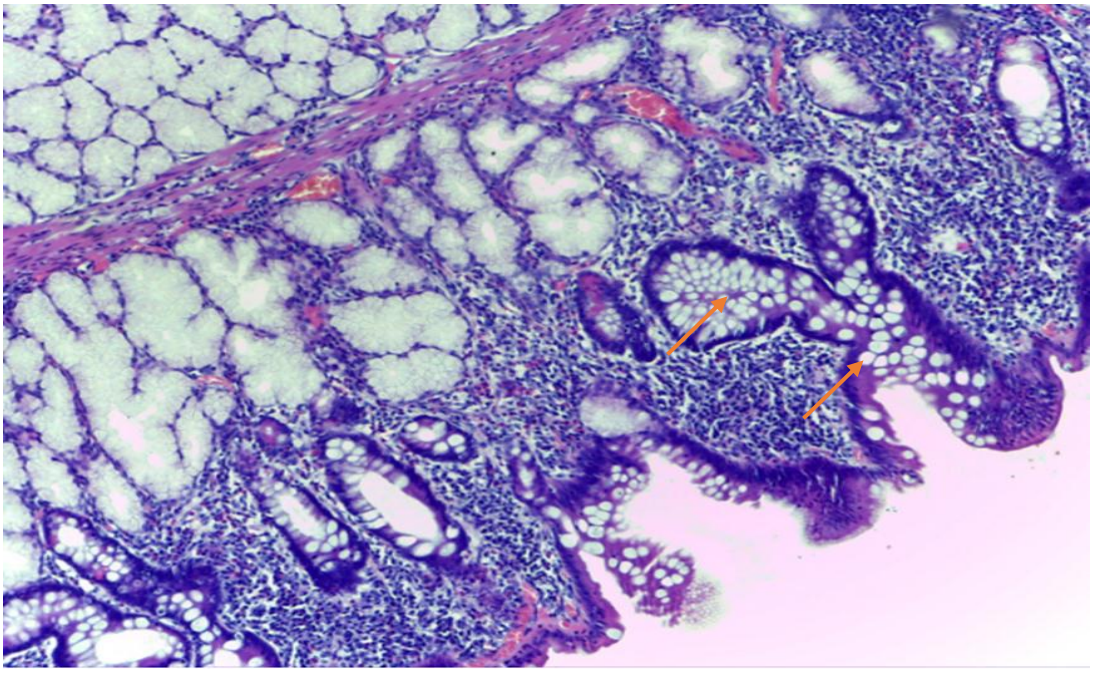


Figure 5: Intestinal metaplasia; partial replacement of gastric mucosa by goblet cells (arrows) of intestinal morphology with lymphocytes infiltration (HE x100).

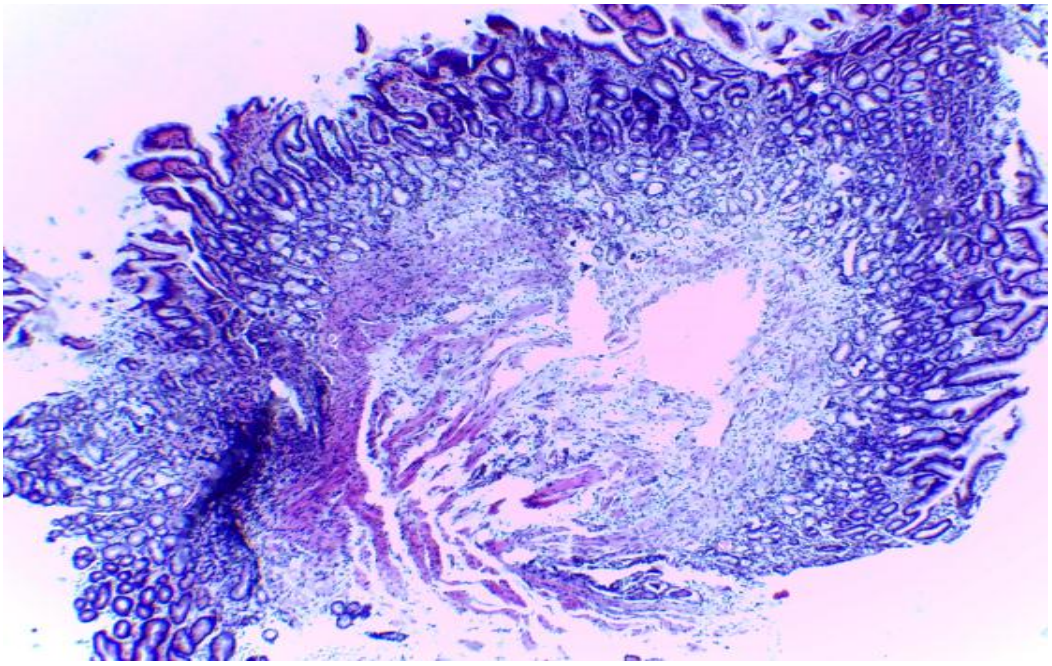


Figure 6: Adenomatous polyp; polypoid projection of dysplastic epithelium overlying dysplastic glands and increased configurations and orientation of glands (HE x40)

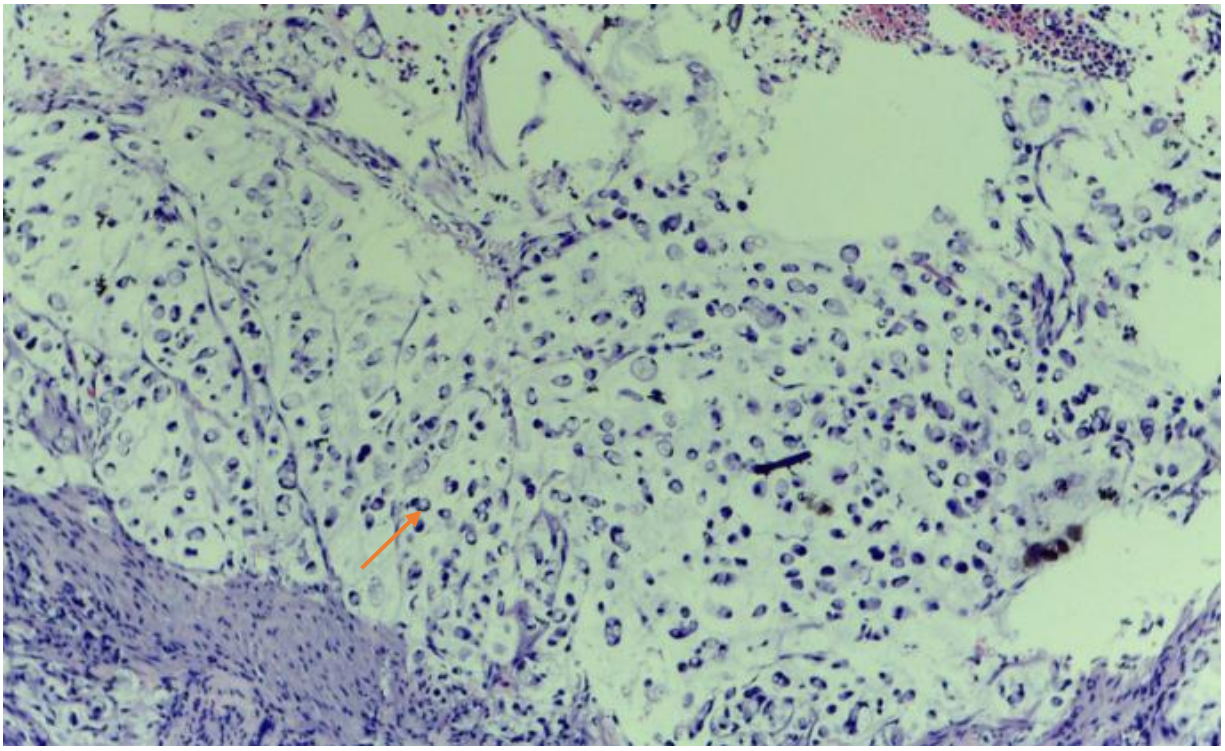


Figure 7: Diffuse Adenocarcinoma (signet ring type); tumour composed of signet ring cells (arrow) in mucinous background (HE x100).

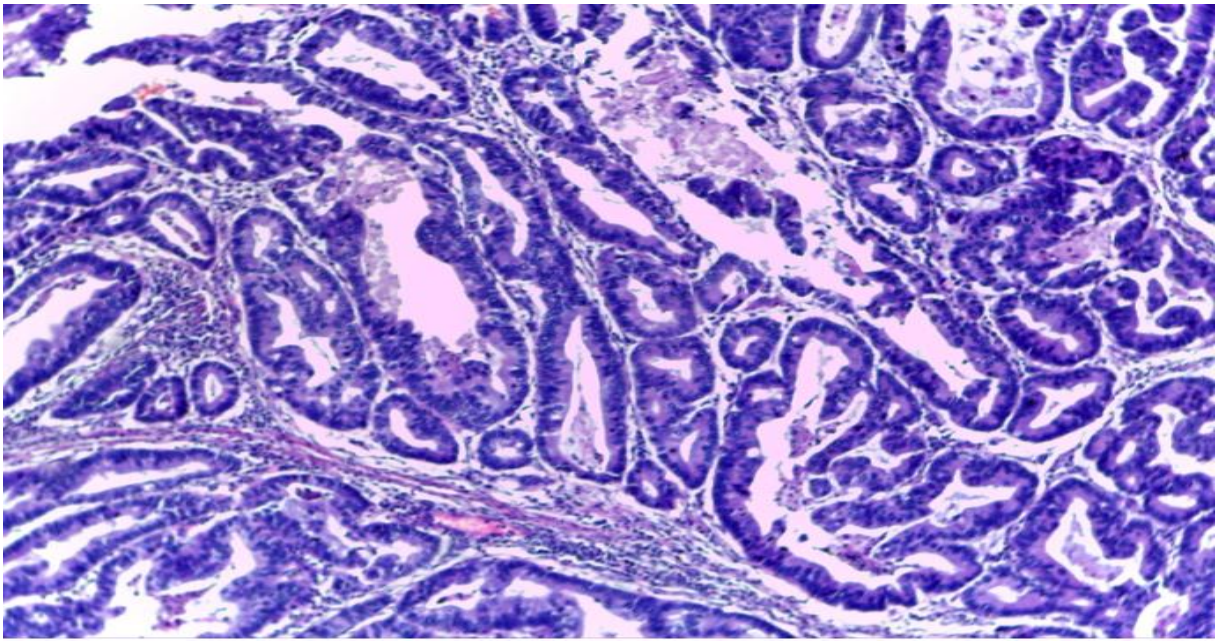


Figure 8: Adenocarcinoma (intestinal type); irregular, different sized glands in the stroma (HE x100).

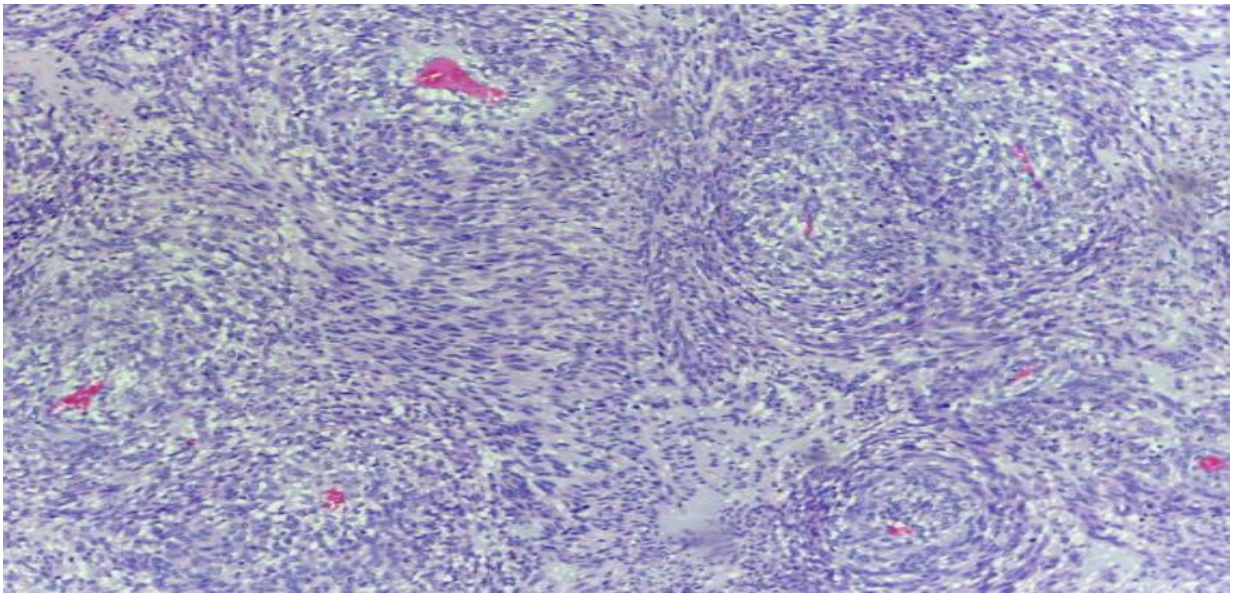


Figure 9: Gastrointestinal stromal tumour; whorls of spindle cells in the stroma (HE x40).

3.3. Association of Gastric lesions with other factors (age groups, sex and anatomical site)

3.3.1. Association of Gastric lesions with age groups

In combined gastric lesions, the majority of the study participantswere 40 years and above (144; 76.2%) while (45; 23.8%) occurred below 40 years. Among patients with gastric cancers, 58/67 (86.6%) occurred in those with 40 and above years of age and only 13.4% were seen below 40 years of age. Intestinal type adenocarcinoma (15.6%) was most frequent malignant lesions in patients below 40 years. Atrophic gastritis, Adenomatous polyp, diffuse type adenocarcinoma and MALT lymphoma were frequently seen in patients above 40 years of age. Chronic gastritis was the most frequent diagnosis in both age groups with proportion of 66.7% and 35.4% in patients with less than 40 years and those with 40 years and above respectively. The results were statistically significant indicating that gastric lesions were more common in advanced age (p=0.019) **(Table 2**and **Figure 11).**

Table 2: Distribution of gastric lesions by age groups (p=0.019)

Category of lesions	Histological diagnosis	Age Groups		Total
		<40 years (%)	≥40years (%)	
Inflammatory lesions	Atrophic gastritis	0	12 (8.3)	**12**
	Chronic gastritis	30 (66.7)	51(35.4)	**81**
	Gastric ulceration	4 (8.9)	10 (6.9)	**14**
Benign lesions	Adenomatous polyp	0	2 (1.4)	**2**
	Gastric epithelial dysplasia	1(2.2)	4(2.8)	**5**
	Intestinal metaplasia	1(2.2)	7 (4.9)	**8**
Malignant lesions	Adenocarcinoma (Diffuse type)	0	12 (8.3)	**12**
	Adenocarcinoma (Intestinal type)	7 (15.6)	39(27.1)	**46**
	Malignant GI stromal tumour	2 (4.4)	4 (2.8)	**6**
	MALT Lymphoma	0	3 (2.1)	**3**
Total		**45**	**144**	**189**

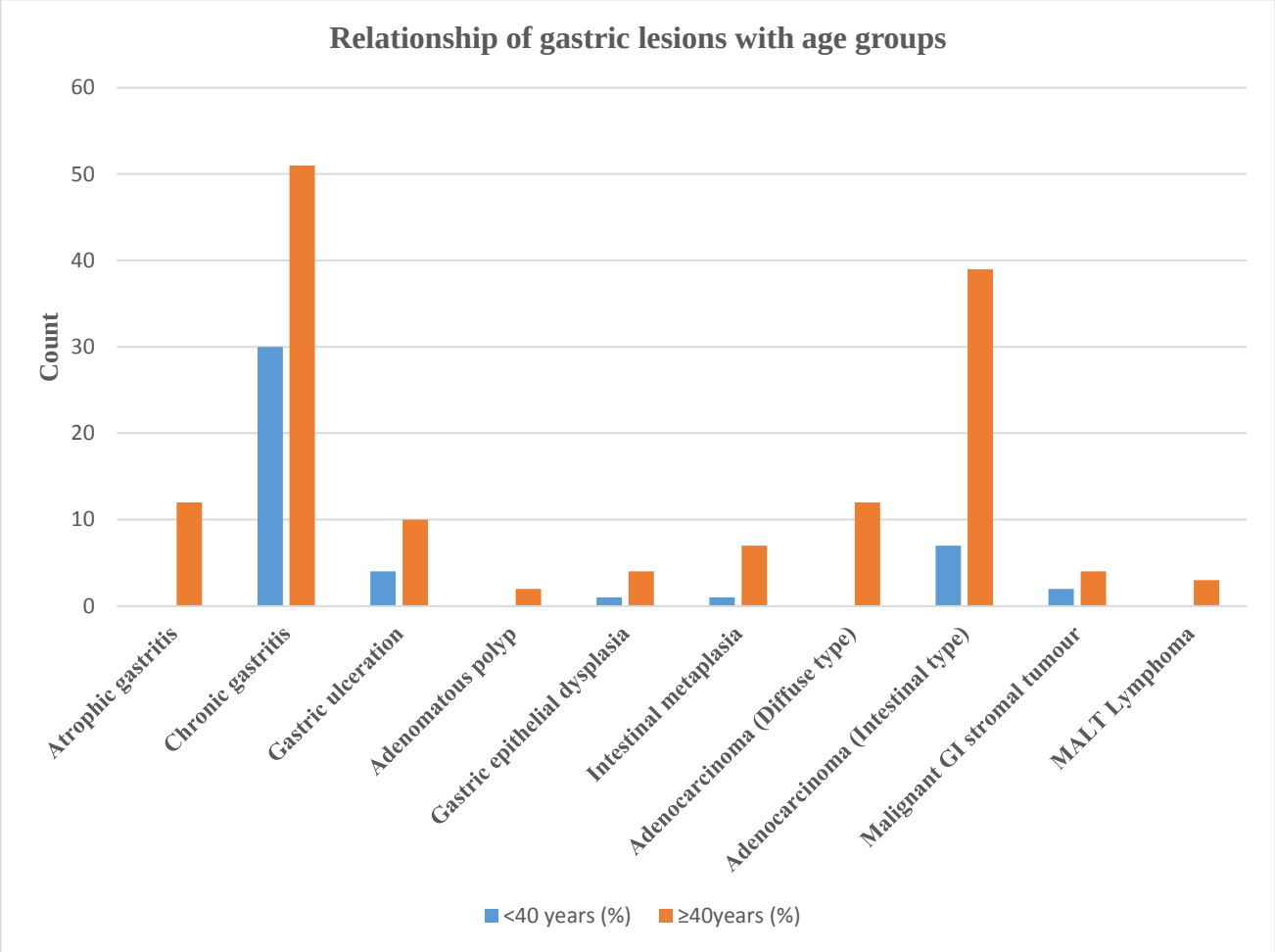


Figure 10: Histological diagnosis with age groups

3.3.2 Association of Gastric lesions with sex

Out of 189 patients with gastric lesions, majority were males (114; 60.3%) while females were 75 (39.7%). Among patients with malignant tumours males were 42/67 (62.7%) and females were 25/67 (37.3%) with a male to female ratio of 1.7:1. Adenocarcinoma dominated in patients with malignant lesions with a proportion of 32.5% and 28.0%) in males and females respectively. Chronic gastritis was the most frequent lesions in both sexes with the proportion of 43.9% and 41.3% in males and females respectively. Among the benign condition adenomatous polyps were confined in females (**Table 3**and **Figure 12**).

Table 3: Distribution of gastric lesions by sex (p=0.405)

Diagnosis category	Histological Diagnosis	Sex		Total N=178
		F (%)	M (%)	
Inflammatory lesions	Atrophic gastritis	7(9.3)	5(4.4)	**12**
	Chronic gastritis	31(41.3)	50(43.9)	**81**
	Gastric ulceration	5(6.7)	9(7.9)	**14**
Benign lesions	Adenomatous polyp	2(2.7)	0	**2**
	Gastric epithelial dysplasia	2(2.7)	3(2.6)	**5**
	Intestinal metaplasia	3(4.0)	5(4.4)	**8**
Malignant lesions	Adenocarcinoma (Diffuse type)	7(9.3)	5(4.4)	**12**
	Adenocarcinoma (Intestinal type)	14(18.7)	32(28.1)	**46**
	Malignant GI stromal tumour	2(2.7)	4(3.5)	**6**
	MALT Lymphoma	2(2.7)	1(0.9)	**3**
Total		**75**	**114**	**189**

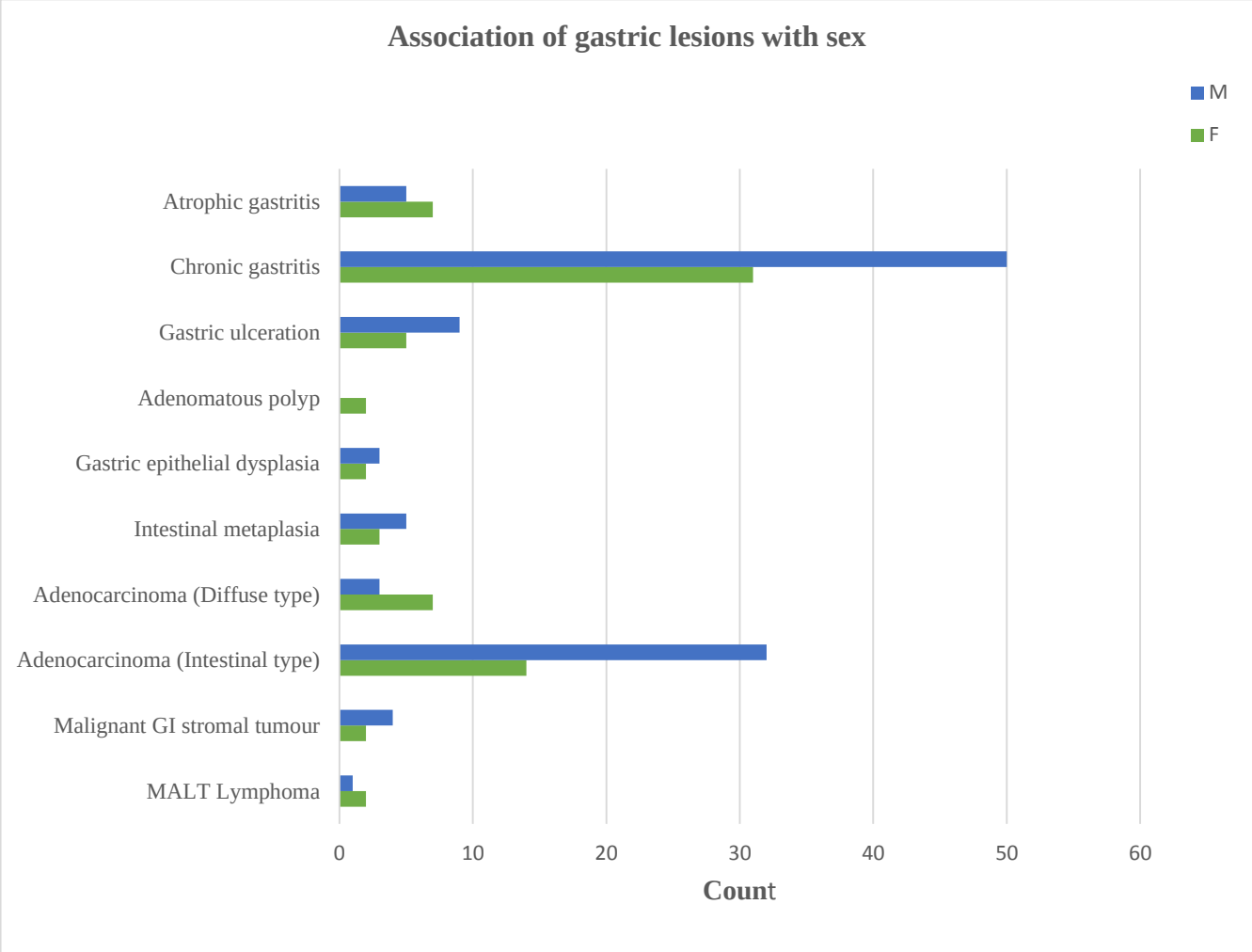


Figure 11: Histological diagnoses by sex

3.3.3 Association of gastric lesions with anatomical site.

Among 189 gastric lesions, 80 (42.3%) were from the antrum making it the most frequent anatomic site for gastric lesions followed by the body (41; 21.7%), pylorus (28; 14.8%), fundus (21; 11.1%) cardia (10; 5.3%) and 9 (4.8%) were diffuse lesions. Among the malignant lesions the antrum was the most site affected with 27/67 (40.1%), followed by body (26.9%), pylorus, (14.9%), diffuse (14.9) cardia (3.0%) and fundus (1.4%). Among the malignant lesions, Adenocarcinoma diffuse type was found most frequently on the body with 8/12 cases,

whereas intestinal type adenocarcinoma was more common to the antrum. Malignant Gastrointestinal stromal tumors were most frequent to the body and the rest were diffuse tumors. Among the inflammatory lesions, chronic gastritis was found in almost stomach anatomical subsites and among benign lesions adenomatous polyps were confided to the antrum. ($p<0.0001$). (**Table 4**and **Figure 13)**

Table 4: Anatomical distribution by anatomical site ($p<0.0001$)

Diagnosis category	**Histological Type**	**Anatomical site**						**Total**
		Cardia (%)	**Fundus (%)**	**Body (%)**	**Antrum (%)**	**Pylorus (%)**	**Diffuse (%)**	
Inflammatory lesions	Atrophic gastritis	0	3(1.6)	1(0.5)	5(2.6)	3(1.6)	0	**12**
	Chronic gastritis	6(3.2)	12(9.3)	16(8.5)	35(18.5)	12(6.3)	0	**81**
	Gastric ulceration	0	3(1.6)	3(1.6)	5(2.6)	3(1.6)	0	**14**
Benign lesions	Adenomatous polyp	0	0	0	2(1.1)	0	0	**2**
	Gastric epithelial dysplasia	1(0.5)	0(0.7)	2(1.1)	2(1.1)	0	0	**5**
	Intestinal metaplasia	1(0.5)	2(1.1)	1(0.5)	4(2.1)	0	0	**8**
Malignant lesions	Adenocarcinoma (Diffuse type)	0	0	8(4.2)	0	0	4(2.1)	**12**
	Adenocarcinoma (Intestinal type)	2(1.1)	0	7(3.7)	25(13.2)	10(5.3)	2(1.1)	**46**
	Malignant GI stromal tumour.	0	0	3(1.6)	0	0	3(1.6)	**6**
	MALT Lymphoma	0	1(0.5)	0	2(1.1)	0	0	**3**
Total		**10 (5.3)**	**21 (11.1)**	**41 (21.7)**	**80 (42.3)**	**28 (14.8)**	**9 (4.8)**	**189 (100)**

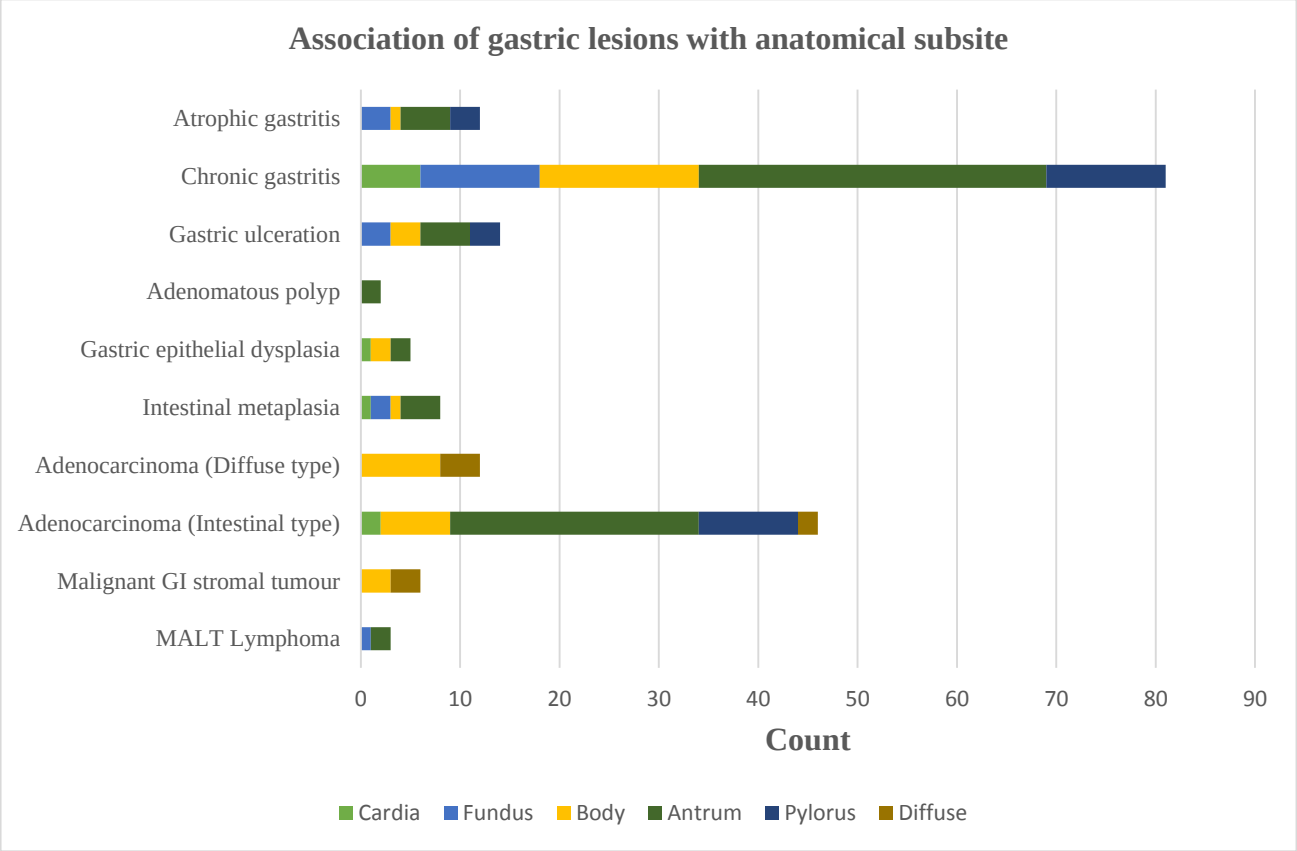


Figure 12: Histological diagnoses by anatomical sub site

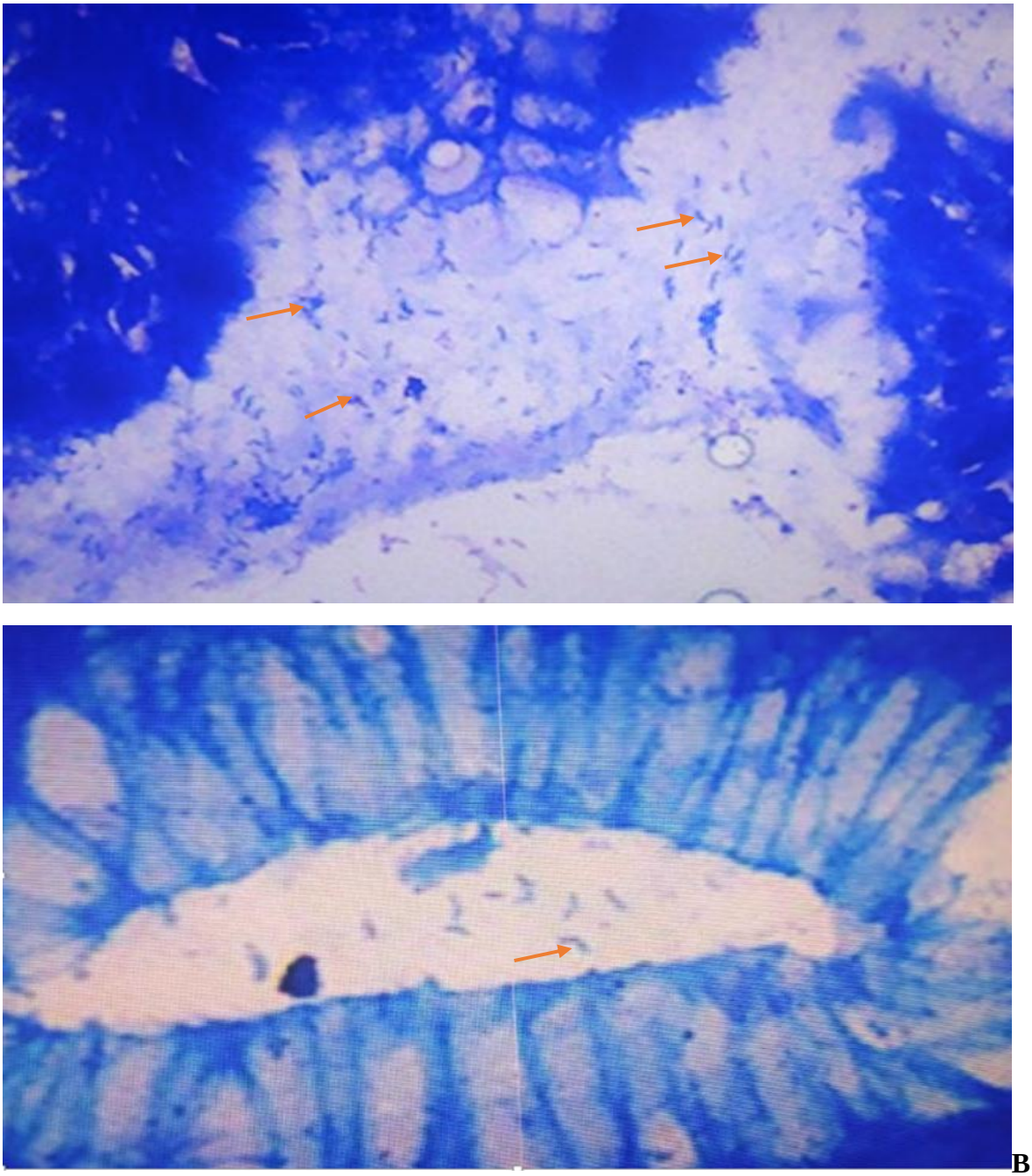
3.4 Helicobacter pylori status in gastric lesions

A total of 189 gastric biopsies were stained for Helicobacter pylori using modified Giemsa stain and among these 47 (24.9%) were positive and 142 (75.1%) were negative for Helicobacter pylori. Out of 47 biopsies which stained for Helicobacter pylori, 43 (91.5%) were inflammatory lesions and only 4 (8.5%) were malignant lesions. Of 67 cases of malignant lesions only 4 (6.0%) stained positive for H. pylori, and all of them were Intestinal type adenocarcinoma. Chronic gastritis with 39(20.6%) was the gastric lesion that expressed much H. pylori. Diffuse type adenocarcinoma, MALT lymphoma did not express Helicobacter pylori (p value was <0.001) (**Table 5**).

Table 5. Proportion of H. Pylori infection in gastric lesions. (p<0.0001)

Diagnosis category	Histological Type	Helicobacter pylori Status		Total
		Negative (%)	Positive (%)	N=178
Inflammatorylesions	Atrophic gastritis	10 (5.3)	2 (1.1)	**12**
	Chronic gastritis	42 (22.2)	39 (20.6)	**61**
	Gastric ulceration	12(6.3)	2 (1.1)	**12**
Benign lesions	Adenomatous polyp	2 (1.1)	0	**2**
	Gastric epithelial dysplasia	5 (2.6)	0	**5**
	Intestinal metaplasia	8 (4.2)	0	**8**
Malignant lesions	Adenocarcinoma (Diffuse type)	12 (6.3)	0	**12**
	Adenocarcinoma (Intestinal type)	42 (22.1)	4 (2.1)	**46**
	Malignant GI stromal tumour	6 (3.2)	0	**4**
	MALT Lymphoma	3 (1.6)	0	**3**
Total		**142(75.1)**	**47 (24.9)**	**189 (100)**

A



B

Figure 13: Helicobacter pylori bacteria (arrows) in gastric lesion showing A. Gastric lesion under study, B. Positive control (Modified Giemsa x400)

CHAPTER FOUR

4.0 DISCUSSION

4.1. Pattern and frequency of Gastric lesions.

Gastric adenocarcinoma was the most frequent diagnosis among the malignant lesions in this study, with a proportion of 86.6% among all malignant lesions. This is closely similar to another study done by Mabula et al., (4) in a teaching hospital in Tanzania with a proportion of 95.1%. This is probably due to similar pathogenic mechanisms as a result of the same risk factors such as H.pylori infection, environmental, dietary, and smoking in Tanzanian patients.

The current study shows a predominance of CG (42.9%) among all gastric lesions similar to the Tanzanian study mentioned above in which CG was predominant (61.1%) (52) and also similar to a study done in India (89%).This indicates that CG is a significant premalignant lesion in different geographical areas, but the frequency of the lesion may vary. The differences in percentages may be explained by the differences in risk factors exposure in a geographic region, the duration and variable methodology of the studies.

4.2 Association of gastric lesions and age groups

The mean age of the study population in the present study was 53.06 years. This finding is closely similar to what was seen in other studies conducted in elsewhere in Tanzania (58.7 years)(53) but higher than patients from a study done in Jordan (36.6 years) (21) and Iran (48 years)(50). The differences in mean ages at diagnosis in Tanzanian patients compared to other studies may be due lack of awareness and inadequate primary health facilities which leads toseeking medical care at late in life, when the disease is already apparent (43).

The finding that Gastric Cancer (GC) was found more common in adults aged 40 years and above (86.6%) is similar to other studies done in BMC (88.4%) (4), and in Korea (86.5%) (3).This implies that patients GC present at late stages clinically (1, 18, and 37). The increased frequency of GC in a young population aged less than 40 years of age (15.6%) seen in the current study corroborates findings in serial studies done in California. The studies revealed that 6% to 15% of patients below 41 years of age had GC (20) and also supported by research in Jordan (21) where 9.7% of participants with GC were under 40 years of age**. These findings can be explained by the fact that the majority of gastric lesions are acquired during childhood as asymptomatic lesions. They then progress in a complex interplay of**

genetic, environmental and host pathogenesis factors before the clinical symptoms manifest (20 - 22).

Diffuse type adenocarcinoma and MALT lymphomas were confined in patients above 40 years of age. This finding is different from that of another study done in India (26), where the two tumours were foundin patients aged less than 40 years. Thedifferences can perhaps be due todifferences in health-seeking behavior in the two countriesleading to diagnosis when premalignant lesions are already transformed, especially in Africa (43).

4.3 Association of gastric lesions with sex.

The large number of male patientswith premalignant lesions in the current study is concordant with other studies in Korea (37) ere males were 65.5%, and females were 34.5. This is also supported by Indian research (26) and others(43,45–50), where approximately 65% of males and 35% of females had gastric lesions.The finding was not easily possible to explain, but other studies have stated that the reason may be due to differences in late health-seeking behaviours among males as compared (41). This could also be due to differences in exposure to the etiological factors between males and females. Similar to premalignant lesions, GCwas frequent in males with a male to female ratio of 1.7:1. This finding supports similar findings elsewhere in Tanzania (4) where males with malignancy were 74.1%, and females were 25.9% and in other areas elsewhere (20,21,27,37,). This could be a result of similar pathogenic mechanisms after exposure to the risk factors such as environmental, nutritional, smoking, social conduct between males and females in different geographical settings (34). For example, men have been historically more likely to smoke tobacco products than females.Sex differences may also reflect physiological differences. Studies have shown that estrogen hormone may protect against the development of GC. In women, delayed menopause and increased fertility may lower the risk of GC, whereas anti-estrogen drugs, e.g., tamoxifen may increase the rates of GC (3, 21, 22).

In the current study, chronic gastritis was observed frequently in both sexes as a premalignant lesion again supporting findings of studies conducted in other regions (15, 31-33).This emphasizes the notion of common etiological risks and pathogenesis in the progression of inflammatory premalignant conditions to GC in both sexes (31-33).

4.4 Association of gastric lesions with the anatomical site of the lesion.

Mabula et al., antrum (56.5%) (4), reported that the antrum and pylorus were the frequent anatomical sites for gastric cancer. Similarly, the current study supports their findings were 56.1% were located in the antrum and pylorus. The increased frequency of cancer in the pylorus and antrum may be due to exposure to dietary risk factors.The finding that the distal part of the stomach was more affected by gastric cancer (94.7%) is similar to results of other studieswhere distal cancers ranged from 56% to 75% (18, 21, 26, 28, 34). The findings further indicate that in developing countries gastric cancer is commonly associated with H.pylori infection which is frequently related to the frequency of distal gastric cancer (3, 21, 25, 28, 34).

4.5 H. Pylori status in patients with gastric lesions.

In general, this study had a low frequency of H. Pylori positive (24.9%) gastric cancer compared to studies done elsewhere where the proportion was slightly higher, ranging from 30% to 60% in different Asian countries (8,33,50,55,56). The difference can be due to the methodologies used and sample sizes, use of antibiotics close to the period of taking a biopsyin this and other studies (55-56).Furthermore, the pathogenesis of some malignant gastric lesions like diffuse-type adenocarcinoma and GIST does not involve the action of H. pylori infection, and these cancers were not considered and omitted in the count. Furthermore, there is a possibility of H. pylori to change its morphology from the usual spiral form during harsh conditions such as antibiotic treatment, hence leading to false-negative results as seen in other studies (26).

Inflammatory lesions, particularly chronic gastritis, accounted for most of the gastric lesions, which expressed Helicobacter pylori infection. These findings were closely similar toother studies (18,45,51), where H. pylori infection was associated with chronic gastritis as one of its major causative factor (10-12). Intestinal type adenocarcinoma, which was the only malignant lesion, associated with H. Pylori (8.9%) in the current study, was the least of all gastric lesions with Helicobacter pylori infection.

In this study, some benign lesions like intestinal metaplasia and gastric epithelial dysplasia whose pathogenesis depend on H. Pylori infection were all negative for Helicobacter pylori. Another research has shown that the loss of H. pylori correlates with the development of intestinal metaplasia and hypochlorhydria, which seem to be hostile environments for H. pylori colonization(20).

CHAPTER FIVE

5.0. CONCLUSION

Gastric lesions are common conditionsin Tanzania's patients commonly manifesting as chronic gastritis and gastric adenocarcinoma for inflammatory and malignant lesions, respectively. They are more frequent in males than females

The age, sex, anatomic site and histomorphological characteristics are similar in **some** regions in Tanzania and similar to those seen in other Africa countries. The frequency and patterns in Tanzania slightly differ from the Far East and Western countries.

Both gastric lesions are found more common in adults with 40 years and above. However, there is an increased frequency of intestinal gastric adenocarcinoma in young age below 40 years of age.

The distal part of the stomach was the most common anatomical site occupied by both malignant and premalignant lesions.

H. Pylori infection in this study was most commonly found in premalignant lesions, especially chronic gastritis.

A significant number of patients with gastric cancer at MNH had precursor inflammatory and benign lesions, which can be diagnosed early and treated before giving rise to cancer.

6.0 RECOMMENDATIONS

Clinicians and pathologists should be aware of the available diagnostic methods for early premalignant lesion, early gastric cancer and H. pylori infection to prevent and initiate early treatment of gastric cancer.

Individuals with inflammatory premalignant lesions like atrophic gastritis should be managed to reverse precancerous lesions by eradicating H. pylori and stop the progression of premalignant lesions to gastric cancer.

Screening programs for gastric cancer, focusing on the people below 40 years of age should be initiated in collaboration with all stakeholders to detectgastric inflammatory and pre-malignant lesions

More studies are required to find the actual frequency of H. pylori and gastric cancer, including pathogenic factors in young and old individuals in Tanzania to plan prevention strategies.

7.0 STUDY LIMITATIONS.

The study time was minimal. More study time is required to make a comprehensive study which will include treatment methods, prognosis and follow up of patients after treatment.

Inadequate funds for purchasing reagents for immunohistochemistry, which would help confirm some diagnosis with controversial.

8.0 DISSEMINATION OF THE FINDINGS

The findings from this study will be presented to the Pathology department at the Muhimbili University of Health, and Allied Sciences and thereafter submitted to the Research and Publication Committee of Muhimbili University of Health and Allied Sciences, the Directorate of Laboratory services where data were collected and studies as well as to the Director of Muhimbili National Hospital. The data will be submitted to the MUHAS library and finally published in peer-reviewed scientific Journal.

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10.0 APPENDICES

10.1 Appendix I: Haematoxylin and eosin staining procedures

i. Retrieve blocks and make tissue sections using microtome [3-5 microns]
ii. Pick the section on a slide from water floatation and place a hot plate 60^0C for 50 minutes.
iii. Deparaffinized sections, hydrate through graded alcohol to water
iv. Remove fixation pigments if necessary
v. Stain in alum haematoxylin for a suitable time.
vi. Wash well in running tap water until section ‘blue’ for 5 minutes
vii. Differentiate in 1% acid alcohol (1% HCl in 70% alcohol) for 5-10 seconds.
viii. Wash thoroughly in tap water until sections are again ‘blue' (10-15 minutes)
ix. Stain in 1% eosin Y for 10 minutes.
x. Wash in running water for 1-5 minutes
xi. Dehydrate through alcohols (70, 95, 100%), clear and mount

10.2 Appendix II: Diff quick stain (Modified Giemsa stain) procedures

i. Dewax sections and bring down to distilled water
ii. Dip the slides in diff quick solution I, 25 dips
iii. Dip slides in diff quick solution II, 25 dips
iv. Rinse quickly in distilled water
v. Differentiate in in 2 change of acetic acid water, 5 dips in each
vi. Rinse quickly in distilled water
vii. Dehydrate in 1 change of 95% alcohol, 15 dips
viii. Continue dehydration with 1 change of absolute alcohol, 5 dips
ix. Clear in 2 changes of xylene
x. Mount with DPX and label the slide

10.3 Appendix III: Data collection Instrument

Serial number	Laboratory number	Age of the patient	Sex of the patient	Anatomical site of biopsy	H&E Diagnosis	H.pylori status