HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR -2 OVEREXPRESSION IN PATIENTS WITH GASTRIC AND OESOPHAGEAL ADENOCARCINOMA- A RETROSPECTIVE STUDY ON GHANAIAN CANCER PATIENTS WHO ATTENDED KORLE BU TEACHING HOSPITAL

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Declaration

Declaration by candidate

I hereby declare that this is the product of my own research undertaken under supervision and has neither been presented in whole nor in part for another degree elsewhere. I am responsible for any flaws in this work

Signature..... Date...../...../.....

(David Larbi Simpong)

Declaration by supervisor

We hereby declare that the practical work and presentation of this thesis were supervised in accordance with guidelines on supervision of thesis laid down by the University of Ghana.

Principal supervisor

Signature..... Date...../...../....

(Professor Richard K. Gyasi)

Co-supervisor

Signature..... Date...../...../.....

(Richard H. Asmah)

Dedication

I dedicate this work to my parents, Mr. Anthony Larbi Simpong and Madam Victoria Boakyewa for their care and support.

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Table of Contents

List of tables

List of figures

List of abbreviations

ADC adenocarcinoma (ADC)

Akt 1- Protein kinase B

ALP Alkaline phosphatise

CR cysteine-rich regions

CT carboxy terminal tail

DPX Distrene, Plasticiser, Xylene

GERD gastro-oesophageal reflux disease

H&E Haematoxylin and eosin

HER-2 Human epidermal growth factor receptor -2

IHC Immunohistochemistry

KBTH Korle Bu Teaching Hospital

LD ligand binding regions

MAPK mitogen activated protein kinase

PI3K phosphatidylinositol 3-kinase

PLCγ phospholipase C-γ

SCC squamous cell carcinoma (SCC)

SD Standard deviation

TK tyrosine kinase domain

TM transmembrane domain

Abstract

Background: Despite improvement in surgical techniques combined with chemotherapy and /or radiotherapy, the prognosis of gastric and oesophageal adenocarcinoma at the advance stage still remained poor. However, there is mounting evidence of the role of HER-2 expression in patients with these cancers.

Aim: To determine the patterns of HER-2 protein expression in patients with gastric or oesophageal adenocarcinoma at the Korle Bu Teaching Hospital (KBTH), Ghana

Method: Retrospectively, records on gastric and oesophageal biopsies received between 2008 and 2012 (KBTH) were reviewed. Ideal tissue blocks were selected for immunohistochemistry analysis. The prevalence of gastric and oesophageal adenocarcinoma, and their significant association with HER-2 protein over-expression were evaluated.

Result: A prevalence of 18.79% gastric adenocarcinoma was observed among gastric biopsies and majority of this cancers occurred in males. Human epidermal growth factor receptor-2 (HER-2) was over-expressed in 41.4% of the gastric adenocarcinomas and was significantly more common in patients older than 55 years and with intestinal type of adenocarcinoma. Though, Squamous cell carcinoma still remains the commonest cancer (31%) type of the oesophagus, compared to oesophageal adenocarcinoma (8.79%), HER-2 was over-expressed in 42.9% of oesophageal adenocarcinoma which is similar to that of gastric adenocarcinoma (41.4%).

Conclusion: Routine testing for HER-2 in gastric and oesophageal adenocarcinoma patients can have significant implication on the management or treatments options offered such patients, which may potentially affect their prognosis.

CHAPTER ONE

1.0 INTRODUCTION

Cancer is a major health problem worldwide with approximately 12% total mortality rate annually (Carl-McGrath *et al.,* 2007). Although breast and lung cancers are the common diagnosed cancers in women and men respectively, gastrointestinal tract cancers involving the oesophagus and stomach contribute significantly to cancer death in general (Carl-McGrath *et al.,* 2007). Gastric cancer is the fourth most commonly diagnosed cancer and the second leading cause of cancer death worldwide (Ahmedin *et al.,* 2010) with approximately 989,600 people being diagnosed annually resulting in 738,000 deaths (Chan *et al.,* 2012). Approximately 482,300 people are diagnosed of oesophageal cancers worldwide, resulting in about 406,000 deaths annually (Chan *et al.,* 2012).

The aetiology and pathogenesis of these cancers are unclear; however several factors have been postulated including gastro-oesophageal reflux disease (GERD) and Barrett's oesophagus. These factors have been suggested to potentiate oesophageal cancer (Wang and Souza, 2011). Other studies have also implicated certain viral and bacterial pathogens (Matsha *et al.,* 2002, Shuyama *et al.,* 2007, Ding *et al.,* 2010, Nagini, 2012) as major risk factors for both gastric and oesophageal cancers. Another study, Carl-McGrath *et al.,* (2007) observed that geographical location also plays a major role in the pathogenesis and the prevalence of gastric and oesophageal cancers (Carl-McGrath *et al.,* 2007). Since 1970, a dramatic change has occurred in the prevalence of oesophageal malignancy, with adenocarcinomas of the oesophagus on the increase while the prevalence of squamous cell carcinoma remains stable (Williams *et al.,* 2006).

Treatment and/or management modalities for gastric and oesophageal cancers include curative surgery, palliative chemotherapy and radiotherapy (Wu *et al.,* 2009). Growing evidence suggests that, the presences of certain molecular biomarkers in these tumours are important for therapy target (Price *et al.,* 2012). One of such targeted receptors for treating gastric and oesophageal adenocarcinoma is human epidermal growth factor receptor 2 (HER-2) (Price *et al.,* 2012).

Human epidermal growth factor receptor 2 (HER-2)**,** which is an 185,000 molecular weight transmembrane tyrosine kinase, has been shown to have therapeutic and prognostic implications in gastric (Walch *et al.,* 2001) and oesophageal (Almhanna *et al.,* 2013) cancers. The HER-2 gene product is involved in the regulation of cell growth, differentiation, migration and apoptosis (Chan *et al.,* 2012). However, HER-2 overexpression has been implicated in the proliferation, invasion and apoptosis of tumour cells during tumourigeneses of many organs (Dang *et al.,* 2012).

Improvement in the prognosis of these cancers has been demonstrated in patients whose tumours over-expressed HER-2 and are administered with biomarker targeted drug, trastuzumab. However, treatment of these cancers in Ghana is still limited to either surgery and chemotherapy or radiotherapy; yet unpublished data from the Korle Bu Teaching Hospital indicate that the survival rate of patients who suffer from these cancers has been generally very poor. Also, these new therapies which turn to target specific genetic alterations and biomarkers, and arguably offer the best chance for improving patient survival have also not been considered for these adenocarcinoma patients in Ghana. The present study intends to find the prevalence of adenocarcinoma in the stomach and oesophagus, and the association of this cancer with HER-2 over-expression.

1.1 Problem statement

Gastric and oesophageal adenocarcinoma contribute significantly to cancer mortality and morbidity in Ghana. Unpublished reports from the Pathology Department, Korle Bu Teaching Hospital (KBTH), in Accra Ghana, indicate that out of the 1,065 gastric and oesophageal specimens received between 2008 and 2012,183 gastric biopsies and 8 oesophageal biopsies were diagnosed as adenocarcinoma. Generally, patients who are diagnosed with adenocarcinoma of the stomach or the oesophagus in Ghana are treated with surgery and chemotherapy or radiotherapy. However, in Europe and USA (Ruschoff *et al.,* 2010, Ross and Mulcahy, 2011, Gencer *et al.,* 2013), treatment of gastric and oesophageal adenocarcinoma had been geared towards certain prognostic and molecular biomarkers. And this has conferred a significant survival advantage in such gastric and oesophageal adenocarcinoma patients. One of these prognostic markers is HER-2 protein, which has gained worldwide acceptance as an important marker in patients with these cancers. However, the role, if any, that HER-2 protein expression plays in patients with gastric or oesophageal adenocarcinoma in Ghana is unknown.

Recently, trastuzumab has emerged as the first targeted drug to improve overall survival when combined with chemotherapy in gastric or oesophageal adenocarcinoma patients who over-expressed HER-2 protein (Pazo Cid and Anton, 2012). However, in Ghana routine screening for HER-2 protein expression is not a common practice among these patients thus making selection for those who will benefit from trastuzumab a major challenge. Moreover, data relating the prevalence and association of these cancers to HER-2 protein in Ghana is also lacking contributing to the less attention given to the importance of HER-2 protein expression

in these patients. This study will contribute to finding solutions to the many unanswered questions relating HER-2 protein expression in adenocarcinoma of either the stomach or oesophagus in Ghana.

1.4 Aim

To determine the prevalence and patterns of HER-2 protein over-expression in patients with gastric or oesophageal adenocarcinoma at the Korle Bu teaching hospital

1.5 Specific objectives

1. To determine the prevalence adenocarcinoma of the stomach and oesophagus among other pathology of these sites for patients visiting Korle Bu Teaching Hospital.

2. To determine whether there is significant association between HER-2 over-expression and adenocarcinoma of the stomach or oesophagus among different age groups, genders and histological subtypes of adenocarcinoma at KBTH.

1.2 Significance of the study

The study proposal aims at a better understanding of using the over-expression of HER-2 as biomarker in the diagnosis, management and treatment of gastric or oesophageal adenocarcinoma. The identification of the association between HER-2 protein over-expression in persons with gastric or oesophageal adenocarcinoma in Ghana will consequently help clinicians in the selection of patients who can benefit from trastuzumab administration or otherwise. Since the level of the over-expressed oncogenic HER-2 protein is the same both in the early and advanced stages of these cancers, suspected adenocarcinomas of the stomach or oesophagus patients can be screened immediately for HER-2 over-expression to aid in the commencement of trastuzumab administration. More importantly, in

situations where patients have not yet over-expressed HER-2, such person can in the future, benefit from HER-2 vaccine which is currently undergoing Phase l clinical trial.

1.3. Hypothesis

There is no significant association between gastric or oesophageal adenocarcinoma and HER2 protein expression in patients with gastric and oesophageal cancer at the Korle Bu Teaching Hospital.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of gastric and oesophageal adenocarcinoma

Cancer of the oesophagus mainly; squamous cell carcinoma (SCC) and adenocarcinoma (ADC) remains the leading cause of cancer mortality worldwide (Kamangar *et al.*, 2009). In 2008, about 482000 new cases of oesophageal cancers were recorded worldwide, with about 83% occurring in developing countries (Ferlay *et al.*, 2010). Most studies conducted earlier on placed more emphasis on SCC compared to ADC, probably because much studies has not been carried out then. However, from the early part of 1970, a dramatic change has occurred in the prevalence of oesophageal malignancy, with adenocarcinomas of the oesophagus on the increase while the prevalence of squamous cell carcinoma remains stable (Williams *et al.*, 2006).

The first estimates of stomach cancer date back to 1975 where it was found to be the most common neoplasm worldwide (Parkin *et al.*, 1984). However, in 2010 stomach cancer drop to become the fourth most commonly diagnosed cancer globally behind lung, breast and colorectal cancers with over 70% of all this cases occurring in developing countries (Ferlay *et al.*, 2010). Contrary to the current evolvement of adenocarcinoma of the oesophagus, adenocarcinoma of the stomach continue to be a leading cause of cancer death worldwide, resulting as the second and fourth most common cancer in males and females respectively (Nagini, 2012). In 2009, it was reported that nearly more than one million new cases of gastric adenocarcinoma will be recorded each year (Wu *et al.*, 2009).

There are several types of gastric tumours, for instance, endocrine tumours, mesenchymal tumours, lymphoma and epithelial tumours (adenocarcinoma), however, gastric adenocarcinoma alone represents over 95% of all gastric tumours (Meireles *et al.,* 2004). As of 1980, gastric cancer was the most frequent neoplasm registered in the world with the highest death rate being registered in Japan, followed by northern Europe and the Andean populations of Latin America (Correa, 1992).

2.2 Prevalence and incidence of gastric and oesophageal cancer

The prevalence of gastric and oesophageal adenocarcinoma varies in different parts of the world, among various ethnic groups and gender (Nagini, 2012, Corley and Buffler, 2001). For instance gastric adenocarcinoma remains the top killing cancer in Asian countries like Japan, Korea, and China (Wu *et al.,* 2009). In Africa, the highest prevalence of stomach cancer countries comprised Rwanda, Burundi, South Western Uganda, Eastern Kivu province of Democratic Republic of Congo and sub-Saharan African countries (Ahmed *et al.,* 2011). A retrospective study conducted in Lagos, Nigeria for 105 gastric cancer cases seen between 1995 – 2007 revealed a prevalence of 90% adenocarcinoma among other gastric cancers (i.e. mesenchymal tumours, small cell non-Hodgkins lymphoma and carcinoid tumour). Males showed higher prevalence compared to females with ratio of 2:1 and mean age of 55.3 (Abdulkareem *et al.,* 2010). Similar retrospective study carried out in Togo for 742 gastrointestinal tumours diagnosed between 1986 and 2005 identified 306 (41.2%) as stomach cancers and about 224 (73.2%) of this number were diagnosed as gastric adenocarcinoma (Amegbor *et al.,* 2008). In Tunis, Tunisia, 84% of 582 gastric cancer cases sampled between 1970 and 1985 were histologically diagnosed as adenocarcinoma, and the patients had an average age 55years (Chadli *et al.,* 1986). In Zambia 8.8% of 2132

diagnosed upper gastrointestinal tumours from 1999–2005 saw adenocarcinoma of the stomach to be the commonest (Kelly *et al.*, 2008). In Senegal, a prevalent of 90% gastric adenocarcinoma was recorded for 220 diagnosed gastric cancer cases between 1984 and 1991 (Niang *et al.*, 1996). Meanwhile, the intestinal type of gastric adenocarcinoma has been found to be prevalent in Plateau state of Nigeria, with about 51.2% of 205 gastric cancer cases recorded from 1985 to 2004 (Mandong *et al.*, 2010). Stomach cancer incidence is known to increase with age with the peak incidence occurring at 60-80 years (Nagini, 2012). The variation in gastric cancer among ethnic groups in the same country is typified by the prevalence of 68.2% of poorly differentiated gastric adenocarcinoma as against 4.5% well differentiated adenocarcinoma in fifty three consecutive patients diagnosed in Kenya between 1987 and 1989 (Ogutu *et al.*, 1991). Incidence rates for gastric cancer are about twice as high in men as in women, ranging from 3.9 in Northern Africa to 42.4 in Eastern Asia for men, and from 2.2 in Southern Africa to 18.3 in Eastern Asia for women (Ferlay *et al.*, 2010).

In 2008, about 482,000 new cases of oesophageal cancers were recorded globally, placing this caner as the eighth most frequently diagnosed cancer and the sixth leading cause of cancer deaths (Ferlay *et al.*, 2010). The highest incidence rates of oesophageal cancer are found in Asia and Sub-Saharan Africa and the lowest rates are found in Europe and North America, with rates much higher in men than in women (Jemal *et al.*, 2010). As of 2010, oesophageal cancer was the 5th commonest cancer in developing countries while in Sub-Saharan Africa, 15,150 cases in males and 7,200 cases in females were estimated to have occurred in 2000 (Kachala, 2010). Indeed, the incidences of oesophageal adenocarcinomas have recently risen more rapidly than any other malignancy in some countries; with average annual incidence

increases of up to 17% for oesophageal adenocarcinoma (Corley and Buffler, 2001). As of 2012, the United State had diagnosed approximately 17,460 oesophageal cancers with an expected death rate of 15,070 from this neoplasm (Almhanna *et al.*, 2013). In Ghana, there is scanty epidemiological data on the prevalence of oesophageal adenocarcinoma. A prevalence of 21.3 % oesophageal adenocarcinoma was reported among 152 samples diagnosed with cancer of the oesophagus from 1992 to 2010 in Ghana (Tettey *et al.*, 2012). However, when a one year cross sectional study was conducted for 287 patients presenting for oesophageal-gastro-duodenoscopy at Mulago hospital, Uganda, a prevalence of 7.3% adenocarcinoma was reported among the 55 diagnosed oesophageal cancers (Ocama *et al.*, 2008).

2.3 Aetiology of oesophageal adenocarcinoma

Extensive investigations into the aetiology of oesophageal adenocarcinoma started only in the 1990s, mainly in Western countries, where larger numbers of this cancer began to be diagnosed (Kamangar *et al.*, 2009). The two major factors that have been identified to have been associated with oesophageal adenocarcinomas are the environment and genetic (Kamangar *et al.*, 2009). Several factors have been associated with the environmental aetiology of oesophageal adenocarcinoma. These include; gastroesophageal reflux disease, obesity, tobacco smoking, hiatal hernia, achalasia, low intake of fresh fruits and vegetables, consumption of carbonated soft drink, use of H2 blockers, non-steroidal anti-inflammatory drugs, drugs that relax the lower oesophageal sphincter, consumption of hot drink, and low socioeconomic status (Rubenstein and Taylor, 2010). Genetically, it has also been noted that constitutive expression of activation-induced cytidine deaminase (AID) in oesophageal cells contributes to the accumulation of somatic mutations in both

CDKN2A and TP53 genes, resulting in a substantial proportion of human Barrett's epithelial and adenocarcinoma (Morita *et al.*, 2011). Also, pancreatic enzymes (Peters and Fitzgerald, 2007) and bile acid reflux plays a critical role in aberrant AID expression in the development of Barrett's oesophageal adenocarcinoma (Morita *et al.*, 2011).

2.4 Aetiology of gastric adenocarcinoma

The aetiology of gastric cancer is multi-factorial, and includes both dietary and non-dietary factors (Nagini, 2012). A hypothesis published in 1975 considered 3 major aetiological factors for gastric carcinogenesis, namely excessively salty foods, low intake of ascorbic acid and carotenoids (Correa, 1992). Although Lifestyle, genetic, socio-economic and others are classified as non dietary contributory factors for gastric carcinogenesis (Nagini, 2012), the crucial role played by *Helicobacter pylori* (*H. pylori*) as the single most common cause of gastric cancer cannot be overlooked (Milne *et al.*, 2009). A meta-analysis concluded that *H. pylori* infection is allied with an approximately two-fold increased risk of developing gastric cancer (Eslick, 2006).

The infection by *H. pylori* varies markedly in different countries with higher prevalence in developing countries than in industrialized, developed nations. At present, about 50 per cent of the world's population is infected by this bacterium (Fock and Ang, 2010). Mishmash involving; a tolerant environment, the virulent form of *H. pylori* and a genetically vulnerable host induced gastric cancer by this bacterium. This Suggest a possible trigger of cascade of events that promote the sequential progression of normal gastric epithelium through atrophic gastritis, intestinal metaplasia, and dysplasia to carcinoma (Kim *et al.*, 2011b, Nagini, 2012).

One important distinguishing factor of the virulent form of *H. pylori* is the ability to induce the production of reactive oxygen and nitrogen species that suppresses the host antioxidant defence mechanisms, resulting in oxidative DNA damage. This oxidative stress and the damage is solely restricted to the gastric mucosa of the susceptible host (Suzuki *et al.*, 2012).

A review conducted by (Nagini, 2012) found out that populations at high risk for stomach cancer are those who consume diets rich in starch and poor in protein quality, fewer intakes of fresh fruits and vegetables. And that, both high starch and low protein diet may errand acid-catalyzed nitrosation in the stomach and grounds mechanical damage to the gastric mucosa (Krejs, 2010). Also, there exist a correlation between dietary salt intake and risk of gastric cancer with progressively increasing risk across consumption levels based on a meta-analysis (D'Elia *et al.*, 2012). Nitrosation of a number of naturally occurring guanidine and L-arginine-containing polypeptides produces mutagenic compounds in stomach. These dietary nitrates are found naturally in foods such as cabbage, cauliflower, carrot, celery, radish, beets, and spinach or may be added during preservation. Other sources of this nitrate are from fertilizers, soil, and water (Suzuki *et al.*, 2005). Cigarette smoking has also been associated strongly with the risk of gastric adenocarcinoma and its precursor lesions (Shikata *et al.*, 2008).

Though, the initial stage of gastric cancer development has been associated with *H. pylori* infection, the intermediate stages in the progression to gastric adenocarcinoma is also linked to ingestion of ascorbic acid and nitrate, and the final stages proposed to be correlated to the supply of B-carotene and with excessive salt intake (Correa, 1992). In the aetiology of gastric adenocarcinoma, a delicate interplay exist between

the environment and the host genes which varies with sex, the individual and their ethnic backgrounds, providing in each individual a unique milieu for cancer progression or suppression (Milne *et al.*, 2009). Thus, the relative importance of environment and genetics can however vary with tumour type, and it is long identified that the brunt of environmental triggers can be seen at gene level (Milne *et al.*, 2009). A further study into the aetiology of gastric adenocarcinoma showed that it could results from the complex gene environment interactions, deregulation of signalling pathways, aberrant DNA methylation patterns, and chromosomal imbalances (Hudler, 2012). Epitomize by the fact that majority of this gastric adenocarcinomas are characterized by genetic instability, either microsatellite instability (MSI) or chromosomal instability (CIN) (Hudler, 2012), inactivation of tumour suppressor genes, activation of oncogenes, and reactivation of telomerase (Zheng *et al.*, 2004).

2.5 Pathogenesis of oesophageal adenocarcinoma

It is extensively established that oesophageal adenocarcinoma develops from Barrett's oesophagus through a series of steps from low-grade dysplasia through high-grade dysplasia to adenocarcinoma which may be followed by metastasis (Peters and Fitzgerald, 2007). The oesophagus is prone to repeated insult from duodeno-gastro-oesophageal refluxate containing acid, bile acids and pancreatic enzymes; trauma from hot and cold foodstuffs, salivary enzymes and the production of nitric oxide from the ingestion of nitrous species (Fitzgerald, 2005). The interaction between these acidic gastric refluxate and nitrite rich saliva activates potentially mutagenic luminal within oesophagus (Suzuki *et al.*, 2005). Furthermore, the reflux of gastric and duodenal contents leads to mucosal injury, cellular proliferation and columnar/glandular metaplasia of the normal squamous mucosal lining (Moyes and

Going, 2011). Oesophageal cells are damage following these interactions, resulting in the recruitment of inflammatory mediators such as cytokines to the site of injury in response to the tissue insult (Wetscher *et al.,* 1997). The free radicals produced by these inflammatory cells tend to induce genetic mutations instead of getting rid of the damaged cells. However, whiles most of these changes will result in cell death, others may confer a survival advantage and lead to a clonal expansion of the premalignant, Barrett's cell type (Peters and Fitzgerald, 2007, Wetscher *et al.*, 1997). Meanwhile, for Barrett's oesophagus to develop, chronic exposure to the earlier mentioned insults seems to be very critical, suggesting that luminal damage of the epithelium is required at the outset (Gillen *et al.*, 1988). However, it is imperative to note that Barrett's metaplasia is likely to have originated from within the oesophageal compartment rather than from overgrowth of nearby gastric tissue (Li *et al.*, 1994). In Barrett's oesophagus the supporting oesophageal stroma becomes infiltrated with inflammatory cells fundamentally different from those found in oesophagitis (Fitzgerald *et al.*, 2002). Barrett's oesophagus has increased levels of T helper 2 (anti-inflammatory) cytokines and a reduction in the ability to signal through the transforming growth factor β (TGFβ) cascade owing to a decrease in the expression of the signalling components TβRII, Smad2 and Smad4 (Onwuegbusi *et al.*, 2006).

A metaplasia-dysplasia-cancer sequence is characteristic of progression to Barrett's oesophagus (replacement of normal squamous mucosa with columnar lined epithelium) (Moyes and Going, 2011). The metaplastic conversion of the oesophageal squamous epithelium to a columnar-lined epithelium might take place from two diverse categories of cell; either direct conversion of differentiated cells in the absence of cell proliferation, or develop from the conversion of a "stem" or

"pluripotential cell" (Schier and Wright, 2005). Barrett's oesophagus is predisposed to more severe acid reflux because of two principal pathological mechanisms; mechanical dysfunction of the lower oesophageal sphincter or the decreased amplitude of distal oesophageal contractions in Barrett's oesophagus patients compared with healthy controls or patients with oesophagitis (Souza *et al.*, 2001).

The transformation of a single Barrett's epithelial cell into a cancer-initiating cell through a series of genetic changes is enough for the development of adenocarcinoma from Barrett's oesophagus (Fitzgerald, 2006). Therefore a number of important molecular biomarkers such as cyclooxygenase-2 (COX-2), HER2/neu (erbB2), mesenchymal epithelial transition factor (MET), and matrix metalloproteinases (MMPs) in the oesophagus acting as promising targets for in vivo cancer detection, risk stratification, and therapeutic intervention (Lu and Wang, 2008); for instance, amplification of HER-2 has been shown to have a link to worse outcome in patients with Barrett's oesophagus-associated adenocarcinoma, while protein over-expression, on the other hand, has been found in 22% of adenocarcinoma patients (Brien *et al.*, 2000). Also, HER2 protein over-expression has been implicated in early feature of the invasive adenocarcinoma pathway, whereas amplification is a late event, and that possible method of protein overexpression may exist (Geddert *et al.*, 2002).

There are a number of important molecular biomarkers in oesophagus that are promising targets for in vivo cancer detection, risk stratification, and therapeutic intervention, including cyclooxygenase-2 (COX-2), HER2/neu (erbB2), mesenchymal epithelial transition factor (MET), and matrix metalloproteinases (MMPs) (Lu and Wang, 2008).

2.6 Pathogenesis of gastric adenocarcinoma

The transformation of a normal epithelial cell to a malignant cell is a multistep progression and results from accretion of multiple gene abnormalities (Chan *et al.*, 1999). Even though the pathogenesis of gastric cancer remains largely unclear, it has been proposed that, the sequence began with chronic gastritis followed by atrophy, then intestinal metaplasia to dysplasia (Correa, 1992).

Helicobacter pylorus has been identified as the most important environmental risk factor associated with sporadic gastric cancer (Nardone, 2003), while the World Health Organization (WHO) has classified these bacteria as a class 1 carcinogenic (1994). Exposure of gastric epithelial cells to this bacterium results in the generation of reactive oxygen species and inducible nitric oxide synthase with possible genetic alterations leading to cancer in a subset of such people (Nardone, 2003). This bacterium is well modified to the gastric environment, withstanding low pH to gain entry to its preferred mucus layer of the stomach and once there, evades local and systemic immune responses. The bacterial factors, which contribute to carcinogenesis, include those that enable the bacteria to effectively colonize the gastric mucosa, incite a more aggressive host immune response and direct virulence factors of the organism (Stoicov *et al.*, 2004). Ulceration and gastritis in the stomach induces the production of collagen (typically type I and III) to facilitate healing. However, *H. pylori* has the ability to secrete collagenase (HP0 169) enzyme and actively transport it to the bacterial cell surface where it either remain, or is secreted into the extra cellular space and functions to digest collagen produced by the host, allowing for bacterial free progression. This may result in the degradation of mucosal immune response, such as IgA antibodies and possible contribute to disease development or create a conducive

environment for other transient organisms likely to colonized the stomach (Kavermann *et al.,* 2003)

Adhesion of the bacteria to the epithelial layer of the stomach by adhesin, BabA forms a scaffold apparatus that allows bacteria proteins to enter host epithelia l cells. Some bacterial strains adhere more tightly to epithelial cells and promote an aggressive phenotype, and are associated with gastric adenocarcinoma (Guruge *et al.,* 1998). Several products secreted by the bacterium cause gastric mucosal damage. Among this product include; urease, protease, phospholipase, Ammonia, and acetaldehyde. *H. pylori* disrupt gastric barrier function via urease mediated myosin II activation (Nagini, 2012). The Urease produced by the bacterium functions to hydrolyze urea into CO_2 and NH_3. The ammonia effectively buffers the immediate surrounding acid environment thereby permitting bacterium survival in such hostile environment of the stomach. Moreover, this urease enzyme activity is tightly controlled by a pH-gated urea channel, UreI, which is open at low pH and closed at neutral pH conditions (Weeks *et al.,* 2000). Another distinguish marker of *H. pylori* is its ability to produce the CagA gene. Once the bacteria adhere to the gastric epithelial cell, it uses this gene, CagA Island which acts analogously to the type IV secretion system and functions to export bacterial DNA onto the host cell cytoplasm. In the cytoplasm, CagA undergoes kinase-mediated phosphorylation and in turn dephosphorylates host cell proteins, activating intracellular signalling pathway (Selbach *et al.,* 2003, Stoicov *et al.,* 2004). A multiple of changes occur within the host gastric mucosa in response to infection leading to the formation of adenocarcinoma. Indeed, this alters the balance between apoptosis and proliferation, and the distortion of cellular composition following parietal and chief cells depletion which subsequently produce metaplastic lineages (Stoicov *et al.,* 2004).

However, it is now apparent that different genetic pathways lead to the different types of gastric cancer (Zheng *et al.,* 2004). The alteration of any specific gene that plays an important role in cellular functions, such as cell adhesion, glycosylation changes, signal transduction, DNA repair, cell differentiation, development and metastasis, have been found in gastric carcinoma (Ebert *et al.,* 2002, Zheng *et al.,* 2004). This is exemplified by the over expression of HER-2, a cell surface receptor of the tyrosine kinase family often in intestinal-type gastric cancer, whereas in diffuse-type gastric cancers, amplification of c-met, a transmembrane tyrosine kinase receptor, and aberrations in the FGFR2/ErbB3/PI3 kinase pathway have been documented (Yamashita *et al.,* 2011).

2.7 HER-2 protein

HER-2 (also known as neu, ERBB2, p185) is a proto-oncogene located on chromosome 17q21 and encodes a 185-kDa transmembrane tyrosine kinase receptor (Hechtman and Polydorides, 2012b). This protein is a member of the epidermal growth factor receptor super family (HER1 and HER2, HER3 and HER4), which, when activated, dimerizes (HER1/HER2, HER2/HER3, and HER2/HER4) (Tai *et al.,* 2010) and regulates at least three pathways of intracellular signal transduction mechanism; including the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K) (Hechtman and Polydorides, 2012b) and phospholipase C-γ (PLCγ) (Tai *et al.,* 2010). Typically, the protein structure as shown in figure1 consist of two ligand binding regions (LD1 & LD2) on the left, two cysteine-rich regions (CR1 & CR2), a short transmembrane domain (TM), a catalytic tyrosine kinase domain (TK), and a carboxy terminal tail (CT) (Moasser, 2007). The circled P indicates the numerous sites of tyrosine phosphorylation within the TK and

CT domains. Transformed or mutated regions are shown by pointed letters on the right in certain naturally occurring or experimentally induced cancers (Moasser, 2007). The site numbered A, is somatic mutations found in tumours arising in some mice; site B, is a 48bp deletion in the naturally occurring human; while site C is the mutation in the neuT oncogene (Moasser, 2007)

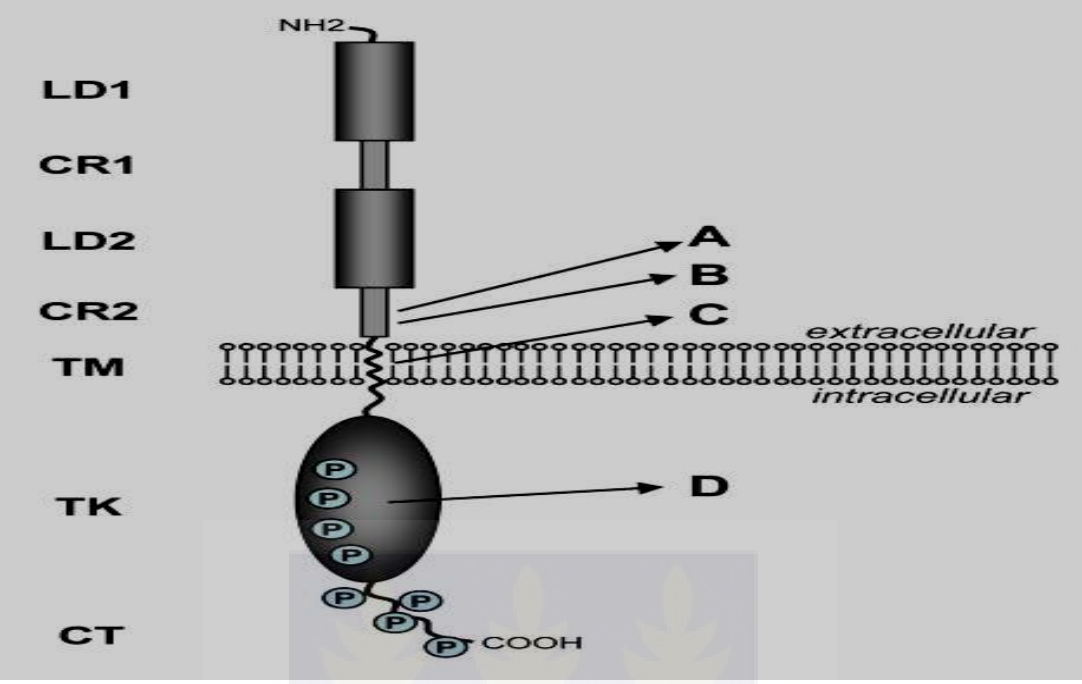


Figure 1: the structure of HER-2 protein (Moasser, 2007)

Indeed HER-2 protein has been associated with a variety of downstream pathway activities (Kim *et al.*, 2011a, Lu and Wang, 2008) and a wide diversity of cellular processes, including increased cell motility, invasiveness, angiogenesis, resistance to apoptosis, and metastatic potential (Ross and Mulcahy, 2011). All the four members of HER family have been shown to migrate into the nucleus (Tai *et al.*, 2010). However, the nuclear localization of HER-2 is likely to be mediated by the transport receptor importin β1 and nuclear pore protein Nup358 (Tai *et al.*, 2010). But , the final target of these pathways is the regulation of gene expression for various proteins that play roles in a multitude of cellular processes such as differentiation, proliferation, and survival (Kim *et al.*, 2011a) as shown in figure 2 below.

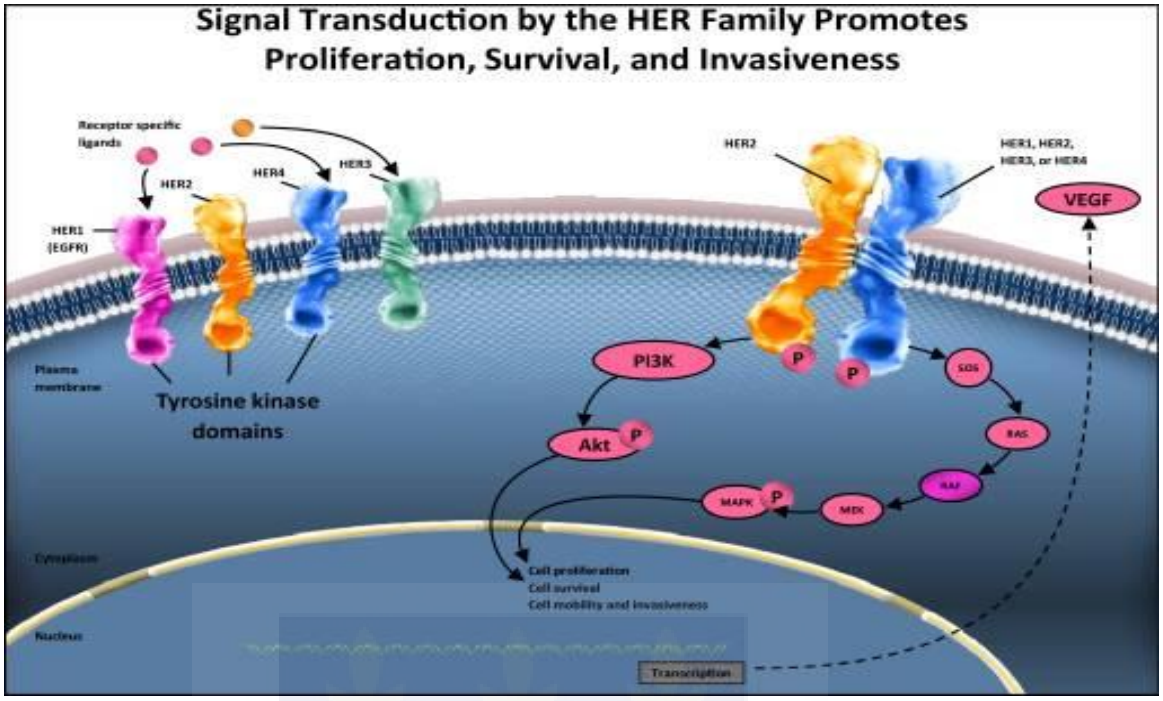


Figure 2: Signal transduction by HER family (adapted from Ross & Mulcahy, 2011)

Human epidermal growth factor receptor 2 is unique because, unlike other members of the epidermal growth factor receptor family, it has no documented direct ligand (Hechtman and Polydorides, 2012b). This receptor may thereby be capable of constitutive activity, producing its biologic response constantly and without the need for a bound activating ligand. The ligand-independent activity of HER-2 suggests that its over expression may be capable of independently inducing malignant transformation and driving tumour growth through increased cell survival and proliferation, even in the absence of growth factor ligands (Hechtman and Polydorides, 2012b). In normal cells, HER-2 plays important roles in all stages of cell development (Tai *et al.*, 2010). However, the mutation of HER-2 could directly lead to tumourigenesis as well as metastasis (Tai *et al.*, 2010). Over-expression of HER-2 in human mammary epithelial cells induces proliferative advantage, transformed characteristics, tumourigenic growth and induces proliferative and antiapoptotic

changes that mimic early stages of epithelial cell transformation (Moasser, 2007). Over-expression of HER-2 can change the composition of HER family dimers, significantly increasing HER-2-containing heterodimers and HER-2 homodimers (Moasser, 2007). Evidence, suggests that these increases can deregulate cell polarity and cell adhesion. In addition, HER-2 containing dimers have prolonged signalling activity and evade signal attenuation increasing signalling potency as shown in figure 3 below. Increased total expression of HER-2 carries with it an increase in expression of subsets of HER-2 including nuclear HER-2 and the rare HER-2 isoform, and the increase in each of these may be functionally relevant to cell transformation (Moasser, 2007)

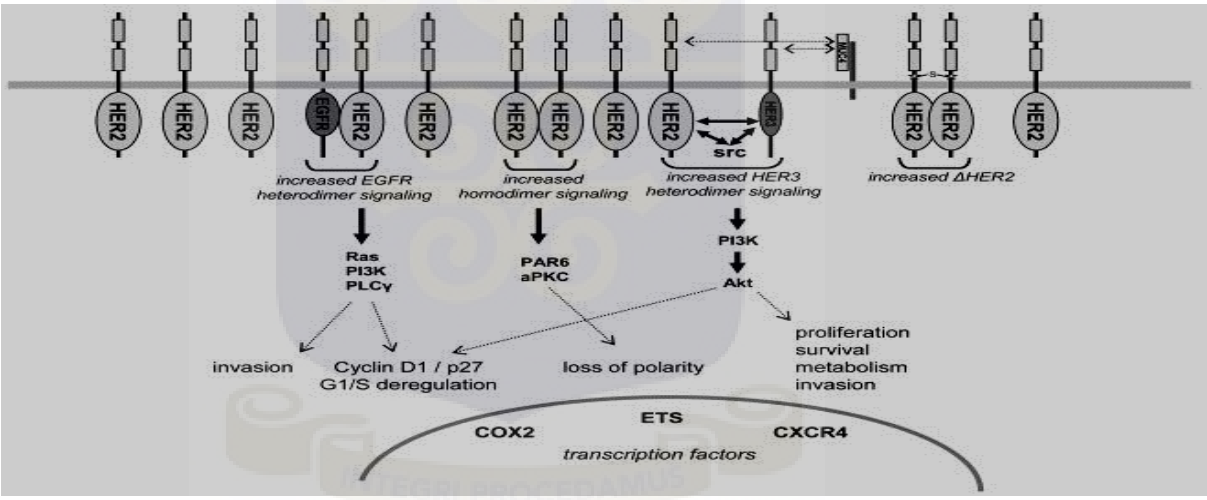


Figure 3: Signalling abnormalities resulting from HER-2 over-expression (adapted from Moasser,2007)

2.8 Prevalence of HER-2 protein in gastric and oesophageal adenocarcinoma

Nearly, about 9-38% of gastric or gastro-oesophageal junction (GEJ) cancers patients over-expressed HER-2 protein, and seems to be associated with poor outcome. For instance, in Madrid, Spain, 10% of 228 gastric adenocarcinoma patients over

expressed HER-2 protein while 25% of 32 oesophageal adenocarcinoma patients were also positive for HER-2 protein over-expression (Grávalos *et al.,* 2011). Meanwhile, in Harvard Medical School, approximately 9% out of 489 gastro-oesophageal patients were found harbouring HER-2 protein (Lennerz *et al.,* 2011). Further reported higher prevalence of HER-2 over-expression in the intestinal type of gastric carcinoma (16%–34%) compared to the diffuse type (2%–7%) (Hechtman and Polydorides, 2012a). Mixed intestinal-diffuse type cancers have HER-2 over-expression rates similar to the intestinal type and, generally, over-expression in these tumours is usually limited to areas of intestinal morphology (Hechtman and Polydorides, 2012a). In Rochester, HER-2 positivity was shown in 17% of resected oesophageal adenocarcinoma (Yoon *et al.,* 2012). Almhanna, citing (Tanner *et al.,* 2005) stated that; of the 100 oesophageal adenocarcinoma patients diagnosed in Finland, 24% were found to be positive for HER-2 protein over-expression (Almhanna *et al.,* 2013). In Spain, 25% oesophageal adenocarcinoma patients were positive for HER2 protein over-expression out of 32 cases (Grávalos *et al.,* 2011). It has been postulated that protein over-expression is an early feature of the pathway to invasive disease, whereas amplification is a late event (Geddert *et al.,* 2002). In one study HER-2 was found in 22% of oesophageal adenocarcinoma, while in another 15% of 110 and 14% of 46 oesophageal adenocarcinoma specimens were found to have over-expressed HER-2 (Peters and Fitzgerald, 2007).

In China, HER-2 over-expression was detected in 23 out of 49 patients with intestinal gastric adenocarcinoma and 9 out of 35 patients with diffuse gastric adenocarcinoma (Dang *et al.*, 2012). Thus, the proportion of patients with HER-2 over-expression was higher in patients with gastric adenocarcinoma of the intestinal-type than those with the diffuse-type (Dang *et al.,* 2012). In addition, statistically significant difference has

been observed among the various age groups; 29 out of 59 patients aged < 60years were HER-2 positive, while 8 out of 25 (32%) patients aged ≥ 60 were HER2-positiv (Dang *et al.,* 2012). However, there was no significant difference with regards to gender for HER- 2 proteins over-expression. A retrospective analysis identified HER-2 as a risk factor for the development of liver metastases and relapse in gastric adenocarcinoma patients (Dang *et al.,* 2012). Different from other cancers, HER2 over-expression rate in gastric cancer varies according to the site of the tumour, as a higher over-expression rate (36%) was found in Gastroesophageal Junction (GEJ) tumours in contrast to 21% in gastric tumours (JLeón-Chong FL *et al.,* 2007)

Most patients with these cancers experience vague and nonspecific symptoms in the early stages of the disease, while the classic triad of weight loss, anaemia, and loss of appetite for meat-based foods becomes apparent only in the advanced stages (Nagini, 2012). Despite advances in diagnosis, these cancers are usually detected after invasion of the *muscularis propria* (Nagini, 2012). Lack of early symptoms of these cancers has also contributed to the delayed detection (Carl-McGrath *et al.*, 2007).

2.9 HER-2 protein and targeted drug

The optimal contemporary management and treatment option for gastric or oesophageal adenocarcinomas are controversial with divided opinion (Keld and Yeng, 2011), and has resulted in diverse treatment options. Treatment with multimodal or conventional chemotherapeutic agents such as 5-fluorouracil, cisplatin, epirubicin, and docetaxel (Hechtman and Polydorides, 2012a) or radio-chemotherapy followed by surgery are the commonly used method to improve the survival of patients with either gastric or oesophageal adenocarcinoma (Berg *et al.*, 2011). However, only 30% to 50% of these patients respond to this treatment (Berg *et al.*, 2011). As the majority

of these cancer patients present with advanced stage, these multimodal therapies (surgery, chemotherapy, and radiotherapy) have limited efficacy to reduce mortality (Wu *et al.,* 2009). Resistance to chemotherapy has also become a major obstacle to treatment of all malignancies including gastric and oesophageal cancers (Almhanna *et al.,* 2013).

Though, numerous molecules have shown potential to target specific pathways for tumour cell growth (Wu *et al.,* 2009). Recently, trastuzumab, a monoclonal antibody, has emerged as an important molecule which targets the protein biomarker, HER-2 in the stomach or oesophagus of patients with adenocarcinoma (Pazo Cid and Anton, 2012). A higher significance in the overall survival rate was observed in gastric and oesophageal adenocarcinoma HER-2 over-expressed patients treated with chemotherapy and trastuzumab compared with those treated with chemotherapy alone (Pazo Cid and Anton, 2012, Al-Momani *et al.,* 2012). However, this same HER-2 performs an important physiological role in the survival mechanism of an injured heart (Munk *et al.,* 2012). An example is seen when, cardiomyocytes terminally differentiated following an injury to the heart is able to reconstitute fully through the role played by HER-2 in the phosphorylation of PI3-K/Art and Ras/Raf/MAPK signalling pathways (Munk *et al.,* 2012). HER-2 therefore appears to exhibit dual functions; either in tumours or in normal physiology. In order to avoid possible knockout of the normally expressed HER-2 in physiological state at the expense of the over-expressed HER-2 in tumour, it is of importance for screening of this protein to identify those who can benefit from trastuzumab

Human epidermal growth factor receptor 2, HER-2 has become an important therapeutic target for gastric and other organ cancers (Almhanna *et al.,* 2013). The

upregulation of HER-2 cause tumouregenesis, typified by the level of HER-2 gene expression in cancer cells compared to that in normal cells. Also, emerging evidence has shown that the level of HER-2 protein over-expression found in either primary tumours and metastasized organs exhibit no significant difference (Tai *et al*., 2010), suggesting the independency of this oncogenic protein in the stage of the tumour. Moreover, HER-2 is the preferred dimerizations partner for other HER receptors in the activation of HER signalling pathways, with HER2 containing heterodimers having the most activated mitogenic potential among the other HER2 family members (Tai *et al*., 2010). Therefore, the inhibition of HER2 dimerization will prevent the activation of several intracellular signalling cascades including the PI3K, MAPK and PLCγ pathway (Tai *et al.*, 2010).

2.10 Technique for demonstrating HER-2 status

Several techniques can be employed for assessing HER2 status in cancer patients. These include; immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), enzyme-linked immunoabsorbent assay (ELISA) and Dot blot analysis (Tan *et al.*, 2011). Immunohistochemistry technique reveal whether HER2 protein is over-expressed or not, FISH is used to assess the level of HER2 gene amplification, ELISA for measuring the level of soluble HER2 receptor in serum, and Dot blot analysis to measure the serum HER2 level and further evaluated their clinical value for predicting tumour HER2 status and tumour progression (Tan *et al.*, 2011).

2.11 Guidelines for HER-2 testing in gastric and oesophageal cancers using immunohistochemistry

It is required that the evaluation of HER-2 protein over-expression in gastric or oesophageal cancers employing the immunohistochemistry technique use the

modified gastric cancer testing protocol, as outlined by Hofmann, and optimized by Rüschoff *et al*, (Almhanna *et al.*, 2013, Rüschoff *et al.*, 2012, Hofmann *et al.*, 2008). In gastric immunostaining, unique features exist compared with breast cancer. This include high incidence of tumour heterogeneity, thus less than 30% of tumour cells staining positive or only focal staining of the tumour cells in up to 30% of HER-2 positive cases (Hofmann *et al.*, 2008, Rüschoff *et al.*, 2012). Again, positive staining of HER-2 in gastric carcinomas are usually of the gland forming intestinal type and may show incomplete, basolateral, or lateral staining contrary to immunostaining in breast cancer (Rüschoff *et al.*, 2012). A minimum of 5 cohesive, unequivocally positive tumour cells should be present when reporting a specimen sample as positive for HER-2; a minimum of 10% of positive tumour cells is required for reporting a positive HER-2 reaction; and the antibodies used should meet the approved US Food and Drug Administration (FDA) recommendation for the selection of HER2-positive gastric tumours (Almhanna *et al.*, 2013). Information from the trastuzumab for gastric cancer study confirmed that patients with tumours that had high levels of HER-2 protein expression derived the greatest advantage from treatment with trastuzumab. Therefore, immunohistochemistry should be the initial testing method while fluorescence in situ hybridization or silver in situ hybridization should be reserve for retesting or confirmation of IHC 2+. In order to ensure accurate and reproducible HER-2 screening results can only be obtained if interpretation of HER-2 results is performed with strict adherence to the scoring criteria specific for gastric cancer as reported in the trastuzumab for Gastric cancer study and the recommended by the panel of expert pathologists (Rüschoff *et al.*, 2012) as shown in table 6, appendix.

Several well-described pitfalls in HER-2 IHC scoring exist for both gastric and oesophageal cancers and should be noted during scoring (Rüschoff *et al.*, 2010). For

instance; HER-2 false positivity may be seen in areas of intestinal metaplasia and reactive atypia near gastric ulcers or cytoplasmic and/or nuclear stains. Also, stain within nonneoplastic lesions as well as "edge" and crushing artefacts of tumour cells represents a non specific stain and should be ignored (Almhanna *et al.*, 2013). It has been shown generally that determining the microscopic magnification at which specific membranous staining is visible overcomes the problem of visual illusions associated with brightness perception. Thus, when observers focus on the identification of the distinct features (i.e. membranous staining) and not only on the intensity of staining, marked improvement are seen among inter-observers. As a result, the 'magnification rule' should always be used in conjunction with the gastric cancer-specific scoring criteria (Rüschoff *et al.*, 2010). Considering the degree of microscopic magnification required to identify membranous staining is helpful in determining the immunohistochemistry score, particularly in borderline, immunohistochemistry 2 +. Immunohistochemistry 3+ staining is defined as any membranous staining visible at low magnification (x 2.5–5). Lateral- or U-shaped membranous staining is typically seen at cell–cell junctions. Immunohistochemistry 2 + membranous staining are visible at x10–20 magnification Immunohistochemistry 2 + cases should be retested using fluorescence in situ hybridization or silver in situ hybridization. Immunohistochemistry 1+ staining is visible only with x40 magnification and should be considered immunohistochemistry-negative (Rüschoff *et al.*, 2012)

2.12 Trastuzumab

Trastuzumab (also known as Herceptin and produced in Genentech Inc., USA) is a humanized recombinant mAb that binds to the extracellular domain of the human HER-2 protein (Fiszman and María, 2011).

2.13 Mechanism of action by trastuzumab

The exact mechanism of actions of trastuzumab is not fully understood (Fiszman and María, 2011). However, it has become widely accepted that by interfering with the dimerization of HER2, inhibits HER2 activation and suppresses Akt phosphorylation. In line with this, it has been proposed that this drug exhibit its mechanism of actions through; internalization and degradation of HER-2, MAPK or PI3K/Akt (Vu and Claret, 2012), and also interferes with cell cycle, inhibition of angiogenesis and DNA repair, and induction of immune-mediated responses (Fiszman and María, 2011). Trastuzumab function to draw immune cells to tumour sites over expressing HER-2 by means of a mechanism called antibody-dependent cellular cytotoxicity (ADCC). Thus, following HER-2 internalization, recruits c-Cbl to its docking site, resulting in the degradation of HER-2. Indeed, while degrading HER-2, trastuzumab turns to inhibit the MAPK and PI3K/Akt pathways leading to cell cycle arrest and possible suppression of cell growth and proliferation (Vu and Claret, 2012). Recently, the first randomized Phase III trial Trastuzumab for gastric cancer (ToGA) showed that trastuzumab in combination with conventional chemotherapy is superior to conventional chemotherapy alone in HER2-positive advanced gastric cancer (Kim *et al.*, 2011a) and oesophageal adenocarcinoma (Almhanna *et al.*, 2013). Therefore, an accurate evaluation of HER2 status in gastric or oesophageal cancers has become increasingly important.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

The work a was retrospective study involving archival specimens properly fixed in formalin, embedded in paraffin wax and well documented in the record books of Pathology Department, KBTH. All the specimens were from individuals who had either oesophageal and/or gastric cancer. Gastric and oesophageal biopsies received at the department from 2008 to 2012 were identified and recorded. Biopsies, noted to have been diagnosed as either gastric or oesophageal adenocarcinoma were noted and were sub-documented. Histologically, one hundred and eighty three (183) cases were found to have been diagnosed as gastric adenocarcinoma, however only 99 of their respective tissue blocks were found to have maintained and preserved the integrity of the tissue. Also, of the ninety one (91) oesophageal biopsies received at the Department, 8 cases were diagnosed as adenocarcinoma.

3.2 Study site description

The study was conducted at the Pathology Department, Korle Bu Teaching Hospital (KBTH), Accra, Ghana, for samples diagnosed as adenocarcinoma of either the stomach or oesophagus. KBTH was selected because it is the leading tertiary hospital and the major referral centre in the country. It also serves as the teaching hospital of the University of Ghana Medical School, Accra. Over 80% of the total cases for histological, cytological and IHC diagnosis are processed through this department, while the remaining are usually sent to other tertiary and private care facilities.

3.3 Study population

The population recruited in this study were made up of Ghanaians of any age group or gender diagnosed with adenocarcinoma of either the stomach or oesophagus between the periods of 2008 through to 2012.

3.4 Inclusion criteria

1. Tissue block processed at the Pathology Department
2. Paraffin blocks with enough tissue to perform all immunostaining required for the study
3. Tissues with well preserved cellular integrity

3.5 Exclusion criteria

1. Patients with secondary tumours of stomach and oesophagus
2. Samples with insufficient tissue, and with microscopically evident fixation problems.

3.6 Sample size determination

The minimum number of specimens was calculated using the equation

$$n = \frac{z^2 p (1-p)}{e^2}$$

where z-the standard score at 95% confidence level = 1.96

p-the known prevalence of gastric and oesophageal cancers = 0.07

e- the allowable error margin= 5%

n- minimum number of samples

Substituting into the equation, the sample size, n =

$$= \frac{1.96^2 \ 0.07\ (1-0.07)}{0.05^2}$$

$$= \frac{3.8416 \times 0.07 \times 0.93}{0.0025}$$

$$= 0.2500/0.0025$$

$$= 100$$

Hence, minimum number of samples require was 100

3.7 Informed consent

The study was approved by the Ethical and Protocol Review Committee, University of Ghana Medical School, College of Health Science, Korle Bu. The protocol identification number of the ethical clearance for this study was MS-Et/M.7-4.5/2012-13 with reference number MS-AA/C.2/Vol.16^{A} (Appendix A3). Consent was sought from the Head, Pathology of Department for the use of archived samples

3.8 Microtomy (Sectioning)

The floating-out water bath and the hot plate were switched on and allowed enough time (at least 1 hour) to attain their optimum operating temperatures (water bath 45^{o}C and hot plate, 60^{o}C). The embedded paraffin blocks were individually mounted in the chuck of a Rotary microtome with an advancing blade that cuts thin sections of the tissue. Each tissue block was trimmed to expose the full surface of the tissue. The cut-surface of the blocks were then cooled on melting ice for at least ten minutes and re-inserted in the chuck of the microtome and sections cut to 4 μm thickness. The sections were picked with forceps and placed on 20% alcohol wet plastic plate to

initiate spreading. The ribbons on the plastic plate were transferred onto the warm floating out bath for complete spreading of the ribbons. The ribbons were split into spindle sections using a forceps. Three of the sections representing a full phase of the tissue block were picked onto a clean grease free super frost glass microscope slide (Fisher Scientific, USA) by lowering the slides vertically onto the water bath to make contact with the section and allowed to drain in a vertical position for a few seconds before it was transferred to a hot oven (60^{o}C) for one hour. This allowed the sections to stick firmly onto the slide and avoid peeling off the section in the subsequent staining procedure.

3.9 Haematoxylin and Eosin (H&E) staining of gastric and oesophageal tissue sections

One microscope slide each from the three sets was arranged in a staining rack so that each rack contained 30 slides. The remaining two sections were kept temporally in a slide box to prevent particles settling on it. Each selected slide with the tissue sections on it was treated in three changes of xylene in glass staining baths for 5 minutes each, to remove wax. They were then treated in three changes of absolute ethanol for 5 minutes each and rehydrated in decreasing grades of ethanol from 70% to 50% to clear the xylene, and then rinsed thoroughly in tap water to bring the sections down to water. The slides were than stained in Mayer's haematoxylin (reagent number RE 7107) for 5 minutes, rinsed in tap water for 30 minutes and then stained in 0.5% alcoholic eosin for 1 minute. The slides were rinsed in two changes of absolute ethanol and cleared in two changes of xylene before mounting in a DPX mountant and cover slips either 24x24 or 24 x32 or 24 x40 depending on the tissue size.

3.10 Screening of H & E stained sections

In haematoxylin and eosin stains, cell nuclei are stained blue under the light microscope while cytoplasm and connective tissues stain shades of pink and red cells stain red. A well stained haematoxylin and eosin section of the oesophagus or stomach tissue has all these features. Stained slides were examined blinded by three (3) pathologists using a light microscope to ascertain the presence or absence of tumour. Slides that exhibit the presence of tumour (adenocarcinoma) were further differentiated into either intestinal or diffuse by the same pathologists. In all, one hundred and seven (107) stained sections were confirmed as adenocarcinoma by the three pathologists. Sections confirmed to have well preserved tumour were selected for immunohistochemistry staining. About six samples from either the stomach or oesophagus, which were identified to express normal epithelia, were also included in the immunohistochemistry technique.

3.11.0 Immunohistochemical staining procedure for HER-2 receptor

3.11.1 Test and Control samples

In the immunohistochemistry staining technique, a batch (i.e. 30 slides per rack) contained 28 test samples, two negative controls. In the negative control, addition of HER-2 antibody was omitted in the sequential staining procedure. The slides were transferred onto a hot air oven set at 60^{o}C for one hour. This was to allow the sections to further adhere onto the slides so that they did not peel off during the several washes in the immunohistochemical demonstration techniques.

3.11.2 Pre-immunohistochemical demonstration procedures

All the reagents which were stored in fridge to maintain their potency were retrieved from the refrigerators and placed on the laboratory bench to attain room temperature. The reagents were; NOVOLINK™ Polymer Detection System containing (Peroxidise block RE7101, protein block RE7102, Post primary RE7111, Novolink™ polymer RE7112, DAB chromogen RE7105, Novolink™ DAB substrate buffer RE7143, haematoxylin RE7107), Epitope retrieval solution x10 concentration ph 6 RE7113, IHC Diluent RE7133, buffers (Tris buffered saline and Citrate buffer), HER-2 primary antibody (appendix A1). Reagent products were obtained from Leica Microsystem Novolink™ UK. Test and control slides were also retrieved from the hot air oven and their labels cross-checked to confirm that they were properly labelled for immunohistochemistry. The sections were dewaxed in three changes of xylene for 5 minutes each, followed by 2 changes of absolute ethanol for 5 minutes to clear the xylene. Sections were then brought down to water by passing them through descending grades of ethanol (starting from 90% to 50%) for 5 minutes each and then to water in glass staining baths for 5 minutes.

3.11.3 Antigen retrieval

A pressure cooker was filled to two-thirds of its volume with tap water and placed on the hot plate. A glass staining dish was filled to two-thirds its volume with citrate buffer (pH 6.0), in the end, both citrate in the dish and water in the cooker were of same volume. The hot plate was turned on and the temperature of both water and citrate buffer were allowed to rise to boiling. Sections were arranged in a staining rack and placed in a glass reagent bath. The lid of the pressure cooker was put in place and the pressure valve on the lid was closed so that the water and citrate buffer in the

cooker heated up to a temperature under pressure to about 120^0 C for 2 minutes. At the end of 2 minutes the hot plate was turned off and the pressure released slowly by opening the pressure valve. The cooker and the slides were allowed to stay on the hot plate for about 10 minutes to cool. This procedure was adopted to avoid instant cooling of the glass dish and slides which could result in breaking. After 10 minutes the sections were removed from the citrate buffer and transferred to tap water and then to distilled water for another 5 minutes to complete the process.

3.11.4 Blocking of endogenous peroxidase activity

The slides were transferred into a glass staining bath containing 250ml peroxidase block solution (3% hydrogen peroxide) for 5 minutes. This was done in turns of 30 slides per batch in the same solution until all the slides were blocked. The slides were then washed in tap water for 5 minutes followed by washing in distilled water for another 5 minutes.

3.11.5 Staining procedure

A staining rack for the immunohistochemistry staining technique was constructed with two long plastic rods lying parallel to one another on a levelled plate to ensure proper balancing. The rods were plastered at both ends to a levelled plate and placed on wet cotton wool to avoid accidental movement of the rods during washing and also to provide a moist environment for the entire staining procedure respectively. After removing the entire set of 30 slides from the distilled water, they were then removed individually from the metal racks and placed on the staining rods. A working Tris buffered Saline, pH 6.0 (section 3.7.2 reagent number RE7113) was prepared from a stock solution of Tris Buffered Saline x10 concentration, Novocastra product number T5912 (appendix A2). With the aid of a Pasteur pipette each slide was flooded with

the TBS for 5 minutes and then drained and flooded with TBS for another 5 minutes, and then drained again.

Allowing for up to 200µl for each section, the reagents from the staining kits were applied in the following sequences and times; Peroxidase block (10 minutes), protein block (10 minutes), primary antibodies (see dilution appendix A1) for 1 hour, post primary block (30 minutes), Novo polymer (30 minutes) and DAB (10 minutes). The primary antibody was applied to the slides excluding the negative control slides where TBS was maintained to avoid drying. Also after each application the slides were rinsed and flooded with TBS for 5 minutes and then repeated for another 5 minutes. This was done for all applications except after the application of DAB where tap water was used to rinse the slides. To label cell nuclei, Haematoxylin was applied for 1 minute and blued in tap water for 5 minutes. Sections were dehydrated by rinsing in ascending grades of ethanol, starting from 70%, 80%, 90% and then to absolute ethanol for 2 minutes in each trough. Sections were then rinsed in two changes of xylene, mounted in Distrene, Plasticiser, Xylene (DPX).

3.12 Examination of immunohistochemical stained slides

Evaluation and scoring of HER-2 protein overexpression was performed according to the criteria for **‘‘HER-2 testing in gastric cancer: a practical approach’’** by Ruschoff *et al* (2012). Indeed, as shown in table 11 and 12 at the appendix, staining patterns for surgical or biopsy specimen from the stomach differs, and also differ from that of breast. However, since all the specimens for this study were biopsies, HER-2 expression was scored as follows: 0 (negative), No reactivity or no membranous reactivity in any tumour cell; 1+ (negative), tumour cell cluster with a faint/barely perceptible membranous reactivity irrespective of percentage of tumour cells stained;

2+ (equivocal), tumour cell cluster with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumour cells stained; 3+ (positive), tumour cell cluster with a strong complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumour cells stained. A tumour was considered to be HER2-positive when a score of 3+ was found and negative when a score of 0 or 1+ was observed.

3.13 Reporting

Slides were screened first by the trained researcher/investigator and were later reviewed independently by a consultant pathologist. Discordant cases were reviewed together and a final consensus was reached.

3.14 Quality control

The right temperatures and timing were used in epitope retrieval and staining. Optimization of the immunohistochemical protocols were followed accordingly while the immunoreactivity was monitored by staining of internal controls, and then, all cases were checked blindly.

3.15 Data analysis

Data was entered into an Excel spread sheet (Microsoft Company) and analysed using SPSS and Minitab, and analysed as follows; Continuous variables such as age of patients were tested for homogeneity of variances and normality before analysis. Appropriate measures of centrality (mean, median) and of dispersion (standard deviation) were calculated. Graphical displays of frequency distributions and pie charts were created where appropriate. Frequencies and percentages were calculated for dichotomous data such as HER-2.

For hypotheses comparing frequencies among groups, X^2 tests or Fisher's exact tests were used to find association between age group and the three biomarkers HER-2(-), Equivocal and HER-2(+). All tests were two sided and p-value less than 0.05 were interpreted as significant

CHAPTER FOUR

4.0 RESULTS

4.1 General pattern of gastric adenocarcinoma prevalence

Over the 5 year period, nine hundred and seventy four (974) gastric primary biopsy cases, including subject's age and gender were retrieved from the histopathology log books. One hundred and eighty three (183) representing 18.79% of these gastric biopsies were diagnosed as adenocarcinoma. As shown in table 1, adenocarcinoma was observed to be the second most common disease of the stomach. The annual distribution of gastric adenocarcinoma and the other diseases of the stomach noted are shown in Table 2.

Table 1: The prevalence of gastric adenocarcinoma among other gastric pathology at the KBTH, from 2008 to 2012

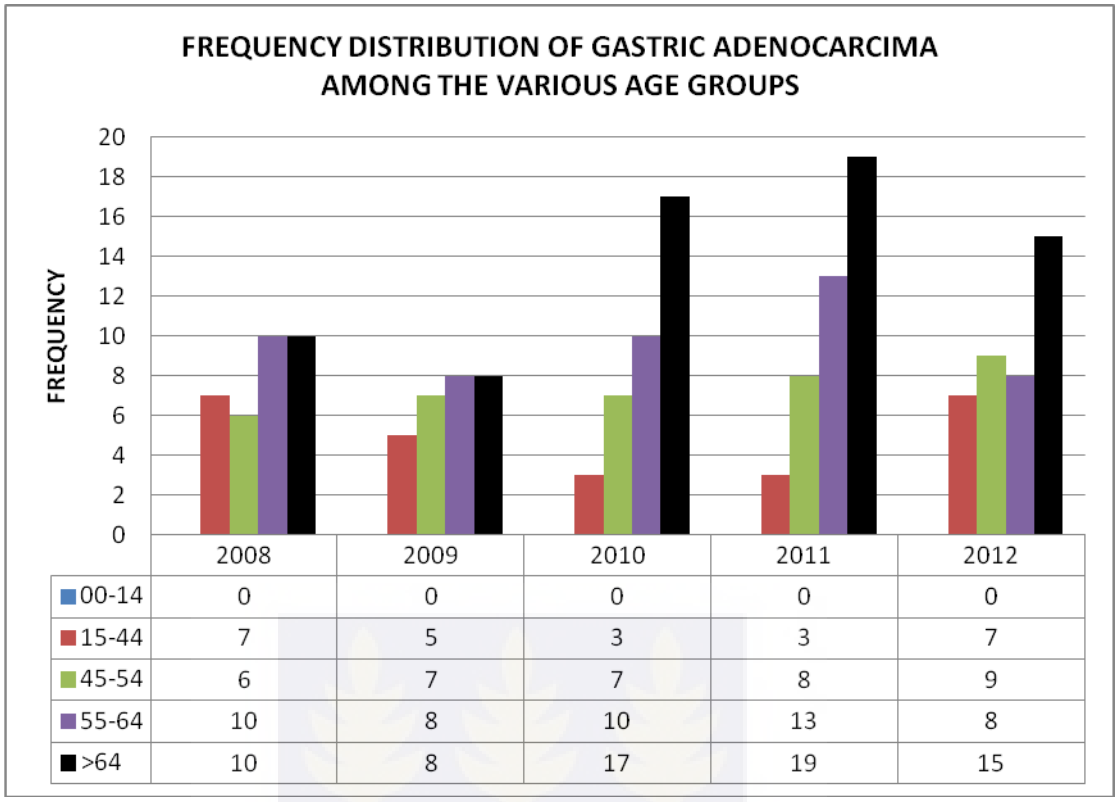
Gastric pathology	Frequency	Percentage (%)
Gastritis	535	54.93
Ulcer	105	10.78
Adenocarcinoma	183	18.79
Others	151	15.50
Total	974	100.00

Table 2: Prevalence of gastric adenocarcinoma among other gastric pathology at the KBTH within a period of five (5) years

Years	Gastritis (%)	Ulcer (%)	Adenocarcinoma (%)	Others (%)
2008	57.36	7.11	**16.75**	18.78
2009	54.74	8.03	**19.71**	17.52
2010	50.58	11.63	**20.93**	16.86
2011	59.78	13.65	**16.61**	9.97
2012	50.52	11.86	**20.1**	17.52

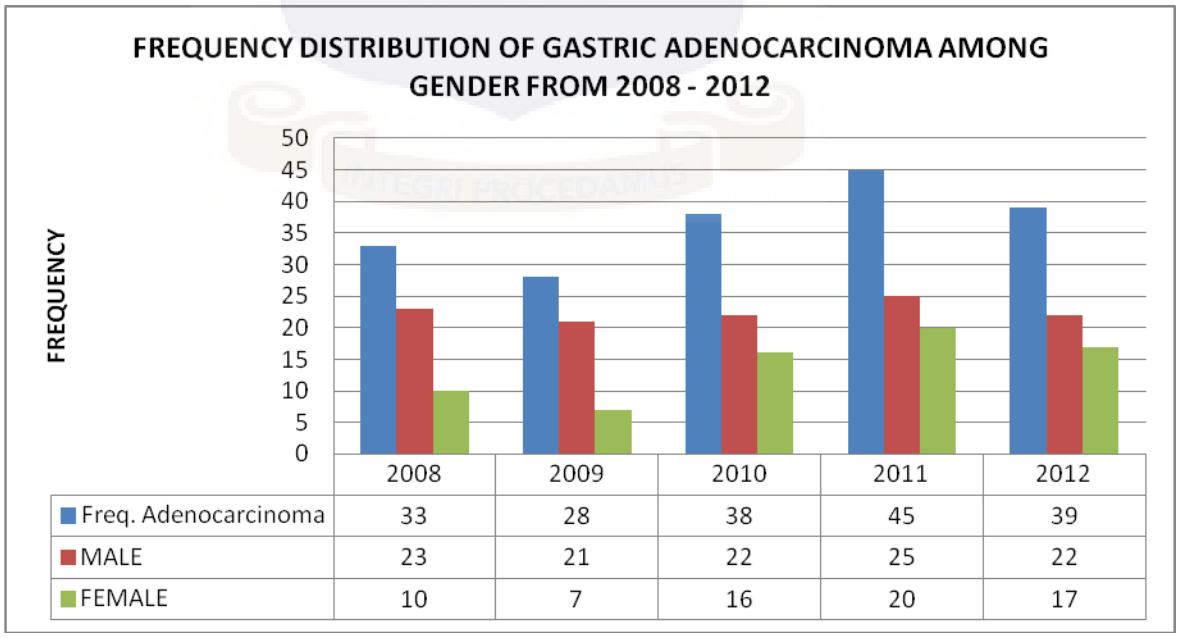
4.1.1 Gender and age distribution of gastric adenocarcinoma

Between 2008 and 2012 a total of 183 gastric adenocarcinoma cases, aged 22 – 100 years (mean age 59.5 ± 13.91 SD) were retrieved. A prevalence of 64.5% was observed among participants aged 55 years and over, while 35.5% was obtained among those younger than 55 years (figure 4). Of the study cases, 61.7% (113 out of 183) were males aged 25 to 80 years (mean 60.04 ± 12.85) whereas 38.3% (70 out of 183) were females aged 22 to100 years (mean 57.9 ± 15.53 SD), as shown in figure 5.



	2008	2009	2010	2011	2012
00-14	0	0	0	0	0
15-44	7	5	3	3	7
45-54	6	7	7	8	9
55-64	10	8	10	13	8
>64	10	8	17	19	15

Figure 4: Age distribution of gastric adenocarcinoma



	2008	2009	2010	2011	2012
Freq. Adenocarcinoma	33	28	38	45	39
MALE	23	21	22	25	22
FEMALE	10	7	16	20	17

Figure 5: The frequency of gastric adenocarcinoma among gender

4.1.2 Distribution of histological subtypes of gastric adenocarcinoma

Of the 183 adenocarcinoma cases recorded in the histopathology log book, only 99 cases had tissue blocks (paraffin wax embedded) that were retrieved from the archives for the subsequent analysis. Seventy seven (77) out of the 99 were sub-classified as intestinal while the remaining 22 were diffuse type of adenocarcinoma (figure 6 and 7)

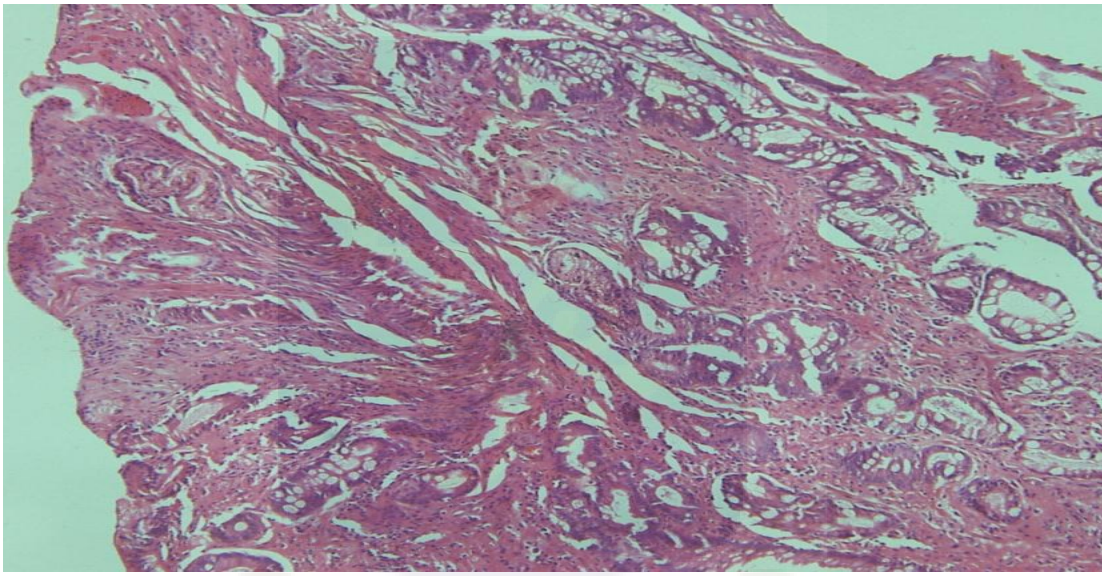


Figure 6: Intestinal type gastric adenocarcinoma showing intestinal metaplasia and invasion of the muscularis propria by moderately differentiated malignant glands. H & E stained (x200).

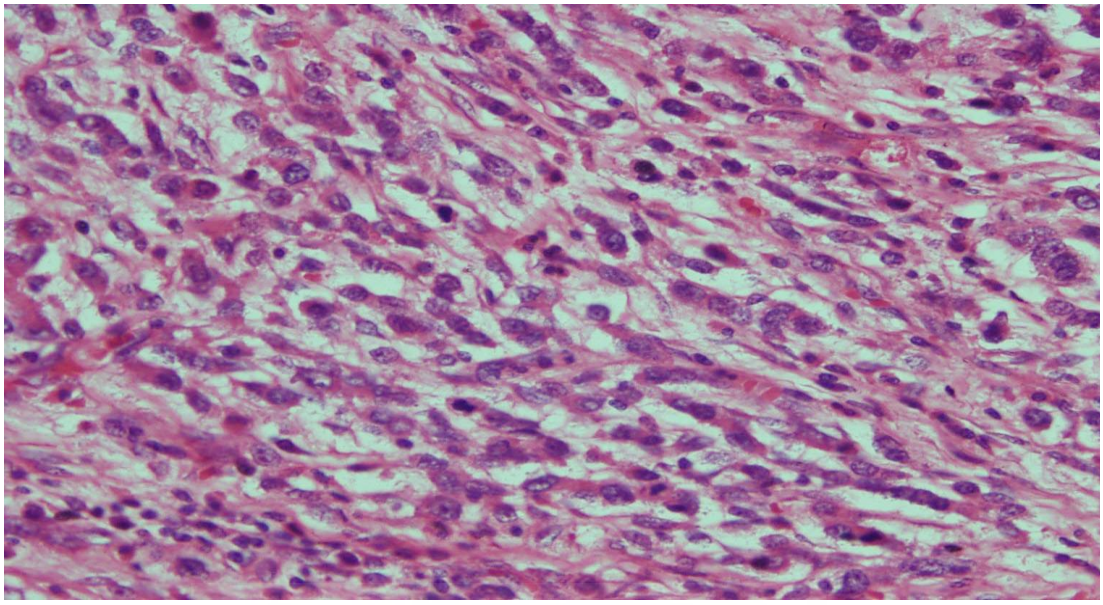


figure 7: Diffuse type gastric adenocarcinoma showing monomorphic tumour cells with an Indian-file pattern of infiltration. H&E (200x).

4.2 HER-2 expression and gastric adenocarcinoma

The demographic characteristics (age, gender and histological subtypes) of 41.4% (41 out of 99) gastric adenocarcinoma subjects who over-expressed HER-2 are shown in Table 3. Also, as shown in figures 8 and 9 are respectively, HER-2 positive (3+) and negative.

Table 3: Human epidermal growth factor 2 over-expression in gastric adenocarcinoma

Parameter	Patients(n)	HER2+ Patients(n)	P - value(α = 0.05)
Age(Yrs)			0.034
0 – 14	0	0	
15 – 44	13	6	
45 – 54	18	7	
55 – 64	29	10	
>64	39	18	
Gender			
Male	40	20	0.655
Female	59	21	
Tumour type			
Intestinal	77	34	0.001
Diffuse	22	7	

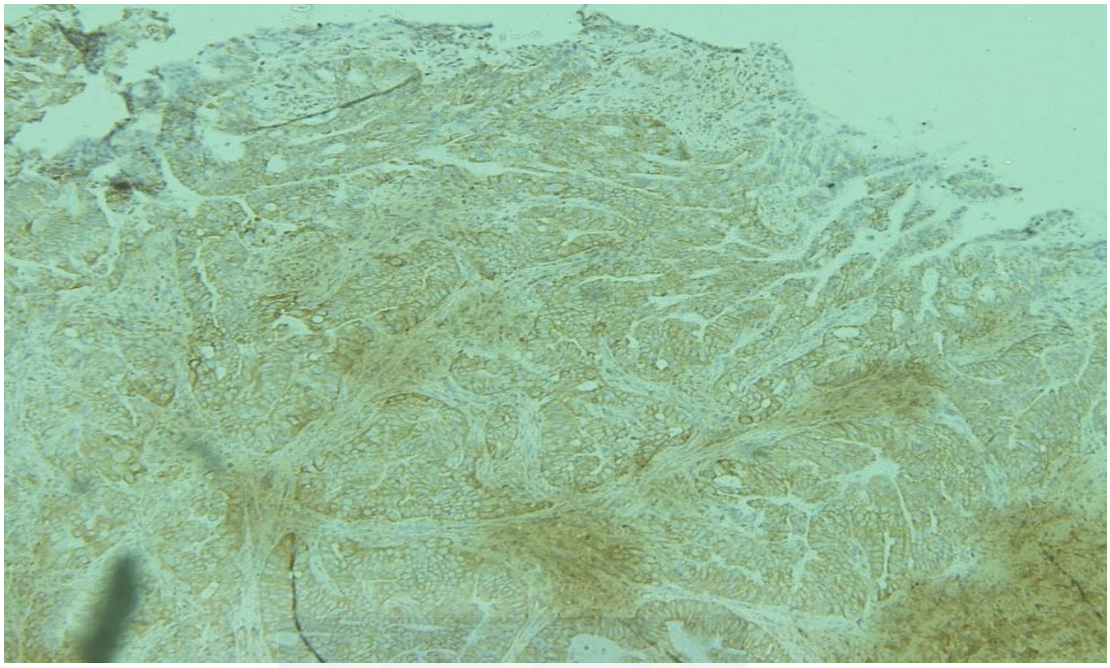


figure 8 HER-2 positive (3+) with tumour cells showing complete membrane staining. IHC (100x)

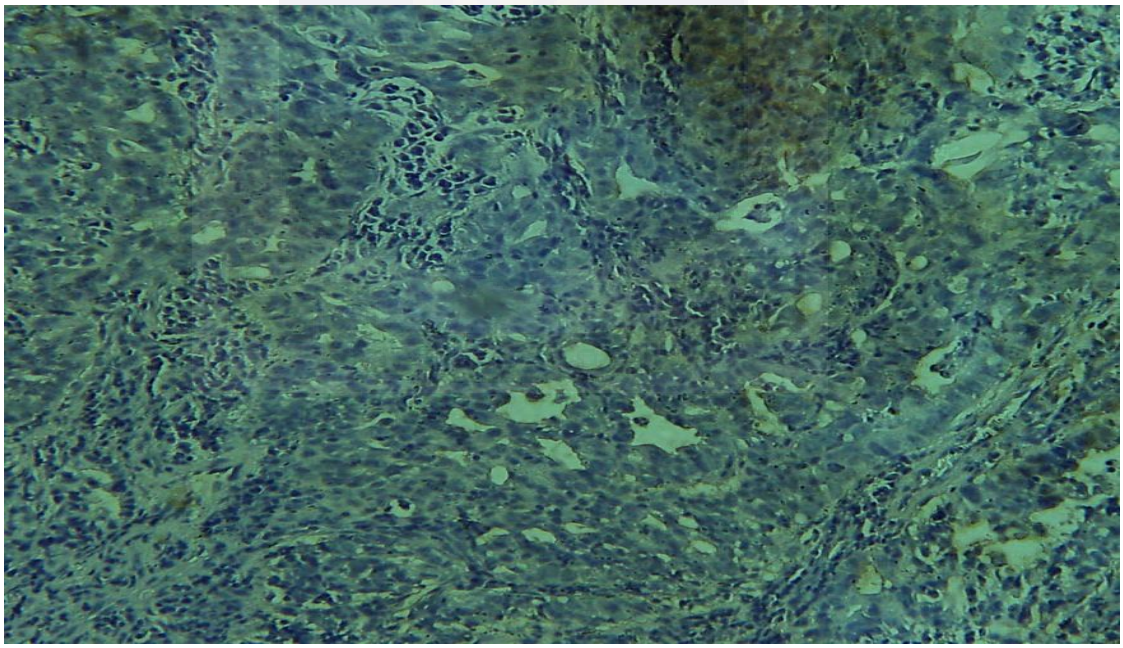


Figure 9: HER-2 negative, showing malignant glands and cells without membrane staining. IHC(200x).

4.3 Association of HER-2 overexpression among age and gender

Sixty eight point three percent 68.3%, (28 out of 41) cases that who over-expressed HER-2 were 55 years old and over, whereas 31.7% (13 out of 41) were less than 55 years. A significant association was observed between subject age groups and HER-2 over-expression ($P<0.05$) as shown in table 3. Twenty (20) out of the 41cases who overexpressed HER-2 were males whereas 21 were females. There was no significant association between gender and HER-2 over-expression, $P > 0.05$ (table 3)

4.4 HER-2 expression and histological subtypes of gastric adenocarcinoma

Of those gastric adenocarcinoma cases who over-expressed HER-2, 82.9% (34 out of 41) were intestinal subtype, whereas the remaining 17.1% were diffuse type. Statistically, a significant association ($p=0.001$) was observed between HER-2 over-expression, and intestinal and diffuse types of gastric adenocarcinoma (table 3).

4. 5 Prevalence of oesophageal adenocarcinoma

4.5.1 General pattern of oesophageal adenocarcinoma

The histopathological characteristics of the 91 evaluable oesophageal samples are shown in Table 4. In particular, eight (8) samples representing 8.79% were diagnosed as adenocarcinoma. Figure 10 shows mucin secreting adenocarcinoma of oesophagus. Table 5 shows the annual distribution of oesophageal adenocarcinoma among other diseases of the oesophagus from 2008 to 2012.

Table 4: Prevalence of oesophageal adenocarcinoma among other pathology of the oesophagus at the Korle-Bu Teaching Hospital from 2008 to 2012

Oesophageal pathology	Frequency	Percentage (%)
Oesophagitis	9	9.89
Squamous cell carcinoma	28	30.77
Adenocarcinoma	8	8.79
Others	46	50.55
Total	**91**	**100.00**

Table 5: The frequency distribution of oesophageal adenocarcinoma and other diseases of the oesophagus at the KBTH from 2008 to 2012

Years	Oesophagitis	Adenocarcinoma	Squamous	Others	Total
2008	4	**1**	7	15	27
2009	0	**0**	0	3	3
2010	0	**1**	0	11	12
2011	4	**3**	11	12	30
2012	1	**3**	10	5	19
Total	9	**8**	28	46	**91**

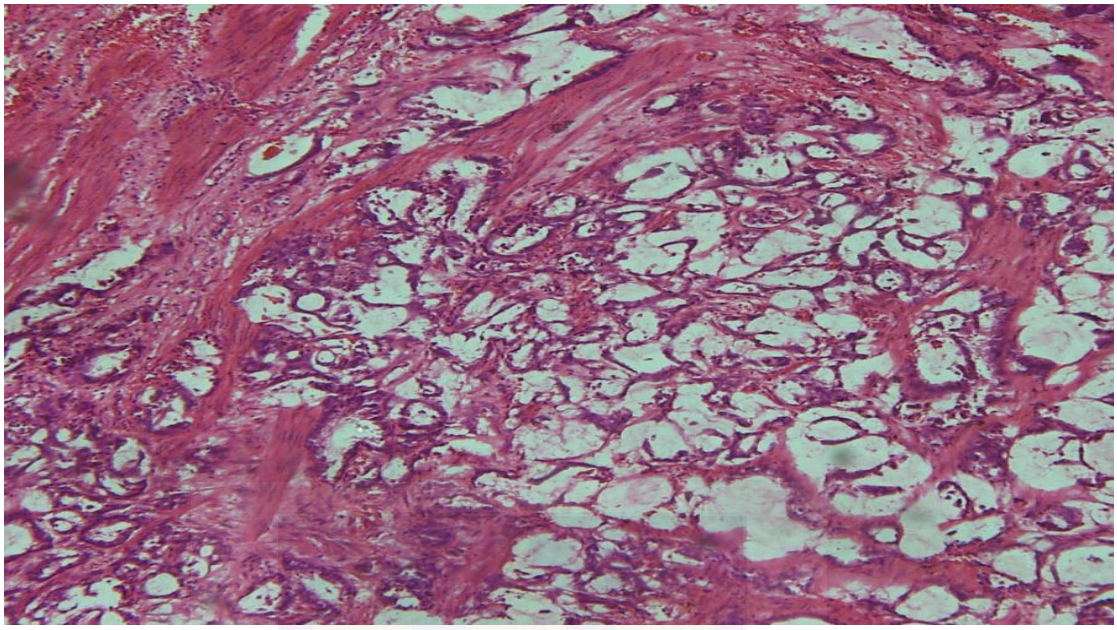


Figure 10: Mucin sereting adenocarcinoma of the oesophagus. Malignant glands invading the muscular wall. H & E (200x)

4.5.2 Age and gender distribution of oesophageal adenocarcinoma

Eight oesophageal adenocarcinoma subjects median age 45 years (36 - 94 years), include 3 females (median age 43 years) and 5 males (median age 48 years). The disease was found to be commonest among men than women (figure 11), and is characterised by a ratio of 1.7 (male): 1(female).

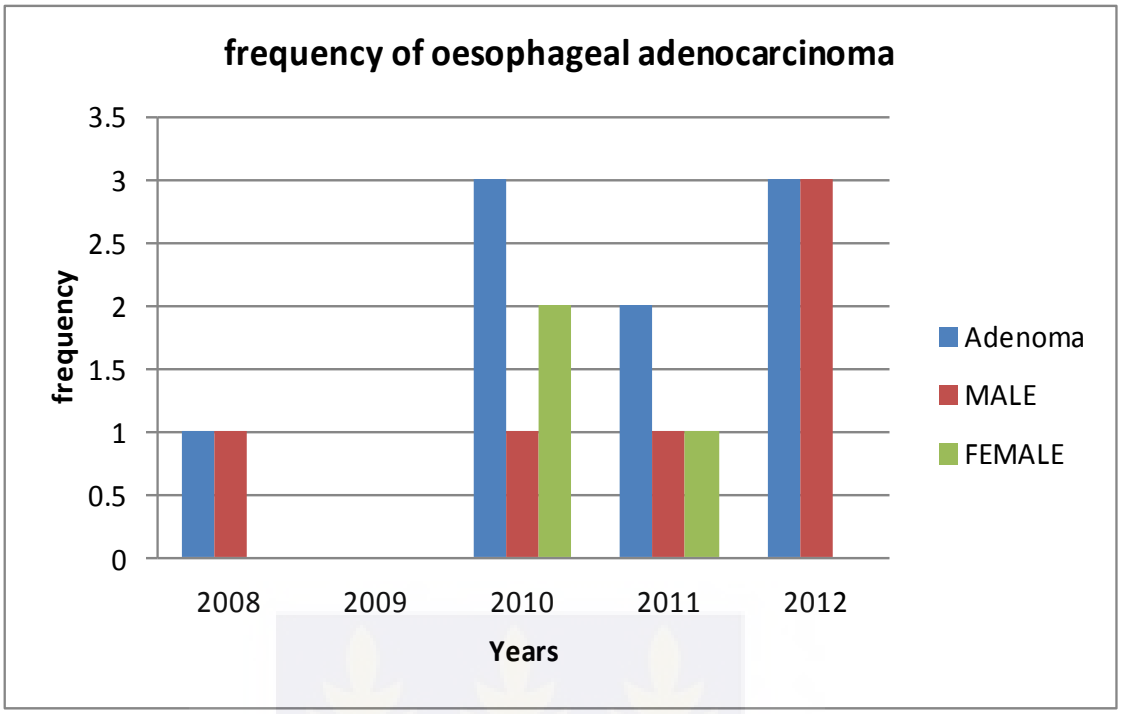


Figure 11: The frequency of oesophageal adenocarcinoma among gender.

4.6 HER-2 over-expression and oesophageal adenocarcinoma

Eight (8) oesophageal adenocarcinoma tissue blocked samples were retrieved for HER-2 analysis. Three (42.9%) were found to have over-expressed HER-2. Of this number, 2 were males and one female, all aged over 40 years. Figure 12 shows a HER-2 positive mucin producing adenocarcinoma of oesophagus. Figure 13 shows HER-2 negative adenocarcinoma of oesophagus.

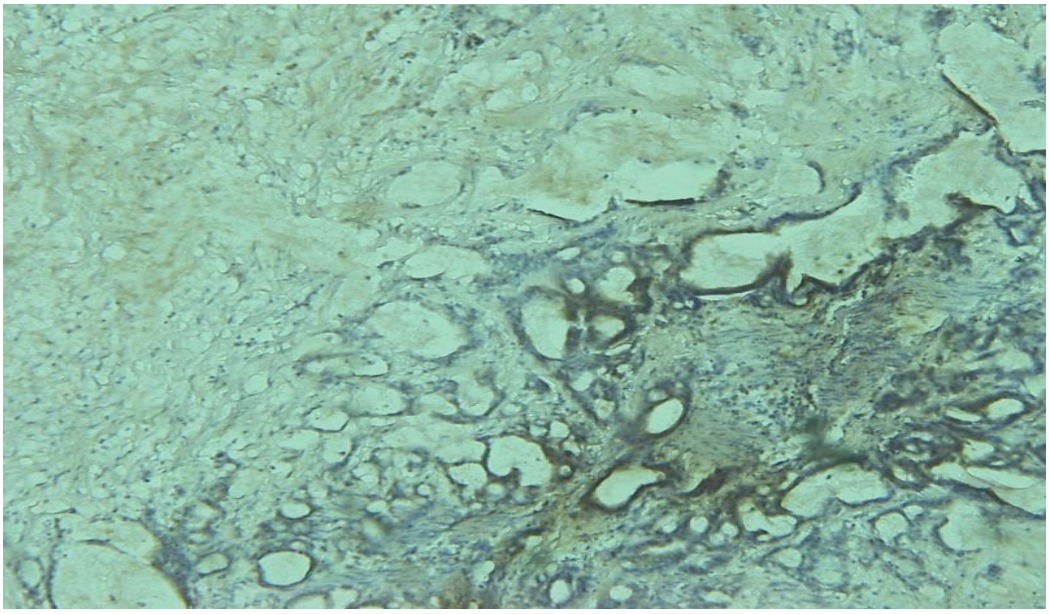


figure 12: HER-2 positive (3+) mucin producing adenocarcinoma of oesophagus. IHC (200x)

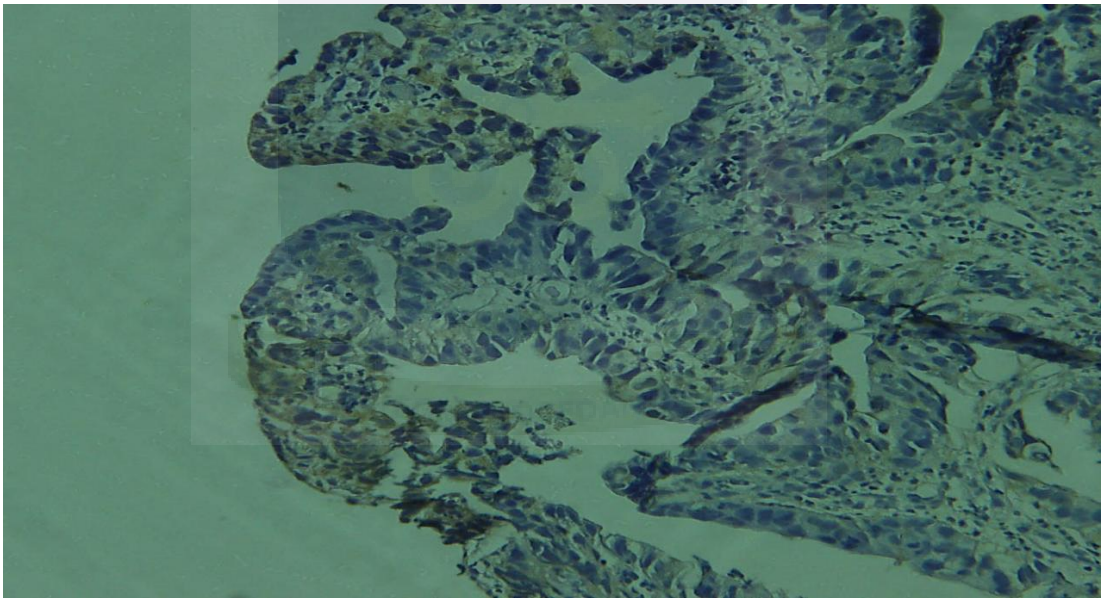


figure 13: HER-2 negative adenocarcinoma of the oesophagus. IHC(200x)

CHAPTER 5

5.0 DISCUSSION

5.1 General pattern of gastric adenocarcinoma

The prevalence of gastric adenocarcinoma over a period of 5 years in a tertiary care hospital in Ghana, West Africa was analyzed. It was established in the study an approximately 18.79% prevalence of gastric adenocarcinoma in a total of 974 stomach biopsies.

In Southern Brazil, a prevalence of 1.6% gastric adenocarcinoma was noted among 20,521 patients who underwent upper gastrointestinal endoscopy (Rampazzo *et al.*, 2012), whereas in Zambia, similar study observed 5.3% (113 out of 2,132) as gastric adenocarcinoma (Kelly *et al.*, 2008). Relative to these previous works done in Brazil and Zambia the obtained prevalence in this study was high. Possible explanations for the variation appear diverse, but one likely reason may mainly reflect differences in the prevalence of certain risk factors such as *Helicobacter pylori*, human papillomavirus or Epstein-Barr virus infection (Parkin, 2006, Ding *et al.*, 2010). It is important to note that sanitation and consumption of salted food, particularly red meat have been associated with gastric cancers (Rampazzo *et al.*, 2012), and adenocarcinoma also constitutes about 95% of all gastric cancers (Meireles *et al.*, 2004); raising possible suspicion that, the observed variation in the prevalence of gastric adenocarcinoma might be associated with subjects' lifestyle.

5.1.1 Gender and age distribution of gastric adenocarcinoma

The mean age (59.95 years) and male predominance in this study are similar to that in other global reports, 60 ± 15SD years (Chadli *et al.*, 1986, Jemal *et al.*, 2010, Nagini,

2012). Throughout the world, stomach cancer is a disease of the elderly population with predominance in men (Rampazzo *et al.,* 2012). The analysed trend in this study showed similar pattern in gender proportions over the 5 year period. The explanation for male predominance in this series is varied, but one likely contributing factor may be the greater exposure of males to smoking (Murata *et al.,* 2010). The intake of salty foods is also a well-established risk factor for gastric cancer, however a recent study found this association only in males (Murata *et al.,* 2010) raising one more possible explanation for the prevalence of gastric adenocarcinoma in men in this study. Although the national prevalence cannot be adequately evaluated, due to the fact that not all gastric biopsies are processed at KBTH, yet, adenocarcinoma of this site appear to be one of the most common cancers in the population diagnosed with gastric cancer at KBTH.

5.1.2 Distribution of histological subtypes of gastric adenocarcinoma

The distribution of histological subtypes of gastric adenocarcinoma (intestinal and diffuse) appears to have similar pattern as reported from other studies. Globally, (Wu *et al.,* 1997, Polkowski *et al.,* 1999, Tanner *et al.,* 2005, Lordick *et al.,* 2007, Lennerz *et al.,* 2011) the intestinal type has been shown to be the common histomorphological subtype of gastric adenocarcinoma. A similar pattern was observed in this study.

5.2 HER-2 expression and gastric adenocarcinoma

Globally, the prevalence of HER-2 over-expression has been noted in several studies to range from 9% - 38% (Penault-Llorca *et al.,* 2011, Daum *et al.,* 2011, Pazo Cid and Anton, 2012). For instance in Spain 10% of 228 gastric adenocarcinoma patients

over-expressed (Grávalos *et al.,* 2011) HER-2, while in UK 9% over-expressed HER-2 out of 489 gastric adenocarcinoma patients (Lennerz *et al.,* 2011). However, in this present study a prevalence of 41.4% was noted among the 99 gastric adenocarcinoma cases to have over-expressed HER-2 protein. The obtained prevalence in this study is relatively higher than the previous studies in Spain and UK (Lennerz *et al.,* 2011; Grávalos *et al.,* 2011). The reasons for the observed difference appears complex, however one possible explanation could be a useful indicator to the prognostic significance of this biomarker, HER-2 in this patients in Ghana.

5.3 HER-2 expression among age group and gender

The association between age group or gender and HER-2 over-expression in gastric adenocarcinoma is varied. This study observed a significant association between HER-2 over-expression and age group, but not gender. Similarly, Dang *et al.,* (2012) found a significant association between HER-2 over-expression and age group but not in gender as well. However, work done by (Gravalos and Jimeno, 2008) found out that there was no significant association between HER-2 expression and either gender or age. The reasons for these differences in the expression of HER-2 in gastric adenocarcinoma among gender and age group are unclear and still need further analysis.

5.4 HER-2 expression and histological subtype of gastric adenocarcinoma

In gastric adenocarcinoma, HER-2 positivity differed significantly by histological subtype (intestinal 34%, diffuse 6%) (Lordick *et al.,* 2007). This was also demonstrated in this current study (intestinal 82.9%, diffuse 17.1%). The higher association between HER-2 expression and intestinal histological type was reported far back in the 1990s by several authors (Wu *et al.,* 1997, Polkowski *et al.,* 1999) and

confirmed in more recent studies (Gravalos and Jimeno, 2008, Tanner *et al.*, 2005). Further studies have also reported that the intestinal type of gastric carcinoma has higher prevalence of HER-2 over expression (16%–34%) compared to the diffuse type (2%–7%) (Tanner *et al.*, 2005, Gravalos and Jimeno, 2008) and, generally, over-expression in these tumours is usually limited to areas of intestinal morphology (Yan *et al.*, 2010).

This trend of intestinal-type histomorphology carrying higher rates of HER-2 over-expression is also true for this study. This study observed a higher rate of HER-2 over-expression in intestinal type (44.16%) of adenocarcinoma than in diffuse type (31.82%). The explanation for this difference in the over-expression of HER-2 among the histologic types is largely unclear. Nevertheless, this finding supports the suggestion that separate genetic alterations occur in different phenotypes in terms of HER-2 over-expression. The association of this HER-2 oncogene with intestinal histological tumour type possibly indicates that certain characteristics (e.g. HER-2 over-expression and intestinal phenotype) may be expressed together preferentially. However, this could not be the only reason for selective over-expression of HER-2 in the intestinal-type of gastric adenocarcinomas in this study and therefore warrants an in depth analysis for further explanation. This is because not all tumours of the intestinal type in this study over-expressed HER-2, suggesting the possible involvement of other factors.

5.5 Prevalence of oesophageal adenocarcinoma

5.5.1 General pattern of oesophageal adenocarcinoma prevalence

Globally, the prevalence of oesophageal adenocarcinoma is up to about 17% (Corley and Buffler, 2001). In Uganda a prevalence of 7.3% was observed among 287 oesophageal-endoscopic patients (Ocama *et al.*, 2008). Results from this study demonstrated a prevalence of 8.79% oesophageal adenocarcinoma among 91 oesophageal biopsies received. The observed prevalence in this work is greater than that found in Uganda (7.3%). The reasons for these variations appear mixed; however it appears to provide evidence that Ghana might be an area of high-risk zone for this cancer and therefore further studies may be paramount.

5.5.2 Age and gender distribution of oesophageal adenocarcinoma

The gender ratio (male to female) all over the world shows significant diversity ranging from 0.85 in Northern Iran (Pedram *et al.*, 2011) to as high as 20.5 in Hispanics (Nordenstedt and El-Serag, 2011) for oesophageal cancers. Although various studies have demonstrated varying figures in prevalence between gender (Parkin *et al.*, 2005, Kachala, 2010, Dawsey *et al.*, 2010), most of their reports focused on squamous cell carcinoma, apparently because it seems to be more common. Nordenstedt and colleague recorded that for oesophageal adenocarcinoma, all races had similar sex specific prevalence patterns (Nordenstedt and El-Serag, 2011).

This study observed eight confirmed oesophageal adenocarcinoma, (5 males and 3 females). In the USA, the prevalence of oesophageal adenocarcinoma among both genders was found to be similar (Nordenstedt and El-Serag, 2011). Reasons for the

differences noted in the previous study in USA compared to the present study may vary; however, one possible explanation may reflect susceptibility state conditioned by individual genetic traits (Brown *et al.*, 2001). Another, possible explanation for the observed higher prevalence in males may be associated with excessive smoking of tobacco. Tobacco has been found to be associated with oesophageal adenocarcinoma and contribute significantly to disease progression (Kollarova *et al.*, 2007). However, the synergistic effect of alcohol and smoking when consumed together may potentiate the relative risk of oesophageal adenocarcinoma in males.

In Sub-Saharan Africa, the disease was prominent among the age group of 45 - 65 years in both sexes (Kachala, 2010). This is similar in both developed and developing countries. Oesophageal adenocarcinoma of the oesophagus is infrequent before 40 years of age, beyond which the prevalence rises with each decade of life (Pickens and Orringer, 2003). This study observed a diverse age range with a mean of 47.7 years. However, due to the small number of cases diagnosed with adenocarcinoma in this study, trend analysis cannot be deduced.

5.6 HER-2 expression and oesophageal adenocarcinoma

Significant expression of HER-2 protein in oesophageal adenocarcinoma has been noted in several studies; globally it has been found to be 32% (Lordick *et al.*, 2007), in the United States it is 17% (Yoon *et al.*, 2012), 24% in Finland (Tanner *et al.*, 2005) and in Germany it is 22% (Geddert *et al.*, 2002). However, in this study HER-2 over-expression in oesophageal adenocarcinoma was found to be 42.9%. The observed variations in the expression of HER-2 in oesophageal adenocarcinoma at different settings appear to be significant. Likely explanations for the variation may include differences in the durations of the study as well as sample size. In the previous

studies carried out in the Germany, Finland and United State (Geddert *et al.*, 2002 Tanner *et al.,* 2005; Yoon *et al.,* 2012) the recorded prevalence of HER-2 over-expression were sampled from over 10 cases of adenocarcinoma of the oesophagus whereas; the present study evaluated seven (8) histologically diagnosed cases.

6.7 CONCLUSION

In this study, a prevalence of 18.79% gastric adenocarcinoma was recorded among gastric biopsies received at the Department of pathology, Korle Bu Teaching Hospital (KBTH) from 2008 to 2012 and majority of this cancers occurred in males. Intestinal variant of adenocarcinoma of the stomach was the commonest compared to the diffuse type of adenocarcinoma. Human epidermal growth factor receptor-2 (HER-2) was over-expressed in 41.4% of the gastric adenocarcinomas. HER-2 over-expression in gastric adenocarcinoma was significantly more common in patients more than 55 years old and in those with intestinal type of adenocarcinoma.

Squamous cell carcinoma was the commoner cancer (31%) type in the oesophagus, compared to oesophageal adenocarcinoma (8.79%). However, HER-2 was over-expressed in 42.9% of oesophageal adenocarcinoma which is similar to that of gastric adenocarcinoma (41.4%).

5.8 Recommendation

Routine testing for HER-2 in gastric and oesophageal adenocarcinoma patients can have significant impact on the management or treatment options offered such patients, which may potentially affect their prognosis. A nationwide prospective study on this protein in these subjects is recommended.

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APPENDICES

A1: HER-2 Antibody description

Human epidermal growth factor receptor -2

Product code: NCL-L-CB11

Lot: 6009335

Clone: CB11

Immunogen: synthetic peptide corresponding to a site on the internal domain of the human c-erbB-2 oncoprotein.

Immunoglobulin class: IgG1

Antibody concentration: Greater than or equal to 23.4mg/L as determined by ELISA

Total protein concentration: 3.9g/L

Total antibody concentration: 26mg/L

Dilution factor of antibody used: 1/40 (one part of antibody against 39 part of the IHC diluents)

Concentration of antibody used: 26mg/L X 1/40 = 0.65mg/L

A2: Reagent preparation

50mM Tris Buffered Saline (TBS)

The following were dissolved in 800ml of distilled water

8g sodium chloride

0.2g potassium chloride

6g Tris base

pH was adjusted to 7.6 with concentration hydrochloric acid

Total volume was made up to 1L with distilled water

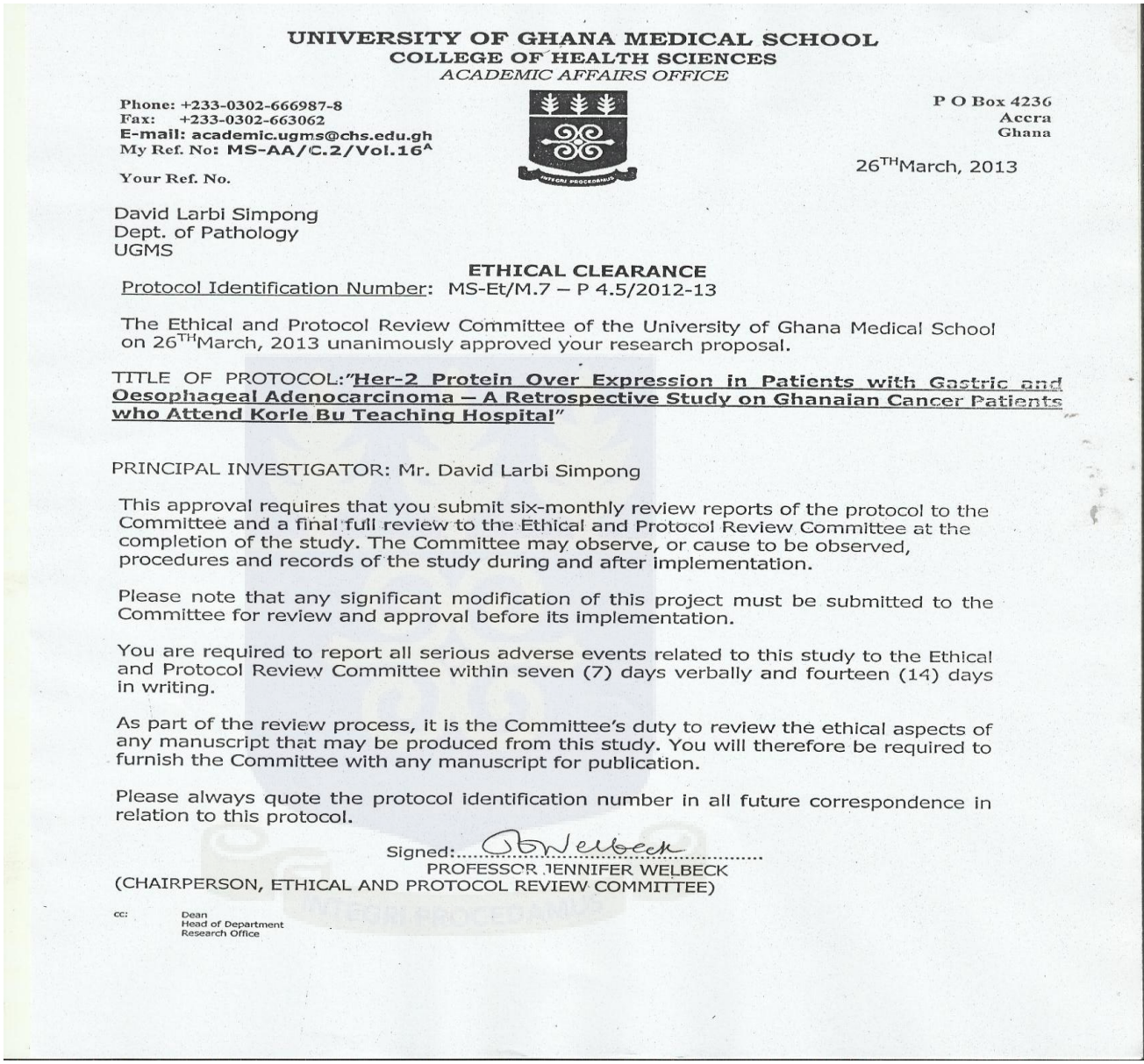
Table 6: Human epidermal growth factor receptor 2 (HER2) scoring criteria for gastric cancer

Score	Surgical specimen-staining pattern	Biopsy specimen-staining pattern	HER2 overexpression assessment
0	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
+1	Faint/barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with a faint/barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
+2	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumor cell cluster with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal
	Strong complete, basolateral, or lateral membranous	Tumour cell cluster with a strong complete, basolateral,	Positive

+3	reactivity in ≥10% of tumour cells	or lateral membranous reactivity irrespective of percentage of tumour cells stained

(Rüschoff *et al.*, 2012)

A3: Ethical Clearance



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ETHICAL CLEARANCE

Protocol Identification Number: MS-Et/M.7 – P 4.5/2012-13

The Ethical and Protocol Review Committee of the University of Ghana Medical School on 26TH March, 2013 unanimously approved your research proposal.

TITLE OF PROTOCOL: "**Her-2 Protein Over Expression in Patients with Gastric and Oesophageal Adenocarcinoma – A Retrospective Study on Ghanaian Cancer Patients who Attend Korle Bu Teaching Hospital**"

PRINCIPAL INVESTIGATOR: Mr. David Larbi Simpong

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: JWelbeck
PROFESSOR JENNIFER WELBECK
(CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE)

cc: Dean
Head of Department
Research Office