PREVALENCE OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR-2(HER-2) OVER-EXPRESSION IN PATIENTS WITH GASTRIC AND GASTRO-ESOPHAGEAL JUNCTION CARCINOMA SEEN AT KENYATTA NATIONAL HOSPITAL

BY

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M.B.Ch.B (U.O.N)

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DECLARATION

I declare that this dissertation is my original work and has not been presented for a degree in any other university

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DEDICATION

This book is dedicated to my parents for their guidance and support throughout my life.

My wife who has been the source of my strength and inspiration and my sons Hassan, Hussein and Mohammad for withstanding the long hours I was away from them.

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ABBREVIATIONS

ABC-HRP	Avidin Biotin Complex-Horseradish Peroxidase.
ANOVA	Analysis of Variance.
ASCO	American Society of Clinical Oncology.
CI	Confidence Interval.
DAB	Diamino-BenzidineTetra hydrochloride.
ERBB2	Erythroblastosis Oncogene B 2.
FFPE	Formalin Fixed Paraffin Embedded.
FISH	Fluorescence in Situ Hybridization.
5-FU	5-Fluorouracil.
GC	Gastric Cancer.
GEJ	Gastro-Oesophageal Junction.
H&E	Haematoxylin and Eosin.
HER-2	Human Epidermal Growth Factor Receptor-2.
IHC	Immuno-Histo-Chemistry.
kD	Kilo Dalton.
KNH	Kenyatta National Hospital.
KNH/UON	Kenyatta National Hospital/University of Nairobi.
OGD	Oesophagoduodenoscopy.
PBS	Phosphate Buffered Saline.
RCPAQA	Royal College of Pathologist of Australia Quality Assurance.
RTU	Ready to Use.
SD	Standard Deviation.
SKBr 3	Sloan Kettering Breast cells.
SPSS	Statistical Product and Service Solution.
TBS	Tri Buffered Slide.

ToGA	Trastuzumabfor Gastric Cancer Trial.
UON	University Of Nairobi.
US	United States.
WASR	World Age Standardized Incidence Rates.

ABSTRACT

Background; Gastric cancer in Kenya is ranked third in both males and females. Most patients present clinically with advanced unresectable disease have poor prognosis despite administration of standard chemotherapy. Human epidermal growth factor receptor – 2(HER-2) over expression in gastric cancer is related to poor outcome. Advances in molecular therapy have identified HER-2 to be an important component in the treatment of advanced gastric cancer. The prevalence of HER-2 in Kenya is unknown.

Objective: To determine the prevalence of HER-2 over expression in patients with gastric and gastro-oesophageal junction carcinoma at Kenyatta National Hospital.

Methodology; Descriptive Cross sectional study on patients with histological diagnosis from endoscopic/resection specimens of gastric or GEJ cancer at KNH. A sample of 66 patients was selected by progressive sampling. Approval was obtained from the KNH/UON ethics and research committee. Data was collected using a pretested questionnaire. All tissue blocks were tested for HER-2 receptor protein using IHC. Data entry and analysis was done via SPSS version 21.0.

Results; Study sample of 66 patients were included in the study with a mean age of 60.7 years and males consisting of 66.7%. 42 specimens were obtained from OGD and 24 from surgically resected specimens. Approximately 91% of the tumours were located in the gastric region. Gastric adenocarcinoma accounted for89.4 %(N=59) mainly intestinal (78.8%, N=52) and diffuse (9.1%, N=6) while 1.5% (N=1) was a denosquamous. HER-2 over-expression was diagnosed in 42.4% (N=28) of patients. HER-2 over-expression was not significantly associated with age (P= 0.844) and gender (P= 0.682). The anatomical site was not significantly associated with HER-2 over-expression (P=1). HER-2 over-expression was found mostly in adenocarcinoma (96.4%) compared

to 3.6% in adenosquamous, with intestinal type showing highest rate of over-expression (87.5%) compared to diffuse (12.5%).

Conclusion: HER-2 over-expression was found to be higher in our study (42.4%) compared to most of the studies. HER-2 over-expression is observed predominantly in intestinal type of gastric and GEJ adenocarcinomas.

INTRODUCTION

Gastric cancer is the second leading cause of cancer death world-wide[1]. The highest incidence is found in Eastern Europe, Eastern Asia and South Africa, the lowest in North America.[2]

The major risk factors for gastric cancer are male sex ,Helicobacter pylori infection ,smoking, high levels of dietary salt and nitrates, medical conditions such as atrophic gastritis and pernicious anaemia, positive family history of gastric cancer and previous gastrectomy[3].Obesity has been associated with gastric cardia and junctional tumours, most likely through increased gastro-oesophageal reflux and subsequent Barrett's metaplasia.[3]Tumours of the gastro oesophageal junction are classified as gastric cancer. Adenocarcinoma of the stomach accounts for 10% of all cancer worldwide[4].

Gastric cancer is more common in males than in females with a distribution rate of approximately 2:1 respectively.

Incidence data from Africa are weak, reliable estimation of cancer incidences is difficult to obtain and few established cancer registers are available.

According to the Nairobi cancer registry, gastric cancer is ranked third in both males and females after cancer of the prostate and oesophagus in males and cancer of the breast and cervix in females. In males, gastric cancer accounts for 7.3% of all male cancers and 9.5% in females[5]. Clinically, most patients present with advanced gastric cancer and prognosis remains poor .The overall 5 year survival rate is about 27%, with stage 1 being 90% but hardly discovered clinically. Stage 2-3 disease is 20%-50% and 5%-10% for stage 4[6].

In non-metastatic disease, surgery is the mainstay of treatment but recurrences are common despite curative resection hence adjuvant radio-chemotherapy is recommended[6].

The discovery of new targeted therapies and chemotherapy agents, together with increasing knowledge of biological pathways underlying GC and the ability to predict which patients or tumours will respond to which treatment, has led to improved GC patient outcomes. Targeted therapies have emerged as a new hope in cancer management during recent years[6].

Molecular targeted therapy advances have identified HER2 as an important target for anti-cancer therapy in gastric cancer. HER-2 over expression in gastric cancer has been reported to range widely from 6% to 45%[7]. HER2 over-expression is a negative prognostic factor in Gastric and GEJ carcinoma, correlating with a poor survival[4].

In Kenya, prevalence of HER-2 over expression has not been established hence the therapeutic utility of targeted therapies still remains unclear. The study aims to determine the prevalence of HER2 over expression in patients with gastric and gastro-oesophageal junction cancer presenting at Kenyatta National Hospital.

LITERATURE REVIEW

Gastric cancer is the fourth most common malignancy and second leading cause of cancer deaths world-wide, henceasignificantglobalhealthproblem[1].

The world age standardized incidence rates (WASR) of gastric cancer of various African population is as follows;

Table 1; World age standardized incidence rate of gastric cancer in African population

Location	Year	WASR males	WASR females
Kenya, Meru[8]	1991-1993	14.3	7.1
Mali, Bamako[9]	1988-1992	19.6	11.1
Zimbabwe, Harare[10]	1993-1995	12.3	11
Uganda, Kyadondo[11]	1991-1994	4.7	3.2
Algeria, Setif[9]	1990-1993	14.4	3.5

Source; stomach cancer in Africa 4.18; 372/cancer incidence in five continents, 8; 94-95

HER-2 (Human Epidermal Growth Factor Receptor-2) is a protein encoded by the ERBB2 gene in humans. ERBB2 gene is a proto-oncogene located at the long arm of human chromosome 17 (17q12). Amplification or over expression of this gene has been shown to play an important role in the pathogenesis and progression of certain aggressive types of cancers such as breast and gastric. HER-2 has become an important biomarker and target of therapy for these cancers.

This gene is translated into 185-kD membrane growth factor receptor protein, which transmits signals regulating normal cell growth, development and survival. The binding of several high affinity ligands to HER receptor-family members leads to receptor dimerization and activation of intracellular signalling through receptor tyrosine kinases.The amplification of

HER-2 gene which translates to over-expression of HER-2 receptor protein on the cell membrane increases the likelihood of receptor dimerization and activation of these signalling pathways [12].HER-2 is associated with excessive dimerization that contributes to cell survival, cell proliferation and tumorigenesis[13].

HER-2 AND GASTRIC CANCER

HER-2 protein over-expression in gastric cancer was first described in 1986 using immuno-histochemistry (IHC) [14].

In gastric and gastro-oesophageal junction carcinoma, HER-2 appears to be an important prognostic factor which is related to poor prognosis. Different studies have found HER-2 over-expression in gastric and gastro-oesophageal adenocarcinoma to be associated with increasing depth of invasion, lymph node involvement, distant metastases and poor survival[15].However, there is conflicting information in this respect and not all studies have shown a clear association between HER2 over-expression and poor prognosis. HER2 protein over-expression and gene amplification are much more heterogeneous in gastric cancer compared to breast cancer hence its implications for the clinical testing of biopsy specimens[16].

Wide range of variation in prevalence of HER-2 has been demonstrated in various population, different histologic types and location of the tumour. Bang et al[17]found HER-2 positivity rate of 22.1% in the Republic of Korea. This was similar with Europe 23.6% and Asia 23.5%[18, 19]. Even higher prevalence of 53% and 91% has been reported[20, 21].

Varying prevalence in tumour site was seen in several studies. In a US population study, HER-2 over-expression was found to be 12% in gastric cancer and 10% in gastro-oesophageal junction cancer[22].

In Finland gastric cancer prevalence was at 12% and GEJ was at 24% [23], similar trend was seen in Spain recording a prevalence of 9.5% in gastric and 25% in GEJ[24]. Lordwick et al did a multinational study and reported a prevalence of 18% in gastric and 32% in GEJ[25].

Variation in prevalence according to histologic types was also reported in over 5 studies. The prevalence of HER2 has been shown to be higher in intestinal than diffuse type of gastric cancer as illustrated in the table below.

Table 2; Variation in prevalence of HER2 according to histological types of gastric cancer.

Author	Population	Histologic types		
		Intestinal (+ %)	Diffuse (+ %)	Mixed/unknown (+ %)
Tanner [23]	Finland	21.5	2	5
Gravalos[24]	Spain	16	7	14
Lordwick[25]	International	34	6	20
Matsubara [26]	Japan	32.5	6	
Park [27]	Korea	8	1	

Source; Connection; HER-2 testing in gastric and oesophageal adenocarcinoma, 2010: 15; 49

HER-2 STATUS ASSESSMENT

Accurate determination of HER-2 status is critical to ascertain which patients might benefit from targeted therapies.HER-2 status is typically measured by immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH) [28, 29]. IHC is frequently utilized for HER-2 assessment due to the wider availability in routine diagnostic testing and cost implications compared to FISH. Moreover, FISH shows higher sample related failure and is very sensitive to fixatives and duration of fixation.

Additionally, FISH documentation is a challenge due to the diminishing fluorescents signals on the slides[30]. A validated scoring system has been developed for HER-2 assessment in gastric cancer[31] based on the study done by Hoffman et al[31]. Consensus was reached and the following was recommended for scoring HER-2 over expression in gastric cancer as illustrated in Table 3 below.

Cells that stain with a score of 0 or +1 are considered negative meaning they don't have amplification of the HER-2 gene thus do not over express the HER2 receptor protein. A score of +3 is confirmed over expression while +2 denotes an equivocal positive score.

Table 3: Consensus panel recommendation of HER 2 evaluation for Gastric cancers

Pattern	Score/classification
No reactivity or membranous reactivity in <10% of the cells	0/negative
Faint/barely perceptible membranous reactivity in>10% of cells; cells are reactive only in part of their membranes	1+/negative
Weak to moderate complete or basolateral membranous reactivity In >10% of cells	2+/equivocal
Moderate to strong complete or basolateral membranous reactivity in >10% of cells	3+/positive
Biopsy samples with cohesive IHC3+ or FISH+ clones, irrespective of size (even if <10%)	3+/positive

Source: connection: HER-2 in gastric and oesophageal cancer; 2010: 15; 50[31]

HER-2 AND TARGETED THERAPIES

The implication of knowing HER-2 status is the use of molecular targeted therapy in the management and prognostication of stomach and gastro-oesophageal cancer[32].

Surgical resection is the mainstay of treatment and can cure early stage cancer, however most patients are diagnosed at advanced stage. For these patients, chemotherapy is the main treatment option. Survival of patients with advanced GC treated with palliative chemotherapy remains low[33], hence a great interest in targeted therapies has emerged and several molecular targeting agents are being tested. So far, trastuzumab is the only targeted therapy that has a proven survival benefit in GC[32].

Trastuzumab is a monoclonal antibody directed against the HER-2 receptor and acts by the inhibition of HER-2 mediated signalling as well as induction of antibody-dependent cellular cytotoxicity [34-36]. Trastuzumab is efficient in breast cancer showing either HER-2 gene amplification or HER-2 membrane protein over-expression, and has become the standard of care in HER-2-positive early and metastatic breast cancer [37-39].

Early studies in GC cell lines which over-expressed HER-2 showed growth inhibition by trastuzumab.Cortes funes et al demonstrated that in advanced GC patients with over-expression/amplification of HER-2, addition of trastuzumab to cisplatin resulted in better response rate (35%) and stable disease (17%) [40].

In the Trastuzumab for Gastric Cancer Trial (ToGA), the efficacy and safety of trastuzumab was evaluated in HER-2 positive advanced GC. Patients were randomized to receive trastuzumab with 5FU or capecitabine and cisplatin versus chemotherapy alone. Preliminary results showed better median survival with the combination (13.5 vs. 11.1 months), with a 26% reduction in risk of death. Patients with high HER-2-positivity by IHC had a trend for better survival in the pre-planned analysis; patients with HER-2 IHC2+/FISH+ or IHC3+ had a longer survival (16 months) with trastuzumab compared to chemotherapy alone (11.8 months)[41].

Nicholas et al[42,] showed that addition of trantuzumab to cisplatin and docetaxel in advanced gastric or GEJ cancer had a response rate of 80%.

KNOWLEDGE GAP

The prevalence of HER-2 over-expression is unknown in our set up, hence the utility of targeted therapies and prognosis of HER-2 positive patients with gastric and gastro-oesophageal junction carcinoma remains unclear.

RESEARCH PURPOSE

The purpose of this study is to determine the prevalence of HER-2 over expression in patients with gastric and gastro-oesophageal junction cancer at KNH, this argues for a possible therapeutic utility of targeted therapies.

RESEARCH QUESTION

What is the prevalence of HER-2 over expression in patients with gastric and gastro-oesophageal junction carcinoma at KNH?

STUDY JUSTIFICATION

Gastric cancer remains the fourth most commonly diagnosed cancer and the second leading cause of cancer-related deaths worldwide [43, 44]. The annual mortality attributed to stomach cancer worldwide is 803000 deaths. In Kenya gastric cancer is the third most common cancer[5].

The overall survival rate of gastric cancer remained poor until the introduction of multidisciplinary approaches and identification of novel targeted agents, which has continued to improve survival outcome. Trastuzumab is a monoclonal antibody that interferes with HER-2 receptor function. It has been shown to improve the survival of patients with advanced gastric cancer and prolong their lives by 2.7 months when added to standard chemotherapy than those patients who received chemotherapy alone [17, 45].

Patients with HER-2 positivity by IHC have been shown by other studies to have a longer survival (16 months) with addition of trastuzumab compared to chemotherapy alone (11.8 months) [41].

HER-2 over expression in gastric cancer has been reported to range widely from 6% to 45%. Due to this wide range of prevalence, our own local prevalence needs to be evaluated. There is paucity of data on the prevalence of HER2 over expression in gastric and gastro-oesophageal junction cancer in the African population.

OBJECTIVES

Broad objectives

To determine the prevalence of tumour HER-2 over-expression among patients with gastric and gastro-oesophageal junction carcinoma at Kenyatta National Hospital.

Specific objectives

1. To determine the demographic pattern of tumour HER-2 over-expression in patients with gastric and gastro-oesophageal junction carcinoma at Kenyatta National Hospital.
2. To determine the histological type and anatomical site of the tumour at which HER-2 over-expression is manifest.
3. To determine the percentage of gastric and gastro-oesophageal junction carcinoma that over-express HER-2.

METHODOLOGY

Study area

The setting of the study was at Kenyatta National Hospital which is a teaching and main tertiary referral hospital in Kenya and University of Nairobi Immunohistochemistry Laboratory.

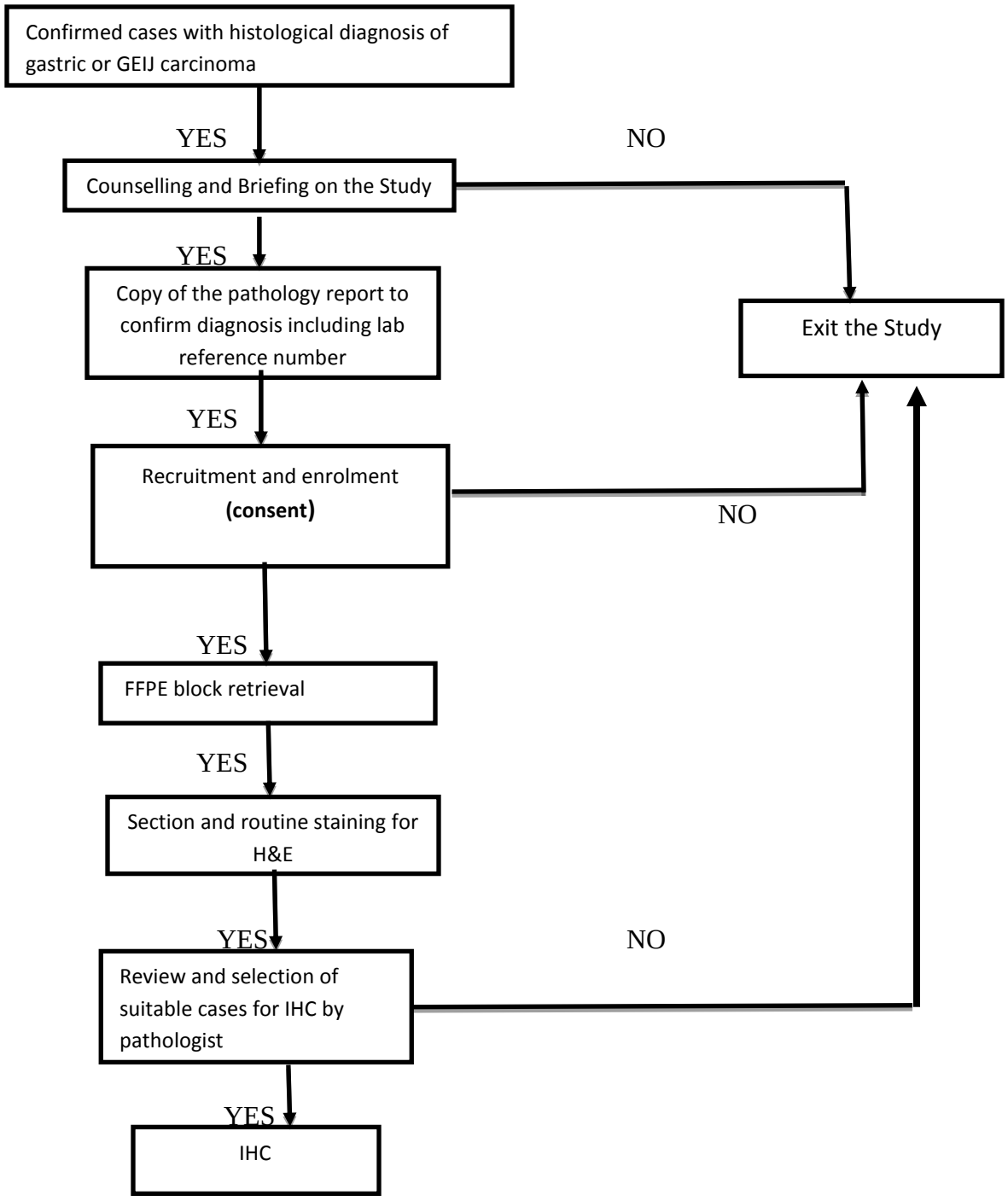
Study population

All patients with confirmed histological diagnosis from endoscopic/resection specimens of gastric or gastro-oesophageal junction carcinoma, admitted in surgical/radioncology wards, attending surgical/radioncology clinics, endoscopy unit, accident and emergency at Kenyatta National Hospital, were evaluated for the study.

Study design

Descriptive Cross sectional study

Recruitment Algorithm



KEY:

- GEJ- Gastro-oesophageal Junction
- IHC- Immuno- histochemistry
- FFPE- formalin fixed paraffin embedded.
- H&E- Haematoxylin and Eosin

Sample Size

Sample size was calculated using the formula below

$$n = \frac{Z^2\alpha \times P\,(1-P)}{e^2}$$

$$n = \frac{1.96^2 \times 0.22(1-0.78)}{0.1^2}$$

$$n = 66$$

n= sample size

e= ± level of precision around estimated HER-2 prevalence (10%)

p= prevalence of HER-2 over- expression in Kenya which is unknown therefore assumed to be 22%

z_{α} = 1.96 area under the standard normal curve representing 95% confidence interval.

1-p= 1 – prevalence of HER-2 expression.

Sampling Procedure

Non-random progressive sampling of patients who meet inclusion criteria until sample size was obtained.

Inclusion criteria

- Patients with confirmed histological diagnosis of gastric or gastro-oesophageal junction carcinoma, with viable histologic tissue blocks at KNH/UON laboratory and consent to participate in the study.
- Patients with radiological diagnosis of gastric or gastro-oesophageal junction tumours awaiting Oesophagoduodenoscopy (OGD) or surgery and consent to participate in the study at KNH , subject to the histopathological examination confirming the diagnosis of carcinoma and a viable tissue block being retrieved from the KNH/UON laboratory.

Exclusion criteria

- Patient who declined consent to participate in the study.
- Patients whose histological diagnosis ruled out carcinoma from OGD specimen.
- Patients with non-viable tissue blocks at KNH/UON pathology laboratory (inadequately Fixed/processed or showing crush/mechanical distortion rendering HER-2 immuno staining difficult or impossible to evaluate).
- Patients whose histology was done in other laboratories other than KNH/UON.
- Patients whose histological diagnosis indicated secondary tumour or distal Squamous cell carcinoma of the oesophagus.

Materials and Methods

The study setting was at the Kenyatta National Hospital surgical/radioncolgy wards, clinics and endoscopy unit, KNH/UON pathology laboratory.

The study commenced upon approval by the Ethical Committee of the Kenyatta National Hospital/University of Nairobi.

Following patients recruitment, informed consent was obtained and their formalin fixed paraffin embedded (FFPE) tissue blocks were retrieved. Sections were made and staining done using routine Haematoxylin and Eosin stain. The slides were reviewed for quality and quantity of material by a pathologist in KHN/UON laboratories. Suitable cases were selected and presented for IHC for HER-2 receptors using anti HER-2antibodies.

Immunohistochemistry

Immunohistochemistry was done using manual method of immuno staining at the University of Nairobi immunohistochemistry laboratory. The details of the immuno staining are outlined in Appendix 3.

HER-2 protein expression was assessed in carcinoma cells by immunohistochemistry (IHC) in paraffin-embedded 3 μm – 5 μm tissue sections according to the manufacturer's instructions (Leica Microsystems Novocastra Ready-to-Use Mouse Monoclonal Antibody c-erbB-2 Oncoprotein Product Code RTU-CB11).

The sectioning and staining was carried out by histo-technologist with a higher National diploma in histology and a wide experience in immunohistochemistry procedure.

Each stained slide was analysed and interpreted by a pathologist using a validated scoring system for HER-2 assessment criteria specific for gastric and GEJ carcinoma. The slides were read by one primary pathologist and reviewed by a second pathologist as part of further quality assurance.

The researcher was actively involved in the logistics of the study including data capture.

Quality Assurance

Measures for quality assurance were put in place to minimize pre analytical, analytical and post analytical variables. This included:

- Pre-Analytical variables of IHC tests; measures were put in place to avoid effects of over or under fixation and over processing of the FFPE blocks.
- Sectioning and staining- the techniques and procedures for reagent preparation, staining and quality control followed the UON standard operating procedures for routine staining and the standard manufacturers guide for HER-2.
- Interpretation and reporting of the results was done by a pathologist using a validated scoring system.
- Following review by a second pathologist all inconsistent cases were reviewed and reported by a third pathologist (tie breaker).

Data was collected using questionnaires as a tool of data collection. Detailed data was documented on the ratio of male to female's HER-2 over expression, percentage of HER-2 over expression in gastric and gastro oesophageal junction carcinoma, histological type and anatomical site of cancer at which HER-2 over expresses.

Data Management and Analysis

A pretested questionnaire was administered for data collection. Consecutive sampling was used to collect data on patients and tissue blocks in the laboratories.

Data was cleaned and entered in SPSS version 21.0 which was used for statistical analysis at the end of data collection. Further data cleaning was done before analysis where errors and inconsistent (conflicting) answers, missing entries and duplicate entries were checked to ensure high quality data. The study population was described using demographic information which was summarized into mean and standard deviations (SD) for age and relative frequencies for sex and residence. Anatomical site and histological type of the tumours was analysed and presented as percentages.

Prevalence of HER-2 over-expression was analysed and presented as a percentage of patients with HER-2 positive results; 95% confidence interval of the prevalence was also presented. Prevalence of HER-2 over-expression was further stratified by the anatomical site and histological type of the tumour. Associations between HER-2 over-expression was done by comparing mean ages across HER-2 status (Positive, Negative or Equivocal) using ANOVA test. Chi square test was used to analyse HER-2 status with categorical variables such as sex, anatomical site and histological type of the tumours. All the tests of associations or comparisons were significant at 5% (p value≤ 0.05) level of significance. The findings were presented using tables and graphs.

Study Limitation

The main limitation for this study was the use of manual method for immuno staining rather than automated machine due to unavailability of the later in the UON laboratory.

Due to cost implications of FISH test, all the equivocal cases (+2) were not concluded for HER-2 status.

Immuno histochemical tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing during paraffin wax block preparation and contamination with other tissues or fluids may produce artefacts, antibody trapping, or false negative results. HER-2 staining is particularly sensitive to fixation time, especially under fixation, Lack of standardization in biopsy specimen fixation and processing before the block is obtained may be a factor in less than optimal results, especially false negative stains.

The problem of under fixation due to suboptimal length of fixation (less than 4 hours) and suboptimal concentration of formalin was assessed during routine histopathological examination and any tissue block showing features of under fixation were excluded from the study. Over fixation on the other hand is not considered a major limiting factor in HER-2 assessment by immunohistochemistry [46]. In addition, HER-2 immunostaining is sensitive to poor processing techniques that the study has no control over, but these was easily identifiable to the pathologist who excluded poorly processed tissue blocks.

Ethical Consideration

The study commenced upon approval by the Department of Surgery (UON) and KNH ethics and research committee. Informed consent was obtained from each participant prior to enrolment in the study. A pre-consent counselling of the participants was carried out. The guardian or next of kin was required to sign consent on behalf of participants who were unable to do so due to unconsciousness, confusion or too sick.

The participants were informed on the purpose of the study and their express permission sought in writing for the use of the tumour tissue in immunostaining for HER-2 in gastric and GEJ carcinoma. The study did not interfere with the usual management of the patient. The details of the consent process are contained in the Consent form (Appendix I).

Confidentiality; access to detailed information was restricted to the researcher and individuals involved in the study.

Feedback of information; all participants were informed of their individual results for immunohistochemistry.

RESULTS

The study examined 66 cases with gastric or gastro-oesophageal junction carcinoma for HER-2 status. Of the 66 cases studied, 42 cases were from biopsied OGD specimens while 24 cases were from surgically resected specimens.

The mean age of patients with carcinoma was 60.7 years (15.0 years SD) with the youngest being 26 years and the eldest was 89 years. More than 60% of patients were between the ages of 50 to 79 years. Majority (66.7%) were males and more than three-quarters (77.3%) resided outside Nairobi. (See table 4, figure 1 & 2).

Table 4: Socio Demographic Characteristics

Variable	**Frequency (%)**
Mean age in years (SD)	60.7 (15.0)
Min-Max	26-89
Age category, n (%)	
Below 40 years	6 (9.1)
40-49 years	9 (13.6)
50-59 years	13 (19.7)
60-69 years	16 (24.2)
70-79 years	13 (19.7)
80-89 years	9 (13.6)
Sex	
Male	44 (66.7)
Female	22 (33.3)

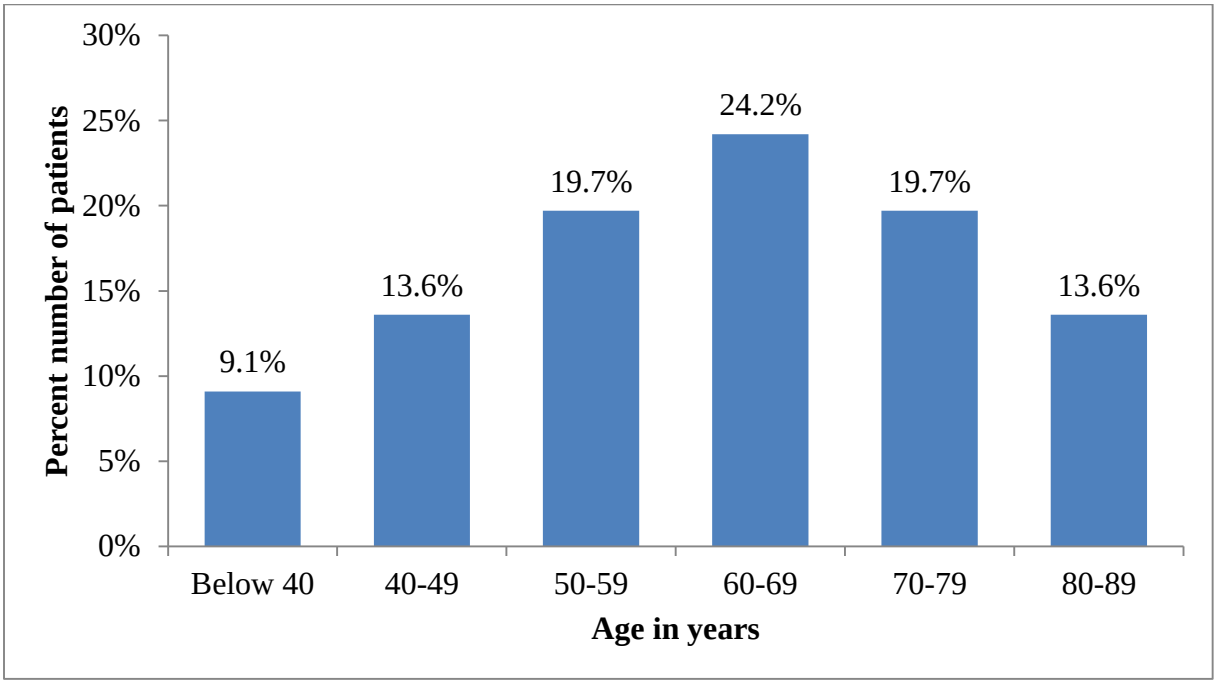


Figure 1: Age distribution.

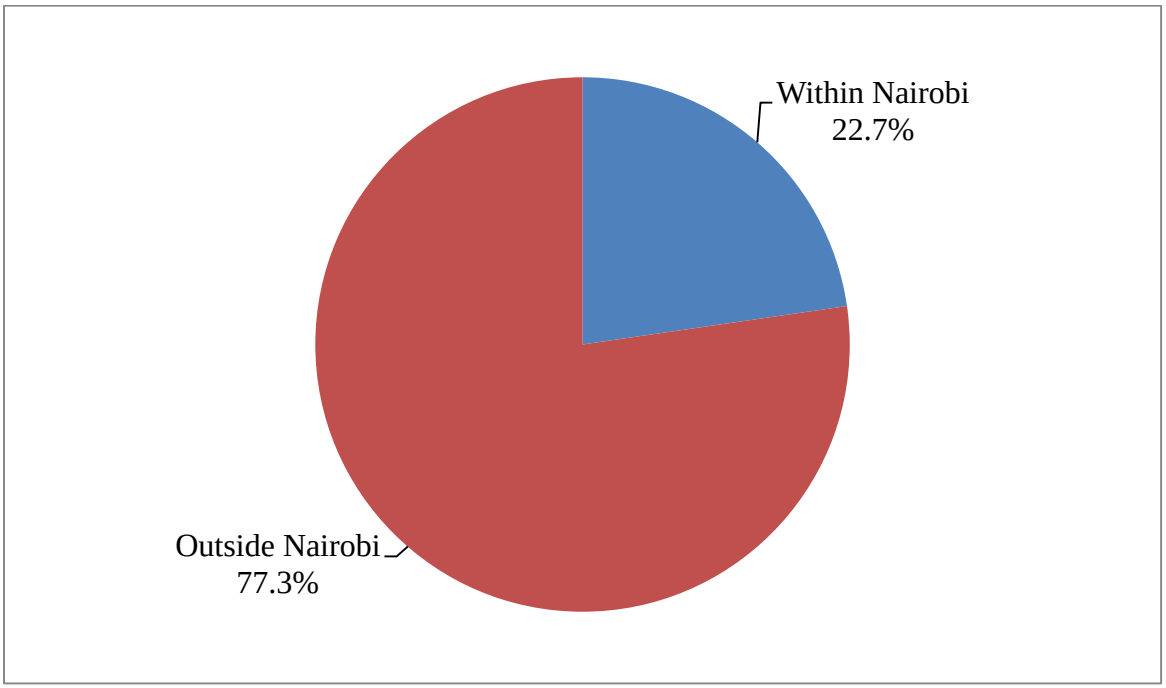


Figure 2: Residence.

All the specimens were interpreted by a primary pathologist for HER-2 status, while the second pathologist was presented with every 5th case (N=13) for confirmation and quality assurance. Discrepancy cases (N=5) were confirmed by a third pathologist (tie breaker) as shown in table 5 below.

Table 5; shows evaluation for 5 discrepancy cases in HER-2 assessment

Case	Primary pathologist	Secondary pathologist	Third pathologist (tie breaker)	Conclusion
Case 1	+3	+1	+1	+1
Case 2	+3	+2	+3	+3
Case 3	+2	+1	+2	+2
Case 4	+1	0	+1	+1
Case 5	+3	+1	+1	+1

The above results was based on the consensus panel recommendation for HER-2 scoring system (see Table 3).

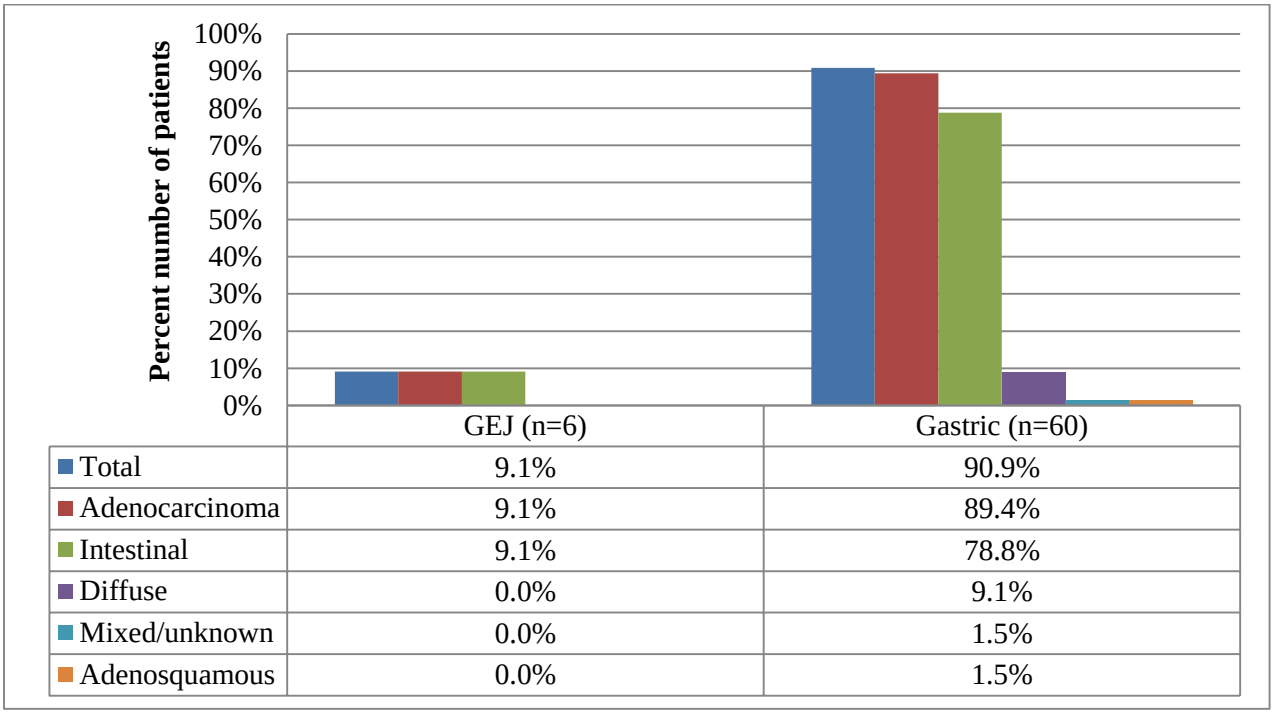
Anatomical Site and Histological Type of the Tumour

Most tumours were located in the gastric region (90.9%, N=60). Majority of the histological types were adenocarcinoma (89.4%, N=59) mainly intestinal (78.8%, N=52) and diffuse (9.1%, N=6) while 1.5% (N=1) was adenosquamous.

Carcinoma of the gastroesophageal junction accounted for 9.1% and all were intestinal adenocarcinomas (N=6). As shown in table 6 and figure 3

Table 6: Anatomical site and histological type of tumors

Variable	**Anatomical site**	
	Gastroesophageal **N (%)**	**Gastric** **N (%)**
Number per anatomical site (n=66)	6 (9.1)	60 (90.9)
Histological type		
Adenocarcinoma	6 (9.1)	59 (89.4)
Intestinal	6 (9.1)	52 (78.8)
Diffuse	0	6 (9.1)
Mixed/Unknown	0	1 (1.5)
Adenosquamous	0	1 (1.5)



	GEJ (n=6)	Gastric (n=60)
Total	9.1%	90.9%
Adenocarcinoma	9.1%	89.4%
Intestinal	9.1%	78.8%
Diffuse	0.0%	9.1%
Mixed/unknown	0.0%	1.5%
Adenosquamous	0.0%	1.5%

Figure 3: Anatomical site and histological type of tumors

HER-2 Over-expression

HER-2 over-expression was diagnosed in 42.4% (N=28) of patients; showing a 95% CI of between 30.9% and 56%. HER-2 negative patients were 48.5% (N=32) while those who portrayed equivocal results were 9.1% (N=6).See table 7 and figure 4.

Table 7: Prevalence of HER-2 over-expression

Variable	**Frequency (%)**
HER-2 status	
Positive (+3)	28 (42.4)
Equivocal (+2)	6 (9.1)
Negative (0, +1)	32 (48.5)
Total	**N=66**

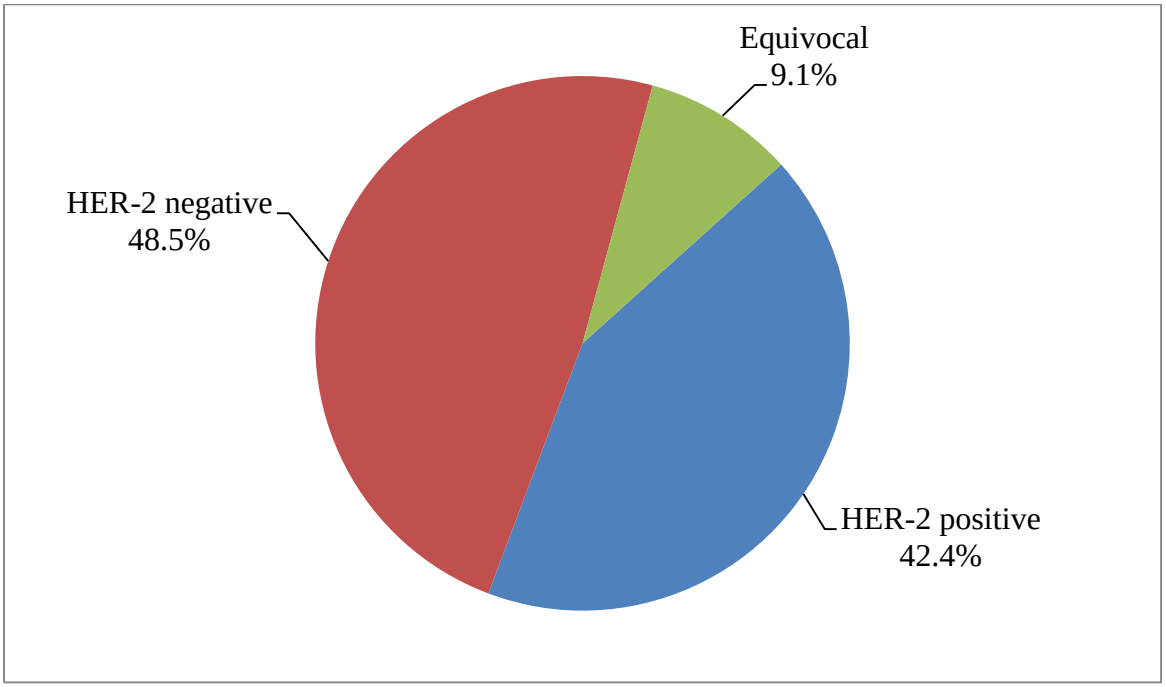


Figure 4:Prevalence.

Demographic Characteristics and HER-2 Over-expression

Patients with HER-2 over-expression had a mean age of 62.3 years. HER-2 over-expression was 43.2% (N=19) in males and 40.9% (9) in females. In overall, HER-2 over-expression was not significantly associated with age and gender (P>0.05). See table 8 and figure 5.

Table 8: Associations between demographic factors and HER-2 over-expression

Variable	**Positive**	**Negative**	**Equivocal**	**P value**
Mean age (SD)	62.3 (11.9)	60.0 (16.0)	60.5 (22.1)	0.844
Sex Male Female	 19 (43.2%) 9 (40.9%)	 22 (50.0%) 10 (45.5%)	 3 (6.8%) 3 (13.6%)	 0.682

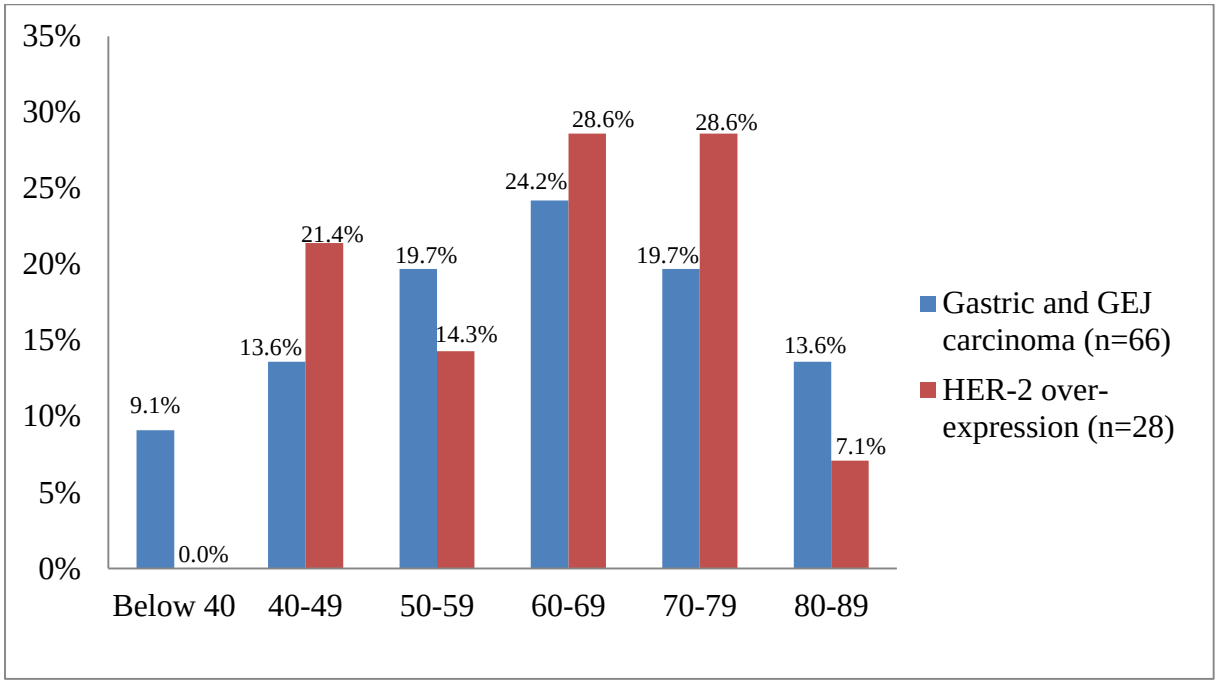


Figure 5: Age distribution and HER-2 over-expression

HER-2 Over-expression and Anatomical Site

As illustrated in figure 6 and table 9 below, 3(50%) out of the 6 cases of GEJ junction carcinoma had HER-2 over-expression and the other half did not over-express. Gastric carcinoma showed HER-2 over-expression in 41.7% (25 out of the 60 cases), while 48.3% were negative and 10.0% were equivocal. The anatomical site was not significantly associated with HER-2 over-expression (P> 0.05).

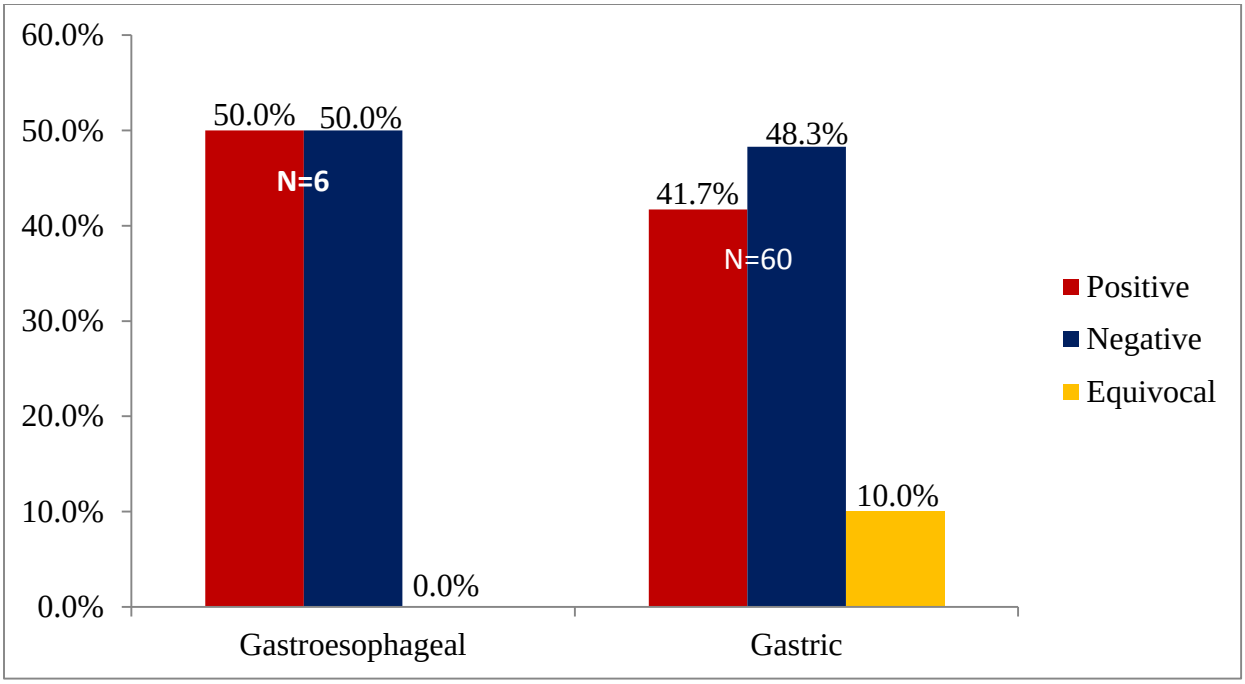


Figure 6: HER-2 over-expression and anatomical sites

Table 9: correlation between anatomical site and HER-2 over-expression

	Anatomical site	
Variable	**GEJ**	**Gastric**
Positive	3 (50.0%)	25 (41.7%)
Negative	3 (50.0%)	29 (48.3%)
Equivocal	0 (0.0%)	6 (10.0%)
Total	6 (100%)	60 (100%)
P-value	1.000	

Comparison in HER-2 Over-expression between Gastric and GEJ carcinoma

In general, out of the 28 cases that showed HER-2 over-expression, 25 cases were found in gastric cancer (89.3%) while the remaining 3 cases (10.7%) were in the GEJ carcinoma. Table 10.

Table 10; HER-2 over-expression between gastric and GEJ tumours

Variable	Positive	Negative	Equivocal	P value
Anatomical site				
Gastroesophageal	3 (10.7%)	3 (9.4%)	0 (0%)	1.000
Gastric	25 (89.3%)	29 (90.6%)	6 (100%)	
Total	28 (100%)	32 (100%)	6 (100%)	

HER-2 Over-expression and Histological Type

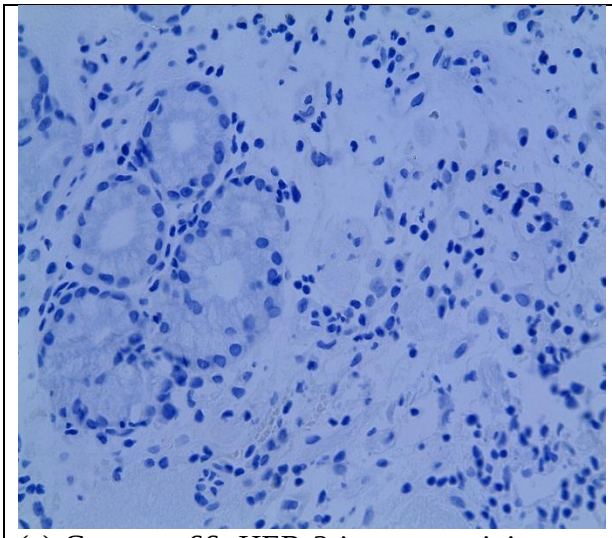
HER-2 over-expression was found mostly in adenocarcinoma (96.4%) as compared to 3.6% in adenosquamous. Intestinal type of gastric adenocarcinoma showed highest rate of HER-2over-expression (87.5%) while 12.5% was of diffuse histological type. In GEJ tumours, all were intestinal type of which half of the cases over-expressed HER-2. See Table 11.

Table 11: Associations between histological type of gastric/GEJ carcinoma and HER-2 over-expression

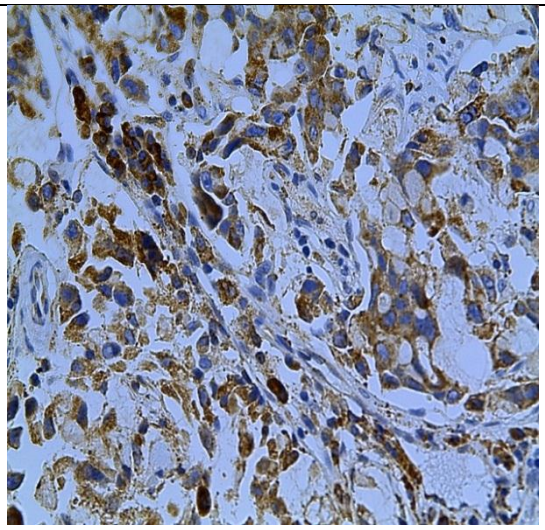
Variable	positive	Negative	Equivocal	P value
Histologic type				
GEJ Site				
Adenocarcinoma • Intestinal	3/28 (10.7%)	3/32 (9.4%)	0/6 (0%)	1.000
Gastric Site				
Adenosquamous • Intestinal	1/28 (3.6%)	0/32 (0%)	0/6 (0%)	0.517
Adenocarcinoma • Intestinal • Diffuse • Mixed/unknown	24/28 (85.7%) 21/24 (87.5%) 3/24 (12.5%) 0/24 (0%)	29/32(90.6%) 27/29(93.1%) 2/29 (6.9%) 0/29 (0%)	6/6 (100%) 4/6 (66.7%) 1/6 (16.7%) 1/6 (16.7%)	0.123

PHOTOMICROGRAPHS

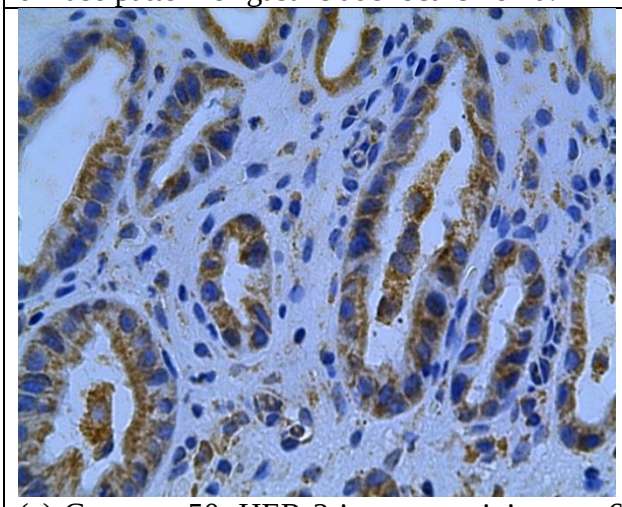
Below are the representative cases for HER-2 immunoscoring.



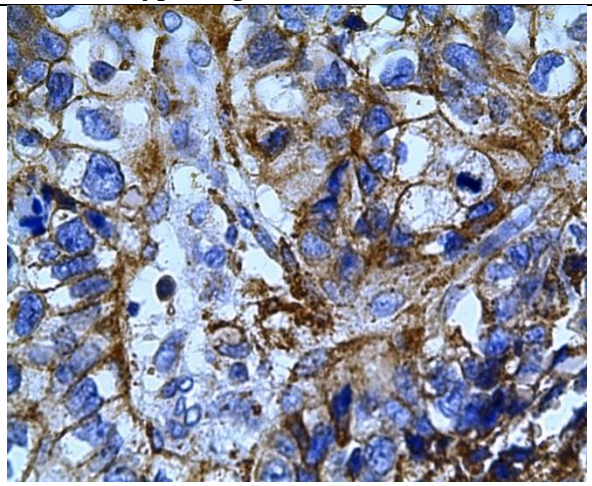
(a) Case no: 66, HER-2 immunostaining at x63 magnification, score of 0 (negative IHC) in a diffuse pattern of gastric adenocarcinoma.



(b) Case no: 2, HER-2 immunostaining at x63 magnification, score of 1+ (negative IHC) in an intestinal type of gastric adenocarcinoma.



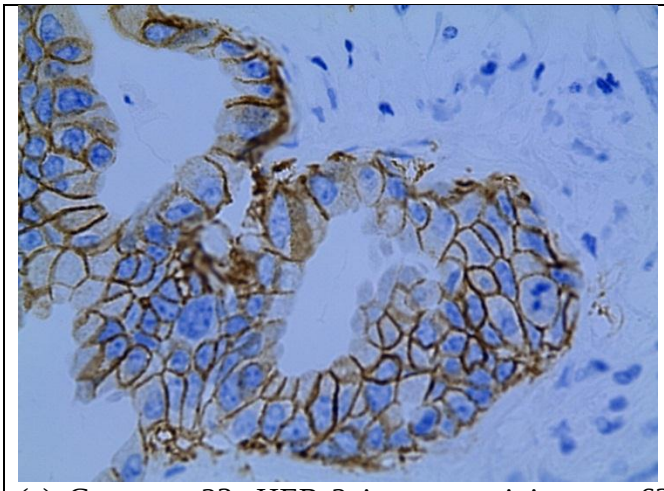
(c) Case no: 50, HER-2 immunostaining at x63 magnification, score 1+ (negative IHC) in an intestinal gastric adenocarcinoma.



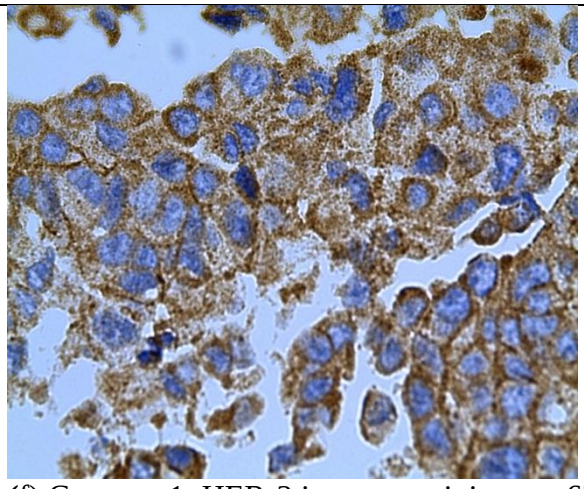
(d) Case no: 11, HER-2 immunostaining, at x63 magnification, score of 2+ (equivocal IHC) in a poorly differentiated intestinal gastric adenocarcinoma.

PHOTOMICROGRAPHS

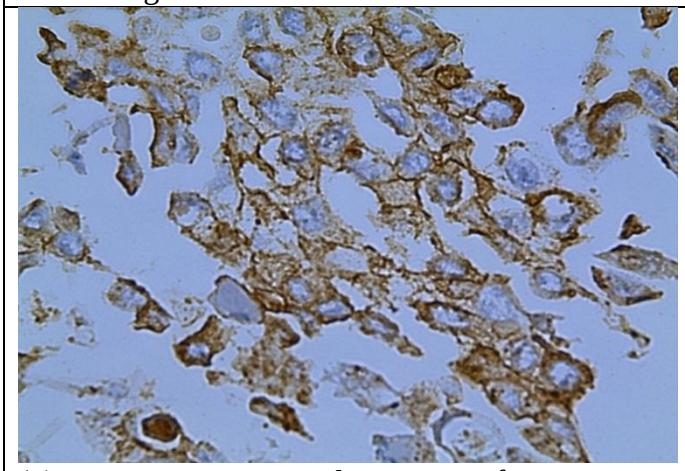
Below are the representative cases for HER-2 immunoscoring.



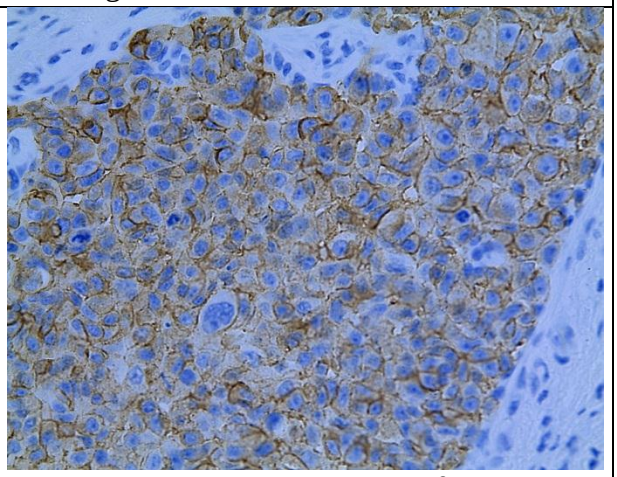
(e) Case no: 23, HER-2 immunostaining at x63 magnification, score of 3+ (positive IHC) in an intestinal gastric adenocarcinoma.



(f) Case no: 1, HER-2 immunostaining at x63 magnification, score 3+ (positive IHC) in a diffuse gastric adenocarcinoma.



(g) Positive control case for HER-2 immunostaining.



(h) Positive control case for HER-2 immunostaining.

DISCUSSION

The main study objective was to assess HER 2 over expression in patients with gastric or GEJ carcinoma seen at KNH. The prevalence of HER-2 over-expression was found to be **42.4%** (N=28). Despite being on the higher side of the wide range for HER-2 over-expression **(6-45%)** [7], most studies have shown a lower rate of HER-2 over-expression **(15%-38%)**[17, 18, 19]. However, over-expression rates of up to 53% and 91% have been observed[20, 21].

The mean age of patients with carcinoma in our study was 60.7 years (15.0 years SD). More than 60% of patients were in the range of 50-79 years. HER-2 over-expression was fairly distributed throughout the age group, with a slight peak at age 60-79 years (28.6%). This is not statistically significant with no association between HER-2 over-expression and age which is also seen in other studies [47.]

Amongst our study subjects, gastric cancer was more common in males (66.7%, N=44) than in females (33.3%, N=22). This correlates with a distribution ratio of 2:1 as seen in the literature. However HER-2 over-expression was 43.2% (N=19) in males and 40.9% (N=9) in females, showing no significant association of HER-2 over-expression and gender. Similar trend is observed in some studies [47].

In view of the above prevalence (42.4%) in our study as compared to other studies (up to 38%), the following explanation is worth mentioning;

Although we cannot entirely exclude the possibility of false positive results given that our specimens were mostly from biopsied OGD specimens (N=42) which have been shown to have higher false positivity [48] as compared to surgically resected specimens (N=24).

Geographic and ethnic heterogeneity of tumour associated aberration which exist in solid tumours may help to explain the differences for HER-2 over-expression in various studies [49 - 52.] In addition there is paucity of data in our African population for HER-2 over-expression with no specific documented prevalence in African population leaving outside the African continent.

Most studies restricted their evaluation to membrane staining only, excluding staining that appeared cytoplasmic.[53-55] However, because membrane staining can project to the cytoplasm in the two-dimensional limitation of the microscopic picture, in our opinion, a definite distinction between an exclusively cytoplasmic and a mainly membranous staining could not be made in some cases. Furthermore, there have been reports of truncated or secreted forms of the HER-2 receptor that are not anchored in the cell membrane[56, 57] and that could have been detected immuno histochemically as a non-membranous staining pattern. [56] In view of these facts, we decided not to restrict our scoring system to membrane staining and, therefore, also considered any case with both membranous and cytoplasmic staining patterns as positive.

In addition, studies with lower rate of HER-2 over-expression were conducted as large cohorts with a larger sample size compared to ours (N=66) hence this might explain the higher rate in our study.

Specimens which demonstrated weak to moderate complete or basolateral membranous reactivity in more than 10% of cells (9.1%, N=6) were classified as equivocal (+2). These cases require to undergo FISH test evaluation to classify them as positive or negative for HER-2 over-expression. FISH was not available in our study as alluded earlier in the study limitation. However in the ToGA trial 26% of the equivocal were FISH positive for HER-2 over-expression, while in a

Chinese cohort study, 28.8% of equivocal turned positive upon FISH evaluation [16, 58]. On extrapolation using the above studies, our HER-2 positivity will be expected to rise to 43.9%.

On assessment of HER-2 over-expression in specific anatomical sites, it was observed that50% (N=3/6) of GEJ tumours and 41.7% (N=25/60) of gastric cancer, over-expressed HER-2. Though the difference is not statistically significant, this trend is similar to other studies which exhibits high HER-2 over-expression in GEJ compared to gastric cancer [17, 23, 47]. HER-2 over-expression may even be higher in oesophageal cancers [17].

In general out of all the 28 cases which revealed HER-2 over-expression, 25 cases were from gastric region (89.3%) and the remaining 3 cases (10.7%) were from the GEJ. This was attributed to fewer cases of GEJ cancer in our study (N=6).

On evaluation of histological pattern for HER-2 over-expression, this study shows higher HER-2 over-expression in intestinal type (87.5%, N=21) compared to diffuse (12.5%, N=3) and mixed (0%, N=0) for gastric adenocarcinoma. This concurs with other studies comparing HER-2 over-expression and histological types of gastric and GEJ carcinomas. [23, 25, 26]. Countries with higher rate of intestinal than diffuse histological type of cancer, had a higher prevalence of HER-2 over-expression [58]. Since the latter is similar to our set up, it may pose a challenge in surgical management outcome and prognosis of our patients.

CONCLUSION

HER-2 over-expression is higher in our study (42.4%) compared to most of the studies, with no correlation to age and gender. Over-expression is predominant in intestinal type of gastric and GEJ adenocarcinomas.

RECOMMENDATIONS

1. All advanced gastric and GEJ carcinomas should undergo HER-2 status evaluation due to high prevalence of over-expression in our study.
2. Cases with HER-2 equivocal results in IHC should undergo FISH analysis to confirm HER-2 status, hence the need to build our local capacity for FISH test.
3. Further studies with larger cohorts need to be conducted to provide more clarity on prevalence of HER-2 over-expression in our set up.
4. Use of automated machines for assessing HER-2 status to eliminate possible human errors.
5. Trastuzumab to be administered as part of combination chemotherapy in patients with advanced gastric and GEJ carcinoma who over-expresses HER-2.
6. Active advocacy and involvement of the Ministry of Health to minimize cost of HER-2 status evaluation and availability of Trastuzumab as part of combination chemotherapy.

STUDY TIME FRAME

Proposal writing and submission for ethical approval.	August2013
Data collection and Analysis	October2013 – April 2014
Dissertation writing	April 2014
Presentation and submission of dissertation	May 2014

BUDGET

Item	Amount (Kshs)
Personal	15,000
Statistician	25,000
Stationery	15,000
Contingencies	10,000
Research fee	2,000
Research assistants	30,000
Printing and binding	20,000
HER-2 receptors staining using anti-HER-2 antibody @ 3500/=	231,000
Total	348,000

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APPENDIX I

Consent form

"PREVALENCE OF HER-2 OVER EXPRESSION IN PATIENTS WITH GASTRIC AND GASTRO-ESOPHAGEAL JUNCTION CARCINOMA SEEN AT KNH."

English version

This Informed Consent form is for patients/Guardian with confirmed diagnosis/suspected gastric or gastro-oesophageal junction carcinoma. The title of the study is "prevalence of HER-2 over expression in gastric and gastro-oesophageal junction carcinoma patients seen at KNH".

Principal investigador: Dr. Ali Hussein Abdulrahman

Institution: School of Medicine, Department of surgery- University of Nairobi

This informed consent has three parts:

1. **Information sheet (to share information about the research with you)**
2. **Certificate of Consent (for signatures if you agree to take part)**
3. **Statement by the researcher**

You will be given a copy of the full Informed Consent Form.

PART I: Information sheet

Introduction

My name is Dr. Ali Hussein, a post graduate student at the University of Nairobi's School of Medicine. I am carrying out a study to determine 'Prevalence of HER-2 over expression in gastric and gastro-oesophageal junction cancer patients seen at Kenyatta National Hospital**'.**

Study purpose

Gastric cancer is the second leading cause of cancer related deaths worldwide; the overall survival outcome of patients continues to improve since introduction of new therapies including identification of targeted agents. This study will therefore help us identify the utility of this new novel discovery in our set up.

I am going to give you information and invite you to be a participant of this research. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will explain. After receiving the information concerning the study, you will be encouraged to seek clarification from myself or my assistant in case of any doubt.

Voluntary participation/right to refuse or withdraw

It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this Hospital will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this hospital for your condition. You have a right to refuse or withdraw your participation in this study at any point.

Procedures and protocol

If you agree to be interviewed you will undergo a brief counselling on the study research. Thereafter you will be free to ask any questions where you do not understand. If you choose to participate in the study, a written consent shall be signed. For those who opt out, this shall be their exit point.

Once consent is given we will follow your biopsy specimen in the laboratory where it will undergo several processing to ascertain its viability. If the biopsy is viable for this study, it will undergo staining procedure for HER-2 receptors status, if not viable, your specimen shall be excluded from the study. Results obtained will be used in the study. Feedback of your results will be communicated to you.

Confidentiality

The information obtained will be treated with confidentiality and only be available to the principal investigator and the study team. Your name will not be used. Any information about you will have a number on it instead of your name. We will not be sharing the identity of those participating in this research.

Sharing the results

The knowledge that we get from this study will be shared with the policy makers in the Ministry of Health and Doctors through publications and conferences. Confidential information will not be shared.

Risks and discomfort

Your involvement in this research will be through an interview and histological assessment of your biopsy specimen. You will not be exposed to any risks or discomfort.

Cost and compensation

There will be no extra cost incurred for participating in this study nor is there compensation offered.

This proposal has been reviewed and approved by UON Department of Surgery/KNH Ethics Committee, which is a Committee whose task is to make sure that research participants are protected from harm.

Who to contact

If you wish to ask any questions later, you may contact:

1. Principal researcher,
   Dr Ali Hussein,
   Department of Surgery, School of Medicine, University of Nairobi
   P.O. Box 19676 KNH, Nairobi 00202
   Mobile no; 0722-772981.

2. University of Nairobi Supervisors

   I. Dr. T.M. Omulo,
   Lecturer, Department of Surgery,
   University of Nairobi, College of Health Sciences.
   P.O. Box 19676 KNH, Nairobi 00202
   Mobile no: 0725-519390.

   II. Professor P.L.W. Ndaguatha,
   Associate Professor, Department of Surgery,
   University of Nairobi, College of Health Sciences,
   P.O. Box 19676 KNH, Nairobi 00202,
   Mobile no; 0722-314533

   III. Dr Emily. A. Rogena,
   Senior lecturer, Department of Pathology,
   University of Nairobi. College of Health Sciences,
   P.O. Box 19676 KNH, Nairobi 00202,
   Mobile no; 0721-674647.

If you have any ethical concerns, you may contact:

- Secretary, KNH/UoN-ERC
  P.O. Box 20723 KNH, Nairobi 00202
  Tel +254-020-2726300-9 Ext 44355
  Email: KNHplan@Ken.Healthnet.org

PART II: Certificate of Consent

I have read the above information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant_____

Date _____

If Non -literate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____

Signature of witness _____

Date _____

Thumb print of participants.

PART III: Statement by the researcher

I have accurately read out the information sheet to the participant, and to the best of my ability made sure that the participant understands that the following will be done:

- Refusal to participate or withdrawal from the study will not in any way compromise the care of treatment.
- All information given will be treated with confidentiality.
- The results of this study might be published to facilitate management and use of targeted therapies in gastric cancer patients.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Name of researcher taking consent……

Signature of researcher taking consent……

Date……
Day/Month/Year

FOMU YA MAKUBALIANO YA KUJIUNGA NA UTAFITI

“PREVALENCE OF HER-2 OVER EXPRESSION IN PATIENTS WITH GASTRIC AND GASTRO-ESOPHAGEAL JUNCTION CARCINOMA SEEN AT KNH”

Kiswahili version

Mtafiti; Dr Ali Hussein

Kituo; shule ya Afya, kitengo ya upasuaji. Chuo kikuu cha Nairobi

Fomu hii ya makubaliano Ina sehemu tatu

1. Habari itayo kukusaidia kukata kauli
2. Fomu ya makubaliano (utakapoweka sahihi)
3. Ujumbe kutoka kwa mtafiti

Utapewa nakala ya fomu hii.

Sehemu 1: Ukurasawahabari

Kitambulizi

Jina langu ni, Dr. Ali Hussein. Mimi ni Daktari ninae somea Upasuaji. Kwa sasa na fanya utafiti kwa anwani ya "Prevalence of HER 2 over expression in Patients with Gastric and Gastro-eosophageal Junction Carcinoma seen at KNH"

Nia YaUtafitiHuu

Utafiti huu utatusaidia kuthamini matibabu mapya katika uuguzi wa ugonjwa wa saratani ya tumbo.Nitakupatia habariyau tafiti huu nakukualika ujiunge na utafit huu. Kukiwepo na ujumbe wowote usio ufahamu. Tafadhali nisimamishe popote patakapo leta tashwishi.

Haki ya kukataa utafiti

Unaweza ukachagua kutoshiriki katika utafiti huu, Nahuduma zote utapewa pasi na pingamizi.Uhusianowakonawafanyikaziwahopspitalihautatiwamashakaniiwapoutakosakujihusisha nautafitihuu. Ni uamuzi wako kama ungependelea kuendelea na utafiti.Uko na hakika kamili ya kujitoa katika utafiti wa kati wowote unapo amua.

UtaratibuwaUtafiti

Uki kubali kutupa nafasi ya kuhojiwa , tuta kueleza kuhusu utaratibu wa utafiti huu.

Tuna kusihi uulize maswali kuhusu chochote ambacho huelewi.iwapo utakubali kuhojiwa na kujiunga na utafiti, utatakiwautiesahihikwafomuyamakubaliano.

Ukitia sahihi kwenye fomu, ina maanisha kuwa umetoa ruhusa tufanyie utafiti Zaidi hicho kinyama kilichotolewa kwenye tumbo lako.

Uchunguzi wa aina ya HER 2 Receptors. Majibui tatu mika Kwa kueleza kiwango ya HER 2 receptor ina yopatikanaKwa wagonjwa wa KNH. Tuta kujulisha juu ya majibu yako yatakotokea.

Tandhima ya siri

Ujumbe kuhusu majibu yako yatahifadhiwa .Ujumbe kuhusu ushiriki wako katika utafiti huu utawezekana kupatikana na wewe na wanao andaa utafiti na wala si yeyote mwingine .Jina lako halitatumika bali ujumbe wowote kukuhusu itapewa nambari badili ya jina lako.

Anwani ZaWahusika

I. Mr T.M. Omulo,
Lecturer, Department of Surgery,
University of Nairobi, College of Health Sciences.
P.O. Box 19676 KNH, Nairobi 00202
Mobile no: 0725-519390.

II. Professor P.L.W. Ndaguatha,
Associate Professor, Department of Surgery,
University of Nairobi. College of Health Sciences,
P.O. Box 19676 KNH, Nairobi 00202,
Mobile no; 0722-314533

III. Dr Emily. A. Rogena,
Senior lecturer, Department of Pathology,
University of Nairobi. College of Health Sciences,
P.O. Box 19676 KNH, Nairobi 00202,
Mobile no; 0721-674647.

Wahusika wa maslahi yako katika Utafiti:

- Secretary, KNH/UoN-ERC
  P.O. Box 20723 KNH, Nairobi 00202
  Tel +254-020-2726300-9 Ext 44355
  Email: KNHplan@Ken.Healthnet.org

SEHEMU YA PILI: FOMU YA MAKUBALIANO

Nime elezewa utafiti huu kwa kina. NakubaIi kushiriki utafiti huu kwa hiari yangu. Nimepata wakati wa kuuliza maswali na nime elewa kuwa iwapo nina maswali zaidi, nina weza kumwuliza mtafiti mkuu au watafiti waliotajwa hapa juu.

Jinala Mshiriki_____

Sahihiyamshiriki_____

Tarehe_____

Kwa wasio weza kusoma na kuandika

Nime shuhudia usomaji Na maelezo ya utafiti hii Kwa mshiriki,Namshiriki alipewa nafasi ya kuuliza maswali. Nathibitisha kuwa mshiriki alipeana ruhusa ya kushiriki bila ya kulazimishwa.

Jina la shahidi_____ Alama ya kidole cha mshiriki.

Sahihi ya shahidi_____

Tarehe _____

Sehemu ya Tatu

Ujumbe kutoka Kwa mtafiti / mwenye kuhusika

Nime msomea mshiriki ujumbe kiwango ninavoweza Na kuhakikisha kuwa mshiriki amefahamu yote yanayo husika katika utafiti huu.Na hakikisha kuwa mshiriki alipewa nafasi ya kuuliza maswali Na yote ya kajibiwa vilivyo.Nathibitisha kuwa mshiriki alitoa ruhusa bila ya kulazimishwa.

Jina la mtafiti: _____

Sahihi ya Mtafiti /Anae chukua ruhusa_____

Tarehe_____

APPENDIX 2

QUESTIONNAIRE

STUDY NUMBER; ……..

PATIENT NUMBER; …….

AGE ……

SEX M ☐ F ☐

RESIDENCE; ……..

CONTACT NO: ……

I. ANATOMICAL SITE OF THE TUMOR

a) GASTROESOPHAGEAL; ☐

b) GASTRIC; ☐

II. HISTOLOGICAL TYPE

a) GASTROESOPHAGEAL ☐

- ADENOCARCINOMA ☐
  - INTESTINAL ☐
  - DIFFUSE ☐
  - MIXED/UNKNOWN ☐
- ADENOSQUAMOUS ☐

b) GASTRIC ☐

- ADENOCARCINOMA ☐
  - DIFFUSE ☐
  - INTESTINAL ☐
  - MIXED/UNKNOWN ☐
- ADENOSQUAMOUS ☐

III. HER-2 STATUS

- NEGATIVE 0 ☐ OR 1+ ☐
- EQUIVOCAL 2+ ☐
- POSITIVE 3+ ☐

APPENDIX 3

IMMUNOHISTOCHEMISTRY STAINING PROCEDURE

Leica Microsystems Novocastra Ready-to-Use Mouse Monoclonal Antibody HER2 Immunostain

Product code: RTU-CB11

Principle of procedure

Immunohistochemical (IHC) staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody and an enzyme complex with a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

Immunogen

Synthetic peptide corresponding to a site on the internal domain of the HER2 oncoprotein.

Specificity

HER-2 oncoprotein (internal domain). Specific binding has been demonstrated by immunoprecipitation with HER-2 oncoprotein SKBr3 cells.

Reagent Composition

RTU-CB11 is a ready to use liquid tissue culture supernatant, presented in 5% horse serum in PBS containing 12 mm sodium azide as a preservative.

Summary of use

Immunohistochemistry on paraffin sections. Incubation of tissue section with primary reagent for 30 minutes at 25^{o}C. High temperature antigen retrieval using 0.01 M citrate retrieval solution (pH 6.0) as recommended. This antibody is pre-titred for use and does not require further dilution when used with the secondary detection system, RE7100-K.

Specimen Preparation

The recommended fixative is 10% neutral-buffered formalin for paraffin-embedded tissue sections.

Reagents

1. Standard solvents used in immunohistochemistry.

2. 0.5% v/v hydrogen peroxide.

3. 50 mm Tris-buffered saline (TBS) pH 7.6.

4. Antibody diluent - optimally diluted normal serum.

5. Normal sera from the species in which the secondary antibody is raised.

6. Secondary biotinylated antibody - prepare as recommended by manufacturer.

7. Avidin/Biotin Complex-Horseradish peroxidase (ABC-HRP) - prepare as recommended by manufacturer.

8. 3, 3’Diaminobenzidinetetrahydrochloride (DAB) - prepare as recommended by manufacturer.

9. Haematoxylin counterstain - prepare as recommended by manufacturer.

10. Mounting medium - use as recommended by manufacturer.

Equipment

1. Incubator set to 37 °C.

2. Microwave for antigen retrieval.

Antigen retrieval solutions

Microwave with PBS buffer at PH 6

Methodology (Steps in immunostaining)

The laboratory is accredited for HER2 staining with technologists who are trained in immunohistochemical techniques. Optimal dilutions for antibodies have been determined. All steps are performed at room temperature (25 °C).

The following are the steps:

1. Cut and mount sections on slides coated with a suitable tissue adhesive.

2. De-paraffinize sections in xylene or xylene substitutes.

3. Re-hydrate through graded alcohols.

4. Neutralize endogenous peroxidase using 0.5% v/v hydrogen peroxide/methanol for 10 minutes.

5. Wash slides in running tap water.

6. Wash sections in TBS for 1 x 5 minutes with gentle rocking.

7. Cover sections with diluted normal serum for 10 minutes.

8. Incubate sections with optimally diluted primary antibody (see Recommendations on Use).

9. Wash in TBS buffer for 2 x 5 minutes with gentle rocking.

10. Incubate sections in appropriate biotinylated secondary antibody.

11. Wash in TBS buffer for 2 x 5 minutes with gentle rocking.

12. Incubate slides in ABC-HRP.

13. Wash in TBS buffer for 2 x 5 minutes with gentle rocking.

14. Incubate slides in DAB.

15. Rinse slides in water.

16. Counter stain with haematoxylin.

17. Dehydrate, clear and mount sections.

Quality Control

To mitigate against differences in tissue processing and technical procedures causing significant variability in results, internal controls and positive in-house controls are used. The positive controls are surgical specimens, formalin-fixed, processed and paraffin wax-embedded.

Negative Tissue Control

Examined to verify the specificity of the labelling of the target antigen by the primary antibody. The variety of different cell types present in most tissue sections frequently offers negative control sites. Non-specific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain non-specifically. False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by endogenous enzymes such as pseudo peroxidase (erythrocytes), endogenous peroxidise (cytochrome C), or endogenous biotin (e.g. liver, breast, brain, kidney) depending on the type of immunostain used. To differentiate endogenous enzyme activity or non-specific binding of enzymes from specific immuno reactivity, additional patient tissues may be stained exclusively with substrate chromogen or enzyme complexes (avidin-biotin, streptavidin, labelled polymer) and substrate-chromogen, respectively. If specific staining occurs in the negative tissue control, results with the patient specimens should be considered invalid.

Negative Reagent Control

A non-specific negative reagent control is used in place of the primary antibody with a section of each patient specimen to evaluate non-specific staining and allow better interpretation of specific staining at the antigen site.

Positive Tissue Control

Used to indicate correctly prepared tissues and proper staining techniques. Positive tissue control with varying staining scores are included for each patient section under the same set of test conditions in each staining run. A tissue with weak positive staining which is more suitable than a tissue with strong positive staining for optimal quality control and to detect minor levels of reagent degradation. If the positive tissue control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

Patient Tissue

Examine patient specimens stained with RTU-CB11 last. Positive staining intensity is assessed within the context of any non-specific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed.

Results Expected

Normal Tissues

HER-2 antigen was not expressed in the membrane of normal tissues evaluated.

Abnormal Tissues (Cancer cells)

The clinical interpretation of any staining or its absence is complemented by morphological studies using proper controls and evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

The results are interpreted based on the scoring scheme proposed by Hofmann *et al*[18] as either negative (score 0 or 1+), equivocal (score 2+) or positive (score 3+).

Criteria for scoring

The criteria specific to gastric and GEJ carcinoma includes 2 parameters:

1. The intensity of complete, basolateral, or lateral membrane staining (0, none; 1, faint; 2, weak to moderate; and 3, strong).
2. The percentage of cancer cells with a given staining intensity. These parameters were used to determine the IHC score according to the validated criteria: high (IHC 3+), strong intensity in 10% or more of the cancer cells; medium (IHC 2+), weak to moderate intensity in 10% or more; low (IHC 1+), faint intensity in 10% or more; absent (IHC 0).

Definition of HER2 overexpression

HER2 overexpression will be considered where HER2 positivity is a score of IHC 3+

The remaining cases (i.e. IHC 2+ or IHC 0-1+) will not be considered as overexpressed on immunohistochemistry.

APPENDIX4: DECLARATION FORM FOR STUDENTS

UNIVERSITYOFNAIROBI

Declaration of Originality Form

This form must be completed and signed for all works submitted to the University for Examination.

Name of Student_____

Registration Number_____

College _____

Faculty/School/Institute_____

Department_____

Course Name_____

Title of the work _____

DECLARATION

1. I understand what Plagiarism is and I am aware of the University's policy in this regard
2. I declare that this_____(Thesis, project, essay, assignment, paper, report, etc) is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.
3. I have not sought or used these services of any professional agencies to produce this work
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own work
5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

Signature_____

Date___

APPENDIX 5: DECLARATION FORM FOR STAFF

UNIVERSITY OF NAIROBI

Declaration of Originality Form

This form must be completed and signed for all scholarly works produced.

Name of Staff _____

Payroll Number _____

College _____

Faculty/School/Institute_____

Department _____

Title and bibliographic details of the work

_____

DECLARATION

1. I understand what plagiarism is and I am aware of the University's policy in this regard.

2. I declare that this _____ scholarly work (Paper, book chapter, monograph, review, etc) is my original work. Where other people's work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

3. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own work.

4. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

Signature _____

Date _____