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HER2/*Neu* Protein Over-Expression in Patients with Gastric and Gastro-Esophageal Junction Carcinoma Seen at Kenyatta National Hospital, Kenya

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Abstract

Background: Gastric cancer in Kenya is ranked third in both males and females. Most patients present clinically with advanced unsusceptible 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 overexpression 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 for 89.4% (N=59) mainly intestinal (78.8%, N=52) and diffuse (9.1%, N=6) while 1.5% (N=1) was adeno-squamous. 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 adeno-squamous, 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.

Keywords: Gastric cancer; HER-2; Anti-cancer therapy; Molecular targeted therapy

Background and Literature Review

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 anemia, positive family history of gastric cancer and previous gastrectomy3. Obesity has been associated with gastric cardiac 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 females5. 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.

Gastric cancer is the fourth most common malignancy and second leading cause of cancer deaths world-wide, hence a significant global health problem [1].

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

Location	Year	WASR Males	WASR Females
Kenya, Meru [8]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

Table 1: World age standardized incidence rate of gastric cancer in Africans. 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 signaling 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 signaling 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 immunohistochemistry (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)

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	

Table 2: Variation in prevalence of HER2 according to histological types of gastric cancer. **Source Connection:** HER-2 testing in gastric and oesophageal adenocarcinoma, 2010 [15, 48].

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 [18] based on the study done by Hoffman et al [18]. Consensus was reached and the following was recommended for scoring HER-2 over expression in gastric cancer. 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 overexpress the HER2 receptor protein. A score of +3 is confirmed overexpression while +2 denotes an equivocal positive score.

Study Justification

Gastric cancer remains the fourth most commonly diagnosed cancer and the second leading cause of cancer-related deaths worldwide [42,43]. 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 [17,44].

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) [40].

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 of the Study

Broad objectives

To 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.

Materials and Methods

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

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 immunostaining 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.

Study setting and sampling

The study setting was at the Kenyatta National Hospital surgical/radioncology 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 immunostaining at the University of Nairobi immunohistochemistry laboratory. The IHC method used was Leica Microsystems Novocastra Ready-to-Use Mouse Mouse Monoclonal Antibody HER2 Immunostain (Product code: RTU-CB11). HER-2 protein expression was assessed in carcinoma cells by immunohistochemistry (IHC) in paraffin-embedded 3 µm–5 µm tissue sections.

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

Each stained slide was analyzed 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 Principal investigator 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 analyzed and presented as percentages.

Prevalence of HER-2 over-expression was analyzed 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.

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 counseling 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. 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.

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)

Table 3: Socio Demographic Characteristics.

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. (Table 3, Figures 1 and 2).

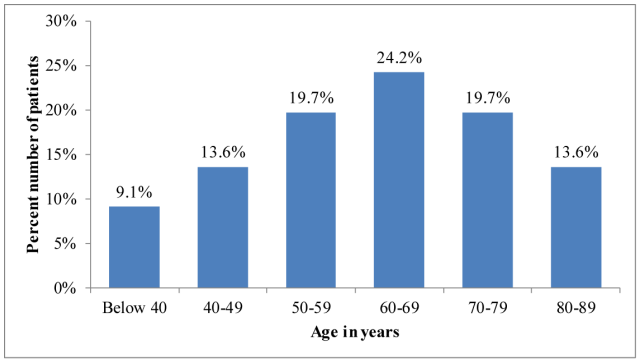


Figure 1: Age distribution.

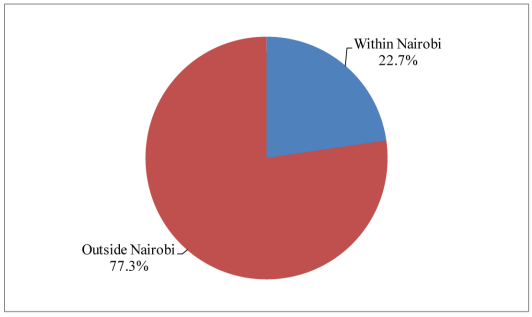


Figure 2: Residence.

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 4 below.

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

Table 4: shows evaluation for 5 discrepancy cases in HER-2 assessment

The above results was based on the consensus panel recommendation for HER-2 scoring system

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) (Table 5 and Figure 3).

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)

Table 5: Anatomical site and histological type of tumors.

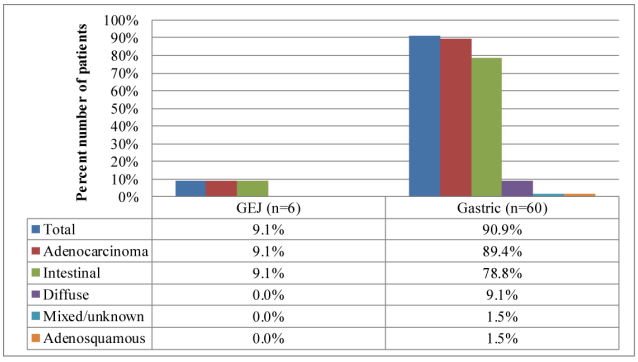


Figure 3: Anatomical site and histological type of tumors

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

Table 6: Prevalence of HER-2 over-expression.

HER-2 over-expression

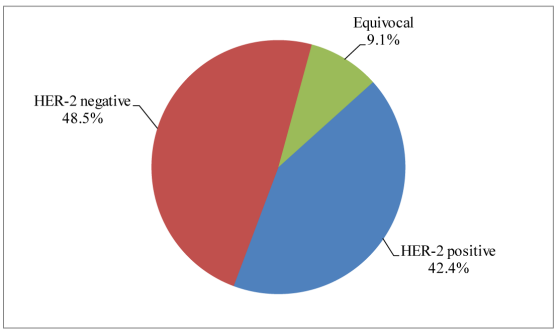


Figure 4: Prevalence.

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 7 and figure 5.

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	19 (43.2%)	22 (50.0%)	3 (6.8%)	0.682
Female	9 (40.9%)	10 (45.5%)	3 (13.6%)	

Table 7: Associations between demographic factors and HER-2 over-expression.

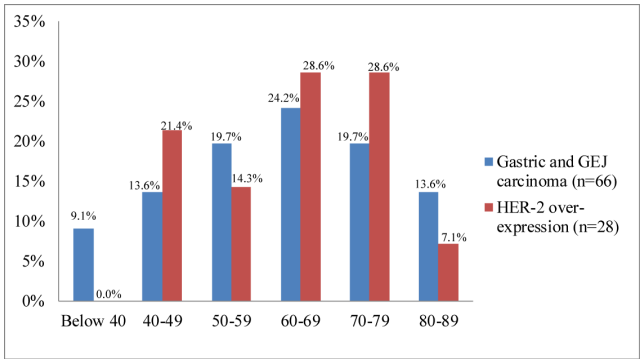


Figure 5: Age distribution and HER-2 over-expression.

HER-2 over-expression and anatomical site

As illustrated in figure 6 and table 8 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).

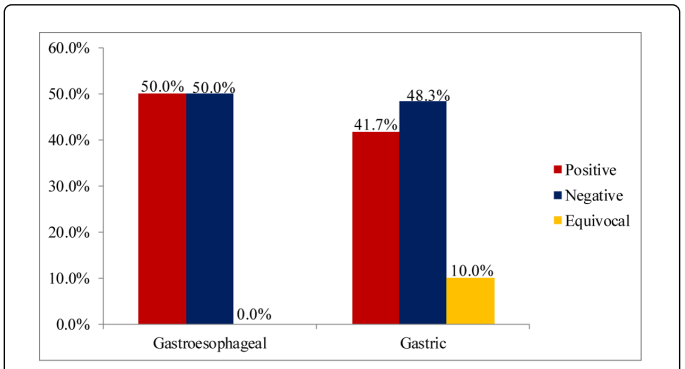


Figure 6: HER-2 over-expression and anatomical sites

Variable	Anatomical site	
	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	

Table 8: correlation between anatomical site and HER-2 over-expression.

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 9.

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%)	

Table 9: HER-2 over-expression between gastric and GEJ tumours

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-2 over-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 10.

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%) [17-19]. 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 [46].

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	24/28 (85.7%)	29/32(90.6%)	6/6 (100%)	0.123
Intestinal	21/24 (87.5%)	27/29(93.1%)	4/6 (66.7%)	
Diffuse	3/24 (12.5%)	2/29 (6.9%)	1/6 (16.7%)	
Mixed/unknown	0/24 (0%)	0/29 (0%)	1/6 (16.7%)	

Table 10: Associations between histological type of gastric/GEJ carcinoma and HER-2 over-expression.

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 [46].

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 [48-51]. 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 [52-54]. 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 [55,56] and that could have been detected immunohistochemically as a non-membranous staining pattern [57]. 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%.

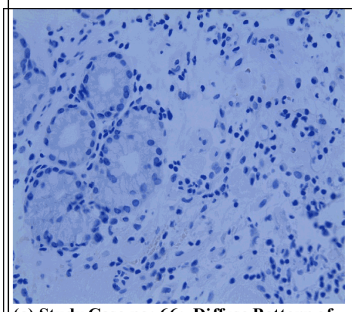
On assessment of HER-2 over-expression in specific anatomical sites, it was observed that 50% (N=3/6) of GEJ tumours and 41.7% (N=25/60) of gastric cancer, over-expressed HER-2. Though the difference is not statistically significant, this trend is similar to other studies which exhibits high HER-2 over-expression in GEJ compared to gastric cancer [17,23,46]. 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).

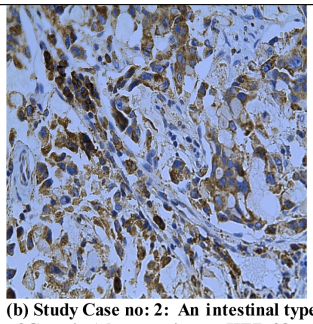
On evaluation of histological pattern for HER-2 over-expression, this study shows higher HER-2 over-expression in intestinal type (87.5%, N=21) compared to diffuse (12.5%, N=3) and mixed (0%, N=0) for gastric adenocarcinoma. This concurs with other studies comparing HER-2 over-expression and histological 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 [57]. Since the latter is similar to our set up, it may pose a challenge in surgical management outcome and prognosis of our patients.

Conclusion

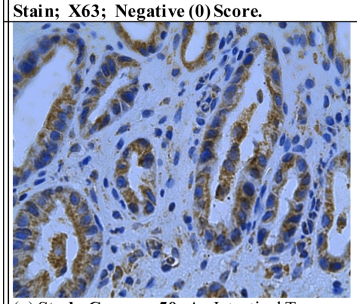
HER-2 over-expression is higher in our study (42.4%) compared to most of the studies, with no correlation to age and gender. Over-expression is predominant in intestinal type of gastric and GEJ adenocarcinomas.



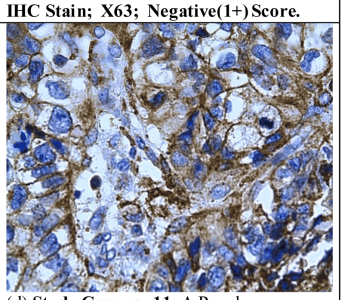
(a) Study Case no: 66: Diffuse Pattern of Gastric Adenocarcinoma. HER-2/neu IHC Stain; X63; Negative (0) Score.



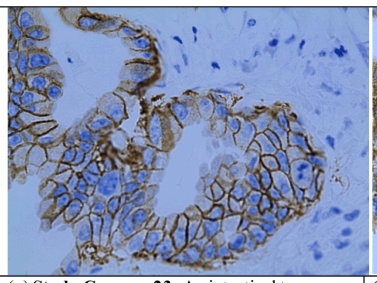
(b) Study Case no: 2: An intestinal type of Gastric Adenocarcinoma.HER-2?neu IHC Stain; X63; Negative(1+) Score.



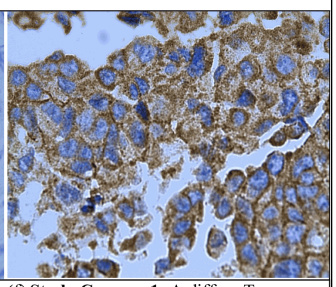
(c) **Study Case no: 50:** An Intestinal Type Gastric Adenocarcinoma. HER-2/neu IHC Stain; X63 magnification; **Negative (1+) Score**



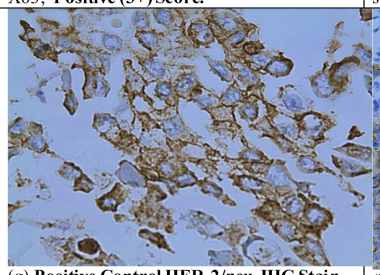
(d) **Study Case no: 11**: A Poorly Differentiated Intestinal type Gastric Adenocarcinoma. HER-2/neu IHC; X63; **Equivocal (2+) Score.**



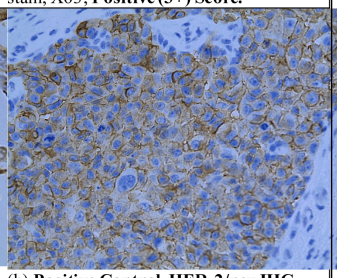
(e) **Study Case no: 23:** An intestinal type Gastric Adenocarcinoma. HER-2/neu IHC; X63; **Positive (3+) Score.**



(f) Study Case no: 1: A diffuse Type Gastric Adenocarcinoma. HER-2/neu IHC stain; X63; **Positive (3+) Score.**



(g) **Positive Control HER-2/neu IHC Stain**



(h) **Positive Control HER-2/neu IHC Stain.**

Photomicrographs: Representative Photomicrographs of Sections Stained for HER-2/neu Protein. (a) Study Case no: 66: Diffuse Pattern of Gastric Adenocarcinoma. HER-2/neu IHC Stain; X63; Negative (0) Score. (b) Study Case no: 2: An intestinal type of Gastric Adenocarcinoma.HER-2?neu IHC Stain; X63; Negative(1+) Score. (c) Study Case no: 50: An Intestinal Type Gastric Adenocarcinoma. HER-2/neu IHC Stain; X63 magnification; Negative (1+) Score . (d) Study Case no: 11: A Poorly Differentiated Intestinal type Gastric Adenocarcinoma. HER-2/neu IHC; X63; Equivocal (2+) Score. (e) Study Case no: 23: An intestinal type Gastric Adenocarcinoma. HER-2/neu IHC; X63; Positive (3+) Score.(f) Study Case no: 1: A diffuse Type Gastric Adenocarcinoma. HER-2/neu IHC stain; X63; Positive (3+) Score. (g) Positive Control HER-2/neu IHC Stain. (h) Positive Control HER-2/neu IHC Stain.

Recommendations

All advanced gastric and GEJ carcinomas should undergo HER-2 status evaluation due to high prevalence of over-expression in our study.

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.

Further studies with larger cohorts need to be conducted to provide more clarity on prevalence of HER-2 over-expression in our set up.

Use of automated machines for assessing HER-2 status to eliminate possible human errors.

Trastuzumab to be administered as part of combination chemotherapy in patients with advanced gastric and GEJ carcinoma who over-expresses HER-2.

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.

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