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Immunohistochemical Profile of Human Epidermal Growth Factor Receptor 2 in Gastric Cancer in Rwanda

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Abstract

Background: Of the cancers diagnosed in Rwanda, stomach cancer is one of the most encountered. In fact, Rwanda belongs to the region where it is most incident in Africa. Most of the patients present with advanced disease. Studies showed that some gastric cancers overexpress Human Epidermal Growth Factor Receptor 2 (HER2/neu) protein and can be treated with Herceptin/Trastuzumab. This targeted therapy improves survival in patients with advanced disease. We conducted a study on Immunohistochemical profile of HER2/neu in gastric adenocarcinomas at two main Rwandan tertiary centers. **Methodology:** We tested for HER2/NEU in gastric adenocarcinomas diagnosed at University Teaching Hospital of Kigali (CHUK) and University Teaching Hospital of Butare (CHUB). Demographic and pathologic parameters were collected. Immunohistochemistry (IHC) for HER2/neu using c-erb/HER-2/neu (clone SP3) Rabbit Monoclonal antibody was done. Using the guidelines established by Hoffman *et al.*, the agreed score between 2 Rwandan pathologists and 1 USA pathologist was considered each time. Data were entered and statistically analyzed using SPSS 22. Descriptive statistical analysis method was

used. P-value calculated with Chi-square analysis for positive vs negative and equivocal negative to correlate HER2/neu overexpression with other variables across both hospitals. **Results:** A total of 286 cases were tested. HER2/neu overexpression (score 3+ or positive) was found in 29 cases (10.1%). 8 cases (2.8%) were equivocal negative (score 2+) while 249 cases (87.1%) were negative (score 0 and 1+). **Conclusion:** HER2/neu is overexpressed in a subset of gastric cancers in Rwanda, a phenomenon that has been reported in other areas of the world. Testing for HER2/neu could identify patients who would get a targeted treatment with Herceptin.

Keywords

Gastric, Cancer, Rwanda, HER2, Immunohistochemistry

1. Introduction

Rwanda, the landlocked Country in East Africa. The year 1994 marked a violent period of genocide against Tutsis that devastated the people and the infrastructure. Steps have been taken to reduce poverty and improve the overall wellbeing of people in order to decrease deaths then related mainly to communicable diseases. However, improved living conditions as days go on with unhealthy diet, some infections, physical inactivity and genetics have led to a rise in non-communicable diseases (NCDs).

Globally, stomach cancer ranks fourth of all diagnosed cancers and the second of all cancer related deaths with approximately 738,000 deaths each year attributed to this disease [1] [2]. The overall incidence of stomach cancer is rising in sub-Saharan Africa [3]. The western region of Africa has the lowest number of new cases, while the incidence is higher in Rwanda, Burundi, Southwestern Uganda, and the Eastern Kivu province of Democratic Republic of Congo [3] [4]. The Research which investigated the frequency of malignant tumors in former southern prefecture of Butare-Rwanda in 1994 revealed that stomach cancer accounted for 9% of all cancers diagnosed [5]. Globally, in 2020, Globocan report showed that gastric cancer was the 4th most incident cancer with 5.6% of all new cases of cancer. A study done in 2016 at the University Teaching Hospital of Kigali (CHUK) showed that gastric cancer was the most diagnosed malignant neoplasm of the gastrointestinal tract (GIT) and accounted for 65.3% of GI malignancies diagnosed in 2015 (Felix Manirakiza: Clinicopathological Characteristics of GIT lesions diagnosed at the Anatomic Pathology unit of CHUK, Thesis for Masters of Medicine in Anatomic Pathology, 2017). A recent study done at CHUB on upper GIT cancers showed that gastric cancer was the most diagnosed comprising 92% of all upper GIT cancers [6].

Surgery yields satisfactory patient outcomes when gastric cancer is diagnosed early; however, most cases seen in Rwanda are diagnosed at an advanced stage which includes inoperable local disease, recurrent disease, and/or metastatic dis-

ease [7]. Delayed medical consultation is thought to be a contributing factor. Once the disease has been deemed distant stage, treatment options are limited and the 5-year survival rate is very low [1] [8]. HER2/neu is a receptor that weighs 185 kDa and belongs to the HER family of receptor tyrosine kinases [9]. A study called the potential for targeted therapies among gastric tumor patients at Kigali University Teaching hospital showed targetable mutation in PTEN gene [10]. HER2 encoding gene is situated on the chromosome 17 and is an oncogene. Its overexpression leads to the development of cancer. This oncogenic mechanism was first identified in breast cancer in 1985. In 1986, the identification of HER2/neu protein in stomach cancer was first described [9] [11] [12]. Subsequently, it has been also found in carcinomas of ovary, lung, colon and prostate [13] [14].

The unfavorable prognosis of people with advanced stomach cancer has led oncology researchers to try to identify an adjunctive therapy that improves patient survival [15] [16] [17]. Targeted therapy refers to the utilization of medicines that are directed against specific errors within cancer cells. Trastuzumab, Ramucirumab, imatinib are some of the drugs used. Subsequently, it has been also found in carcinomas of ovary, lung, colon and prostaste [18] [19].

Many researches have showed HER2/neu protein overexpression and its gene amplification in 4% - 53% (median 18%) of gastric and gastroesophageal junction cancers [20] [17] [18]. A study done in Kenya at Kenyatta National Hospital showed that 42.4% of cases of gastric and gastro-esophageal junction carcinoma overexpressed HER2/neu while a study done in South Africa about HER2/neu in gastric carcinoma showed HER2/neu positivity in 12% of examined cases [12] [18]. A multi-national study demonstrated HER2/neu overexpression of 20% in gastric GEJ cancers [20]. The research study Trastuzumab for Gastric Cancer (ToGA) is in its phase three, and revealed increased survival in people with HER2/neu positive advanced gastric cancer when Trastuzumab/Herceptin is added to previously established chemotherapy regimens [9]. Many other countries now perform routine testing of HER2/neu on all cases of gastric adenocarcinoma (GAC), as well as utilize trastuzumab for treating of HER2/neu positive advanced stomach cancer [9]. In Africa there is insufficient research regarding HER2/neu testing in stomach and gastroesophageal junction carcinomas. No substantial research on IHC profile of HER2/neu in GACs has been done in Rwanda. Our goal was to evaluate HER2/neu protein overexpression in GAC patients in two main tertiary centers of Rwanda to identify the percentage of patients that could experience improved survival with advanced gastric cancer using Trastuzumab treatment.

2. Methods

Study design and cases selection

We performed a mixed retrospective and prospective, descriptive study of patients diagnosed with GAC on gastric biopsies or resections at CHUK from Jan-

uary 2015-December 2016 and at CHUB from January 2017-March 2022.

Settings

CHUK and CHUB are the two main tertiary University Teaching Hospitals in Rwanda, with a combined capacity of 1000 beds and 1200 workers. 6000 histopathology and 3000 cytopathology cases are diagnosed per year. Butaro District Hospital is a hospital in the North of the country and is a cancer center of excellence equipped with digital pathology services.

Data extraction and IHC Process

Hematoxylin and eosin (H&E) stained slides, paraffin blocks, pathology examination request forms, and pathology reports were retrieved from the archives of the anatomic pathology units at both hospitals. Demographic and pathologic information was retrieved from each patient's pathology examination request forms and open clinic records. The H&E stained glass slides were reviewed on light microscopy by three Rwandan pathologists (one at CHUK and two at CHUB) in order to confirm the diagnosis and type of GAC (according to Lauren *et al.*) and select appropriate block for HER2/neu IHC staining. Once the paraffin block was chosen for each case, they were used to cut unstained sections for HER2/neu IHC. For cases from CHUK, the process of manual IHC was done in the anatomical pathology laboratory of Butaro Cancer Center of Excellence in 2018 while cases from CHUB were manually performed at CHUB in 2022. Both laboratories utilized HER2/neu IHC with c-erb/HER-2/neu (clone SP3) Rabbit Monoclonal antibody Cat.#RM-9103-S0, -S1, or-S (0.1 ml, 0.5 ml, or 1.0 ml Supernatant) by DAKO (Santa Clara, California, USA). Each time, IHC slides were read by 2 Rwandan pathologists and then use digital slide scanner to send them for review by 1 USA based pathologist. Using the guidelines of Hoffman *et al.* [17]. The agreed score between these 3 pathologists through regular discussion was retained.

Statistical analysis

The data collected using a premade questionnaire were entered into an excel spreadsheet and analyzed using SPSS version 22. Descriptive statistical analysis method was used to describe age, gender, type of specimen, type of adenocarcinoma, stage of disease and HER2/neu overexpression in frequency and percentage for each hospital. P-value calculated with Chi-square analysis for Positive vs negative and equivocal negative to correlate HER2/neu overexpression with other variables across both Hospitals.

Strength

This is the first study of its kind to be carried out in Rwanda. We feel it has opened the door to larger and more in-depth studies of HER2/neu expression in gastric adenocarcinoma, as well as lead to the potential initiation of routine HER2/neu immunohistochemically testing in all cases of gastric adenocarcinoma. Identification of patients who are eligible for treatment with Herceptin will hopefully lead to increased survival in a subset of people with advanced stomach adenocarcinoma.

Limitations

The in-situ hybridization (ISH) technique which normally helps to sort out equivocal cases, is not available in our laboratories. Accordingly, some cases of HER2/neu positive gastric adenocarcinoma may have been missed.

Most of this study is retrospective, there is no way we could remedy some of the inconveniences that might have been caused by some errors in pre-analytical phase like prolonged fixation and ischemic time.

3. Results

The total number of tested cases was 286, including 143 cases from CHUK and 143 cases from CHUB. A total of 29 cases were positive (10.1%) (Figure 1), a total of 8 cases were equivocal negative (2.8%) (Figure 2) while a total of 249 cases were negative (89.7%) (Figure 3, Table 1).

Among positive cases; 26 (89.7%) were biopsies while 3 (10.3%) were resection specimens, 18 (62.1%) were intestinal type as per Lauren and 7 (24.1%) were diffuse while 4 (13.8%) were mixed type, 1 (3.4%) was early gastric cancer, 2 (6.9%) were locally advanced gastric cancer and 26 (89.7%) were not staged, 11 (37.9%) were males while 18 (62.1%) were females, 10 (34.4%) were aged less or equal to 50 years old while 19 (65.6%) were more than 50 years old (Table 2).

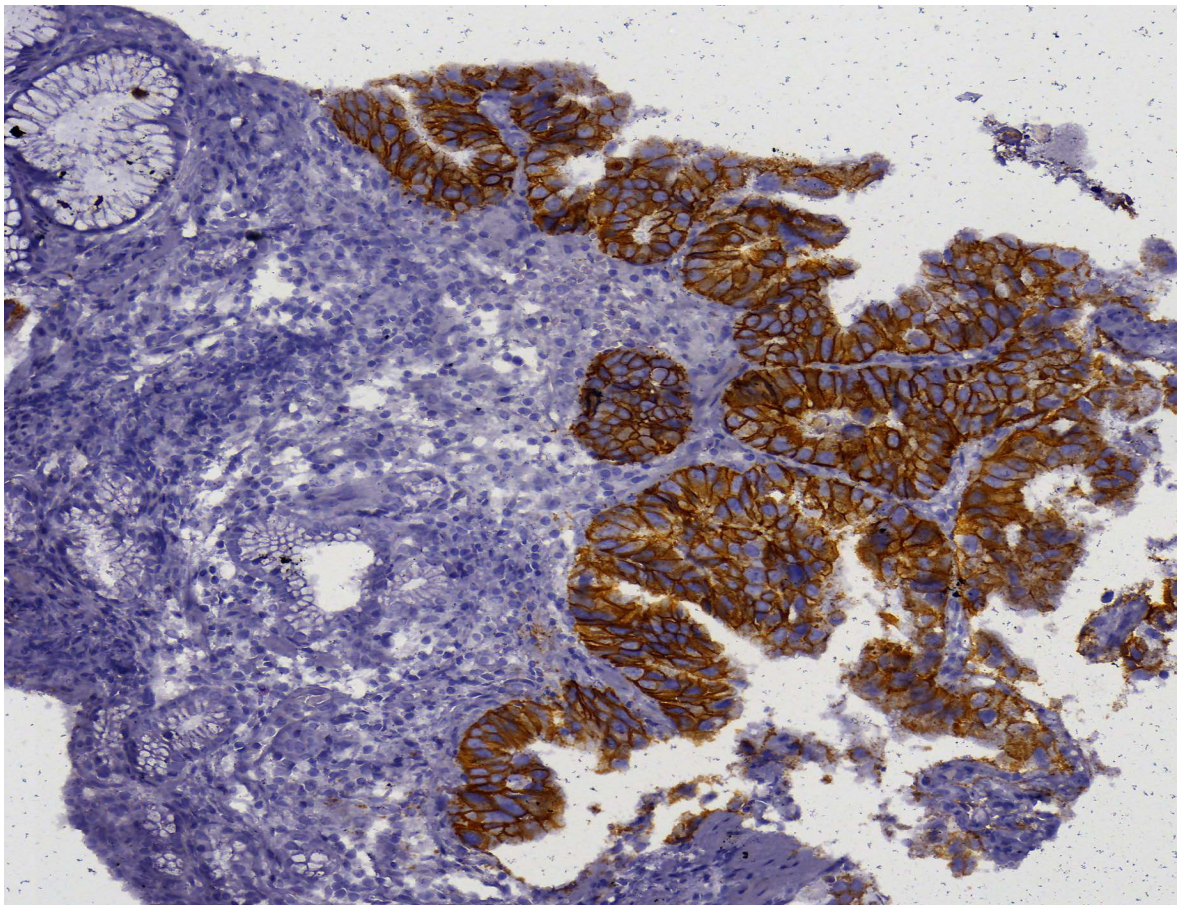

Figure 1. Immunohistochemistry stain. HER2/neu score 3+: Positive/Overexpression.

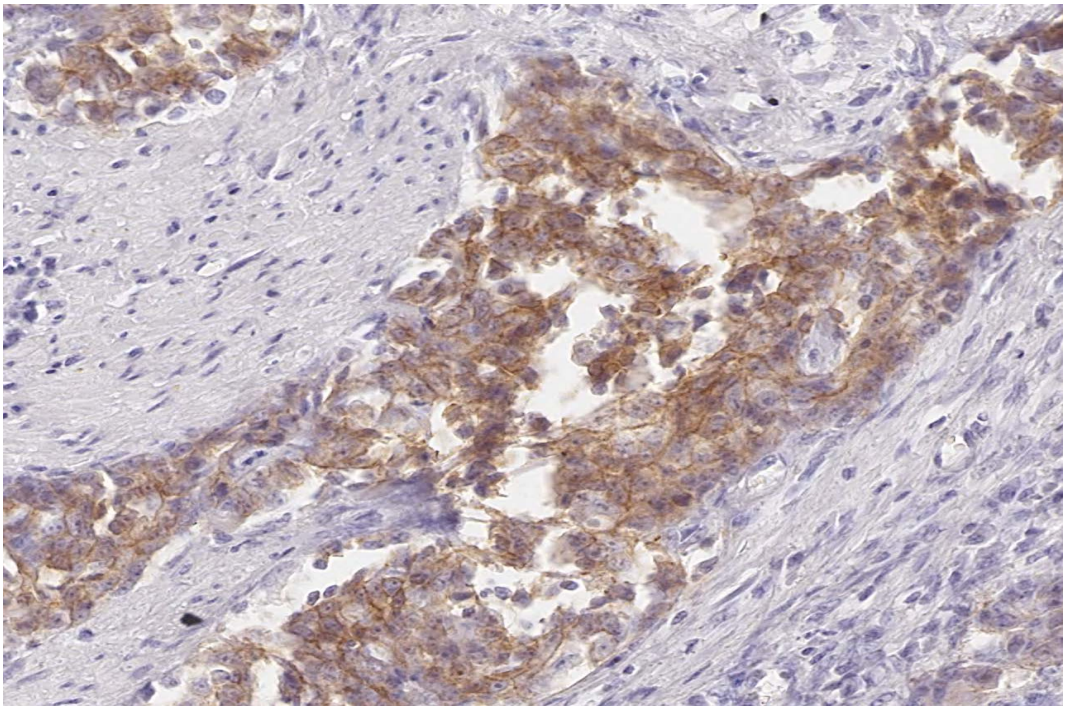

Figure 2. Immunohistochemistry stain. HER2/neu Score 2+: Equivocal negative.

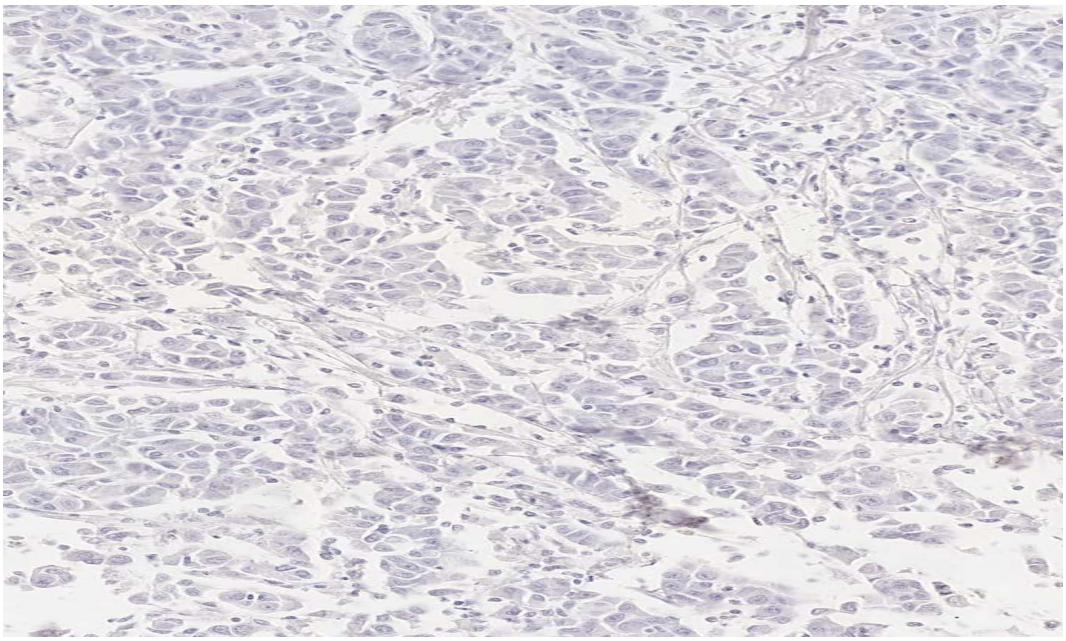

Figure 3. Immunohistochemistry stain. HER2/neu Score 0: Negative.

Table 1. HER2/neu overexpression.

HER2/neu Overexpression	CHUK n = 143 (%)	CHUB n = 143 (%)	Total N = 286 (%)
Negative	126 (51)	123 (49)	249 (89.7)
Equivocal Negative	7 (88)	1 (12)	8 (2.8)
Positive	10 (34)	19 (66)	29 (10.1)

Table 2. Association between demographic and clinical pathology characteristic with Her2/neu overexpression.

Variable name N = 286		HER2/neu overexpression (CHUK) n = 143			HER2/neu overexpression (CHUB) n' = 143			P-value
		Positive	Equivocal negative	Negative	Positive	Equivocal Negative	Negative	
Age (years)	≤50	5	0	42	5	1	37	0.359
	>50	5	7	84	14	0	86	
Gender	Male	2	5	63	9	1	59	0.225
	Female	8	2	63	10	0	64	
Type of specimen	Biopsy	9	6	113	17	1	109	0.928
	Resection	1	1	13	2	0	14	
Type of adeno-carcinoma	Intestinal	5	5	65	13	0	64	0.08
	Diffuse Mixed	4 1	2 0	52 9	3 3	2 0	53 6	
Stage	Early	0	0	0	1	0	3	0.565
	Locally advanced	1	1	13	1	0	10	
	Not applicable	9	6	113	17	1	110	

P-value calculated with Chi-square analysis for Positive vs negative and equivocal negative.

4. Discussion

Our study showed that 10.1% of gastric adenocarcinomas overexpressed HER2/neu protein in addition to other targetable PTEN mutation described by Oscar [10]. This percentage is within the widely variable range of HER2/neu overexpression described in various population across the globe ranging from as low as 4% described in England in 2010 by Grabsch [21] to as high as 53.4% described in Germany by Allgayer in 2000 [11] [22]. In India, a study done By Indu Rajagopal showed that 22.4% of the tumors overexpressed HER2/neu while another done by Prachi SPatil *et al.* revealed 7% of cases overexpressing HER2/Neu [17] [23]. A systematic analysis comprising 17,338 cases published in 48 articles has showed a mean HER2/neu overexpression at 17.9% [24]. A study done at Kenyatta National hospital showed HER2/neu overexpression in 42.2% of 66 cases while a study from South Africa showed HER2/Neu overexpression in 12% of 97 cases studied [12] [18].

The association between HER2/neu overexpression and age was not statistically significant in our study (P-value: 0.359). This is probably due to few positive cases in our research. No significant association with age found also in a study done at Kenyatta National Hospital as well as the study done in Brazil by Renato Santos Laboissiere [12] [25].

No relationship between gender and HER2/neu overexpression in our study (P-value: 0.225). This finding is the same as what was found in a study done at Kenyatta hospital, in Brazil by Renato and in china by SD Xie [12] [25] [26].

No association between HER2/neu overexpression and type of specimen (P-value: 0.928). However, many of HER2/neu positive specimens were biopsies than resection specimens the same as described in the research done in India by Indu Rajagopal [17].

No statistical relation between type of adenocarcinoma and HER2/neu overexpression (P-value: 0.08). This is again thought to be related to the small number of positive cases in this study. This finding is different from many studies like in India by Rajagopal, in South Africa by Roberts and in Brazil by Renato, where significance was found [17] [18] [25]. On the other hand, there was a greater number of intestinal type HER2/neu positive cases compared to diffuse and mixed types, the same as found in a study done at Kenyatta Hospital [12].

No statistical significancy found also between HER2/neu overexpression and the cancer stage (P-value: 0.565).

5. Conclusion

Stomach cancer is deadly worldwide and belongs among the most frequently diagnosed cancers in Rwanda. Its late diagnosis at advanced stage leads to a poorer prognosis due to limited treatment options. Our study has demonstrated that 10.1% of tested cases had HER2/neu protein overexpression. Hence, some of them may benefit from targeted therapy. Immunohistochemistry for HER2/neu in gastric adenocarcinomas can be technically done and interpreted in Rwanda, however In situ hybridization is needed to sort out equivocal cases.

Data Availability Statement

The dataset is available from the author whenever there is a reasonable need.

Funding Statement

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Ethics Approval Statement

After approval in pathology departments, we got approval from institution review boards of CHUK and CHUB

Patient Consent Statement

No consent was signed by patients as we got approvals by both hospitals to retrieve data in their archives.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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