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RESEARCH

The expression status of human epidermal growth factor receptor 2 in epithelial ovarian cancer in Ibadan, Nigeria

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Background: It has been proposed that the overexpression of the human epidermal growth factor receptor 2 (HER2/*neu* proto-oncogene) could be a possible therapeutic target in epithelial ovarian cancer, as has been the case in breast carcinomas. However, there is lack of knowledge on the status of the gene in neoplasms which occur in black women. The objective of this study was to determine HER2/*neu* expression status in EOC in black women.
Method: Ninety cases of EOC were evaluated for HER2/*neu* protein expression using immunohistochemistry.
Results: HER-2/*neu* expression was observed in 33 of the 90 cases (37%), of which 15 EOC cases (17%) were weakly or moderately positive, and 18 (20%) strongly positive. A significant association was not found between HER-2/*neu* expression and age, International Federation of Gynecologists and Obstetrics (FIGO) stage, grading and histological subtypes (*p*-values of 0.463, 0.360, 0.975 and 0.168, respectively). However, there were more cases of advanced-stage disease (III/IV) with HER-2 expression than early-stage EOC (I/II). In this study, 21%, 36% and 42% of HER2/*neu*-positive tumours were grades 1, 2 and 3, respectively. A higher proportion of serous carcinomas (as opposed to mucinous carcinomas) was also observed to be HER2/*neu* positive.
Conclusion: HER2/*neu* expression was observed to increase with advanced stages of cancer, and was more commonly seen in serous, rather than in mucinous, carcinomas.

Keywords: EOC, epithelial ovarian cancer, HER2/*neu,* Immunohistochemistry

Introduction

Epithelial ovarian cancer (EOC) is often diagnosed in the advanced stages of the disease, usually after distant metastasis has occurred.[1–4] Lack of effective screening methods to detect the disease at an early stage has resulted in a high number of disease recurrence in patients.[5,6] An improvement in the survival of patients with ovarian cancer was initially shown with the introduction of cisplatin-based chemotherapy. The percentage of recurrent disease is still high, even in patients who achieve a complete response to chemotherapy. Presently, the five-year survival of such patients is less than 20%.[7,8]

Human epidermal growth factor receptor 2 (HER2/*neu*) in EOC have been proposed in targeted oncological therapy as a way forward in the treatment of ovarian cancer, as it is in Her/*neu* positive breast cancer.[8] *HER2/neu*, also known as the c-*erb*-B2/*neu* proto-oncogene, encodes a 185 000 Da transmembrane receptor protein, which is related structurally to the epidermal growth factor receptor that has intrinsic tyrosine kinase activity. Although studies of HER2/*neu* in ovarian neoplasm have been sporadic, the oncogene has been found to be overexpressed in approximately 20–30% of EOC, and it has been suggested that increased HER2/*neu* expression within the tumour tissue is associated with poor prognosis in patients. However, the reported results are based on a relatively small number of cases and remain controversial.[9–12] It has been suggested that HER2/*neu*-positive EOC benefits from treatment with an anti-HER2/*neu* monoclonal antibody, both at an advanced, and at an early, stage of the disease. Therefore, it is important to determine the distribution of HER2/*neu* throughout the different clinical stages of EOC and to establish the prognostic value of HER2/*neu* overexpression before treatment with anti-HER2/*neu* may be considered.[9,13,14] However, a study in which the expression pattern of this proto-oncogene in ovarian cancer patients has been examined has not been conducted to date on our local African population. This study aimed to address this issue.

Method

A retrospective study was performed on paraffin-embedded tissue blocks of 90 cases of histologically diagnosed EOC seen at the Department of Pathology, University College Hospital, Ibadan, Nigeria, over a seven-year period. Nonepithelial primary ovarian cancer, metastatic cancer in the ovary and primary ovarian epithelial neoplasm, in which slide sections were unsuitable, or tissue blocks were unavailable, were excluded from the study. The demographical data and clinical history of these cases were obtained from the case notes, surgical daybooks, surgical pathology request forms, post-mortem records and cancer registry data. Biopsies were performed in patients undergoing total abdominal hysterectomy, total abdominal hysterosalpingo-oophorectomy, bilateral and unilateral salpingo-oophorectomy, omentectomy and also on post-mortem cases. The haematoxylin- and eosin-stained histopathology slides of each of the cases were reviewed to confirm the original diagnosis, and to assess the histological grade of the neoplasm. The Shimizu-Silverberg three-grade histological grading system was used, which assesses architectural pattern, nuclear pleomorphism and mitotic activity.[15] Histological classification of the EOC was based on the 2013 World Health Organization classification of ovarian tumours.[16] The International Federation of Gynecologists and Obstetrics (FIGO) staging of the cases used for this study was extracted from the case notes of the patients.

HER2/*neu* expression was evaluated using the HercepTest™ kit (Dako, USA), which uses a polyclonal antibody to detect

HER2/*neu*. Staining was performed according to the protocol highlighted in the manufacturer's guide which accompanied the kit. Following deparaffinisation and rehydration, serial sections were cut at 5 µm from each paraffin-embedded tumour block. These sections were pretreated in 1 M Tris buffer, pH 9. The primary antibody [MBO/TEG (Dako, USA)], diluted 1:800, was incubated for 20 minutes. Antigen retrieval was performed prior to immunostaining. The polyclonal rabbit antihuman antibody reacts with the cytoplasmic membrane. The Labelled Streptavidin-Biotin-Peroxidase/DAB kit® (K5001) (Dako, Denmark) was utilised as a detector system for the HER2/*neu* antigen. Staining intensity for HER2/*neu* was graded in accordance with the modified HercepTest™ protocol system used by previous workers:[1,14,17–19]

- *0:* No staining or incomplete membrane staining which is faint or barely perceptible, and within ≤ 10% of the tumour cells.
- *1+:* Incomplete membrane staining which is faint or barely perceptible, and within > 10% of the tumour cells.
- *2+:* Circumferential membrane staining which is incomplete and/or weak or moderate, and within >10% of the tumour cells; or complete and circumferential membrane staining which is intense and within ≤ 10% of the tumour cells.
- *3+:* Circumferential membrane staining which is complete and intense, and within > 10% of the tumour cells.

Samples scored as 0 or 1+ were considered to be negative for HER2/*neu* expression, 2+ weakly positive and 3+ strongly positive.

Known positive HER2/*neu* breast cancer tumours were used as the positive control. As the negative control, tumour specimens pretreated in MBO/Tris buffer solution were immunostained under the same conditions without the primary antibody.

The slides were reviewed independently, and discordant scores resolved through consensus scoring.

The obtained data were subjected to statistical analysis using Statistical Package for Social Sciences® version 20. Statistical analysis was used to evaluate statistical associations between the expression of the HER2/*neu* protein and the clinicopathological parameters, i.e. age, stage, grade and histological subtypes. Continuous variables were compared using Student's *t*-test, and categorical variables using the chi-square test. The level of significance was set at $p < 0.05$.

This study was performed according to the Declaration of Helsinki (Article II) and was approved by the joint University of Ibadan/University College Hospital Ethical Review Committee.

Results

All of the biopsies were from black women. The age range of the 90 patients was 16–82 years, with a mean age of 52.2 ± 12.6 years. Forty-seven (52%) of the patients with EOC were classified as advanced FIGO stage III/IV, and 43 (48%) patients as early FIGO stage I/II. Seven (8%) of the early cases were classified as FIGO stage I with localised disease, while regional disease was reported in 36 cases (40%) (FIGO stage II). Twenty-eight (31%) of the patients with advanced disease were determined to be FIGO stage III, and 19 (21%) FIGO stage IV. Nineteen (21%) of the tumours were grade 1 neoplasms, 34 (38%) grade 2 neoplasms, and 37 cases (41%) grade 3 neoplasms. The association between FIGO stage and the microscopical grading of the ovarian carcinomas was statistically significant (*p* 0. 000) (Table 1).

HER2/*neu* positive staining was observed in 33 (37%) of the EOC, while 57 (63%) were negative (i.e. a score 0 or 1+) (Figure 1). Eighteen (20%) of those in whom positive staining was recorded were strongly positive (a score of 3+), while 15 (17%) were weakly or moderately positive (a score of 2+) (Figure 2). However, a significant association between strength of HER2/*neu* staining and FIGO stage was not noted. There was also no significant association between grade and HER2/*neu* expression (*p* 0.973) (Table 2). Although 20 (61%) of the neoplasms with HER2/*neu* expression were found in patients with advanced cancer (FIGO stage III/IV), a significant association was not found between FIGO stage and HER2/*neu* expression (*p* 0.360) (Table 3). The majority of cases of serous carcinoma and mucinous carcinoma (59%) were negative for HER2/*neu* (Table 4). An association was not found between HER2/*neu* expression and histological subtyping (*p* 0.168).

Table 1: The relationship between the staging and grading of epithelial ovarian cancer (*p* 0.000)

Staging	Grading (*n*, %)			Total (*n*, %)
	1	2	3	
Local	5 (5.6)	2 (2.2)	0	7 (7.8)
Regional	9 (10.0)	18 (20.0)	9 (10.0)	36 (40.0)
Advanced	5 (5.6)	14 (15.6)	28 (31.1)	47 (52.2)
Total	**19 (21.1)**	**34 (37.8)**	**37 (37.8)**	**90 (100.0)**

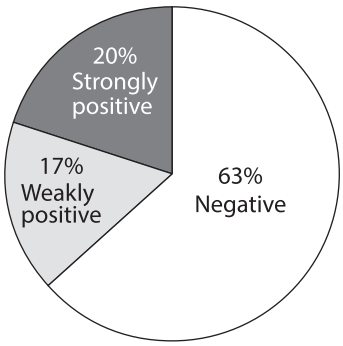

Figure 1: The distribution of HER2/*neu* expression in epithelial ovarian cancer in this study.

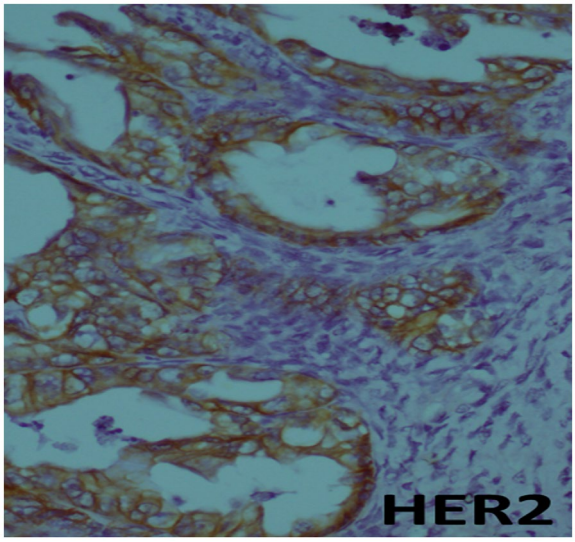

Figure 2: Photomicrographs showing strong membrane immunostaining for human epidermal growth factor receptor 2 (x 400).

attributable to the type of materials analysed (fresh or paraffin embedded) and technique applied, as well as to differences in the specificity of the antibodies used. Paraffin-embedded tissue blocks were used in the current study to determine HER2/*neu* expression by polyclonal antibody. Similar results were obtained from authors who used paraffin-embedded tissue by immunohistochemistry (Table 5).[1,14,21,22] Tuefferd et al. used the more sensitive fluorescence in situ hybridisation technique, in addition to immunohistochemistry on fresh frozen tissue samples which yielded lower values when compared to those obtained in the present study.[24] However, Singleton et al., whose immunohistochemical technique was validated by various molecular techniques, obtained higher values more comparable with those reported in the present study.[19]

Although HER2/*neu* expression was not significantly associated with FIGO stage in this study, more cases of advanced-stage disease (III/IV) were reported with HER2/*neu* expression, than early-stage EOC cases (I/II). Similar results were reported by Seidman et al., who found overexpression in 15% of early-stage and 37% of advanced-stage cancer.[25] Overexpression was apparent in 5% of patients with

Table 2: Relationship between grading and human epidermal growth factor receptor 2 expression

Markers	Histological grade (*n*, %)			Total	*p*
	1	2	3		
HER2/*neu*					
Negative	12 (63.2)	22 (64.7)	23 (62.2)	**57**	
Positive	7 (36.8)	12 (35.3)	14 (37.8)	**33**	
Total	**19**	**34**	**37**	**90**	**0.975**

HER2/*neu*: human epidermal growth factor receptor 2

Table 3: Relationship between International Federation of Gynecologists and Obstetrics staging and human epidermal growth factor receptor 2 expression

Markers	FIGO stage (*n*, %)			*p*
	I/II	III/IV	Total	
HER2/*neu*				
Negative	30 (69.8)	27 (57.4)	57 (63.3)	
Positive	13 (30.2)	20 (42.6)	33 (36.7)	
Total	**43 (100.0)**	**47 (100.0)**	**90 (100.0)**	**0.360**

FIGO: International Federation of Gynecologists and Obstetrics, HER2/*neu*: human epidermal growth factor receptor 2

Table 4: Relationship between histopathological sub-types and HER2/*neu* expression

Histological subtypes	Serous carcinoma, *n* (%)	Mucinous carcinoma, *n* (%)	Endometrioid carcinoma, *n* (%)	Malignant Brenner tumour, *n* (%)	Total	*p*
HER2/*neu*						
Negative	37 (58.7)	19 (79.2)	1 (50.0)	0 (100.0)	57 (100.0)	0.168
Positive	26 (41.3)	5 (20.8)	1(50.0)	1 (100.0)	33 (100.0)	
Total	**63 (100.0)**	**24 (100.0)**	**2 (100.0)**	**1 (100.0)**	**90 (100.0)**	

HER2/*neu*: human epidermal growth factor receptor 2

Discussion

The positive expression rate of HER2/*neu* in this study was 37%. This is comparable to the rate of HER2/*neu* positivity in EOC reported in the literature, which ranges from 7–50%.[1,14,19–23] The variation in HER2/*neu* expression rate in different studies may be

early-stage and 17% of patients with advanced-stage disease in the study by Kacinsky et al.[26] Haldane et al. found no association between HER2/*neu* overexpression and disease stage. Overexpression was reported in five of the 35 patients classified as FIGO stage I/II, and four of the 69 patients classified as FIGO stage III/IV.[27]

Table 5: A comparison of the present study with other studies

References	Location of study	Number of cases	Method	HER2 positive (%)
Tuefferd et al.[24]	France	320	Immunohistochemistry with FISH	6.6
Berchuck et al.[14]	USA	73	Immunohistochemistry	32.0
Singleton et al.[19]	USA	56	Immunohistochemistry	18.0
Felip et al.[21]	Spain	106	Immunohistochemistry	21.7
Verri et al.[1]	Italy	194	Immunohistochemistry	27.3
Slamon et al.[30]	USA	72	Immunohistochemistry	50.0
Nielsen et al.[23]	Denmark	783	Immunohistochemistry	35.0
Present study	Nigeria	90	Immunohistochemistry	36.7

FISH: fluorescence *in situ* hybridisation, HER2/*neu*: human epidermal growth factor receptor 2

EOC, in which positive HER2/*neu* expression is demonstrated, has been associated with an unfavourable prognosis, similar to breast cancer findings.[1,14,19,21] In addition, a higher proportion of HER2/*neu* expression was seen in the high-grade tumours in our study. This latter finding was consistent with what was reported in the literature.[14,21,22] Interestingly, the expression rate of HER2/*neu* in ovarian cancer obtained in this study was comparable with the reported frequency of HER2/*neu* overexpression of 15–40% reported for breast cancer.[28–30]

HER2/*neu* expression was found in all of the histological subtypes. This finding was similar to those reported by some authors.[14,19] A higher proportion of serous (41%) than mucinous (21%) carcinomas was observed to be HER2/*neu* positive in our series. Also, the only case of malignant Brenner tumour was HER2/*neu* positive. Furthermore, in this study, 21%, 36% and 42% of HER2/*neu*-positive tumours were grades 1, 2 and 3, respectively. There appears to be increasing HER2/*neu* positivity with higher grades of ovarian carcinoma.

A statistically significant correlation was noted between FIGO staging and the microscopical grading of ovarian carcinomas. This finding is in agreement with those in other studies in which it was demonstrated that grade and stage were important prognostic indicators; comparable in EOC.[14,19,21]

Conclusion

HER2/*neu* expression was not significantly associated with any of the EOC biological parameters. However, HER2/*neu* expression was observed to increase in the advanced stages of cancer. A higher proportion of serous compared to mucinous carcinomas was also observed to be HER2/*neu* positive. Therefore, it is recommended that studies are performed to determine the efficacy of HER2/*neu* monoclonal antibodies in the treatment of EOC.

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Conflict of interest – The authors declare that they do not have a conflict of interest.

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