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IMMUNOHISTOCHEMICAL EXPRESSION PATTERN OF HUMAN EPIDERMAL GROWTH FACTOR TYPE 2 (HER2) AND EPIDERMAL GROWTH FACTOR (EGFR) IN FEMALE BREAST CANCER PATIENTS IN BAYELSA STATE

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ABSTRACT
Knowing the molecular features of breast cancer is crucial for developing individualised treatment plans for this devastating disease. Using tissue samples from patients diagnosed with breast cancer at the Niger Delta University Teaching Hospital in Okolobiri and the Federal Medical Centre in Yenagoa, this study compares the immunohistochemical expression of Human Epidermal Growth Factor Receptor type 2 (HER2) and Epidermal Growth Factor Receptor (EGFR). Seventy-five breast cancer patients' samples were analysed. Tumour samples were analysed for EGFR and HER2+ expression using immunohistochemistry. Immunohistochemical analysis of breast cancer samples showed no connection between EGFR and Her2 expression. Even while Her2 was significantly associated with stage II breast cancer patients, it was not significantly associated with tumour grade, stage, or hormone receptor status (p 0.05). In conclusion, the expression of HER2+ and EGFR was not significantly linked in this research of women with breast cancer in Bayelsa State. These results help fill in the gaps in our knowledge of the molecular subtypes of breast cancer that are most common in this area, which might lead to more effective targeted therapy strategies. To further understand the processes and possible therapeutic consequences of this non-co-expression pattern, it is advised that additional research be conducted on a larger sample size.

KEYWORDS: Her2, EGFR, Breast cancer, Immunohistochemical, Tumor stage, tumor grade.

INTRODUCTION
Breast cancer is a major health issue across the world, and efforts to better understand its molecular peculiarities are crucial in the search for individualised treatment strategies. .Around 1.5 million women globally (or 25% of all cancer patients) receive a breast cancer diagnosis each year.[1] Breast cancer is a diverse illness from a molecular perspective, and molecular characteristics include activation of the human epidermal growth factor receptor 2 (HER2, encoded by ERBB2), activation of the oestrogen and progesterone receptors, and/or BRCA mutations.[2] Breast cancer is the most common cancer in women worldwide, and the stage of diagnosis has a significant role in prognosis. Delay in therapy and advanced stage diagnosis are major issues in low- to middle-income nations, including Nigeria. .25% of all tumours in women are breast cancer, making it the most prevalent malignancy. Both incidence and mortality of breast cancer are rising substantially throughout Africa.[3] For the creation of breast cancer management strategies and decision-making, understanding of hormone receptors and human epidermal growth factor receptor-2 (HER-2) expression is essential. The amount of instances of these biomarkers varies greatly by area, especially across African nations.[4] The popular cancer biomarker HER2 (human epidermal growth factor receptor type 2) is overexpressed in a variety of tumours and is used as a biological target for therapeutic intervention.In individuals with breast cancer, an increased level of the human epidermal growth factor receptor type 2 (HER-2) is a standalone predictive factor for a poor prognosis [31]. 15-20% of all breast cancers are HER2-enriched, which is referred to as HER2-positive, which is indicated by the overexpression of the HER2 receptor and the lack of the oestrogen and progesterone receptors. Anti-HER2 and cytotoxic chemotherapy are unable to reverse the aggressive nature of the HER2 subtype, which has an increased mortality rate.[5] This biomarker, when compared to Her2-negative breast tumours, predicts more aggressive tumours with characteristics such drug resistance, quick spread, and increased metastasis.[6] PTEN/Akt/mTORC1 signalling increases the number of cancer stem cells in primordial carcinomas of the breast and suggests poor clinical

outcomes, both of which are triggered by HER2 overexpression, which occurs in around 20% of cases.[7] By using immunohistochemistry (IHC), epidermal growth factor receptor (EGFR, ERBB1) expression in breast cancer has been extensively examined. The frequency of EGFR overexpression in breast cancer is highly diverse, ranging from 7 to 43%.[8] Hormone receptor negativity, big tumour size, high histologic grade, and poor clinical prognosis are all correlated with epidermal growth factor receptor overexpression. In patients with HER2-positive breast cancer, coexpression of human epidermal growth factor receptor 2 (HER2) and EGFR is specifically linked to poorer survival.[8] Numerous epithelial malignancies have a pronounced overexpression of EGFRs, and some immunohistochemistry analysis investigations have shown that this overexpression is related to a poor prognosis.[9] . The aim of this study is to determine the relationship between the immunohistochemical expression of Human Epidermal Factor type 2 (HER2) and Epidermal Growth Receptor Factor (EGFR) in female breast cancer patients in Bayelsa state.

MATERIALS AND METHODOLOGY

Study area

A comprehensive knowledge of the molecular aspects of breast cancer is necessary for the development of effective, tailored ways to treating the illness.. The study was conducted at the Niger Delta University Teaching Hospital in Okolobiri, and the Federal Medical center Yenagoa Bayelsa State. the south south of Nigeria,which also serves as a referral centre. Bayelsa State is located between latitudes 4.15IN and 5.23South, long. 5.221 and 6.51East of the equator, and is bordered by the Atlantic Ocean to the south of Nigeria. Bayelsa state may have the highest population of Ijaw tribes in all of Nigeria. The state is the second-biggest producer of crude oil in Nigeria and has the largest gas reserve and oil well in the country. The majority of its residents are farmers and public servants.

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Ethical clearance

The ethical committee of Niger Delta University Teaching Hospital Okolobiri and Federal Medical center Yenagoa granted permission.

Sample collection

Seventy five (75) Paraffin-embedded tissue blocks from the Histopathology departments at Niger Delta University Teaching Hospital Okolobiri and Federal Medical Center Yenagoa were collected under the supervision of the chief Medical laboratory scientist. From the medical charts, we extracted information such as patient demographics, medical histories, and diagnoses.

Sample preparation

The microstructural properties were examined and interpreted using the method of Eruvwahwe et al,[10] which was applied to all of the samples after they were correctly processed.

Tissue processing

The fixed samples were dried, cleaned, infiltrated, and embedded according to a timetable that was tailored to their size in order to create tissue blocks suitable for sectioning.

Sectioning of blocks

Using a microtome the blocks are divided into sections with tissue that is adequate for making slides at a thickness of 3-5 microns.

Slide preparation

By carefully adhering the tissues to the slide after removing it from the hot water bath, the tissues are prepared on a slide.

Methodology

Histological characterization and morphological analysis were performed utilising haematoxylin and eosin staining, the gold standard method. In order to do the EGFR and HER2+ immunostaining, we employed antibodies that we acquired directly from the manufacturer.[10]

Coloration progression and background staining may be observed by using haematoxylin as a counterstain and 3,3-diaminobenzadine as the chromogen. Using strict negative controls that did not use main antibodies, the immunostaining results were confirmed.

In order to more precisely classify the study subjects, they were split into four categories depending on the percentages they carried of each gene. To better describe the various molecular and genetic expressions in each of these subgroups, we used particular histochemical dyes.[11]

Protocol

Tissues that had been treated were placed in a water bath at 70 degrees Celsius for an hour before being sliced into 2-micron-thick sections using a Leica rotary microtone. The pieces were dissolved into water using two cycles of xylene, three cycles of alcohol (Absolute 1, Absolute 2, 95%, 80%, 70%), and two cycles of water. To undertake antigen retrieval, the sections were microwaved in a citric acid solution at 1000C for 15 minutes. The solution's pH level was 6.0.After the components had reached equilibrium, the hot citric acid was flushed out by gradually adding cool water. This took at least 15 minutes.Short exposure to hydrogen peroxide (H202) at a concentration of 3% for 15 minutes inhibited the sections' peroxidase activity.Sections were treated to a 15 minute protein blocking with Avidin after being washed in phosphate-buffered saline (PBS).Sections were biotin-blocked for 15 minutes to prevent endogenous biotin from binding after being washed in PBS.After being rinsed in PBS, sections were incubated with primary

antibodies at the relevant dilutions for the indicated times (for instance, ER antibody diluted 1:100 for 60 minutes).

The primary antibody was washed away with PBS for 15 minutes before the secondary antibody (LINR) was applied.Horseradish peroxidase (HRP) labels were applied to washed pieces, and they were let to sit for 15 minutes.One millilitre of DAB substrate was added to 20 microns of DAB chromogen to achieve this concentration. After rinsing the sections for 5 minutes in PBS to eliminate any residual HRP, they are treated with this potent solution. The brown reaction has just lately emerged in response to a good aim.Both the DAB solution and the precipitate are discarded in a standard drain cleaning.Once the sections have been blued for a few seconds, they are counterstained with a Haematoxylin solution for at least two minutes.Alcohol is used to dry the sections, then xylene and DPX are used to clarify and mount them.

Observation

Positive cells have nuclei that are embedded in the cell membrane and cytoplasm that is a darker shade of brown. Haematoxylin-stained cells without any brown pigmentation receive poor marks. The cells and connective tissue that have brown artefacts on them, as well as any binding that isn't particular, are thrown away.

Statistical analysis

Prism 5.0 was used to analyze the data, and the results were reported as mean, SD, and percentage.

RESULTS

Table 1: Analysis of Her2+ Receptors Stratified by Clinicopathologic Parameters among Breast Cancer Cases

Variable	Group	Frequency (%)		X^2	*p*-value
		Positive	Negative		
		n = 35	n = 39		
Age	≤50	24 (68.60)	23 (59.00)	0.73	.39
	>50	11 (31.40)	16 (41.00)		
Stage	I	2 (5.70)	1 (2.60)		
	II	22 (62.90)	26 (66.70)	0.50	.78
	III	11 (31.40)	12 (30.70)		
	IV	0 (0.00)	0 (0.00)		
Grade	Well Differentiated	10 (28.60)	7 (17.90)		
	Moderately Differentiated	18 (51.40)	27 (69.20)	2.45	.29
	Poorly Differentiated	7 (20.00)	5 (12.90)		
Histologic type	Invasive Ductal carcinoma	27 (77.10)	35 (89.70)		
	Invasive Lobular carcinoma	7 (20.00)	4 (10.30)	2.64	.27
	Mucinous cancer	1 (2.90)	0 (0.00)		

The above table presents a stratified study of Her2 receptor in breast cancer according to clinicopathological parameters. There was no statistically significant correlation between Her2 expression and age in these breast cancer patients (see table, which shows that 11 patients, or 31.4%, are aged 50 or older). Her2 positivity was higher in Stage II (n=22, 62.9%), followed by Stage III (n=11, 31.4%), and finally Stage I (n=2, 5.7%). Her2 expression in breast cancer tumours was not correlated with disease progression.

Immunohistochemical photomicrograph plates.

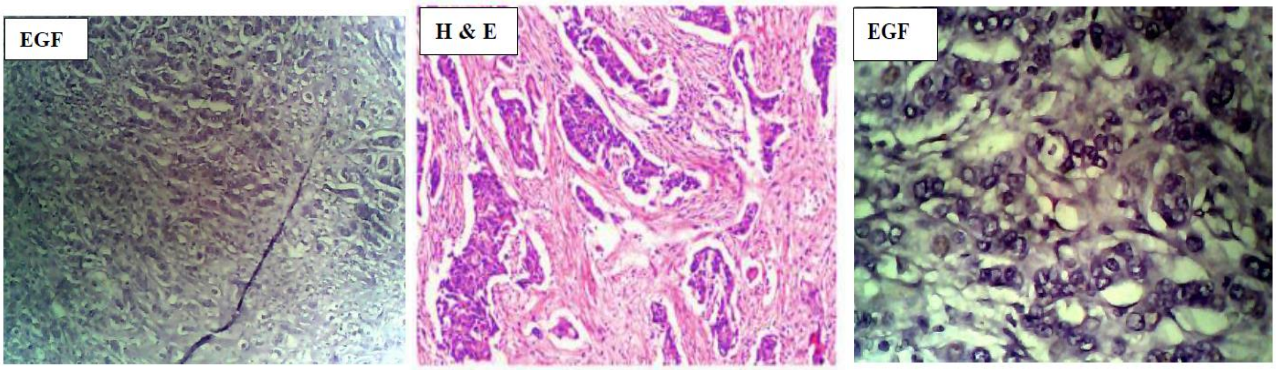

Plate 1: Shows the morphology of the breast after staining with immunohistochemical staining technique and viewed at x10 and x40 magnification. Sections shows breast tissue negative for EGFR.

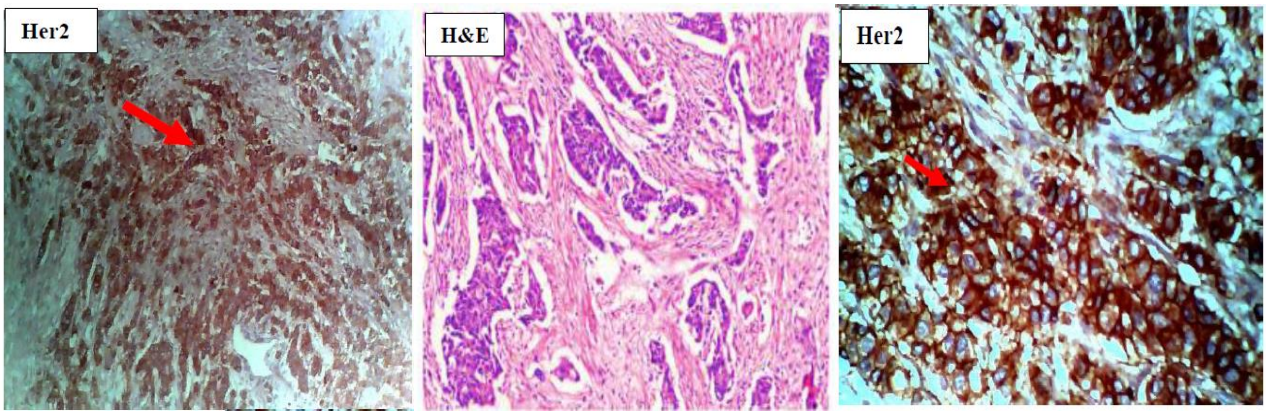

Plate 2: Shows the morphology of the breast after staining with immunohistochemical staining Technique and Viewed at x10 and x40 magnification. Sections show breast tissue positive (arrow) for Her2(++).

DISCUSSION/CONCLUSION

According to our data, the average age of a cancer diagnosis is 45. Previous research in Nigeria and elsewhere in Africa have also come to this conclusion.[12] Women in the early or middle phases of menopause were more likely to seek medical help than those in later stages. Previous investigations in the South-Western and South-Eastern areas of Nigeria yielded results of 66.7% and 69%, respectively, which are consistent with our findings.[13] These findings bolster the growing body of research suggesting an increased incidence of breast cancer in younger women in African countries. Breast cancer has a high mortality rate, however research shows that it may be effectively treated if caught early.

Breast cancer is complicated by a number of factors,[15] such as its histological and molecular subtypes and physiological features.Two competing molecular explanations have been presented for the development of breast cancer. Flat epithelial atypia, atypical ductal hyperplasia, lobular neoplasia, including atypical lobular hyperplasia and lobular carcinoma in situ, and low-grade ductal carcinoma in situ (DCIS) are all considered precancerous lesions that may progress to invasive breast cancer. The oestrogen receptor (ER) and progesterone receptor (PR), but not the human epidermal growth factor receptor 2 (HER2), are part of the molecular profile of these cancers.[16]

There are two forms of breast cancer that can progress to a high stage: HER2-enriched and basal-type. Immunohistochemistry for HER2 will be positive in tumours that lack ER and PR but have high HER2. Triple-negative (no ER, PR, or HER2 expression) Basal cytokeratins are detected by immunohistochemistry in basal-type breast cancers.[16]

Though low-grade DCIS can progress to high-grade DCIS and ultimately develop into high-grade invasive carcinoma, it is well-known that high-grade DCIS is implicated in the evolution of high-grade tumours.[16] Due to their aggressive nature, basal-type breast cancers almost never have an intraductal component and quickly spread beyond that stage.

Diagnosis at an early stage, luminal subtype, and survival are all improved in Caucasian women with breast cancer when low-grade ductal carcinoma in situ (DCIS) is present.[18] There is only around a 15% incidence rate of basal-type carcinoma in this region.[15] Basal-type tumours, which fall under the triple-negative category of breast cancer, occur more often in black women. Some estimates.[19] put the percentage of African women who develop breast cancer at 60%.

The Luminal A subtype was found to include over 60% of the tumours we examined, with an intermediate nuclear grade. DCIS, or ductal carcinoma in situ, was also significantly linked to these tumours (association rate of 72.0%). These findings are at odds with those of a study by Ikeri et al.[15] which found that African-Americans had a higher risk of developing high-grade, nuclear-type, triple-negative malignancies due to a shared genetic predisposition. However, research by Titiloye et al.[20] found that non-basal type triple-negative cancer is more common in southwest Nigeria.In addition, out of 57 patients studied in 2016 at Obafemi Awolowo University Teaching Hospital (OAUTH) in Ile-Ife, Nigeria, researchers found that 43.5% of tumours were triple negative. Of them, 53 (40.5%) tested positive for the oestrogen receptor (ER), while 43 (32.8%) tested positive for HER-2.According to the data, HER2+ people made up just 32.8% of all cases, making them the group with the lowest incidence.

According to the AJCC study, 72 people (87.8%) were classified as having an advanced stage of illness. Research outside of Nigeria, but still in a poor country, uncovered similar trends.[22] Stages III and IV were often discovered late, which is a problem for many individuals. Compared to our results, the percentage of those diagnosed with metastatic illness in the research by

Ntekim et al. and Agbo et al. is much more concerning.[23]

Breast tumours that express the human epidermal growth factor 2 (HER2) are more aggressive and tend to spread more quickly.It is estimated that between 25 and 30 percent of breast cancers are HER2+[32] The prevalence of p53 mutations in 75% of these tumours is largely responsible for the increased aggressiveness, early recurrence and metastasis, and poor prognosis of HER2+ breast cancer.

HER2/neu aids cancer cell proliferation and survival, hence contributing to tumour development and resistance to therapy. About 12% to 20% of individuals had HER2/neu overexpression, which significantly worsens the effects of inflammatory breast cancer. According to criteria established by the American Society for Clinical Oncology/College of American Pathologists,[25] a positive result for HER2/neu is defined as 10% or greater membrane staining.Consistent with the findings of a previous research performed in Sudan[4], the primary histological conclusion of this investigation is that invasive ductal carcinoma is the most common histological subtype.

Patients older than 50 with a positive HER2/neu test, with the majority being HER2 positive at Grade II (12.0%).We found that most HER2+ patients were indeed of grade III, in contrary to the 2020 findings of Usman & Musa.[26] There was no statistical evidence linking Her2+ status to a higher grade of cancer.Her2+ positive tumours accounted for 18.3% of breast cancer cases in a study conducted at Lagos State University Teaching Hospital (LUTH)[27], suggesting that HER2+ may not serve as a reliable prognostic biomarker for tumour grade in the specific population examined. We discovered that HER2+ expression did not correlate with cancer grade within the scope of this investigation.Multiple factors, including prognosis, treatment options, and patient outcomes, highlight the significance of analysing HER2+ receptors in breast cancer patients categorised according to clinicopathologic criteria. Since its FDA approval in 1998,[28] trastuzumab has been effectively used to treat metastatic HER2-overexpressing breast cancer.Patients with HER2-positive breast cancer had a considerable improvement in disease-free survival (DFS) after treatment with trastuzumab.[33] While HER2-positive early-stage breast cancer responds well to trastuzumab,[29] around a quarter of patients will have a recurrence during the first decade after therapy.Patients who have developed early resistance to trastuzumab as a single agent may benefit better from combination chemotherapy regimens based on trastuzumab. Resistance to trastuzumab has been seen in individuals with both advanced and early-stage disease.[30]

CONCLUSION

EGFR (epidermal growth factor receptor) immunohistochemistry expression was shown to be correlated with HER2+ expression in breast cancer patients at NDUTH Teaching Hospital, Okolobiri. EGFR expression was shown to be unrelated to HER2+ status in breast cancer patients.

The findings of this study shed light on the molecular features of breast cancer and open the way for further investigation and potential use in therapeutic settings. If we are to effectively treat patients with breast cancer, it is essential to draw connections between this study and others, including patient demographics, drug alternatives, and therapeutic targets. Both the study's reliance on a single location for data collection (NDUTH Teaching Hospital, Okolobiri) and its relatively small sample size are potential sources of error. limitations in applying findings to other populations or clinical contexts.

Recommendation

Future studies with bigger sample sizes and more thorough molecular profiling approaches may help us better understand the connection between EGFR expression and HER2+ status in breast cancer patients.

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