Original Research Article

IMMUNOHISTOCHEMICAL EXPRESSION PATTERN OF BRAC 1 AND PROGESTERONE RECEPTORS AMONG FEMALE BREAST CANCER PATIENT IN BAYELSA STATE.

ABSTRACT

Breast cancer is a diverse disease that exhibits a variety of clinicopathological features that influence how it behaves and how it is treated. This study looked at progesterone receptor (PR) expression in breast cancer cases and its associations with sociodemographic and clinicopathological factors in the state of Bayelsa. Examining a total of 75 female breast cancer patients, we considered age, histologic type, cancer stage, and tumourgrade.The mean age of diagnosis is 45 years. The most frequent histological type, according to general trends, was invasive ductal carcinoma (72%). Although the PR expression was generally more positive in older patients, there was no statistically significant difference. Invasive lobular tumours showed a higher PR positive rate. Earlier studies on this subtype of cancer patients' features have been confirmed. Although there were differences in the percentage of PR positive patients depending on the stage of the disease, no statistically significant associations between PR expression and cancer stages were discovered. Tumours with moderate differentiation tended to express more PR, according to analysis of tumour grade. Notably, there was found to be a statistically significant link between the existence of a BRCA1 mutation and PR expression (p 0.001), suggesting that there may be a connection between BRCA1 mutations and PR expression in breast cancer. The results of this analysis help us understand the characteristics of breast cancer in the studied population. By studying the relationship between PR expression and demographic and clinicopathological traits, we provide a full understanding of PR's role in breast cancer. These results show the usefulness of using a range of factors to comprehend breast cancer behaviour and guide tailored treatment approaches. To validate these associations and enhance the therapy of breast cancer, more extensive studies are needed.

KEY WORDS;Breastcancer,BRCA 1,Progestrone,Tumor grade,Histologic subtypes,

INTRODUCTION

Worldwide, breast cancer is the most common type of the illness and the leading cause of death among women from cancer. 1.38 million new cases of breast cancer were discovered in 2008, while Kuchenbaeckeret al.[1]report that 60% of breast cancer fatalities and more than 50% of breast cancer patients live in developing countries. For hereditary breast cancers, two of the key genes are BRCA1 and BRCA2. DNA repair processes and The BRCA1 protein plays well-

known cancer suppressor roles. Bado et al.[2] determined that BRCA1 is situated on chromosome 17q21. It encodes a nuclear protein of 1863 amino acids. It partially regulates the control of cell-cycle checkpoints, apoptosis, DNA repair, transcriptional activation, and chromosomal remodelling. According to Parkin et al.[3], BRCA1 is a well-known tumour suppressor gene in familial breast cancer. Women with deleterious BRCA1 mutations that result in loss of BRCA1 function usually develop breast and ovarian malignancies[1]. Gene changes passed down from parents account for 5–10% of breast cancer cases, on average. Hereditary breast cancer is most frequently caused by a genetic mutation in the BRCA1 or BRCA2 gene. women's cancer. The odds of a woman developing breast cancer over her lifetime are statistically increased by 55–65% by a BRCA1 mutation [4]. Since ER causes the production of the progesterone receptor (PR), researchers have looked at it. in place of an ER activity marker and as an additional indicator of how well hormone therapy works for treating breast cancer[5]. According to studies [6], progesterone increases the activity and division of mammary stem cells. Progestrone raises the risk of breast cancer in postmenopausal women, according to a number of comprehensive clinical studies on hormone replacement therapy. Progestrone plus oestrogen significantly increased the risk of invasive breast cancer when compared to oestrogen alone in these studies. Additionally, research shows that progestrone in vitro in the PR-positive breast cancer cell lines show proliferative effects[7].The aim of this study is to evaluate the immunohistochemical expression pattern of BRAC 1 and progesterone receptors in female breast cancer patients in Bayelsa state.

MATERIALS AND METHODS

Study Area

The study was carried out in two tertiary healthcare facilities in Bayelsa state, namely the Niger Delta University Teaching Hospital and the Federal Medical Centre Yenagoa South-south, Nigeria. The facilities serve as a referral point for the state of Bayelsa and a training ground for students in the medical and associated scientific departments at the state university. Bayelsa State and the Atlantic Ocean share a border in the southern part of Nigeria, which is defined by latitudes 4.15IN and 5.23South and longitudes 5.221 and 6.51East of the equator.

Ethical Clearance

It received approval from both the Federal Medical Centre Yenagoa and the Niger Delta University Teaching Hospital Okolobiri ethical committees

Study Population

The study's target population included all incidences of breast cancer reported between 2020 and 2022 by patients at the Federal Medical Centre in Yenagoa and the Niger Delta University Teaching Hospital in Okolobiri.

Sampling Method

Because it was practical, non-probability sampling was utilised. For immunohistochemistry investigation, 75 cancer tissues was chosen.

Inclusion /Exclusion Criteria

All breast cancer samples that were obtained during the course of the investigation period were included, but other tumours were disregarded.

Preparation of Samples

A rotary microtome was used to trim the tissue blocks. After being chosen using glass slides while floating in a water bath, the sections were dewaxed in order to be ready for immunohistochemistry and hematoxylin and eosin analyses**.**

Methodology

Hematoxylin and Eosin (H & E) staining technique[8]

Principle

Based on the chemical affinity between the dye and the tissue, the H and E stain functions. The basic dye hematoxylin creates a blue-purple contrast in basophilic structures, especially those

that contain nucleic acid moieties like chromatin, ribosomes, and cytoplasmic regions rich in RNA. An acidic eosin counter stains basic substances in a variety of pink, orange, and red hues, including RBCs, cytoplasm, muscle, and collagen**.**

Procedure

The tissues were dewaxed in xylene, hydrated by putting them through increasingly stronger alcohol, rinsed in water, drained, and then put in a solution of hematoxylin (the primary stain), where the nuclei stained blue for 20 minutes. The slides were immediately differentiated with 1% acid alcohol for two seconds, rinsed to stop the reaction, then blued in Scott's tap water to restore the nuclear stain. The sample was then dehydrated in two different alcohol concentrations (95 and absolute) and stained with 1% Eosin (a cytoplasmic stain), washed in xylene, and mounted in DPX after being dehydrated in three different alcohol concentrations (70, 95, and absolute).

Immunohistochemical Method and Procedure

Using the Avidin Biotin Complex for Immunohistochemistry Staining. The approaches used by Eruvwahwe et al.,[9]were adopted. The Avidin Biotin Complex (ABC), also known as the Avidin Biotin Immuno-Peroxidase Method, was applied to the formalin fixed paraffin embedded sections (FFPE).

Principle

The fundamental principle of immunohistochemistry is the imaging of antigen-antibody interactions by a marker enzyme through the localization of antigens in tissue sections by the use of labelled antibodies as specific reagents. It was visualised using 3,3-diaminobenzidine (DAB).

The major antibodies were specific to the oestrogen receptor antigen. The antibody was used, and it was diluted 1:100.

Procedure

The formalin-fixed, paraffin-embedded tissue was cut into 2 mm thick sections, which were then heated to 70 °C for an hour using Leica's rotary microtome. Sections were put through two cycles of xylene, three cycles of alcohol (Absolute 1, 95%, and 70%), and then two cycles of water to get them down to water. For 15 minutes, the sections were heated in a 6.0 pH citric acid solution in an effort to extract the antigen. The pieces were allowed to cool for five minutes after progressively substituting cool water for the heated citric acid. Simply covering the slices with 3% hydrogen peroxide (H2O2) for 15 minutes prevented the peroxidase enzyme from working. The sections for the study were washed in Phosphate Buffered Saline (PBS). 2-minute period. the use of protein blocking Avidin and biotin were used to halt endogenous biotin production for 15 minutes. The sections were then washed with phosphate buffer saline and diluted with primary antibody for 60 minutes. Extra antibody was removed using PBS. The slices were marked for 15 minutes using horseradish peroxidase (HRP). Visualisation was performed utilising DAB. After two minutes of hematoxylin counterstaining, sections were swiftly blued. After being cleaned in xylene and dehydrated in alcohol, sections were mounted with DPX. The characteristically dark cytoplasm and cell membrane of positive cells were visible. Negative cells were those with little to no brown colour or with poor brown colouring[9].

Observation

The antigenic sites define positive cells as having membrane-bound nuclei and a certain shade of brown in the cytoplasm. Hematoxylin stained cells without any indication of a brown hue receive

negative ratings. Ineffectively bound or brown artefact-containing cells and connective tissue are removed.

Statistical analysis

Graph Prism 5.0 was used to examine the data, and the results were presented as mean, standard deviation, and percentage.

RESULTS

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

Variable	Group	Frequency (%)		X^2	*p*-value
		Positive n = 48	Negative n = 26		
Age	≤50	29 (60.40)	18 (69.20)	0.57	.45
	>50	19 (39.60)	8 (30.80)		
Stage	I	3 (6.30)	0 (0.00)		
	II	25 (52.10)	23 (88.50)	9.99	.01*
	III	20 (41.70)	3 (11.50)		
	IV	0 (0.00)	0 (0.00)		
Grade	Well Differentiated	10 (20.80)	7 (26.90)		
	Moderately Differentiated	36 (75.00)	9 (34.60)	17.03	.00**
	Poorly Differentiated	2 (4.20)	10 (38.50)		
Histologic type	Invasive Ductal carcinoma	36 (75.00)	26 (100.00)		
	Invasive Lobular carcinoma	11 (22.90)	0 (0.00)	7.76	.02*
	Mucinous cancer	1 (2.10)	0 (0.00)		

Key: X^2 = Chi-square statistic, ** = Significant association observe, p< .01, * = Significant association observed, p< .05

The table above show the analysis of progesterone receptors stratified by clinicopathological parameters among breast cancer subject .The age of breast cancer subject ≤50 had more positive cases for progesterone receptors than the age >50 19(39.6%). However there was no significant association between age and progesterone expression in breast cancer.Stage II breast cancer subject had more positive cases for progesterone receptor 25(52.10%) followed by the stage III subject 20(41.7%) and the stage I 3(6.30%).

There was a significant association between the stage of tumor in breast cancer and progesterone expression $p<0.05$, $x^2 = 9.9$.

The moderately differentiated cancer grade has more of the progesterone positive subjects 36(75%), next is the well differentiated cancer subject 10(20.8%) and then the poorly differentiated cancer subject 2(4.2%). There is a significant association between tumor grade and progesterone expression in breast cancer at $p<0.01$,$x^2 = 17.03$.

The invasive ductal carcinoma had more progesterone positive subject 36(75%) followed by the invasive lobular carcinoma 11(22.9%0 and then the mucinous cancer 1(2.1%). There is a significant association observed between histological breast cancer type and progesterone expression $p<0.05$, $x^2 = 7.76$.

PLATE 1IMMUNOHISTOCHEMICAL PLATES

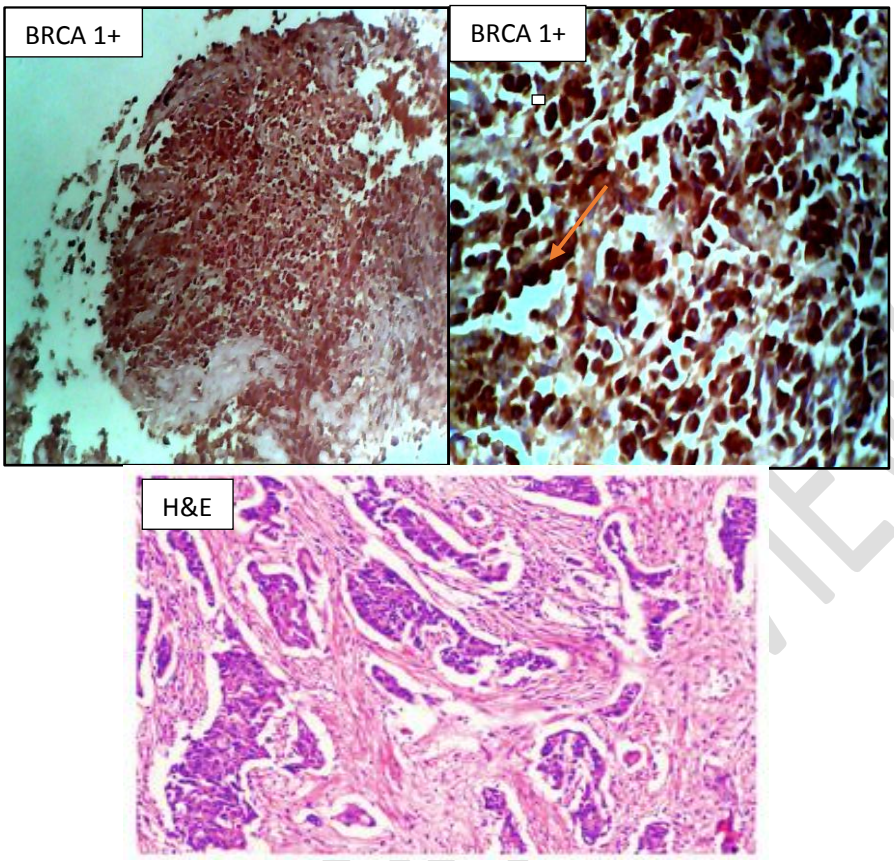

Plate 1. Shows the morphology of the breast after staining with immunohistochemical staining technique and viewed at x10 magnification. Sections shows breast tissue positive(++) for BRCA1(arrow)

PLATE 2

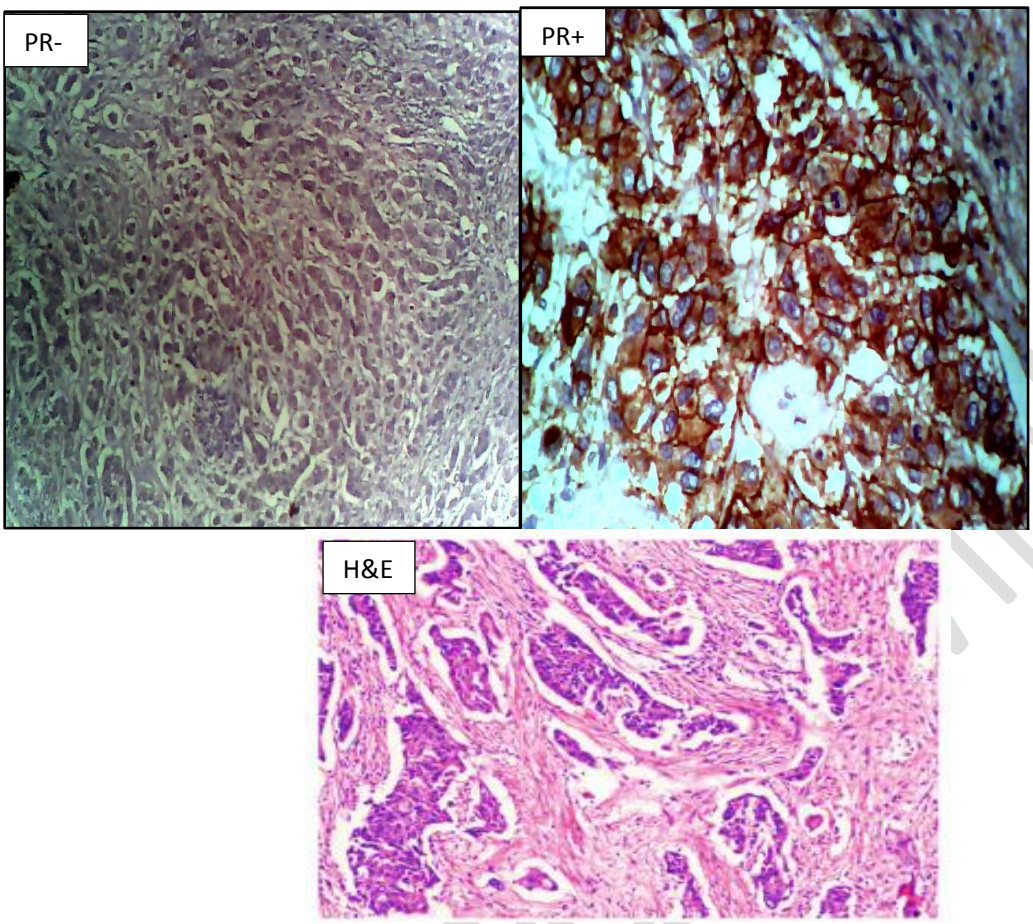

Plate 2. Shows the morphology of the breast after staining with immunohistochemical staining technique and viewed at x40 magnification. Section shows breast tissue negative for Progesterone recptors(PR-) and also positive for Progesterone(PR++) receptors .

DISCUSSION

In this study, we investigated the progesterone receptor (PR) expression in breast cancer cases and its associations with various sociodemographic and clinicopathological variables.The study population's demographic makeup was consistent with previous research's findings. 98.3% of the total cases we looked at included females, which is similar to the study by Uzoigwe et al. [10]. Our participants' average age was 45.1 years, which falls within the Uzoigwe et al [10]. mentioned range of 20 to 70 years. According to Veronesi et al. [11], invasive ductal carcinoma

(IDC) is the most common type of breast cancer. Most cases (around 80%) include carcinomas. Some of the subtypes covered in the IDC classification of breast cancer include tubular carcinoma, medullary carcinoma, mucinous carcinoma, papillary carcinoma, and cribriform carcinoma. Additionally, in line with the findings of Ntekin et al. [12] research, our study supports the notion that invasive ductal carcinoma is the most prevalent histological type in breast cancer cases.

In numerous large-scale clinical trials of hormone replacement therapy, progesterone has been associated with an elevated risk of breast cancer in menopausal women, according to a 2011 study by Liu et al [7]. According to these studies, the interaction of Pg and oestrogen significantly increased the incidence of invasive breast cancer. as contrasted with oestrogen alone, cancer. Additionally, Pg has been shown to have proliferative effects in breast cancer cell lines that are PR-positive in vitro [7]. When we investigated stratified PR expression by age in breast cancer patients, we found no statistically significant difference between those 50 years of age or younger and those older than 50 years. Our findings, which revealed a tendency towards greater PR positivity in older people, are consistent with the results reported by Uzoigwe et al. [10]. These findings suggest that the expression of PR in breast cancer may be influenced by ageing, but stronger conclusions call for future studies with larger sample sizes. In terms of histologic type, our research confirms Usman'set al.,[13] findings that invasive ductal carcinoma is the most prevalent kind. subtype. Additionally, we found enhanced PR positive in cases of invasive lobular carcinoma, which is consistent with past studies emphasising the higher PR expression frequency in this specific subtype. Additional support for the need of considering tumour heterogeneity in breast cancer comes from the link between histologic type and PR expression[19].

The correlation between PR expression and stage continues to be studied in breast cancer research. Although our analysis did not find any statistically significant associations between PR expression and different stages, we did find differences in the percentages of PR positive between stages. These results support Uzoigweet al.[10]findings that underline that early-stage cases showed increased PR positivity. But Ntekin et al. [12] found in their research a higher proportion of advanced-stage cases, suggesting that stage distribution may vary at the population level. More research with larger cohorts are needed to study the complex connections between PR expression and stage in breast cancer.

It is well known that tumour grade affects the prognosis of breast cancer. Although we did not detect statistically significant correlations between tumour grade and PR expression, we did find a tendency towards greater PR expression in moderately differentiated tumours. Usman et al.,[13] views are supported by this Finding, which also emphasises the need for more studies with larger sample sizes to shed light on any potential relationships between cancer grade and PR expression. Our findings were in agreement with Uzoigwe et al. [10] findings regarding PR expression prevalence rates. in instances of body cancer. Our work builds upon these results by analysing the associations between PR expression and additional clinicopathological parameters. To provide a more complete picture of PR expression in breast cancer, factors such as age, histologic type, stage, and grade are considered.The chi-square test was applied to ascertain the statistical significance of the observed distribution. The computed chi-square value of 23.55 (p 0.001) indicates a strong relationship between the existence of the BRCA1 mutation and PR

expression. Margolin et al.,[14] study from 2020 looked at the clinicopathological traits and prognosis of Latin American breast cancer patients with BRCA1 and BRCA2 mutations. A significant link was found by the researchers. between a BRCA1 mutation and a functioning progesterone receptor (PR) . This suggests that germline BRCA mutations have a high likelihood of being undetected. In various articles [15], the chromosomal rearrangements that potentially impact the BRCA1 and BRCA2 genes are compiled. In DNA repair and checkpoint activation, a pleiotropic DNA damage response protein by the name of BRCA1 is implicated. According to Roy et al.,[16] and [17], BRCA2 mediates homologous recombination. Some of the biological processes that BRCA1 is engaged in when it comes to carcinogenesis include the transcriptional regulation of DNA repair-related genes, heterochromatin formation on the X chromosome, double strand break repair, and ubiquitination. BRCA1, BRCA2, TP53, and RAD51 interact with many proteins involved in the cell cycle and the response to DNA damage. DNA double strand breaks must be repaired. pathways. Lacking a functional BRCA1 protein prevents cells from arresting in the G2 phase of the cell cycle in the case of DNA damage, and transcription-coupled repair is also hindered [18]. Furthermore, BRCA1 interacts with H2AX to alter the chromatin's structure, enabling DNA repair proteins to access damaged areas [19]. BRCA2 participates, like BRCA1, in DNA double strand break repair by recombination and the preservation of chromosomal integrity. Following numerous divisions, unforeseen chromosomal abnormalities

including doublestranded, tri-radials, and quadri-radials, such as tri-radials and quadri-radials, appear due to BRCA2 loss[18].

Conclusion

Luminal A was found to be the most common molecular subtype, and Her2 was shown to be the least common. Patients with breast cancer in Bayelsa state most frequently had an Idiopathic Ductal Carcinoma (IDC). The expression of the progesterone receptor (PR) and the grade of the tumour did not substantially correlate. However, there was a strong association between the existence of a BRCA1 mutation and PR expression. These results highlight the importance of considering PR expression and BRCA1 mutation status when deciding how to diagnose and treat breast cancer. To fully grasp the clinical implications of these findings and look into targeted therapies for breast cancers associated with BRCA1, more research is necessary.

Recommended

It is important to conduct more study on the relationship between PR expression and BRCA1 gene carrier status in order to understand the underlying mechanisms and clinical implications.

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