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Level of Adherence to Breast Cancer Molecular Subtyping among Women with Breast Cancer Attending Tertiary Health Facilities

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Abstract

Background: Breast cancer is a genetically and clinically heterogeneous disease with multiple subtypes. The classification of these subtypes has evolved over the years. The most common and widely accepted classification of breast cancer is from an immunohistochemical perspective, based on the expression of the following hormone receptors: Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth Factor (HER2). Accordingly, the following four subtypes of breast cancer are widely recognized—Luminal A, Luminal B, HER2 Enriched and Triple Negative. Breast cancer management approaches include surgery, chemotherapy, radiotherapy and targeted hormone therapy necessitated by molecular subtyping. **Aims:** This study aimed to determine the level of adherence to breast cancer molecular subtyping among women with breast cancer attending tertiary health facilities in Imo State. **Methodology:** Immunohistochemistry reports of women with breast cancer attending tertiary health facilities in Imo State were retrieved from patient's case files. Tissue blocks were also retrieved from tissue block archives of both hospitals for women who did not take up immunohistochemistry services after their initial diagnosis and also those whose immunohistochemistry reports were not found in their case files. **Results:** Among the 121 women that participated in the study, there were in all 74 (61.2%) had molecular subtyping of their tumour blocks. Up to 45 (37.2%) did not go for molecular subtyping of their tumour blocks while 2 (1.7%) were not sure whether they had or not. **Conclusion**: It, therefore, depicts that the rate of uptake was found as 61.2% among the participants and there is a need to create more awareness of the

importance of molecular subtyping, which necessitates the use of targeted hormone therapy.

Keywords

Breast, Cancer, Subtypes, Immunohistochemistry, Estrogen, Progesterone, Human Epidermal Growth Factor 2, Receptor

1. Introduction

Breast cancer is a disease in which abnormal breast cells grow out of control and form tumours. If left unchecked, the tumours can spread throughout the body and become fatal (WHO, 2023) [1]. Female breast cancer is the most commonly diagnosed cancer in women worldwide with an estimated 2.26 million new cases diagnosed worldwide in 2020[1]. The incidence and mortality rates are increasing rapidly with over 500,000 deaths recorded each year, making it the leading cause of cancer death in women [2].

Breast cancer accounts for 25% of all cancers in females worldwide. In Africa, the incidence of breast cancer is relatively low compared to the Western developed countries, however, morbidity and mortality rates are alarmingly high, reflecting the relatively poor survival from the disease in the continent [3] [4] [5]. In 2020, 685,000 deaths were recorded globally. As of the end of 2020, there were 7.8 million women alive who were diagnosed with breast cancer in the past 5 years, making it the world's most prevalent cancer. Breast cancer occurs in every country of the world in women at any age after puberty but with increasing rates in later life (WHO, 2023) [1]. The American Cancer Society (ACS) estimated an average of 93,600 new cases of breast cancer annually in Africa with about 50,000 deaths. In Nigeria, breast cancer is recognized as a major cause of morbidity and mortality with an incidence rate ranging from 36.3 to 50.2/100,000 women [6]. Also, in Imo State, breast cancer has been reported to be the most common cancer among women [7].

The mainstay of breast cancer management and treatment approach is surgery when the tumour is localized, followed by chemotherapy (when indicated), radiotherapy and targeted hormonal therapy (when hormone receptors status is known) [8]. However, of these four approaches, the use of hormonal therapy stands out because of its increased survival advantage. It has been estimated to be responsible for 35% - 72% of the reduction in mortality [9]. The use of hormone therapy can only be employed when the hormone receptor status of a breast cancer patient is known and this can only be achieved through molecular subtyping using Immunohistochemistry (IHC). IHC is used to characterize intracellular proteins or various cell surfaces in all tissues. Individual markers or more often panels of various marker proteins can be used to characterize various tumour subtypes, confirm tissue of origin, distinguish metastatic from primary

tumour and provide additional information, which may be important for prognosis, predicting response to therapy or evaluating residual tumour post-treatment. There is a growing list of available products (antibodies) or antigen retrieval techniques, which all contribute to the broader utility of immunohistochemistry for solving diagnostic problems or for determining prognosis and response to therapy in breast pathology. Diagnostic and prognostic markers are described although some of them can be included in both [10]. A growing list of available antibodies, improved antigen retrieval techniques and a better understanding of biology have all contributed to the broader utility of IHC for solving everyday diagnostic problems in breast pathology [10]. The use of immunohistochemistry to further characterize breast cancer globally has introduced a new dimension to the knowledge of the disease. Breast cancer can no longer be regarded as a single entity and morphological features alone cannot completely predict the behavior of breast [11]. The three immunohistochemical markers currently in routine diagnostic use in most countries are Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth Factor 2 (HER2). These markers determine which tumours are likely to respond to hormonal therapy and Herceptin treatment 12. It is generally acknowledged that breast cancer is a heterogeneous disease with a wide spectrum of clinical, pathologic and molecular features. The molecular classification is becoming the gold standard for the complete characterization of breast cancer and the underlying technology has already generated gene-profiling models to predict outcomes [12].

According to Cancer Treatment Centers of America (2021) [13], breast cancer has four primary molecular subtypes defined in large part by hormone receptors (ER, PR) and other types of proteins involved (or not involved) in each cancer: Luminal A (ER/PR+, HER2−), Luminal B (ER/PR+, HER2+), Triple Negative (ER/PR−, HER2−), and HER2 Enriched (ER/PR−, HER2+). World Health Organization (WHO), American Society of Clinical Oncology (ASCO) and the College of American Pathologist (CAP) guidelines on treatment of breast cancer recommend that all women diagnosed with breast cancer should have their tumour blocks tested for Estrogen, Progesterone and Human Epidermal growth factor receptors status using immunohistochemistry diagnostic services. This should provide oncologist bases for administering targeted hormonal therapy to women with breast cancer aimed at helping them recover, thus reducing morbidity and mortality associated with the disease.

2. Material and Methods

Ethical approvals were obtained from the research ethical committee of both tertiary health facilities (Federal Medical Centre and Imo State University Teaching Hospital). Available Immunohistochemistry reports were retrieved from the hospital's case files. Formalin Fixed Paraffin wax-Embedded (FFPE) tissue cassettes of breast cancer patients whose reports were not found because they did not go for molecular subtyping of their tumour blocks after their initial diagno-

sis were retrieved from Histopathology department tissue block archive of both tertiary health facilities and sent with fees paid by researcher for Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER-2/neu) over expression to Labnetwork Pathology and Medical Laboratory Ltd, Abuja for molecular subtyping. FFPE tissues were sectioned serially into 4um and placed in frosted microscopic slides and deparaffinized in series of xylene. Antigen retrieval was performed using a water bath in 10 mM citrate buffer (pH 6.0) at 95˚C for 45 min. Then washed with Tris Buffered saline and blocked with 3% hydrogen peroxide in phosphate Buffered Saline. After that, tissue sections were blocked with background snipper using a blocking agent (Biogenex UK). Then incubated for 1 hr with primary antibodies at room temperature: anti-ER (clone EPR703, Biogenex UK), anti-PR (clone PR88, Biogenex Ltd, UK), and anti-HER2 neu (clone CB11), followed by biotinylated horse anti-mouse or goat anti-rabbit secondary antibodies. Staining was visualized using Diaminobenzadine (DAB) and counterstained with haematoxylin. ER and PR were considered positive if >1% nuclei of tumour cells were stained (Figure 1 and Figure 2) as per the American Society College of Oncology/College

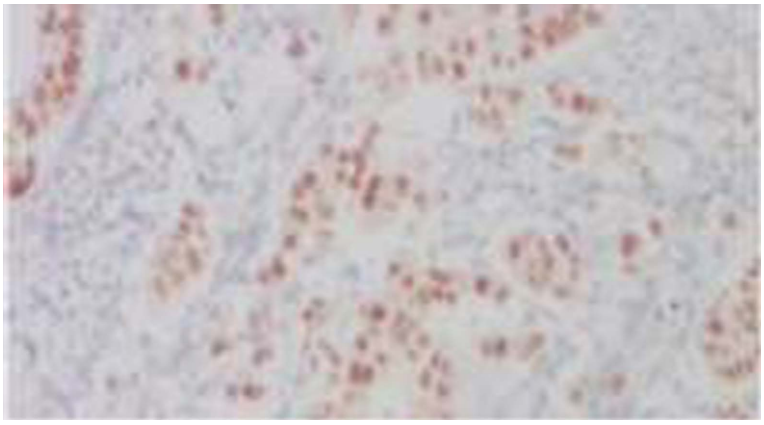

Figure 1. Immunohistochemical demonstration of oestrogen nuclear receptors in a breast cancer section (×40).

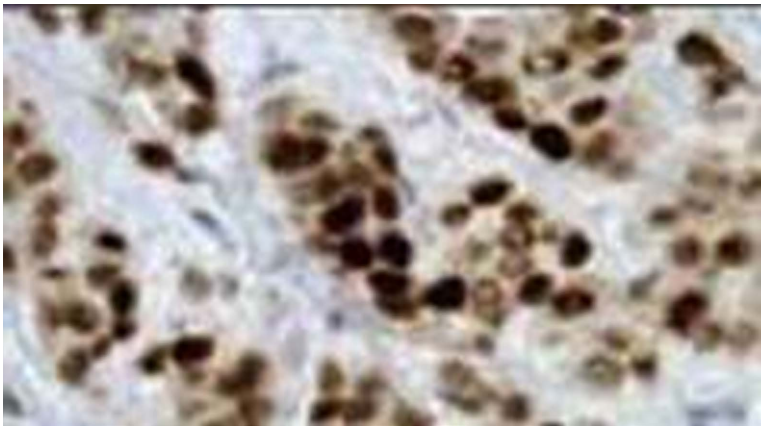

Figure 2. Immunohistochemical demonstration of progesterone receptors in breast carcinoma (×40).

of American Pathology (ASCO/CAP) guidelines for women [14]. HER2 was scored as 0, 1+, 2+, 3+. A zero score defines tumours with no staining or membrane staining in less than 10% of the tumour cells (**Figure 3**) while 1+ refers to tumours with a faint membrane staining in more than 10% of the tumour cells. A weakly positive result characterized by weak to moderate complete membrane staining in more than 10% of the tumour cells is represented by a 2+ score, while a strongly positive result defined as strong complete membrane staining in more than 10% of the tumour cells is represented as 3+ (**Figure 4**). Scores of 0, 1+ was classified as negative while a score 2+ and 3+ was regarded as positive [14].

Molecular breast cancer subtypes were defined using combination of these IHC markers as follows: Luminal A-like (ER positive and/or PR positive and HER2 negative), Luminal B-like (ER positive and/or PR positive/PR negative and HER2 positive), HER2 enriched type (ER negative, PR negative and HER2 positive and Triple negative (ER, PR, and HER2 negative).

Data analysis was performed using SPSS 21.

3. Results

A study of 121 respondents shows in **Table 1**, that few of the participants 16 (13.2%)

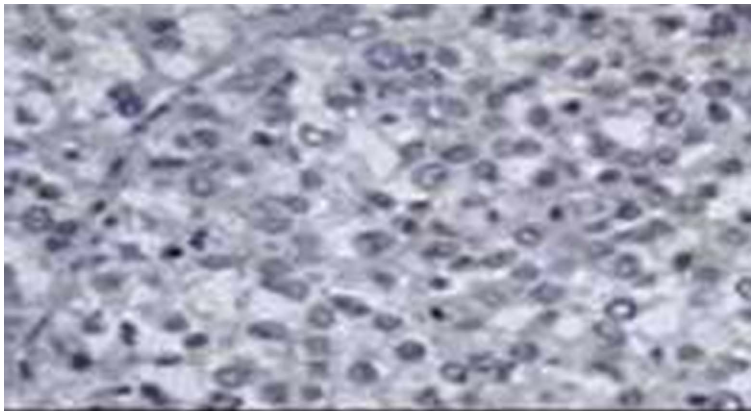

Figure 3. Section of the breast cancer showing lack of any of the aforementioned receptor markers (×40).

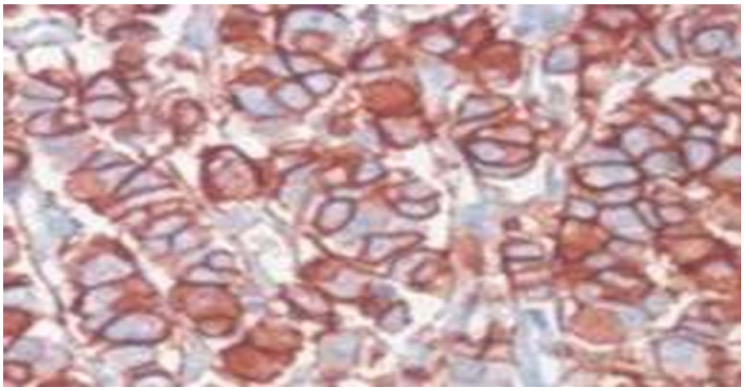

Figure 4. Immunohistochemical demonstration of strong circumferential membrane staining of HER2 over-expression of breast cancer (×40).

Table 1. Distribution of participants by characteristics of uptake of IHC services for subytyping.

Adherence characteristics	Frequency	Percent %
When were you diagnosed of breast cancer		
Less than 6 months	16	13.2
6 - 11 months	25	20.7
12 - 17 months	47	38.8
18 - 23 months	5	4.1
24 months and above	24	19.8
Not sure	4	3.3
If doctor requested IHC services		
Yes	88	72.7
No	10	8.3
Not sure	13	10.7
If respondents took up IHC services		
Yes	74	61.2
No	45	37.1
Not sure	2	1.7
When after diagnosis did respondents took up IHC services		
Less than 6 months		
6 - 11 months	18	24.3
12 - 17 months	44	59.5
18 - 23 months	8	10.8
24 months and above	4	5.4
Reasons for not taking up IHC services		
I couldn't afford the cost of IHC services	31	65.9
I was scared of the result outcome	7	14.9
I resorted to prayer for divine healing	4	8.5
I was advised by significant order not to take IHC services	2	4.3
Nobody advised me to go for IHC services	3	6.4
If IHC services is beneficial		
Yes	67	90.5
No	7	9.4
Not sure	0	0
If prompt uptake of IHC is beneficial		
Yes	68	91.8

Continued

No	6	8.1
Not sure	0	0
If prompt uptake of IHC services is essential for better mgt		
Yes	68	91.8
No	6	8.1
Not sure	0	0

were diagnosed of breast cancer within less than 6 months, followed by 25 (20.7%) within 6 - 11 months, 47 (38.8%) within 12 - 17 months, 5 (4.1%) within 18 - 24 months, 24 (19.8%) within 24 months & above and 4 (3.3%) were not sure when diagnosis was made. Among the 121 respondents that participated in the study, there were in all 74 (61.2%) that had molecular subtyping of their tumour blocks which was necessary for breast cancer treatment. Up to 45 (37.2%) did not while 2 (1.7%) were not sure. It, therefore, depicts that the rate of uptake was found as 61.2% for the respondents. Also, most of the respondents 88 (72.7%) averred that their Doctor requested them to take up IHC services while 10 (8.3%) claimed otherwise and 13 (10.7%) were not sure if their Doctor requested them to take up IHC services. On the other hand, out of 52 (42.9%) respondents who were diagnosed of breast cancer less than 6 months, only 18 (24.3%) took up IHC within the period (less than 6 months). The implication of this result is that those among the respondents who took up IHC services within less than 6 months will have a higher recovery rate compared to the other groups. Molecular subtyping is recommended immediately after diagnosis and so the earlier the better the management. Among participants that did not go for molecular subtyping of their tumour blocks, more than half 31 (65.9%) couldn't afford the cost, 7 (14.9%) were scared of the result outcome, 4 (8.5%) and so resorted to prayers for divine healing, 2 (4.5%) were advised by a significant order not to while 3 (6.4%) were not advised by anybody to go for molecular subtyping. Among all respondents 74 (61.2) that did molecular subtyping, only 67 (90.5%) attested to the fact that it benefited them while 7 (9.4%) did not benefit, however, no participant claimed not to be sure of the benefits. Concerning benefits of prompt adherence and its essential for management, majority of the participants 68 (9.8%) concurred, 6 (8.1%) didn't attest while none was in doubt.

It can be observed from **Figure 5** that more than half 74 (61.2%) did molecular subtyping of their tumour blocks while 45 (37.2%) did not and 2 (1.7) were not sure if they did.

4. Discussion

The level of uptake of Imunohistochemistry (IHC) services among women with breast cancer was found to be slightly higher compared to that of 59% in a meta-analysis of 40 studies [15]. This finding is abysmal especially now that is a

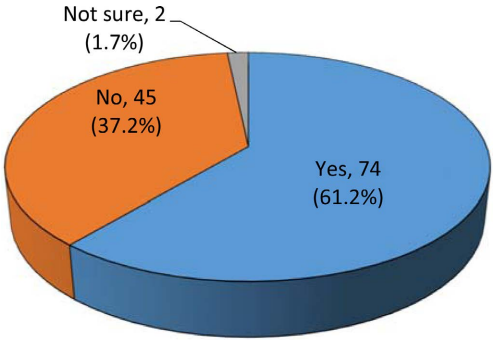

Figure 5. Pie chart showing distribution of respondents by uptake of Imunohistochemistry (IHC) services.

gold standard set by WHO to have all diagnosed breast cancer tumour blocks subtyped. The benefits from this cannot be over-emphasised. In a study carried out by Mariangel *et al.* (2008), it was estimated to be responsible for 35% - 72% of the reduction in mortality

Also, another meta-analysis study showed a poor level of adherence of 16.3% with high heterogeneity of 98.9% [16]. It is, however, obvious that no clear adherence pattern can be obtained in this study. That could be as a result of differences in recommendations on adherence by the medical personnel or as a result of differences in hospital visits. In our study, being recommended by the doctor is a significant factor of adherence. It might also mean that many women with breast cancer are faced with inconsistency of information about molecular subtyping. This study opined the participants reported not getting adequate motivation from family members and other health personnel that could have encouraged them towards adherence. There is dearth of research studies on the level of adherence to molecular subtyping in study area.

5. Conclusion

It, therefore, depicts that the rate of uptake was found as 61.2% for the respondents and there is a need to create more awareness of the importance of molecular subtyping.

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Conflicts of Interest

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

References

[1] World Health Organization (2023) Breast Cancer.

https://www.who.int/news-room/fact-sheets/detail/breast-cancer

[2] Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. and Jemal, A. (2018) Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA*: *A Cancer Journal for Clinicians*, **68**, 394-424. https://doi.org/10.3322/caac.21492

[3] Siegel, R.L., Miller, K.D. and Jemal, A. (2016) Cancer Statistics, 2016. *CA*: *A Cancer Journal for Clinicians*, **66**, 7-30. https://doi.org/10.3322/caac.21332

[4] Forman, D., Bray, F., Brewster, D.H., Mbalawa, G.C., Kohler, B., Piñeros, M., *et al.* (Eds.) (2014) Cancer Incidence in Five Continents Volume X. IARC Scientific Publication No. 164, International Agency for Research on Cancer, Lyon.

[5] Ferlay, J., Ervik, M., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., *et al.* (Eds.) (2012) GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 v1.0. IARC CancerBase No. 11, International Agency for Research on Cancer, Lyon.

[6] Fregene, A. and Newman, L.A. (2005) Breast Cancer in Sub-Saharan Africa: How Does It Relate to Breast Cancer in African-American Women? *Cancer*, **103**, 1540-1550. https://doi.org/10.1002/cncr.20978

[7] Anele, A.A., Okoro, I.O., Oparaocha, D.C. and Igwe, P.O. (2009) Pattern of Breast Disease in Owerri, Imo State, Nigeria. *Port Harcourt Medical Journal*, **4**. https://doi.org/10.4314/phmedj.v4i1.52368

[8] World Health Organization (2008) Cancer Control: Knowledge into Action: WHO Guide for Effective Programmes: Module 4: Diagnosis and Treatment. https://fctc.who.int/publications/i/item/9789241547406

[9] Ciccarese, M., Lorusso, V. and De Laurentis, M. (2008) Controversies in Adjuvant Endocrine Therapy for Pre- and Post-Menopausal Women with Breast Cancer. *European Journal of Cancer Supplements*, **6**, 4-9. https://doi.org/10.1016/j.ejcsup.2008.06.016

[10] Zaha, D.C. (2014) Significance of Immunohistochemistry in Breast Cancer. *World Journal of Clinical Oncology*, **5**, 382-392. https://doi.org/10.5306/wjco.v5.i3.382

[11] Bauer, K.R., Brown, M., Cress, R.D., Parise, C.A. and Caggiano, V. (2007) Descriptive Analysis of Estrogen Receptor (ER)-Negative, Progesterone Receptor (PR)-Negative, and HER2-Negative Invasive Breast Cancer, the So-Called Triple-Negative Phenotype. *Cancer*, **109**, 1721-1728. https://doi.org/10.1002/cncr.22618

[12] Berry, D.A., Cirrincione, C., Henderson, I.C., Citron, M.L., Budman, D.R., *et al.* (2006) Estrogen-Receptor Status and Outcomes of Modern Chemotherapy for Patients with Node-Positive Breast Cancer. *JAMA*, **295**, 1658-1667. https://doi.org/10.1001/jama.295.14.1658

[13] Cancer Treatment Center of America (2021).

[14] Hammond, M.E., Hayes, D.F., Dowsett, M., Allred, D.C., Hagerty, K.L., Badve, S., Fitzgibbons, P.L., Francis, G., Goldstein, N.S., Hayes, M., Hicks, D.G., Lester, S., Love, R., Mangu, P.B., McShane, L., Miller, K., Osborne, C.K., Paik, S., Perlmutter, J., Rhodes, A., Sasano, H., Schwartz, J.N., Sweep, F.C., Taube, S., Torlakovic, E.E., Valenstein, P., Viale, G., Visscher, D., Wheeler, T., Williams, R.B., Wittliff, J.L. and Wolff, A.C. (2010) American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer. *Journal of Clinical Oncology*, **28**, 2784-2795. https://doi.org/10.1200/JCO.2009.25.6529

[15] Ropka, M.E., Wenzel, J., Philips, E.K., Sladaty, M. and Phibrick, J.T. (2006) Uptake Rates for Breast Cancer Genetic Testing: A Systematic Review. *Cancer Epidemiol-*

ogy, Biomarkers & Prevention, **15**, 840-855.
https://doi.org/10.1158/1055-9965.EPI-05-0002

[16] Smith, S.G., Sestak, A., Forster, A., Partridge, L., Side, M.S., Wolf, R., Horne, J. and Cuzick, J. (2016) Factors Affecting Uptake and Adherence to Breast Cancer Chemoprevention: A Systematic Review and Meta-Analysis. *Annals of Oncology*, **27**, 575-590.
https://doi.org/10.1093/annonc/mdv590