

TRIPLE NEGATIVE BREAST CANCER IN MAIDUGURI, NORTH EAST NIGERIA: A FIVE YEAR REVIEW

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Abstract

Introduction: Breast cancer is the most common malignant neoplasm and a leading cause of cancer related deaths among women worldwide. Traditional breast cancer classification based on histopathologic features has limited prognostic and predictive value compared to the molecular classification. Currently, three biomarkers are commonly used to classify breast cancer including estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2). Assessing these markers help to select patients appropriately for treatment targeted on these major molecular pathways of the disease. A breast cancer that fails to express ER and PR, and has absence of HER-2 overexpression is designated Triple Negative. Paucity of information on triple negative breast cancer from North Eastern Nigeria prompted this study. **Methods:** The study was a hospital based, descriptive and cross sectional one. One hundred and thirteen (n=113) archived tissue blocks from confirmed cases of breast cancer that fulfilled the inclusion criteria for the study were retrieved along with their request cards. Immunohistochemistry was performed on the tissue blocks using monoclonal mouse antibodies to human ER, PR and HER-2 (Novocastra post primary, Leica Biosystems REF7111, 7102 and 7101 respectively). The slides were evaluated using the J score. Equivocal HER-2 cases had FISH done on them at Unistel medical laboratories, South Africa using the IQFISH protocol. The slides were evaluated using the ASCO/CAP 2013 guidelines. **Results:** Out of the 1415 cancers diagnosed at the Department of Histopathology, University of Maiduguri Teaching Hospital (UMTH) over the study period, breast cancer accounted for 276 (19.5%) cases. Of the 113 breast cancer cases analysed by immunohistochemistry for the study, 78(69%) were triple negative breast cancer. TNBC was predominantly invasive carcinomas, 72 (92.3%) cases. The histological Grades 1, 2, and 3 recorded 1 (0.9%), 43 (38.1%), and 56 (49.6%) cases, respectively. **Conclusion:** The present study buttresses the fact that breast cancer in Nigeria is predominantly triple negative (69%) and have a high unfavourable molecular subclass (Basal-like). It also shows that triple negative /basal like tumours have high cases of grade 3 carcinoma (53.8%) which may partly explain the poor treatment outcomes being experienced. It also shows that FISH if available, can influence management decisions in cases that are HER-2 equivocal by immunohistochemistry.

Key words: Triple Negative Breast Cancer(TNBC), immunohistochemistry, FISH, Maiduguri

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Introduction

Breast cancer is a clinically heterogeneous disease.¹ It is the most common cancer among women worldwide.¹ More than two million cases occur annually worldwide.² GLOBOCAN 2022 data indicated that breast cancer is the most frequently diagnosed female cancer worldwide accounting for 11.6% of all diagnosed cancer.² It is also the most frequently diagnosed cancer in Africa (16.8%)³ and Europe (13.1%)⁴. An estimated 2.9 million women live with breast cancer in the United States of America.⁵ The incidence in the USA is higher among non-Hispanic whites but mortality is higher among African Americans. In Africa, breast cancer accounts for 27.6% of all cancers in females.⁵ The incidence varies with regions across the continent. In Nigeria, breast cancer has been reported to be the most common cancer in females in many centres. Ezenkwa *et al*⁶ in a recent study in Northeastern Nigeria showed that breast cancer is the leading cancer accounting for 22.9% of all cancers in the region. Despite much research work in the field of cancer research, it is still a mystery why a neoplasm arising in an organ readily accessible to self-examination and clinical diagnosis, continues to exert such a heavy toll.⁷ Late presentation, lack of systemic screening programs, ignorance of the nature of disease, economic, sociocultural factors for breast cancer among many other limiting factors has hindered the management of breast cancer, with up to 90% of cases diagnosed at stage III-IV of the disease in Africa.⁸ Seeing that patients with early breast cancer can present with metastasis, breast cancer is now considered a systematic disease from the outset and the approach toward clinical assessment and management of breast cancer has substantially changed over the past few decades to reflect the emerging reality.⁸ The trend is toward a more conservative surgical approach and use of chemotherapy and hormonal therapy. The use of adjuvant therapy has clearly proven beneficial by helping eradicate metastatic deposits.¹ Breast cancer has traditionally

been classified on the basis of its histopathological features which have been shown to have low prognostic and predictive value.⁹ A more recent molecular classification that is based on hierarchical clustering analysis of gene expression profiling has been proposed. This microarray based molecular classification has better prognostic value and is of predictive value therapeutically.¹⁰ The molecular classification has been shown to bear striking similarity to those of immunohistochemically defined schemes using antibodies to known breast markers.¹¹ These markers are used as surrogates for the molecular classification. Immunohistochemistry as an adjunct in assessing molecular pattern of breast cancer has contributed to prognostication of the disease.¹² Currently, three biomarkers are commonly used to classify breast cancer including estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2)¹² Assessing these markers help to select patients appropriately for treatment targeted on these major molecular pathways of the disease.

A breast cancer that fails to express ER and PR, and has absence of HER-2 overexpression is designated “Triple Negative.”¹³ Triple negative breast cancer (TNBC) compared with other groups of breast cancer describes a subtype of this malignant disease with worse prognosis and dismal survival outcomes.¹⁴ It is associated with unfavorable and aggressive clinicopathological features including onset at a younger age, high grade, pushing borders, poorer Nottingham prognostic index, much frequent node positivity, higher incidence of recurrence, distant metastasis, and poorer survival.¹⁵ The TNBC is described also as a heterogeneous disease because molecular panels such as basal keratin and other markers (Ck5/6, Ck14, Ck17, 34BE112, CD117, EGFR, P53, and SMA) have been used in subtyping TNBCs into two groups.¹⁶ Positivity for these stains describes the basal TNBCs, to differentiate it

from the non-basal subtype. The former constitute the majority of TNBCs and has a worse prognosis than the latter.¹⁷ This study is aimed to determine the proportion of TNBCs in 113 patients who had immunohistochemical studies (ER, PR and HER-2) and FISH (fluorescent in situ hybridization) as well as their relationship to histological type and histological grade.

Methods

The study was conducted at UMTN, Maiduguri which is the apex referral centre in the Northeastern region of Nigeria. The study was a hospital based and descriptive. One hundred and thirteen (113) breast cancer cases that fulfilled the study criteria had their archived tissue blocks along with their request cards retrieved (over a five-year period, 2010-2014). The histologic diagnosis and tumour grade were retrieved from the reports.

Immunohistochemistry was performed on the tissue blocks using monoclonal mouse antibodies to human ER, PR and HER-2 (Novocastra Post Primary Leica Biosystems REF711, 7102 and 7101 respectively).¹⁸

Sections from the tissue blocks were initially stained with haematoxylin and eosin (H&E) to determine their adequacy for immunohistochemistry. Four micrometer thick sections were taken from each tissue block and mounted on adhesive coated glass slides preparatory to immunohistochemical staining.

Sections were deparaffinised in xylene for 10 minutes, rehydrated in grades of alcohol followed by blocking of endogenous peroxidase activity using 0.5% hydrogen peroxide. Heat induced epitope retrieval was achieved using microwave incubation in citrated buffer (0.1M: PH6.0). Antigen localisation was achieved by incubating sections with primary antibody followed by application of primary antibody enhancer. The indirect avidin-biotin complex procedure using 3,3-aminobenzidine tetrahydrochlorate

(DAB) as substrate chromogen was applied for detection of bound antibodies.¹⁹

The scoring system for ER and PR status was based on J score which score the percentage of tumor cells with nuclear staining: Score 0, no stained cells; Score 1+, stained cells $\leq 1\%$; Score 2+, $1\% < \text{stained cells} < 10\%$; and Score 3+, stained cells $\geq 10\%$. Scores 0 was classified as negative; Scores 1 and 2 as intermediate; and Score 3 as positive. HER-2 staining was scored based on membrane staining pattern (intensity and completeness)-Hercept test: score 0, when no staining is observed, or is seen in $< 10\%$ of tumor cells; Score 1+, when staining is faint/barely seen in $> 10\%$ of tumor cells; Score 2+, when a weak/moderate complete staining is observed in $> 10\%$ of tumor cell; and Score 3+, when a strong complete staining is detected in $> 10\%$ of tumor cells. Score 0 and 1+ were classified as negative; Score 2+ as inconclusive (weakly positive); and Score 3+ as positive.²⁰ Specimens that fail to be positive for ER, PR, and HER-2 earned the triple negative designation.

Equivocal HER-2 cases had FISH done using the standard IQFISH protocol at Unistel Medical Laboratory, Stellenbosch University, South Africa and reported using the 2013 American Society of Clinical Oncologists /College of American Pathologists (ASCO/CAP) guideline.²¹

Results

A total of 113 cases of breast cancers were eligible for the study having fulfilled the inclusion criteria and all had immunohistochemistry done on them. Out of these, a total of 78 (69%) cases were negative for all three immunohistochemical markers and included in the study. Two biopsies were initially equivocal by immunohistochemistry and over amplified on doing FISH.

Invasive carcinoma (NST) constituted the vast majority of histological types of breast cancer, accounting for 100(88.5%) cases. The TNBC was predominantly invasive

carcinomas, 72 (92.3%) cases. The remaining 6 (7.7%) cases of TNBC were invasive lobular carcinomas. (Table 2)

The histological Grades 1, 2, and 3 recorded 1 (0.9%), 43 (38.1%), and 56 (49.6%) cases, respectively (Table 3). The association between histological grade and TNBC status for Grades 1, 2, and 3, were 1 (1.4%), 28 (38.8%), and 42 (58.3%) of cases, respectively.

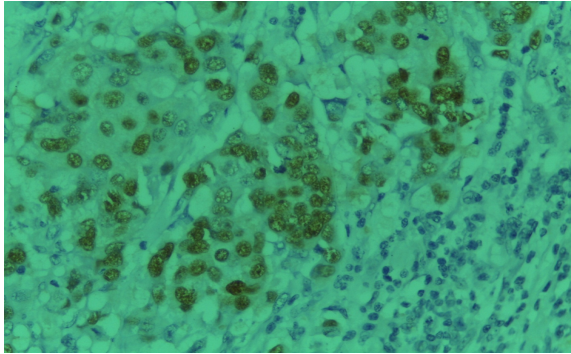


Figure 1: Photomicrograph of ER positive breast cancer
The section shows nuclear staining in about seventy percent of the tumour cells (J- Score) (X200)

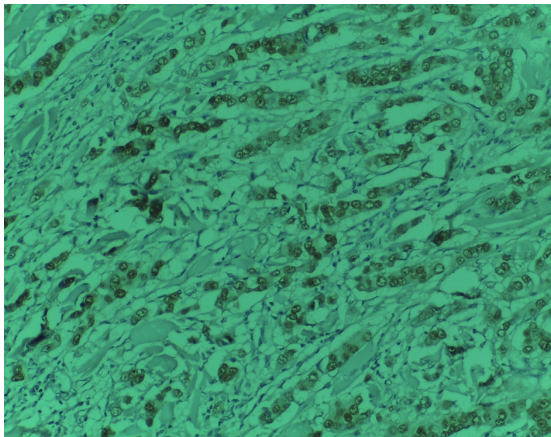


Figure 2: Photomicrograph of PR positive breast cancer
The section shows nuclear staining that is visible in sixty percent of the tumour cells (J-Score) (X200)

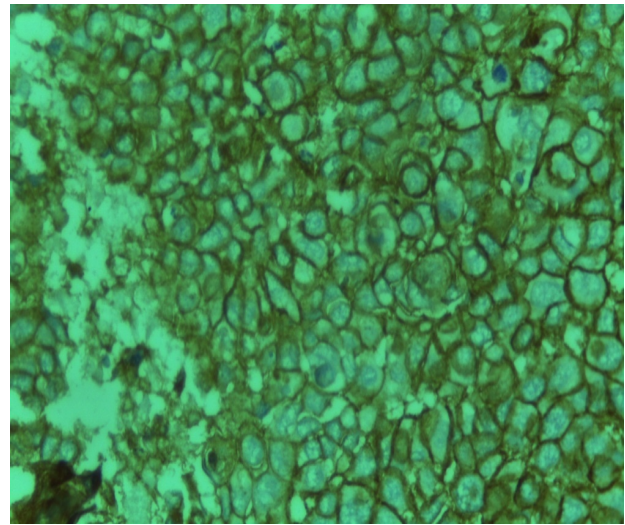


Figure 3: photomicrograph of HER-2 positive breast cancer
The section shows complete, strong membrane staining in 90% of tumour cells. (X200)

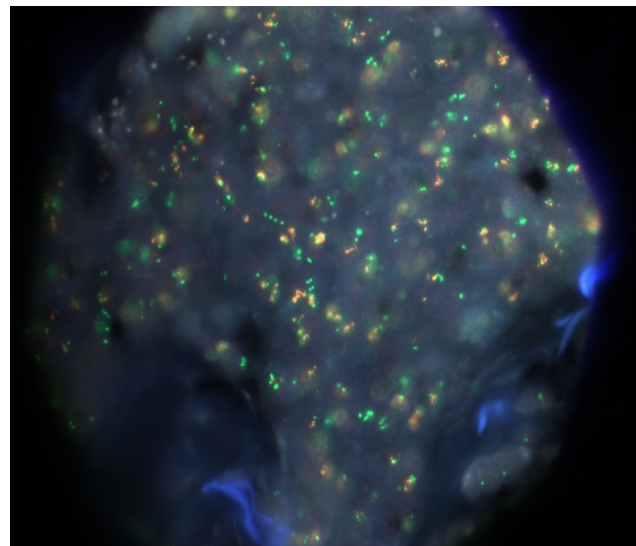


Figure 4: fluorescent in situ hybridization photomicrograph.
The photomicrograph shows amplification of HER-2. The orange signal represents *HER2* and the green signals chromosome 17(*CEP17*). The HER-2/cep 17 ratio was 3.49 and the average HER-2 copy number equals 12.05

Table I: Frequency Distribution of ER, PR and HER-2 among individual Breast Cancer Cases (N=113)

Immunohistochemical markers	Frequency distribution	Percentage (%)
ER, PR, HER-2 negative	78	69
ER, PR, HER-2 positive	0	0
ER, PR positive	10	8.8
ER, HER-2 positive	1	0.9
PR, HER-2 positive	1	0.9
ER positive	12	10.6
PR positive	1	0.9
HER-2 positive	10	8.8
Total	113	100.0

Table II: Frequency Distribution of Breast Cancer according to histologic types

Histologic Type	Frequency(%)	Frequency in TNBC(%)
Invasive Carcinoma NST	100(88.5)	72(92.3)
Invasive lobular Carcinoma	12(10.6)	6(7.7)
Mucinous Carcinoma	1(0.9)	-

Table III: Frequency Distribution of Breast Cancer according to histologic grade

Histologic Grade	Frequency(%)	Frequency in TNBC(%)
1	1(0.9)	1(1.4)
2	43(38.1)	28(38.8)
3	56(49.6)	42(58.3)
Special Types	12(10.6)	1(1.4)

Discussion

There were a total of 1415 histologically diagnosed cancers in the diagnosis register of Histopathology department UMTH during the five year study period (2010-2014). Out of this number, breast cancer constituted 276 cases accounting for 19.5% of all cancers diagnosed during the study period. This figure is higher than the 13.9% reported by Nggada *et al.*²² in the same centre 2001-2005. This may be attributable to more awareness on breast cancer and its screening methods as well as increased access to health care facilities by the rural dwellers in the region that were displaced by insurgency to Maiduguri the state capital.

The Index study observed the frequency of hormone receptors (ER and PR) and HER-2/neu (as shown in table I to be 24 (21.2%) for ER, 11(9.7%) for PR and 13 (11.5%) for HER-2 while 78 (69%) were negative for all three markers. The total frequencies are in agreement with most studies from other parts of Nigeria that show a low hormone receptor and HER-2 positivity and high triple negativity.²³⁻²⁹ Minoza *et al.*²⁵ in the same facility in 2016, reported triple negative breast cancers of 52.6% while Mandong *et al.* in Jos recorded TNBC of 41.3%.⁸

Stark *et al.*¹² in Ghana compared hormone receptor and HER status between White Americans, African Americans and Africans (Ghanaians) breast cancer patients and recorded ER of 22.8%, PR of 30.8% and HER-2 of 5.3% among Ghanaians. Their findings show a low hormone receptor and HER-2 positivity similar to the index study.

In two comparative studies between Nigerian and British breast cancer women, Agboola *et al.*^{26,27} reported ER of 72% and 51%, PR of 58.3% and 38.7% and HER-2 of 12.5% and 14.7% respectively among the British cohort. These findings are at variance with the index study and most other studies from Africa.

The results for hormone receptors ER and PR in the index study are similar to other studies

in Nigeria and Africa where breast cancer cases are more likely to be hormone receptor negative.²³ Some studies have shown that there is significant difference in oestrogen signaling and levels between Blacks and Caucasians with blacks having a decreased level of the protective *ERα* isoform.^{23,30}

The index study observed that invasive carcinoma NST was the most prevalent histological variant accounting for 100(88.5%) cases out of which 72(92.3%) are TNBC. This finding is similar to those of other researchers in Nigeria. Mandong *et al.*⁸ in Jos, recorded 92.3% of triple negative breast cancer cases being invasive carcinomas. Ohehe-Yeboah *et al.*³¹ recorded TNBC/basal-like subtype of 42.7% in Ghana,

Using the modified SBR (Nottingham) grading system, the index study observed that out of the 113 cases analysed, there was 1 (0.9%) case of grade 1 carcinoma, 43 (38.1%) cases of grade 2 carcinoma and 56 (49.6%) cases of grade 3 carcinoma as shown in table IV. Thirteen (11.5%) cases were special type carcinomas out of which 12 (10.6%) cases were invasive lobular carcinoma and 1 (0.9%) case a mucinous carcinoma. The findings from this study corroborates those of other studies from other centres in Nigeria and Africa where breast cancers from this part of the world are predominantly high grade and poorly differentiated. Minoza *et al.*²⁶ reported 63.2% of grade 3 cancers in Maiduguri. Other studies observed 60%, 69.7 70%, and 65% and in Uganda, Ife, Jos, and Gombe respectively.^{23,24,32,33} Studies from Switzerland, India and Oman reported lower prevalences of grade 3 cancers accounting for 27.1%, 22.9% and 30.2% respectively.^{34,35,36} Their findings are similar to those of Caucasians who are more likely to present with lower grade tumours (grade 1 and 2) which in this study accounted for 0.9% and 38.1% respectively. Studies have shown that histological grade alone or as a component of Nottingham prognostic index can be used to predict biological behaviour and outcome of breast cancer management.³⁵ Grade 3

tumours have been shown to have poor prognosis. Preponderance of grade 3 cancers in this study may further assert findings from other studies in Nigeria as cited above, that breast cancers in our setting are mainly high-grade tumours.

Studies have observed significant differences among molecular subtypes relating to tumour grade.³⁵ The index study showed that triple negative/Basal-like tumours showed high prevalence of grade 3 tumours 58.3% with well and moderately differentiated tumours constituting 1.4% and 38.8% respectively. Spitale *et al.*³⁵ reported similar findings with 75.9% of TNBC/basal-like tumours being high grade. They also alluded to the fact that other studies in Carolina, California and Poland showed poorly differentiated carcinoma constituting 82%, 71.1% and 72% of basal-like subtype respectively.³⁵

Immunohistochemical panels such as basal keratin Ck5/6 have been used in subtyping TNBCs into two groups.¹⁶ Positivity for these stains describes the basal TNBCs while negativity describes the non-basal subtype. The current study did not assess for CK5/6. It is also known that majority of TNBC arise in women with BRCA1 mutation.⁸ However, our study did not assess the status of BRCA1.

Conclusion

This study buttresses the fact that breast cancer in Nigeria is predominantly triple negative. The results show low hormone receptor and HER-2 positivity with an over representation of triple negative breast cancer which predicts poor response to hormonal therapy. The study suggests that empirical use of anti-hormonal therapy without prior receptor status testing in this environment be discouraged. It also shows that triple negative/basal like tumours have high cases of grade 3 carcinoma (53.8%) which may partly explain the poor treatment outcomes being experienced.

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Conflict Of Interest

The authors declare no conflicts of interest with respect to this research.

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