Clinicopathological features and molecular markers of breast cancer in Jos, Nigeria

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Summary

Background: **Several studies have suggested that breast cancer in black women is associated with aggressive features and poor survival. This study examines molecular markers along with clinical stage and pathological grade in breast cancer material from Jos, Nigeria.**

Study design: **The histological diagnoses of 178 consecutive Nigerian patients with breast cancer were retrieved from their hospital records.**

A subset of 36 patients was staged and their tumours typed and graded. Immunohistochemical staining of sections from paraffin wax embedded tissues from these cases for the expression of oestrogen receptor (ER), progesterone receptor (PGR), Human ERBB2 (or HER2/neu), p53 and cyclin D1 (CCND1) was carried out using the avidin biotin complex (ABC) procedure.

Results: **A majority of the cases were invasive ductal carcinoma (92.7%), high grade (grade 3, 70.6%) and of late clinical stage (stages III and IV, 58.3%). Only 25% and 27.8% of cases expressed ER and PGR respectively. The ERBB2 and CCND1 antigens were expressed in 25%, and 5.7% of cases respectively. The p53 protein was the most frequently expressed in this study (47.2% of cases). High grade tumours were significantly more likely to be ER and PGR negative (P = 0.006 and P = 0.002 respectively).**

Conclusion: **There is predominance of high grade, invasive ductal carcinomas which are likely to be ER and PGR negative but p53 positive. These features suggest a biologically aggressive form of breast cancer in Nigerian women with the possibility of poor response to both hormonal therapy and chemotherapy**

Key-words: *Breast cancer, Grade, Stage, Molecular markers, Jos.*

Résumé

Introduction: Plusieurs études avaient suggéré que cancer du sein chez des femmes noires est associé aux traits agressifs et une survie mauvaise. Cette étude examine les marqueurs moléculaire avec une étape clinique et grade pathoiogique dans le matériel du cancer du sein de Jos, Nigéria.

Plain d'étude: Le diagnostique histologique de 178 patientes nigérianes consécutives, atteintes du cancer du sein ont été tirés le leur dossiers dans l'hôpital. Un sous-ensemble de 36 patientes ont été mis en scène et leurs tumeurs ont été déterminées et calibrées. Les section de la teinuire d'immunohistochimique de la paraffine solide tissus intégrés de ces cas pour l'expression de récepteur oestrogène (ER), récepteur progestérone (PGR), ERBB2 humain (ou HER2/neu), p53 et cyclin D1 (CCNDI) était effectué a travers l'utilisation de la procédure Complexe Avidin Biotin (CAB).

Résultats: Le plus grand nombre des cas sont les suivant: carcinome invasif ductal (92,7%) niveau élevé (3,70.6%) et une étape clinique retardée (etape III et iv, 58,3%) seulement 25% et 27,8% des cas avaient exprimé ER et PGR respectivement. Le ERBB2 et CCND1 antigens ont été exprimés en 25% et 5,7% des cas respectivement. La p53 protéine était la plus fréquemment exprimée dans cette étude (47,2% des cas). Tumeurs de niveau élevé, étaient plus sensiblement vraisemblable d'être ER et PGR négatif (P = 0,006 et P = 0,002 respectivement).

Conclusion: Il y a une prédominance du niveau élevé, carcinome ductal invasif qui sont probable d'être ER et PGR négatif mais p53 possitif. Ces traits suggèrent une forme agressive biologique du cancer du sein chez des femmes nigérianes avec la possibilité d'une mauvaise réponse à la thérapie hormonale et chimiothérapie les deux.

Introduction

There is a worldwide variation of up to five folds in the incidence and mortality from breast cancer [1]. The incidence rate in Nigeria has steadily increased from 15.3 per 100,000 in 1976 to 33.6 per 100,000 in 1992 [2]. The prevalence rate is presently estimated at 116 cases per 100,000 women per year[3]. The 5-year survival after a diagnosis of breast cancer as reported by various studies in Nigeria is between 8-15%[4,5,6]. Studies among white and black American women have found similar poor outcome in black women even after adjustment for confounding factors like socioeconomic status and access to healthcare [7,8].

The use of molecular markers along with the traditional biological markers is being explored to produce a better index for the prediction of breast cancer risk, assessment of prognosis and selection of appropriate treatment[9,10,11]. For example, trastuzumab (Herceptin), a monoclonal antibody against ERBB2 has shown clinical benefit in 15-20% of ERBB2 positive breast cancer patients[12]. This study aims to investigate the molecular markers of the reported aggressive phenotype of breast cancer in Nigeria. It is also important to define the molecular characteristics of the African tumours so that African patients with breast cancer can benefit from these and other recent advances in molecular genetics.

Materials and Methods

The age and specific histological diagnosis of 178 consecutive Nigerian patients with breast cancer seen at the Jos University Teaching Hospital were retrieved from their hospital records.

A subset of 46 archival formalin fixed, paraffin wax embedded breast cancer tissue blocks from consecutive female patients were selected for immunohistochemistry. Sections from these blocks were first stained with haematoxylin

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and eosin to determine their adequacy for immunohistochemical staining. They were also regraded by the same pathologist. Ten were excluded from the study because the amount of tissue was either inadequate or necrotic. From the remaining 36, serial paraffin tissue sections were taken from each selected tissue block and mounted on adhesive coated glass slides for ER, PGR, ERBB2, p53 and CCND1 staining as follows:

Sections were deparaffinised by heating at 60°C for ten minutes followed by three washes in xylene. They were rehydrated in graded alcohol. The slides were placed in 0.5% hydrogen peroxide for 10 minutes to quench endogenous peroxidase and washed in tap water. Antigen retrieval was achieved by microwave oven incubation for five minutes in citrated buffer (0.01M: pH 6.0) testing for ER, PGR, ERBB2 and p53 oncoproteins and in 1mM EDTA (pH 8.0) for CCND1. Antigen localisation was achieved by incubating sections with primary antibodies at various dilutions (see table 1). The indirect avidin-biotin complex (ABC) procedure using 3,3-diaminobenzidine tetrahydrochloric (DAB) as the substrate chromogene was applied for detection of bound antibody.

Immunostaining was assessed by two independent observers. Nuclear staining of at least 10% of malignant cells was considered to indicate a positive result for ER, PGR, CCND1, and p53. Cell membrane staining of at least 5% was used as the cut off point for ERBB2 positive staining.

The Chi-Squared and Fisher's exact tests were used for statistical analysis.

Results

Age at presentation: The mean age at diagnosis was 44 years (SD 12.4; range 14-90) with a median of 42 years

Clinical stage: There was no clinical stage I tumour while 41.7% were stage II disease. Clinical stages III and IV made up 38.9% and 19.4% respectively of the cases.

Histological type (larger group): In the larger group of 178 patients, invasive ductal carcinoma (not otherwise specified NOS) made up 92.7% of all the cases. There were no lobular carcinomas. Special types of invasive ductal carcinoma were seen in 5.1% of cases, of which medullary and mucinous carcinomas made up 1.7% each. Rare types of cancers like squamous cell carcinoma, malignant phyllodes tumour and rhabdomyosarcoma were only seen in 2.2% of the cases (see table 2)

Table 1 Dilutions for antigen localisation for ER, PGR, ERBB2, p53 and CCNDI

Antibody	Dilution	Makers
Polyclonal rabbit antihuman ERBB2	1 : 1000	Dako, Glostrup, Denmark
Monoclonal mouse antihuman (ER6F11)	1 : 15	Novocastra, Newcastle, UK
Monoclonal mouse antihuman PGR (PGR636)	1 : 50	Dako, Carpinteria, USA
Monoclonal mouse antihuman p53 (DO - 7)	1 : 50	Dako, Glostrup, Denmark
Monoclonal mouse antihuman CCNDI (P2D11F11)	1 : 20	Novocastra, Newcastle, UK

Table 2 The histological types of breast cancer seen in this study

Histological types		Jos	Percentage
Invasive ductal carcinoma Not otherwise specified (NOS)		165 (92.7%)	92.7%
Invasive ductal carcinoma Special types	- Mucinous	3	1.7%
	- Medullary	3	1.7%
	- Comedo	2	1.1%
	- Papillary	1	0.6%
	Subtotal	9	5.1%
Rare cancers	- Squamous cell	1	0.6%
Malignant phyllodes tumour		1	0.6%
Rhabdomyosarcoma		2	1.1%
	Subtotal	4	2.2%
	Total	178	100%

Table 3 The result of grading and immunohistochemistry for ER, PGR, ERBB2, p53 and CCND1

	Nigerian samples x/n	Percentage positivity
Molecular markers		
ER	9/36	25%
PGR	10/36	27.8%
ERBB2	9/36	25%
CCND1	2/35	5.7%
P53	17/36	47.2%
Clinical stage		
Stage I	0/36	0%
Stage II	15/36	41.7%
Stage III	14/36	38.9%
Stage IV	7/36	19.4%
Histological grade		
Grade 1	2/34	5.9%
Grade 2	8/34	23.5%
Grade 3	24/34	70.6%

Key
ER: Oestrogen receptor
PGR: Progesterone receptor
x: Positive cases
n: Number of cases analysed

Histological type and grade (smaller group): In the smaller group of 36 patients, all the tumours studied by immunohistochemistry were of the invasive ductal type. Two of the samples could not be graded because the amount of tumour present was too small. Of the remaining 34, two (5.9%), 8 (23.5%) and 24 (70.6%) were grades 1, 2 and 3 respectively (see table 3).

Molecular Markers: One slide did not stain satisfactorily for CCND1 and was excluded from the analysis. Immunohistochemistry staining for ER and PGR was positive in 25% and 27.8% of cases respectively. The ERBB2 and CCND1 antigens stained positive in 25%, and 5.7% of cases respectively. The p53 protein was the most frequently expressed in this study (47.2%of cases).

Oestrogen receptor positive tumours also significantly over expressed PGR (P = .0001) while ER and PGR negative

Table 4 Association between the molecular markers shown by P-value

Variable	ER*	PGR*	ERBB2 *	p53*	CCND1	Grade** 1,2,3	Stage* 1 & 2 3 & 4	Age* 49 & < 50 & >
ER	X	0.0001	1.000	0.128	0.061	0.006	0.252	1.000
PGR	0.0001	X	1.000	0.065	0.076	0.002	0.142	0.689
ERBB2	1.000	1.000	X	0.451	1.000	0.454	0.705	0.006
p53	0.128	0.065	0.451	X	1.000	1.717	0.736	0.717
CCND1	0.061	0.076	1.000	1.000	X	0.012	0.496	1.000
Grade	0.006	0.002	0.454	0.717	0.012	X	0.507	0.664
Stage	0.252	0.142	0.705	0.736	0.496	0.507	X	0.468
Age	1.000	0.689	0.006	0.717	1.000	0.664	0.468	X

Keys
ER: Oestrogen receptor
PGR: Progesterone receptor
X: Not comparable
*Fisher's Exact Test**
*Chi-Square Test***

tumours were significantly likely to be of higher histological grade than ER or PGR positive ones (X^2=10.238, P = 0.006) and (X= 12.325, P = 0.002) respectively. Although the p53 positive tumours tended to be ER and PGR negative, this was not statistically significant (P = 0.128 and P = 0.065 respectively).

The two CCND1 positive tumours were grades 1 and 2 (X=8.410, P = 0.015) and were both ER and PGR positive (P = 0.061 and P = 0.076 respectively).

Women aged 50 years and above (N= 10) were more likely to express ERBB2 compared to women 49 years and below (N = 26) (P = 0.006) (see table 4).

Discussion

The mean age at presentation of 44 years is similar to previous studies in Nigeria[13,14]. A number of studies comparing Africans and Caucasians have reported this tendency for breast cancer to be diagnosed in relatively younger Africans[15,16]. The reason for this is not clear. In Nigeria, this may be explained partly by the very young age distribution of the Nigerian population[17].

We have found a late clinical stage at presentation, a high rate of invasive ductal carcinoma (IDC) and a high frequency of grade 3 tumours. Various studies have made similar observations[2,5,18]. The late clinical presentation has been attributed to socioeconomic factors, patronage of native doctors and low utility of health facilities[4,13,19]. All the tumours in this study were invasive; this finding probably reflects the absence of a national breast screening programme and late detection of cases in Nigeria. This is in contrast to the situation in Europe and America where screening mammography is routine and ductal carcinoma in situ (DCIS) which previously made up less than 5% now accounts for 15-20% of all breast cancers[20,21]. There were no invasive lobular carcinomas (ILC) in our study. Walker[22], studying breast cancer in young British women did not find any case of ILC in women less than 40 years of age. In our study, the average age was 44 years; this relatively young age distribution may explain the absence of ILC[22]. Since IDC is known to be more aggressive than ILC, the predominance of IDC is one factor which predicts a poorer prognosis for the Nigerian patients[23].

With regards to hormone receptors; our results are similar to most previous studies which have shown that Africans are more likely to have hormone receptor negative tumours[24,25,26]. In some of these studies[25,26], there is a positive correlation with late stage at presentation, but this does not agree with our finding. Poola et al[27] found that there was a significant difference in oestrogen signaling between blacks and whites in America. The protective ERâ isoform was significantly decreased in the blacks. They believe that this is partly responsible for the aggressive clinical course of breast cancer in this ethnic group. These observations are of critical clinical importance. Firstly, the use of antioestrogens and other hormonal therapies that have revolutionized breast cancer treatment in Europe are less likely to be effective in the Nigerian patients[28,29]. Secondly, there is evidence pointing to the fact that much of the epidemiology of breast cancer is linked to endogenous hormones, particularly oestradiol[30] and altered oestrogen metabolism caused by polymorphisms in various enzymes in its biosynthesis[31,32].

The 53 gene was the most frequently over expressed marker in this study. Several studies have found mutations and over expression of this gene in black populations associated with younger age at presentation, ER negativity and other tumour features of aggressive biological behavior[16,33]. Though the p53 positive tumours showed a tendency to be ER and PGR negative in our study, this was not statistically significant (P=0.128 and P=0.065 respectively). In addition, we did not observe any significant association between p53 expression and age at diagnosis or histological grade. Shiao et al[34] found that black Americans with p53 gene alterations had a significantly poorer survival compared to white Americans with p53 gene mutation. Tumours expressing p53 have been found to be resistant to chemotherapy (which is thought to arise from impaired apoptosis, a mechanism the drugs use for inducing cytotoxicity)[35].

Only two of the tumours expressed CCND1 and both were ER and PGR positive. These results are in agreement with other studies which have found that tumours that express ER and PGR are more likely to express CCND1[36,37]. The low expression level of CCND1 in our study is however, at variance with the finding of Joe AK et al[38].

The expression of ERBB2 in this study was lower but not significantly different from that seen in other studies and in studies comparing blacks and whites[8,39]. Interestingly, in this study, tumours in older women were more likely to express ERBB2. This may define a subset with a worse prognosis that would benefit from additional adjuvant treatment[13].

The major limitation of this study is the small number of tumours studied by immunohistochemistry. This may reduce the strength of the correlation between the molecular markers, tumour grade and types and the overall conclusion. Despite this, our study has agreed with many larger studies on the subject.

Conclusion

It is of concern to observe that apart from presenting late with higher tumour grades, breast cancer in Nigerian women has low expression levels of ER and PGR which may suggest possible poor response to hormonal therapy. Furthermore, the high level of p53 expression suggests possible resistance to chemotherapy. Molecular genetic and epidemiological studies are needed to clarify these observations and correlate them to clinical outcomes. There is need to search for the most appropriate and effective treatment option for the Nigerian women with breast cancer in view of these observations.

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