IMMUNOHISTOCHEMICAL EXPRESSION OF P53 PROTEIN, HISTOLOGICAL TYPE AND GRADE IN INVASIVE BREAST CARCINOMAS

ABSTRACT

Background: Breast cancer still remains the commonest cause of cancer related deaths in women. The aim of this study is to determine the proportion of p53 expression in breast cancer cases in OAUTHC Ile-Ife and to compared with histological grades and types.

Method: Eighty-five cases that were diagnosed as breast cancer within a 2-year (2018-2019) period were retrieved. The tumours were graded using Nottingham grading system and the histological types were stated. Immunohistochemistry for p53 was performed on retrieved representative tissue blocks and its level of expression was scored as positive or negative. The association of p53 expression and the histological types and grades were sort using the chi square statistical test to compare variables and p value < 0.05.

Results: The Invasive Ductal Carcinoma-Not Otherwise Specified (IDC-NOS) was the commonest histological types (92.9%). The histological grade 2 and 3 predominated accounting for 48.2% each. Of all the breast carcinoma cases analysed, p53 positive expression was found in 52.9%. The median age of 50.6 years was observed in p53 positive patients and 49.4 years in p53 negative patients. Even though most high-grade tumours were p53 positive, statistical analysis showed no significant association between p53 positivity and histological grades and histological types.

Conclusion: Many of our series show expression of p53 and the high grade tumours bare high level of p53 expression. This finding though not statistically significant may suggest aggressive behaviour of tumours.

INTRODUCTION

The incidence of cancer is rising worldwide, putting a huge burden on the existing health resources. The incidence of breast cancer had remained constant for many years but has gradually increased in the last decade.[1] A cancer data base released by the Descriptive Epidemiology Group of the International Agency for Research on Cancer (GLOBOCAN 2018) put the incidence of breast cancer in West Africa at 37.3/100,000 female.[2] The incidence seems to be rising in developing countries such as Nigeria, Ghana and South Africa.[2,3] In Nigeria, the latest number of new cases of breast cancer in females is 26,310 (37%).[2] The rising incidence has been attributed to improved diagnosis, access to good health care and changes in life-style.[3]

Tumour size, grade, lymphovascular invasion, number of axillary lymph nodes involved, hormone receptors status and Her-2 status are predictive and prognostic indicators of breast cancers. Studies have shown that mutation in p53 is more common in patients of African descent irrespective of their location and its over expression has also been identified as a poor prognostic marker in breast cancer amongst other factors.[4,5] Similarly, p53 is closely related with clinicopathological findings like lymph node metastasis, high histological grade and Her2 overexpression.[6,7] The clinical course of patients with breast carcinoma varies greatly and this depends on many of these factors.[7] The knowledge about the impact of p53 on these factors is also controversial. Therefore, this study aims to identify the proportion of breast cancers that express p53 protein and to compare them with histological type and grade.

METHODOLOGY

Eighty-five cases of histologically diagnosed breast cancer from the in the Department of Morbid Anatomy and Forensic Medicine Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Osun State within a 2-year period (2018- 2019) were for selected the study.
The inclusion criteria are all samples diagnosed with breast carcinoma from biopsy, lumpectomy or mastectomy specimens are included in the study. Non-epithelial tumours of the breast, all cases in which the tissue blocks could not be retrieved and any lumpectomy or mastectomy case which had a previous biopsy diagnosis of breast carcinoma to prevent double accession were excluded.

The age, gender, nature of specimen and other relevant clinical information were obtained from the records. Representative sections from Formalin Fixed Paraffin Embedded (FFPE) tissues blocks were obtained for Haematoxylin and Eosin (H&E) stain and immunohistochemistry. The slides were reviewed and graded using the Modified Bloom and Richardson grading system.
Immunohistochemical staining for p53 was done on the cases using mouse monoclonal antibody by DAKO. An indirect immunoperoxidase method was used according to standard laboratory protocol. Tonsilar tissue was used for p53 positive control while negative control was obtained by replacing the primary antibody with non-immune serum.

P53 was considered positive where there was greater than or equal to 5% positive nuclear staining regardless of the intensity.[8]
The overall expression was further evaluated and graded according to Evaluation of IHC for p53 as follows: (0-5% of p53 staining are evaluated as negative and graded 0 while 6-25%, 26-50%, 51-75%, 76-100% staining are evaluated as positive and graded 1+, 2+, 3+ and 4+ respectively.)
The data generated was analysed using SPSS version 20.

Chi-square test statistics was used to determine association between p53 expression, the patients' biodata and histopathological features outlined earlier. The level of significance was set at $P<0.05$.

Ethical approval was obtained from the Ethics and Research Committee of the Obafemi Awolowo University Teaching Hospitals Complex with assigned number-ERC/2019/08/06

RESULTS

Demography of Patients with Breast Carcinoma.

The total number of cases seen during the study period was eighty-five and all were females. The age range of patients was 32 to 76 (mean age = 50.07 $\pm$ 9.79 years, modal age = 47 years, median age = 50 years). The majority of the cases were within the fifth and sixth decade age group and accounted for 70.6% of the of cases while 10.6% of the cases were above sixty years (Table 1)

Table 1: Showing Age group distribution of the Breast Carcinoma cases.

Age group	Frequency	Percentage (%)
31-40	16	18.8
41-50	30	35.3
51-60	30	35.3
61-70	5	5.9
71-80	4	4.7
Total	85	100

Histological Types and grades of breast carcinoma

The histological types of breast carcinoma seen were Invasive Ductal Carcinoma 79 (92.9%), Metaplastic carcinoma 5 (5.9%) and Lobular carcinoma 1(1.2%). The photomicrograph of the histological types is depicted in Figures i,

The histological grade 1 tumour, accounted for 3(3.5%) grade 2 and 3 histological tumours, accounted for 41(48.2%) each of the total number of cases.

Immunohistochemistry Expression Profile of p53 in Breast Carcinoma

Among the cohort of breast carcinoma, forty-five cases showed positive expression of p53 while forty cases did not express p53. In the positive breast carcinoma cases, based on the percentages of cells positive for p53, 17 (20.0%), 10(11.8%), 6(7.1%) and 12 (14.1%) of the cases were scored (+1), (+2),

(+3) and (+4) respectively. The photomicrographs of score 0, 1+ and 4+ and p53 positivity in invasive ductal and lobular carcinoma are demonstrated in Figures ii.

.**Comparison of p53 protein expression with Histologic Types and Grade of Breast Carcinoma.**
The p53 expression in these histological variants were 88.9%, 8.9% and 2.2% for the Invasive Ductal Carcinoma, Metaplastic carcinoma and Lobular carcinoma. histological types respectively.

Histological Grade 3 tumours were responsible for the highest number of cases 23 (51.1%) with p53 immunoreactivity. Whereas grade 2 and grade 1 tumours were positive for p53 in 19 (42.1%) and 3 (6.7%) cases respectively**.** There was, however, no statistically significant difference in the p53 staining pattern in these histological types and grades (p=0.283). (Table 2).

Table 2: Expression of p53 in different histological types and grades of Breast Carcinoma.

Histological Type/Grade of breast Carcinoma	p53 expression		Total frequency
	Negative(%)	Positive(%)	
Invasive Ductal Carcinoma	39(97.5)	40(88.9)	79
Lobular Carcinoma	0(0)	1(2.2)	1
Metaplastic Carcinoma	1(2.5)	4(8.9)	5
Total	40 (100%)	45 (100%)	
Histological grade			
1	0(0)	3(6.7)	3
2	22(55)	19(42.1)	41
3	18(45)	23(51.1)	41
Total	40(100)	45(100)	

DISCUSSION

Demography of Patients with Breast Carcinoma

A mean age of breast carcinoma diagnosis observed in this study is similar to that reported by other studies conducted in Nigeria,[9] Malaysia, and Iran[10,11]. This also correlates with findings by Ohene-Yeboah et al[12] in Ghana and Kallel et al in Tunisia who reported a mean age of 49.19 and 50 years respectively.[13] The percentage of the study population reported is similar to Adelusola's study and other previous studies from this Institution. In most studies in Africa, the highest peak age of breast cancer is seen in the 5th decade, unlike in this study where we observed a peak in the 5th and 6th decade.[14] Some other studies also showed similar peak age group.[1,15,16] Adelusola et al[17] in Ile-Ife observed peak age groups of 40-49 and 60-69 years. Literature have also supported that, in Africa, breast carcinoma occurs more in the premenopausal period unlike in Europe and America.[18]

Histological Types and Grades of Breast Carcinoma.

Invasive Ductal Carcinoma (Not Otherwise Specified) of the breast was the commonest histological type. This is not different from observation by other authours.[19,20,21] This is similar to Rambau and colleagues[22] in a Tanzanian retrospective study that used a larger sample size (328), and obtained a frequency of 91.5%. Other studies in South West Nigeria by Daramola et al[23] in Lagos and Titiloye et al[19] in Ile-Ife have also reported Invasive Ductal Carcinoma (Not Otherwise Specified) as the commonest histological type, even though lower frequencies of 63.5% and 87.3% were reported respectively in their series.

Metaplastic carcinoma was the second most common type of breast carcinoma cases in this series. One of the metaplastic carcinoma cases was initially diagnosed as Invasive Ductal Carcinoma (NOS). This is similar to Daramola et al[23] in Lagos who also had metaplastic carcinoma as the second commonest in their series, but with a much higher frequency of 14.8%. This difference may be due to sampling errors of the biopsy cases used in this study as metaplastic carcinomas have classical pathologic features, where their ductal component may be partially or totally replaced by non ductal (non glandular) components[24] and this can be affected by sampling errors. It may also be due to the possibility of special types of invasive ductal carcinomas being under-diagnosed in the cases reviewed.

The number of cases that were graded 2 and 3 in this study cohorts outweighs the low grade tumours. High histological grade is commonly seen in blacks generally.[25,19,10] A similar trend has been observed

in a previous study in Ile-Ife by Titiloye et al and in Ghana by Ohene Yeboah et al. Some of the reasons are said to be related to genetic factors and late presentation.[18,22] In particular, BRCA1/2 gene mutations which are associated with breast cancer are described in black populations.[26,27,28] However, other mutated genes associated with breast cancer have also been identified in white populations such as HER2, APOBEC3B, RAD50 and SMAD4 genes.[29] Emphasising further, late presentation may make tumours de-differentiate into higher grade tumours overtime. Our observation agrees with others that higher grades are seen more in African Blacks than their European counterpart.[19,18,30]

p53 Expression in Breast Carcinomas.

This study is unique being the first study of such in our centre to determine the p53 expression of breast carcinoma and relating it to other known prognostic indices. The result of p53 immunohistochemical staining revealed this finding is similar to the study by Sirvent et al[21] who worked on Spanish patients. However, his value was slightly lower. The reason for the difference is not clear. Many researchers have supported the fact that p53 expression in tumours usually signifies a more aggressive behaviour,[31-32] thus we may conclude that there is an intrinsically more aggressive tendency of tumours in our local environment as evidenced by the higher p53 expression. A lower percentage (29.6%) was reported in Malaysia, although, the sample size of the Malaysian study was higher at three hundred and eighty-two.[10]

We also observed that the younger age groups had higher expression of positive p53 expression. This may be difficult to explain from this study, although many studies have observed that p53 protein expression and Tp53 gene mutation are associated with poor prognosis.[31,33] Few reports have mentioned the relationship of p53 with early onset of breast cancer in ages less than forty years,[34] except in Li-Fraumeni syndrome in which in addition to early onset of breast cancers, patients also present with other childhood tumours.[4,35,36]

Relationship of p53 Expression, Histological Types and Histological Grades.

The histological type of breast carcinoma is a known prognostic factor that is related to tumour biology. This study showed a greater percentage of Invasive Ductal Carcinoma (NOS), however, metaplastic and lobular carcinoma of special types showed a high expression of p53 immunostaining. Observations from other studies have shown that some special histological types (Metaplastic, micropapillary subtypes) are associated with bad prognosis.[4,33,37,38]

Furthermore, high histological grades and p53 mutation in invasive carcinoma is an indicator of bad prognosis.[31-32] Different observations are recorded on p53 expression in association with grade and other traditional prognostic markers in breast carcinoma. In this cohort our findings agree with what is in the literature, that higher grade tumours generally tend to have high p53 expression.[39,10,21] More so, most cases that expressed the p53 protein were grade 3 (high grade) tumours and all the low grade still expressed p53. This is in contrast to what might be expected of these low grade tumours and this may mean that p53 expression alone cannot be used as a sole factor in prognosticating breast carcinomas.

Olufemi and colleagues in Lagos noticed a similar trend in which 89.6% of breast carcinomas positive for p53 were seen in high grade tumours[25]. The high grade in their series corresponds to grade 2 and 3 in this study. The smaller sample size used in this study may explain the higher percentage observed in our study. The high level of p53 expression in this study may support the aggressive nature of breast cancers, in addition to late presentation, poor access to quality health delivery system and financial constraint experienced in a resource-poor country like ours.

The histological grade is one of the factors considered to indicate the clinical course of breast carcinoma. In our study, there was no association between p53 expression and histological grade of breast carcinomas. Similar to us, Olufemi et al[25] from Lagos and Robab et al[40] from Iran reported that there was no statistically significant association between p53 expression and the histological grades. Sirvent et al[21] and Joudi et al[10] noticed a statistically significant association between p53 expression and histological grade. Keiichi et al,[41] also found a strong association between p53 expression and histological grade and linked this association to bad prognosis. The reason for this discordant finding may be attributed to inter-observer variability associated with grading especially the intermediate grade 2. It may also mean that many other factors including other genetic mutations determine the grades of breast cancer asides Tp53 gene mutation in ours

5.2 Conclusion

In conclusion, a key finding of this research was that the majority of our study population were in their 5th and 6th decade. This same age group was found to have the highest percentage of p53 expression. Most of the cases that showed immunoreactivity to p53 had high histologic grade. We did not find any statistically significant association between the histological types and the different histological grades with their expression of p53.

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Appendix 1: Histological types and p53 staining pattern

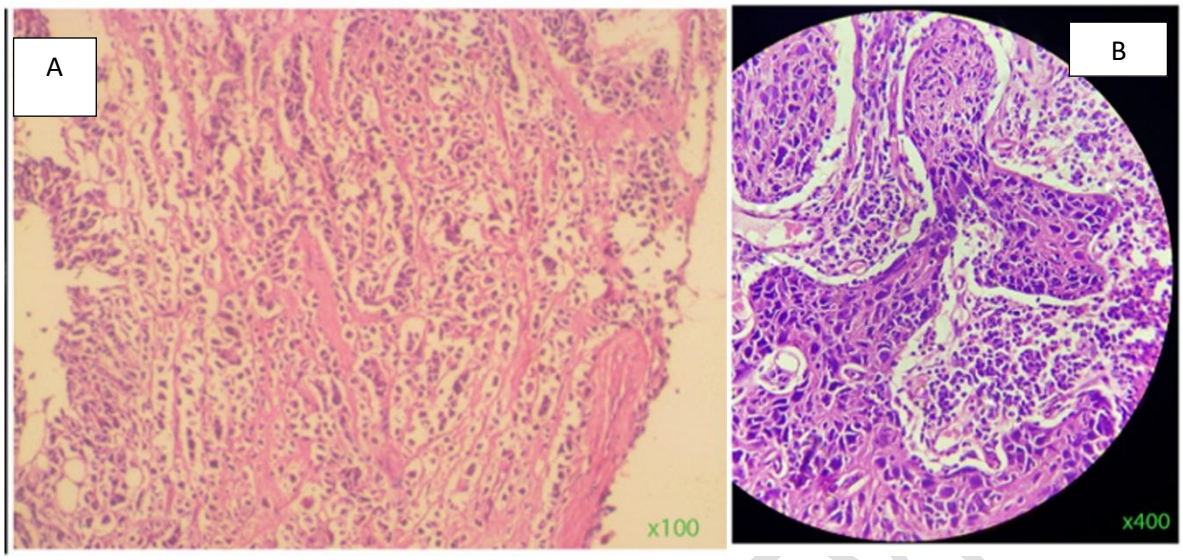

Figure 1: A: Lobular carcinoma B Metastatic carcinoma (Hematoxylin and Eosin stain)

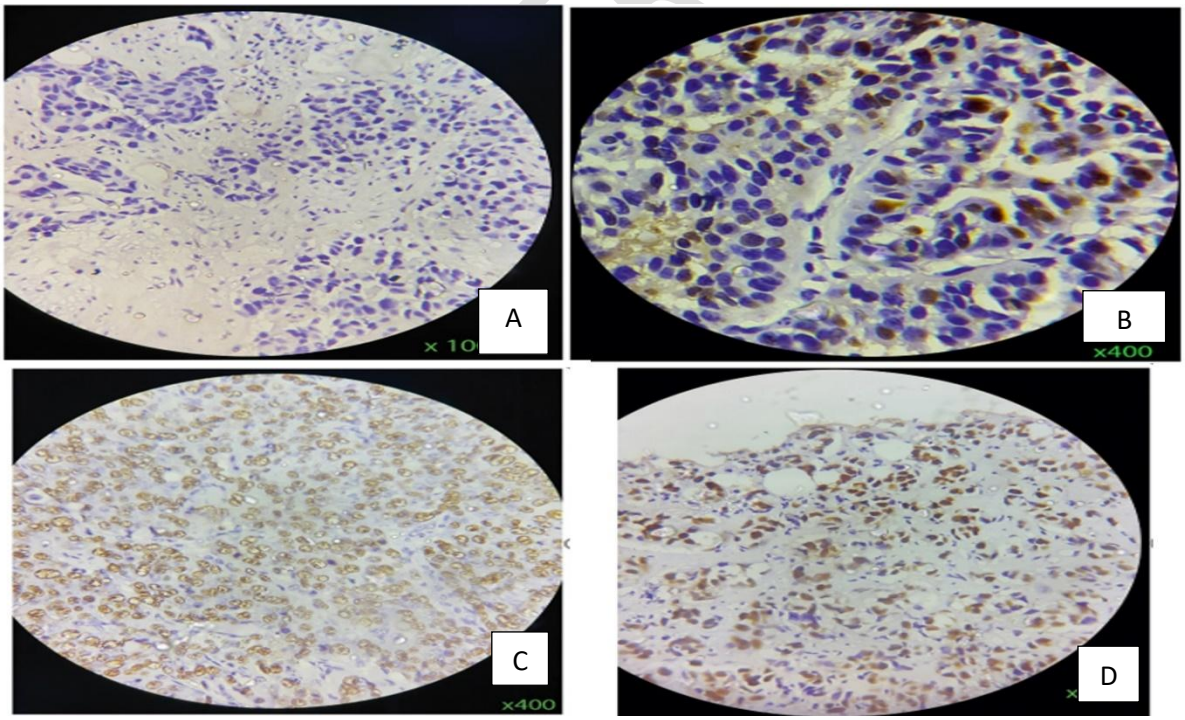

Figure 2: p53 score (A : p53 negative, B, C and D are p53 positive staining