Original Article

Correlation of p53 Expression with Clinicopathological Characteristics of Breast Carcinoma

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Keywords: *Breast Cancer, Invasive Ductal Carcinoma, Lymph Node, Nucleus, P53 Over Expression, Immunohistochemical.*

ABSTRACT

Background: Breast carcinoma has become the most common malignancy in the female population. The p53 gene is a breast cancer progression gene that regulates the cell cycle and DNA repair and it's over expression associated with a worse prognosis. The importance of studying the various prognostic factors in breast carcinoma so as to identify patients at high risk of early recurrence and thus to more effectively target aggressive adjuvant chemotherapy, radical mastectomy & intensive follow up protocols.

Methods: The prospective study was conducted in the department of Pathology, M.K.C.G. Medical College, Berhampur from 2013 to 2015. Immuno-histochemical evaluation of a total 72 Patients was conducted who were confirmed to have breast carcinoma histologically.

Result: Our study showed that majority of 64 cases was positive for p53 expression. Maximum no. of T1, T2, T3 tumors showed moderate & high p53 expression. Maximum number of cases showed moderate & high p53 expression in patients with N2 & N3 lymph node involvement. 50% of patients showed high p53 expression in patients with N3 lymph node involvement. 47% of Grade I tumors showed moderate p53 expression. Maximum no. of Grade II tumors and 41% of Grade III showed moderate to high p53 expression. Invasive Ductal carcinoma (Not Otherwise Specific) showed maximum of moderate to high p53 expression.

Conclusion: A significant correlation of p53 with tumor grade and also with lymph node status was found, but not with tumor size. In breast cancer, we suggested that the over expression of p53 protein in the nucleus is an indicator of poor prognosis.

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Introduction

Breast carcinoma has become the most common malignancy in the female population, effecting one in eight women and is one of the leading causes of mortality among women in developing countries.[1] The number of tumor-related features available to predict the prognosis of patients with breast cancer has grown markedly in recent time period. Lymph node status, tumor size and histological grade are now supplemented with measurements of steroid hormones receptors, proliferation index, tumor suppressor genes, and growth factors, oncogenes and oncogenes products. Tumor size and axillary lymph node status are most important classic variables in the predicting the prognosis of breast cancer. Several investigators have shown that the 5 year recurrence rates in patients with axillary node negative cases varies from 11% for those with tumor size <2 cm to 24% for those with tumor size >5 cm.[2,3] In node-negative breast cancer cases, the single most important prognostic factor is tumor size and one of the strongest predictors for dissemination & rate of relapse in these cases.[4] However, axillary node status is the single most important prognostic factor for patients with early breast cancer. Many studies had shown that treatment outcome was very poor in cases which had axillary lymph node metastasis as compared to node negative breast cancer cases.[2] Recent attention has been directed singularly at molecular classification of breast cancer. While molecular and genetic testing is very elegant, prognostic and predictive, they are expensive and not yet widely available. [5] The p53 tumor suppressor gene, located on the short (p) arm of chromosome 17, is a another proved breast cancer progression gene that regulates the cell cycle and DNA repair .[6,7] Unlike normal p53, nonfunctional mutated p53 accumulates in the nucleus of tumor cells, and therefore, it can be detected by immunohistochemical analysis. Multiple studies have shown that p53 over expression in breast cancer is associated with a worse prognosis.[6] Recent studies have suggested that p53 status might have a different predictive value for the efficacy of anthracycline/alkylating agent based chemotherapy regimen between triple negative & non triple negative breast cancers.[8] The above facts reassert the importance of studying the various prognostic factors in breast carcinoma so as to identify patients at high risk of early recurrence and thus to more effectively target aggressive adjuvant chemotherapy, radical mastectomy & intensive follow up protocols.

Materials and Methods:

The present study was conducted in the department of Pathology, M.K.C.G. Medical College, Berhampur. Study duration was from 2013 to 2015. Immuno-histochemical evaluation of a total 72 Patients was conducted who were confirmed to have breast carcinoma histologically. The haematoxylin & eosin (H&E) stained slides of the cases were retrieved & screened for confirmation of diagnosis followed by selection of the appropriate paraffin blocks. The representative neoplastic tissue blocks (paraffin embedded) were cut at 3.0_{μ} on poly-L-Lysine coated slides. One of the sections was routinely stained with H&E. The histological grading of the tumor was done on H&E stained sections according to Modified Bloom & Richardson Grading. Patients included in our study were mastectomy specimen with axillary clearance and needle biopsy, Incision biopsy, Enucleation & simple mastectomy, those who did not give consent for IHC, Inadequate tissue samples, Improperly preserved tissues were excluded. Breast carcinoma was used as a positive control. Tumor cells with nuclear staining were accepted as positive. The extent of positive p53 was graded semi quantitative for intensity and distribution. p53 overexpression were taken as Negative, Low, Moderate, High when less than 5%, 5% - 19%, 20% - 50%, >50% of cells were positive for p53. Tumor size was divided into three groups < 20mm, 20 mm – 49mm, >49mm on gross examination.

Statistical Method: Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean± SD (min-max) and results on categorical measurement are presented in number (%).

Significant figures of P value:

- + suggestive significance (P value: $0.05 < P < 0.10$)
- * Moderately significance (P value: $0.01 < P \leq 0.05$)
- ** strongly significance (P Value: $P \leq 0.01$)

For all such categorical data chi-square test was applied using graph pad prism software version 5.0. P value <0.05 was considered as the minimum level of significance.

Result

A prospective clinical correlation study of 72 patients with breast cancers over a period from 2013-2015 was undertaken in Department of Pathology to study immunohistochemical detection of p53 & its correlation with tumor size, sub types, histological grade and lymph node involvement.

In the present study, Age ranged from 21- 73 years & the mean age was 50 yrs. Majority, 40 cases (55.6%) belonged to 41-60 yrs. No pre-pubertal cases were encountered during the study period. Most of the patients were postmenopausal 38 cases (52.8%). Most of the cases presented with Breast Lump which was the commonest symptoms in 56 cases (77.8 %). Followed by breast lump with nipple discharge

in 6 cases (8.4 %), breast lump with pain & breast lump with skin involvement involving 4 cases (5.5%) each & 2 cases (2.8%) had breast lump with ulcer. Majority 41 (56.9%) cases showed tumor in upper outer quadrant, followed by 16 cases (22.3%) in upper inner quadrant. Only 7 cases involved the central breast. 5 and 3 cases involved lower outer & inner quadrant respectively, no tumor found to involve entire breast. In the present study, SBR grade II was the most common grade having 33 cases (45.8%). Followed by Grade III with 22 cases (30.6%) & Grade I with 17 cases (23.6 %). Present study showed that Predominant histologic subtype is infiltrating Ductal Carcinoma (NOS) accounting for 62 cases (86.1%). The other histological subtype encountered were 4 cases (5.5%) of medullary carcinoma and 4 cases (5.6%) of lobular carcinoma. We encountered 1 case (1.4%) of mucinous carcinoma & metaplastic carcinoma each. Majority 58 cases (80.6 %) had lymph node metastasis of tumor. 14 cases (19.4%) had either no lymph node in mastectomy specimen or no lymph node metastasis. We had 58 cases with lymph node involvement. Out of which majority 26 cases (36.1%) had 1-3 lymph node involvement. Followed by 24 cases (33.3%) having 4-9 lymph node involvement. 8 cases (11.1%) had ≥ 10 lymph node involvement.

Our study showed that majority of 64 cases (88.9%) were positive for p53 expression of which maximum 26 cases (36.1%) showed > 50% of P53 expression. 28 cases (38.9%) had 20-50% of p53 expression (**Table 1**). Maximum no. of T1, T2, T3 tumors showed moderate & high p53 expression (**Table 2**). 50% of patients with N0 patients were negative for p53 & rest showed varying p53 expression. Maximum number of cases showed moderate & high p53 expression in patients with 1-3(N2) & 4-9(N3) lymph node involvement (**Table 3**). 47% of Grade I tumors showed moderate p53 expression (Figure 1a, b). Maximum no. of Grade II tumors showed moderate to high p53 expression (Figure 2a, b). 41.0% of Grade III tumors showed high p53 expression (Figure 3a, b) **(Table 4)**. IDC (NOS) showed maximum of moderate to high p53 expression. Out of 4 cases of LCA, 2 showed moderate & 2 showed low p53 expression (Figure 4a, b). One case of mucinous carcinoma and metaplastic carcinoma each showed high p53 expression (Figure 5a,b ; 6a,b).

Table 1: P53 Expression

P53 expression	Number of patients	Percentage %
Negative (<5%)	8	11.1 %
Positive (5-19%)	10	13.9 %
Positive (20-50%)	28	38.9%
Positive (>50)	26	36.1 %
TOTAL	**72**	**100.0 %**

Table 2: Correlation of P53 with tumor size

Tumor size (mm)	Number of patients	P-53 expression			
		<5%	5-19%	20-50%	>50%
< 20 mm (T1)	12(16.7%)	3	2	4	3
20 – <50 mm (T2)	46(63.9%)	3	6	19	18
>50 mm (T3)	14(19.4%)	2	2	5	5
Total	**72(100%)**	**8**	**10**	**28**	**26**

Table 3: Correlation of p53 with Lymph node status

Lymph node status	Number of patients	P53 Expression			
		<5%	5-19%	20-49%	>49%
Negative (N0)	14	7	2	3	2
Positive (1-3)	26	0	5	12	9
Positive (4-9)	24	1	1	11	11
Positive (>9)	8	0	2	2	4
Total	**72**	**8**	**10**	**28**	**26**

Table 4: Correlation of P53 with tumor grade

Histological (SBR) grade	Number of pt (n=72)	P53 expression			
		<5%	5-19%	20-49%	>49%
Grade I	17	3	5	8	1
Grade II	33	3	3	13	16
Grade III	22	2	2	7	9
Total	**72**	**8**	**10**	**28**	**26**

TABLE 5: Correlation of P53 & Lymph node status with other studies

Number of lymph node involved	Ivkovic-Kapicl T. et al[22]	Jeong Han et al[23]	Banu Lebe et al [19]	Present study
N0- (0 lymph node)	P<0.05 Significant association	P> 0.5 No significant association	P> 0.05 No significant association	P = 0.0002 *** **significant association**
N1- (1- 3 lymph node)				
N2- (4-9 lymph node)				
N3- (≥ 10 lymph node)				

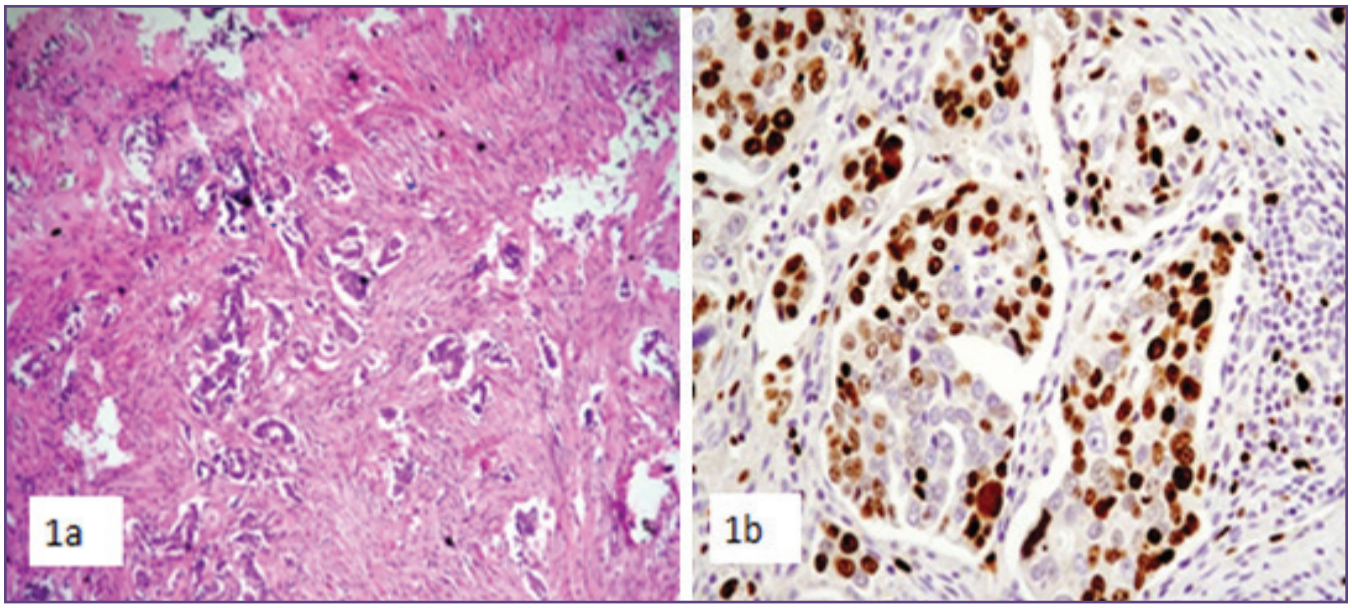

Fig. 1a: Microphotograph of Grade I, IDC (NOS) showing prominent tubular formation (H&E, ×400).

Fig. 1b: Microphotograph showing High (95%) P53 expression (IHC, ×400)

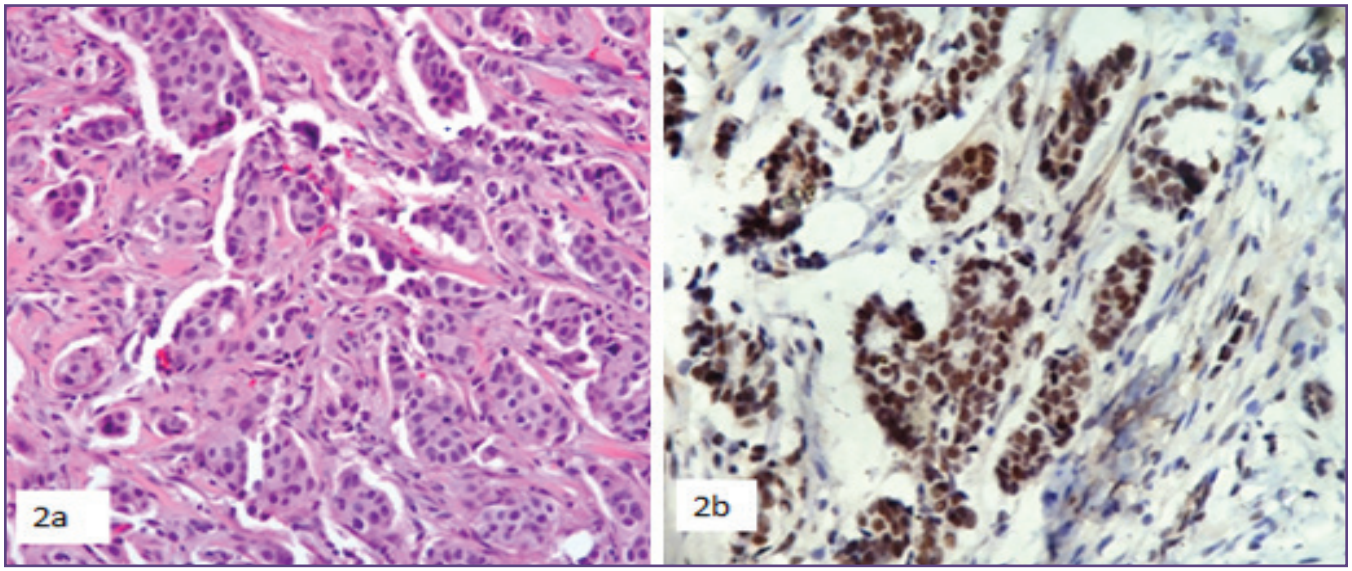

Fig. 2a: Microphotograph showing a case of grade II (IDC-NOS) with tumor cells arranged in nests and cords (H&E, ×400).

Fig. 2b: Microphotograph showing moderate P53 expression. (IHC, ×400)

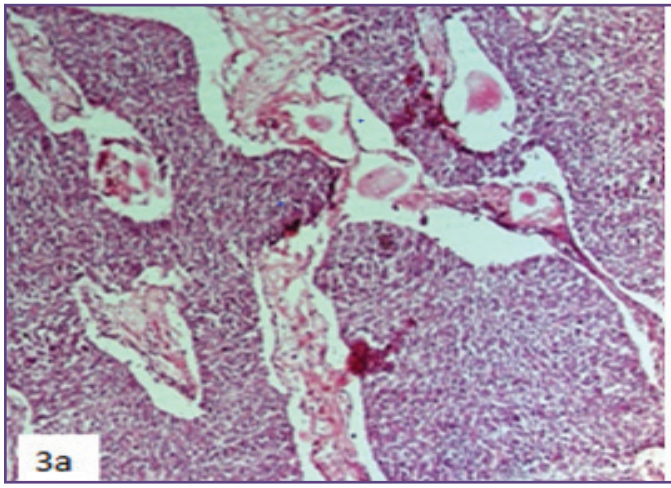

Fig. 3a: Microphotograph of Grade III (NOS) showing pleomorphic tumor cells in sheets (H&E, ×400).

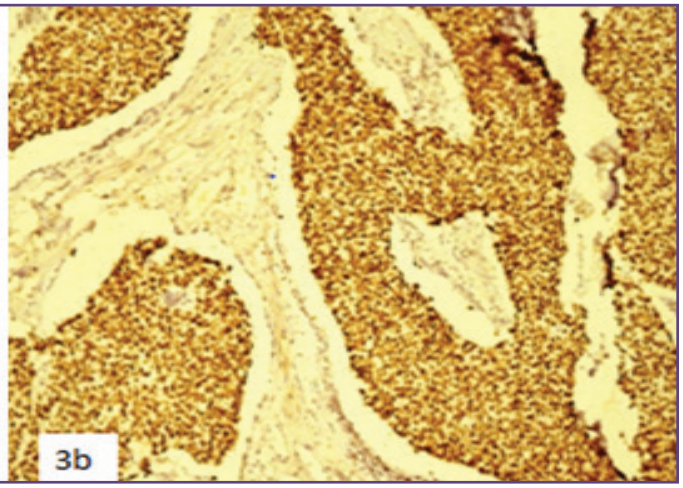

Fig. 3b: Microphotograph showing high p53 expression (IHC X 400)

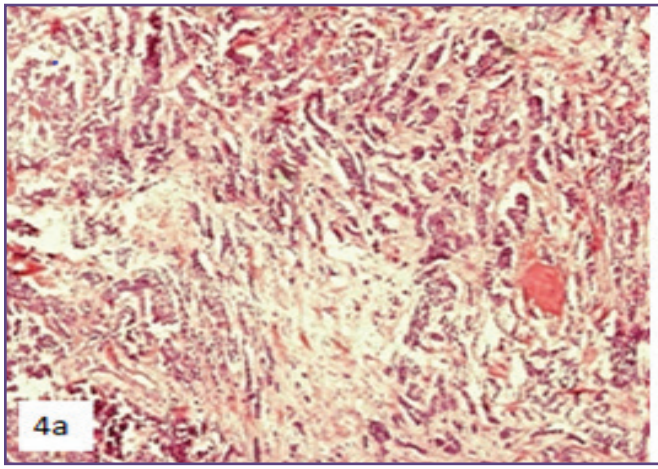

Fig. 4a: Microphotograph of Lobular carcinoma showing predominant tumor cells which are of low grade & less pleomorphic in Indian file pattern. (H&E, ×100, ×400).

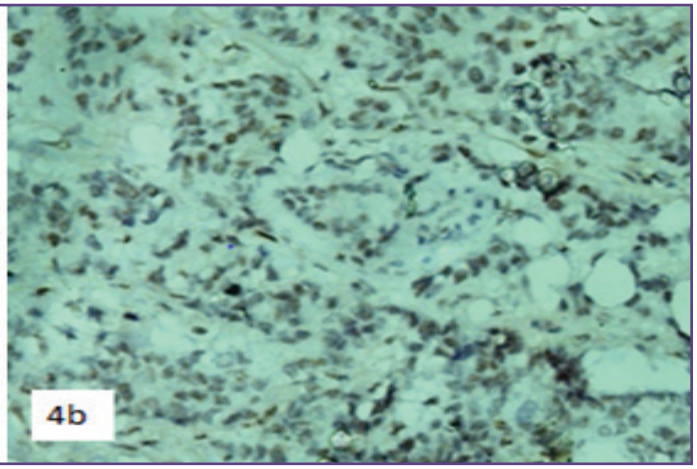

Fig. 4b: Microphotograph showing High p53 expression (IHC x400)

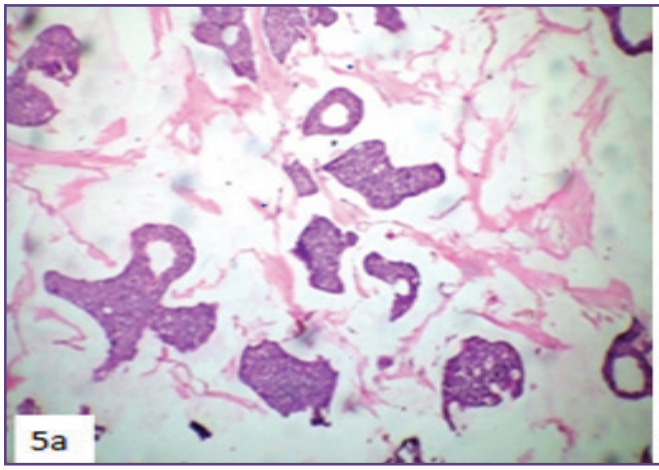

Fig. 5a: Microphotograph showing tumor cells in a pool of extracellular mucin (H&E, ×400).

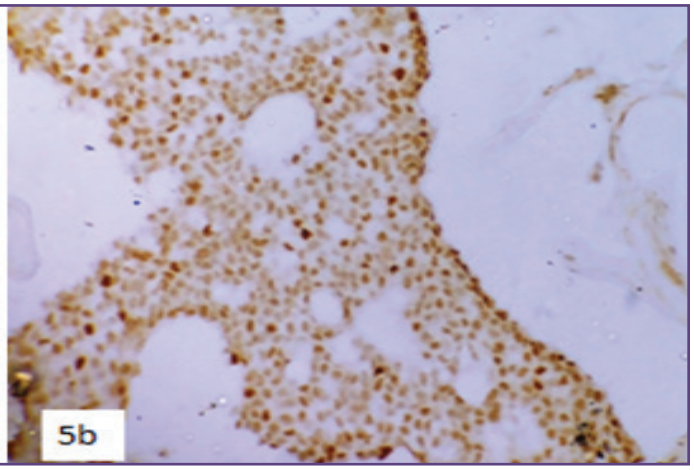

Fig. 5b: Microphotograph showing high p53 expression (IHC x 400).

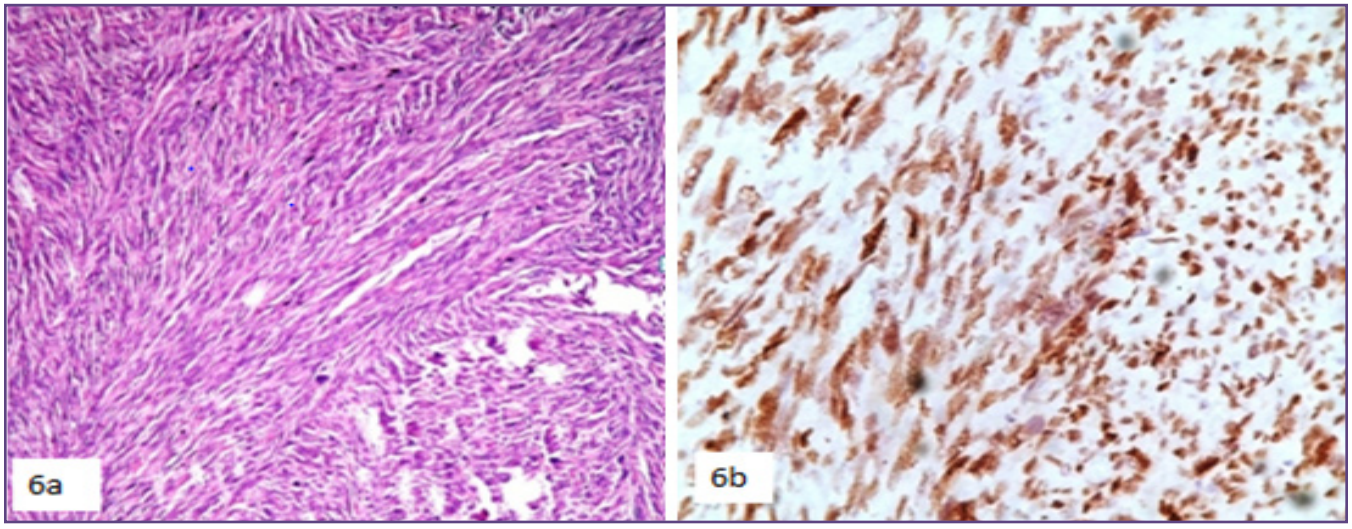

Fig. 6a: Microphotograph of metaplastic carcinoma Showing sheets of spindle shapes cells with no morphologic epithelial differentiation. Inset: showing spindle cells with atypical mitosis.(H&E, ×400).

Fig. 6b: Microphotograph showing high p53 expression (IHC x 400)

Discussion

Breast carcinoma is a disease with a tremendous heterogeneity in its clinical behaviour. Pathological variables such as tumor size, histological type, histological grade, lymph node metastasis, vascular space invasion, tumor cell proliferation, extent of ductal carcinoma in situ are the predictors of prognosis & for the need of adjuvant therapy. Biomarkers such as ER, PR, HER- 2, expression represent the most acceptable ones for predicting prognosis, response/resistance to treatment and in deciding the use of newer drugs such as transtuzumab in the case of HER-2 over expression.

It is a documented fact that advancement of age increases the risk of breast cancer and most women are over the age of 60 yrs when diagnosed.[9] Although there is evidence that Indian women are more likely to develop breast cancer at earlier ages than their Western counterparts.[10] The age of presentation in our study ranged from 21 to 73 years with mean age of 50 years. Similar observation was made by Christy BA.[11] The early age of presentation as compared to Robab et al [9] & Costa M et al [10] was seen because of low socioeconomic status in general population. In the present study, 56.9 % (41 cases) were in upper outer quadrant, which was slightly higher compared to studies by Meena et al [12] (54%), Costa M et al [10] (54.1%) & Christy BA [11] (50%). The relatively high proportion of carcinomas arising in the upper outer quadrant of the breasts is argued to support the hypothesis that underarm cosmetics cause breast cancer. The standard hypothesis is a reflection of the greater amount of breast tissue in this quadrant.[13] Tumor size is one of the most powerful predictors of tumor behaviour in breast cancer. Larger tumor size has poor 5 year survival rate. In present study, Maximum number of tumors was (T2) 20-50 mm size which was similar to study conducted by Raina et al[14] and Badwe et al.[15] But a major difference we found was 19.4 % tumors were of size > 5 cms, possibly includes those cases presented late to the clinics or because of lack of awareness among the population. Since most of the breast cancer mass are relatively painless & are ignored by the patients till they reach a significant palpable size or cause complications like skin or nipple involvement, till then it remains undiagnosed.

In the present study, 86.1% (62 Cases) were IDC (NOS). Similar observations were made by Zfarani B et al[16], Peiro G et al.[17] Other types of carcinoma had varied incidence in different studies. Zafarani et al[16] reported no other types of carcinoma whereas we got 9.7% of cases which included medullary carcinoma, mucinous carcinoma, & metaplastic carcinoma. Grade of any tumor is based on the fact that degrees of malignancy of tumor are reflected in their morphological structure. Our study showed that Majority of studies including our study have reported majority of carcinomas to be histological grade 2; Grade 1 tumors were variable in different studies. Tumor grade is the description of a tumor based on how abnormal the tumor cells and tumor tissue look under a microscope and indicates how quickly the tumor is likely to grow and spread. It differs depending on the type of cancer and one of the factors considered when planning treatment for a patient. It is a well established fact that the larger the tumour diameter,

the greater the number of axillary lymph nodes metastatic, also the worse the outcome.[18] In our study, Since there were tumors of >2 cm or more in 83.3% of cases, a higher lymph node involvement by the tumor cells was found in 80.6% of cases when compared to other studies. The p53 gene appears to play a prime role in controlling cell proliferation and apoptosis, and in DNA repair. The genetic changes most commonly found in breast cancer are alterations in the p53 tumor-suppressor gene, with an incidence ranging from 15 to 50% in different series. Our study Showed no significant association between p53 with tumor size. Similar observations were made in study conducted by Banu Lebe et al.[19] These variations can be explained by the quality of the tissue used (frozen, fixed, stored for a long time, etc), the number and type of antibody used, and also the interpretation of the results; it is well known that some positive cells do not take the stain, which often happens. [20]. It may also depend on the number of cases of each histologic type in a given series, since the accumulation of p53 protein is more common in high grade ductal carcinoma and medullary carcinoma.[21]

The most important prognostic factor for breast cancer is lymph-node status. Nevertheless, numerous attempts have been made to find other parameters that will aid in predicting the clinical outcome more accurately and in selecting the most appropriate therapy for each case. In our study, there was a significant association of P53 with Lymph node involvement & similar observations were made in study conducted by Ivkovic-Kapicl et al.[22] Feki et al.[20] found a correlation between p53 and other prognostic factors. But P53 expression was not shown to be an independent prognostic factor in disease-free interval or ten-year survival. No significant association was found in study conducted by Jeong Han et al [23] & Banu Lebe et al. [19] It is also possible that the p53 protein plays an important role in the progression of malignant human tumors.[23] In breast cancer, immunohistochemical positivity is found in up to 25% of in situ carcinomas, which suggests that they may occur in early stages of the disease, before it becomes infiltrating. The staining pattern of metastatic lymph nodes are usually similar to those of primary tumors; only very rarely does a positive stain for p53 occur in a node when the tumor is negative. [24, 25]

In our study, We found a significant association between tumor grade and p53 expression. Our finding coincides with studies conducted by Yamashita et al[26] and Jamaica D. Cass et al (**Table 6**).[27] The p53 alteration may reflect a greater degree of tumor progression and a higher proliferation rate, as well as a greater probability of micro metastases. Mutation and the over expression of p53 protein are directly related to histological grade and cell-proliferation fraction. Cases positive for p53 could be interpreted as those which have lost a mechanism for controlling the inhibition of cell proliferation and have gained an activator for malignancy potential.[21] In our study we had maximum number of IDC (NOS) cases and very few numbers of other histopathological types; hence we could not find correlation of p53 with histological type of tumor. But study conducted by Sirvent et al [21] showed p53 expression distribution by histological type highlighted the absence of any preference for p53 positivity and/or negativity in the case of ductal carcinoma, negativity in lobular carcinoma and strong positivity in medullary carcinoma.

TABLE 6 Correlation of P53 & tumor grade with other studies.

Histological (SBR) grade	Yamashita et al.[26]	Jamaica D. Cass et al[27]	Present study
● Grade I	P<0.0001 Significant association	P=0.032 Significant association	P = 0.0342 Significant association
● Grade II			
● Grade III			

Tumor grade, a parameter although easily assessed on core biopsies, but is not sufficient to define prognosis and it cannot be assessed optimally in post neoadjuvant settings.[28] Furthermore, as more conservative surgeries and staging techniques increasingly are introduced into the management of breast carcinoma e.g., increasing use of fine needle aspiration over tissue biopsies, much useful prognostic information, including tumor size, tumor grading, vascular invasion and lymph node involvement, will not be available. In this setting new markers such as p53 can be applied on small samples and they may be of prognostic significance which will be invaluable.[29] There are studies contradicting our findings and the differences may be due to heterogeneous group of population, different methods for assaying p53, or different cut offs to designate high or low.

Conclusion

In conclusion; we found a significant correlation of P53 with tumor grade and also with lymph node status, but not with tumor size. Breast cancer aggressiveness appears to be directly related to the percentage of p53 positive cancer cells. The p53 alteration reflects a greater degree of tumor progression and a higher proliferation rate and hence over expression of both proteins is directly related to histological grade and cell-proliferation fraction. Cases positive for p53 could be interpreted as those which have lost a mechanism for controlling the inhibition of cell proliferation and have gained an activator for malignancy potential. In breast cancer, we suggested that the over expression of p53

protein in the nucleus is an indicator of poor prognosis. We are of the opinion a large scale, standard multivariate studies to determine correlation between high p53 index and other prognostic markers in breast carcinoma patients.

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Competing Interests
None Declared

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