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Original Research Article

Association of p53 expression in various molecular subtypes of invasive breast carcinoma NOS

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ABSTRACT

Introduction: Breast cancer is the second most common cause of mortality for women after heart disease. Consequently, stopping it is difficult at all times. Metastasis frequently occurs to distant organs such as the liver, lung, and brain.

All specimens from lumpectomies, simple and modified radical mastectomies, and trucut biopsies identified as Invasive Ductal Carcinoma Not Otherwise Specified at the Central Diagnostic Laboratory of the Pathology Department at tertiary care centre, were included in the study. Immunohistochemical markers were utilised and haematoxylin and eosin sections were examined.

Aim: Association of p53 expression in various molecular subtypes of invasive breast carcinoma NOS.**Materials and Methods:** All lumpectomy specimens simple and modified radical mastectomy, trucut biopsies specimens diagnosed as invasive carcinoma breast not otherwise specified, submitted to the Department of Pathology, AJIMS, Mangalore for histopathological study are included in this study.**Result:** Statistical analysis of the data was performed using SPSS 20.0. The continuous variables were presented as mean±SD. Categorical variables were presented in frequency and percentage. Categorical variables were analysed using Chi square test. A p value<0.05 was considered statistically significant.**Conclusion:** This study is on the expression of p53 in invasive ductal carcinoma of the breast type NOS and correlating it with different histological grades, tumour sizes, and molecular subtypes. This study showed expression of p53 is independent of molecular subtype.This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.For reprints contact: reprint@ipinnovative.com

1. Introduction

The second most prevalent cause of mortality for women is breast cancer, which is the most common malignancy in females. Breast carcinogenesis is a multi-step process involving various cell types. Its prevention is never simple. Frequently, it spreads to distant organs like the liver, lung, and brain.¹

Long-term use of oral contraceptives, a low body mass index (BMI), and a large intake of animal fat in the diet are the modifiable risk factors. Genetic mutations

and family history are two non-modifiable risk factors. Adverse pathological variables include high grade tumours, hormone receptor negativity, and HER2neu overexpression are linked to breast cancer in young women. Recurrence and survival rates are thus unfavourable. Breast cancer patients' prognosis and treatment choices are still heavily influenced by their age upon diagnosis. As a result, managing breast cancer in young women entails paying close attention to poor gene signatures, long-term follow-up following breast conserving therapy, and surgical negative margins.²

Breast cancer patient staging provides a wealth of knowledge about each patient's prognosis. According to the TNM classification system, staging is done. Despite

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significant advancements in the disease's management, 80 to 90% of women still live five years after receiving their initial diagnosis. Additionally, ongoing efforts are done to identify biomarkers for breast cancer prognosis.³

The first tumour suppressor gene discovered, P53, prevents the growth of malignant cells by eradicating them and inhibiting their proliferation. The most frequent genetic alteration in human neoplasia is still a mutation. It has a negative impact on overall survival and is linked to aggressive illness in breast cancer. However, compared to other solid tumours, breast cancer has a lower frequency of p53 mutations. The predictive and diagnostic value of molecular pathological characterization of particular p53 pathway components in breast cancer is likely.

2. Materials and Methods

All lumpectomy specimen, simple and modified radical mastectomy, trucut biopsies specimens diagnosed as invasive carcinoma breast not otherwise specified, submitted to the Department of Pathology, AJIMS, Mangalore for histopathological study. A total of 100 cases are included in this study.

The specimens were received in the Pathology department in 10% formalin. After a detailed specimen description, multiple sections were taken. After conventional processing, paraffin sections of 5µm thickness were stained by hematoxylin and eosin (H & E) for histopathological study. In addition, 3µm sections were cut from a paraffin block of tumor tissue and taken on glass slides coated with adhesive Poly-L-Lysine for immunohistochemistry (IHC).

2.1. Immunohistochemistry procedure

1. 3-4 µm sections were cut from paraffin blocks and mounted on glass slides coated with Poly-L-Lysine.
2. Sections were incubated at 40°C overnight. Next day sections were again incubated at 60°C for 1 hour.
3. De-paraffinization with fresh Xylene (5 minutes) and absolute alcohol (5 minutes) was done and later slides were washed in distilled water.
4. Antigen retrieval was done in citrate buffer (pH 6.0) in temperature-controlled antigen retriever. Microwave technology was used and 4 cycles were run for 8 minutes on 95° C and cooled at room temperature.
5. Slides were washed with TBS (Tris-Buffer-Saline) for 5 minutes in consecutive 2 changes.
6. Sections were covered with peroxide block (3% H₂O₂) for 5 minutes to quench the endogenous peroxidase and later washed with two changes of TBS for 5 minutes each in consecutive 2 changes.
7. Sections were incubated with peroxide block for 5 minutes and later washed with two changes of TBS for 5 minutes in consecutive 2 changes.

8. Sections were incubated with primary antibody (ER, PR, HER2/neu, Ki67, p53) for 60 minutes and later washed with two changes of TBS for 5 minutes in consecutive 2 changes.
9. Sections were incubated with Post Primary block for 30 minutes and later washed with two changes of TBS for 5 minutes each in consecutive 2 changes.
10. Sections were incubated with NovoLink™ Polymer for 30 minutes and later washed with two changes of TBS for 5 minutes each consecutive 2 changes.
11. Peroxidase activity was developed with DAB (diaminobenzidine) working solution (DAB chromogen 50 µl with 1 ml of DAB buffer) for 5 minutes later slides were rinsed in running tap water.
12. Counter staining was done with Meyers Hematoxylin for 30 seconds and later slides were rinsed in running tap water.
13. Sections were dehydrated with graded alcohol and cleared with Xylene.
14. Mounting was done using DPX.

3. Results

Statistical analysis of the data was performed using SPSS 20.0. The continuous variables were presented as mean±SD. Categorical variables were presented in frequency and percentage. Categorical variables were analysed using Chi square test. A p value<0.05 was considered statistically significant.

The present study was conducted on 100 invasive ductal carcinoma breast patients with an average age of 57.04 ±10.581 years. Majority (39%) of the patients in this study were of age group 49-58 years followed by 23% each of <=48 years and 59-68 years. 15% belonged to the age group 69- 78 years.

In the present study, 91% of the patients were ER positive and 9.0% with ER negative.

In the present study, 89% of invasive ductal carcinoma patients were PR positive and 11% were PR negative.

In the present study of breast cancer patients, 89% were Her2/neu negative followed by 11% were Her2/neu positive.

Among the 100 invasive ductal carcinoma breast patients included in the study, 59% had high level of Ki67 followed by 32% with intermediate level and 9% with low level of Ki67.

The present study included 78% patients with negative P53 and 22% with positive P53. 38.5% of p53 negative patients belonged to the age group of 49-58 years. 40.9% of p53 positive patients were of age group 49-58 years.

The study included 88% of invasive ductal carcinoma breast patients with tumor size 2-5 cm and 12% with more than 5 cm. 36.4% of the patients with tumor size (2-5cm) belonged to the age group of 49 -58 years. 58.3% of the patients who had tumor size more than 5 cm, were of the age group 49-58 years.

Table 1: P53 on the basis of histological grading

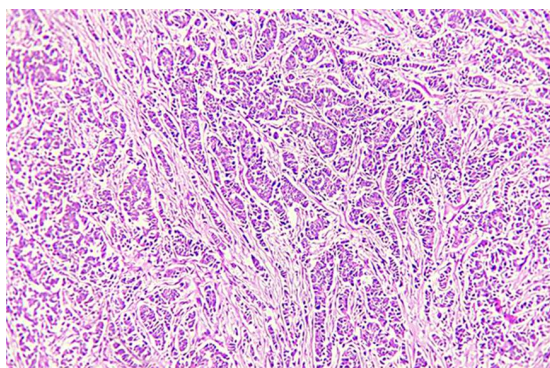
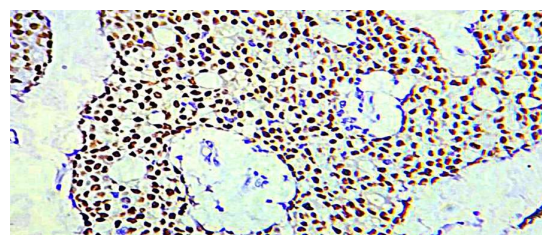
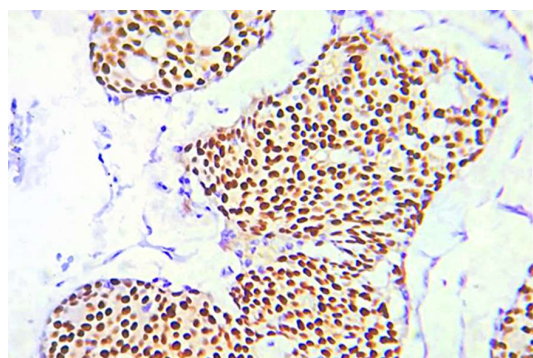
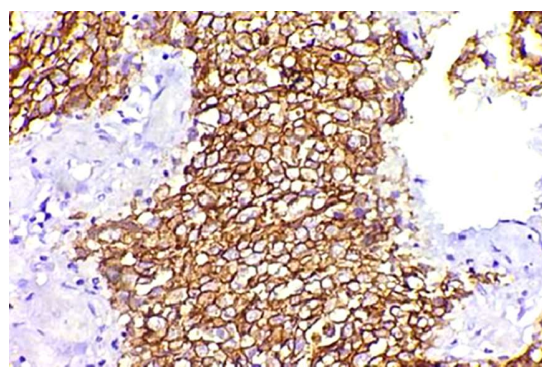
Histological grade	Grade II	P53		Total
		Negative	Positive	
		78	22	100
		78.0%	22.0%	100.0%
Total		78	22	100
		78.0%	22.0%	100.0%

Table 2: Cross tabulation of p53 with tumor size

		Tumor size		Total	Chi square value	p value
		2-5 cm	>5 cm			
P53	Negative	69	9	78	0.072	0.789
	Positive	19	3	22		
		88.5%	11.5%	100.0%		
		86.4%	13.6%	100.0%		
Total		88	12	100		
		88.0%	12.0%	100.0%		

Table 3: Molecular subtype with histological grade

Histological Grade	Molecular sub type				Total
	Triple Negative	Luminal A	Luminal B	Her2+	
Grade II	7	82	9	2	100
	7.0%	82.0%	9.0%	2.0%	100.0%

**Figure 1:** Infiltrating ductal carcinoma (H&E, X100)**Figure 3:** Positive staining for PR in tumor cells in breast (IHC PR, X100)**Figure 2:** Positive staining for ER in tumor cells in breast (IHC ER, X100)**Figure 4:** Positive staining for Her2neu in tumor cells in breast (IHC Her2, X100)

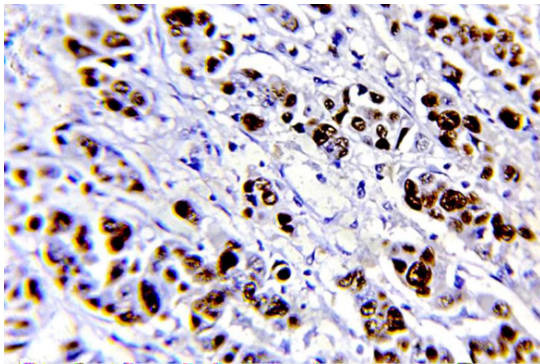


Figure 5: Positive staining for p53 in tumor cells (IHC p53 X100)



Figure 6: Gross appearance of a modified mastectomy specimen



Figure 7: Cut surface of mastectomy specimen

In the present study, The Luminal A was the most common sub type of Breast carcinoma (82%) followed by Luminal B (9%), Triple negative (7%) and Her2+ (2%).

The present study indicated that 33.3% of ER negative were from the age group 49 to 58 years and 39% of ER positive group were from the age group 49 to 58 years with chi square = 4.31 and $p > 0.05$. Analysis showed no significant association between age and ER status.

Cross tabulation of age with PR status in this study showed, 45.5% patients with PR status negative were of age 49 to 58 years. 38.2% patients with PR status positive belonged to the age group of 49 to 58 years. The present

study showed chi square equal to 0.465 and $p > 0.05$. The analysis showed no significant association between age and PR status.

The analysis showed no significant association between age and Her2/neu with chi square=10.256 and $p > 0.05$. The tables depicted 75% of positive 3+ Her2 status were in the age group 49 to 58 years, 38.2% of negative Her2 status in 49 to 58 age group and 50% of equivocal 2+ in the age group ≤ 48 years.

The analysis indicate no significant association between age and Ki67. In all the age group majority were from high level Ki67. 56.5% in ≤ 48 years, 64.1% in 49-58 years, 56.5% from 59-68 years and 53.3% from 69-78 years.

The analysis depicted no significant association between p53 and tumor size with chi square=0.071 and $p > 0.05$. In the present study 88.5% of p53 negative group had tumor size 2-5 cm and 86.4% of p53 positive group had tumor size of 2-5 cm.

The study shows 78% of p53 negative and 22% of p53 positive in Grade II patients. (Table 1)

In the present study, all the 100 patients belong to histological grade II, among them 82% are of molecular subtype Luminal A, 9% with molecular sub type Luminal B, 7% with triple negative and 2% with Her2+.

In the present study 81.8% of patients who had tumor size 2-5 cm and 83.3% of patients who had tumor size > 5 cm belonged to molecular sub type Luminal A. Over all it shows out of 100 patients 72 of them with Luminal A had smaller tumor size (2-5 cm). Analysis shows no significant association between molecular sub type and tumor size with chi square=3.699 and $p > 0.05$

4. Discussion

In the present study, 100 cases of breast carcinoma were studied. Immunohistochemistry analysis for ER, PR, HER2/neu, p53 and ki67 were performed.

4.1. Age

In the present study, the patients belonged to the age group of 28 years to 78 years with an average age of 57 ± 10.581 years. In this study, 39% belonged to the age group of 49-58 years. 23% to 28- 48 years, 23% to 59-68 years and 15% to the age group of 69- 78 years. In the study done by Chand P et al,⁴ among 100 cases studied, 45% were from the age group of 24-80 years with an average age of 55.28 years. Similarly, in the study done by Ahmed et al,⁵ 157 cases were studied out of which 40% belonged to the age group of 16-80 years with an average age of 43.75 years.

4.2. ER/PR, Her2/Neu, Ki-67 status

In the present study 91 cases were ER positive and 89 cases were PR positive. Her2neu positivity was seen in 5 cases. Similarly, in a study done by Chand P et al,⁶ among the 100

cases studied 7% of the cases showed Her2Neu positivity. However, in a study done by Bansal C et al⁶ with a sample size of 120 cases, 40.7% showed Her2Neu positivity.

4.3. Hormonal status according to age groups

In our study, higher (39.6%) ER positivity, (Figure 2) higher (38.2%) PR positivity (Figure 3) and higher (100%) Her2Neu positivity (Figure 4) was noted among women who were of the age group 49-58 years. According to study by Mohla et al⁷ and Rana et al⁸ found that woman aged around 49-58 years showed higher ER positivity (46%) which was also seen in present study. Similarly, Alvarez Goyanes et al⁹ examined 1509 tumors from Cuban women diagnosed with breast cancer and showed that ER expression was greater in patients with >50 years. In a study done by Chatterjee S et al¹⁰ and Rilke et al¹¹ Her2neu over expression was more seen in patients aged 45-60 years which was similar to our study. In study done by Huang Huei et al¹² showed that in estrogen positive breast cancer, Her2neu and PR are inversely associated and confirmed age related inverse relationship was seen between Her2neu and PR only in women >45 years.

4.4. Ki-67 status

Among the 100 invasive ductal carcinoma breast patients included in this study, majority (59%) had high level (>25%) of Ki67 followed by 32% with intermediate level (10-25%) and 9% with low level (<10%) of Ki67. In a study done by C. Inwald et al,¹³ among 70 cases studied, Ki-67 positivity was seen in 28 cases (40%). Out of 28 positive cases 19 cases showed high (>25%) and intermediate level (10-25%) of Ki-67.

4.5. Tumour size

In present study 88% of cases were tumor size 2-5 cm. (Figure 6,7) Similar results were observed by Sofi et al,¹⁴ Orang et al¹⁵ and Saleh F et al¹⁶ in their study. While study from western country, Taucher S et al¹⁰ reported that the tumors were predominantly ≤ 2 cm size, this could be due to awareness among patients and early cancer detection programs. In India owing to the lack of awareness of this disease and in absence of appropriate facilities, the majority of breast cancers are diagnosed at a relatively advanced stage. A ten-year survival rate was seen in 90% of cases in a study done by Carter CL et al¹⁷ in patients with tumor size <1 cm.

4.6. p53 status

4.6.1. p53 with tumor size

In the present study, p53 negative group (88.5%) had tumor size 2-5 cm and p53 positive group (86.4%) had tumor size 2-5 cm (Table 2, Figure 5). There was no significant

association between p53 and tumor size (chi square=0.071 and $p>0.05$) in the present study. In a study done by Bharat Jindal et al⁹ tumor size ranges from 1.5 cm to 15 cm and majority of breast tumors (29/50, 58%) were of size >2 cm to 5 cm followed by 15/50 (28%) tumors with size >5 cm and least (6/50, 12%) were tumor of size ≤ 2.0 cm. Almost similar observations have been reported in other studies such as Sharma M et al (2016),² Gupta K et al (2016)⁹ and Pan Y et al (2017).⁸

4.7. P53 with histological grading

In the present study, majority of cases were of grade II (Figure 1) according to Nottingham Modified Bloom Richardson System, among which 78% were p53 negative and 22% were p53 positive. A study done by Bharat Jindal et al,⁹ also showed majority of cases of breast carcinoma of grade II according to Nottingham Modified Bloom Richardson System, with no statistically significant association between p53 overexpression and tumor grade.

4.8. Molecular subtype

In the present study, all the 100 patients belong to histological grade II, among them 82% were of molecular subtype Luminal A, 9% with molecular sub type Luminal B, 7% with triple negative and 2% with Her2neu+. Figures 2 and 3 and 4 showing strong positivity of ER, PR and Her2neu. A study done by Engström MJ et al,¹⁶ showed high tumor grade to be mainly associated with non-luminal subtypes. Interestingly, the study showed that there was no difference in survival according to subtypes for those who had survived the first 5 years because of adjuvant-therapy.

5. Conclusion

This study is on the expression of p53 in invasive ductal carcinoma of the breast type NOS and correlating it with different histological grades, tumor sizes, and molecular subtypes. This study showed expression of p53 is independent of molecular subtype.

Tumor size and molecular subtype did not significantly correlate in this study (chi square=3.699, $p>0.05$). Tumor size and p53 did not significantly correlate (chi square=0.071, $p>0.05$). The current investigation found no evidence of a connection between p53 and specific molecular subtypes ($p>0.05$). Consequently, p -value > 0.05 demonstrated that p53 expression was unrelated to tumor size, molecular subtype, or grade. Study included only cases of invasive ductal carcinoma with a tumor size greater than 2 cm and a histological grade of 2 or more for p53 expression. It is not associated with higher histological grade and larger tumor size. p53 is independent marker.

6. Source of Funding

None.

7. Conflict of Interest

None.

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