Association of p53 Overexpression with Hormone Receptor Status and Triple Negative Breast Carcinoma

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ABSTRACT

Objective: To determine association of p53 overexpression with hormone receptor status in breast carcinoma.
Study Design: Descriptive cross-sectional study.
Place and Duration of Study: Department of Pathology in collaboration with Department of Oncology, King Edward Medical University, Lahore, from January 2017 to January 2018.
Methodology: All adult female patients coming to Department of Oncology with reports of breast cancer biopsy and receptor status were included. Their age, type of surgery, grade of cancer, stage of cancer, and hormone receptor status were noted from records. P53 immunomarker was applied on tumor containing tissue blocks. Pearson Chi-square test was run for strength of association between the variables using SPSS V. 22.
Results: Mean age of 91 patients at diagnosis was 48 years ±12.49. Fifty-five (60.4%) patients had ER positive tumors, 36 (39.6%) were ER negative, 53 (58.2%) had PR positive status, and 38 (41.8%) were negative. Same number was seen in HER2 neu staining. Out of 91 cases, 31 (34.1%) were p53 positive and 60 (65.9%) were negative. Out of 55 (60.4%) ER positive cases, 11 (12.1%) were positive for p53 and 44 (48.4%) were p53 negative ($p<0.001$). PR positive cases (53/58.2%) showed p53 positivity in 7 (7.7%) and negativity in 46 (50.5%) cases ($p< 0.001$). HER2 positive cases were positive for p53 stain in 8 (8.8%) and negative in 45 (49.5%) cases ($p<0.001$). Fifteen (16.48%) biopsies were negative for all three hormone receptors. P53 was positive in all of these hormone receptor negative tumors (15/15, 100%), although 9 (60%) showed strong positivity and 6 (40%) exhibited weak staining intensity of p53.
Conclusion: P53 overexpression is less frequent in hormone receptor positive breast cancers. However, hormone receptor negative (triple negative) tumors overwhelmingly overexpress p53 protein in tumor cells. P53, detected either immunohistochemically or serologically, can serve to predict poor prognosis and survival in breast cancer patients, unless newer anti-p53 treatments are introduced in Pakistan.

Key Words: *Breast carcinoma, Hormone receptors, p53, Triple negative.*

INTRODUCTION

Breast cancer is the most common cancer in women all over the world and it is one of the leading causes of cancer related mortality in females.[1] Worldwide, breast cancer accounts for 23% of the total cancer cases and 14% of the cancer deaths.[2] Pakistan is amongst those countries which have one of the highest breast cancer incidence and mortality in the world.[3,4] About 20 - 30% of all breast cancer cases are considered as familial, but only 10% occur sporadically and are attributed to somatic changes in genome.[5]

P53 is an important tumor suppressor which may lead to development of many cancers including sarcomas, breast cancer, brain tumors, leukemia, and adreno-cortical carcinomas.[6] In breast cancer, the frequency of p53 gene mutations is approximately 20% to 30%.[7,8] Acquiring a p53 mutation has been suggested to be an early event in breast cancer development and it is related to poor prognosis and chemo-resistance.[9]

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Few studies have been done showing independent prognostic significance of different hormonal (Estrogen receptor (ER), Progesterone receptor (PR) and Human epidermal growth receptor (HER2 neu) and molecular characteristics of breast tumors and their response to systemic and endocrine therapies.[10] Similarly, a few studies showing relationship between different genetic mutations like p53, Ki 67 and molecular varieties of breast cancers have also been conducted.[11] There is controversial data regarding association of p53 with triple negative breast cancer; and hence, poor prognosis.

The objective of this study was to find out the frequency of p53 expression in various subtypes of breast cancer and its association with receptor status which has prognostic and predictive significance.

METHODOLOGY

It was a descriptive cross-sectional study conducted at Department of Pathology, in collaboration with Oncology Department, King Edward Medical University, Lahore, from January 2017 to January 2018. A total of 91 patients were included with the help of non-probability purposive sampling.

All female patients were over 18 years, diagnosed with breast cancer having reports of receptor status confirmed

with immunohistochemistry for Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth factor receptor (HER2/ neu). All patients had to give written witnessed informed consent including permission to contact patient at her address during follow-up. Patients who did not have immunohistochemical reports of hormone receptors, had insufficient or inconclusive biopsy results or male breast cancer patients were excluded from the study population.

Data was collected, after ethical approval from Board of Research of King Edward Medical University, from records of Oncology Department, KEMU/Mayo Hospital, Lahore, for the study period. All adult female breast cancer patients were enrolled in this study after taking informed consent. Their age, type of surgery, grade of cancer, stage of cancer, and immunohistochemical profile of hormone receptor status were noted from records. If any patient's initial biopsy was trucut or wedge-performed at our institution, and mastectomy was performed later on at any other hospital, patient was contacted to get tumor rich blocks.

Biopsy blocks and their reports were retrieved from Pathology Department for grading and typing of tumor. Staging was performed one tumor rich section per case was selected for immunohistochemical analysis. Immunoperoxidase labelling was performed with the automated XT iVIEW DAB V.1 procedure on the BenchMark XT IHC/ISH Staining Module, Ventana with anti-p53 (clone Bp53-11, prediluted, Ventana). Antigen retrieval was carried out with CC1 (Ventana). Sections were incubated with primary antibodies for 16 minutes at 37°C. All slides were reviewed by two pathologists. Nuclear staining was considered as positive reaction when present in more than 10% of tumor cells. Negative was labelled when staining was absent or seen in less than 10% of tumor cells.[11] For the sake of analysis intensity was labelled as focal (1+, 2+): weak positive and if diffuse (3+) positivity seen: strong positive was labelled. Staging was done with the help of pre-treatment mammography, ultra sound and CT scans using TNM method.

Data was entered and analysed retrospectively in SPSS (Statistical Package for Social Sciences) version 22. Mean and ±SD was calculated for quantitative variables, like age. Frequency and percentage was calculated for qualitative variables like type of surgery, tumor type, grade, stage of tumor, hormonal and p53 status (positive, negative). Pearson Chi-square test was used with the help of cross-tabulation to study association between p53 and hormone receptors (ER, PR, Her2neu) keeping the $p \leq 0.001$ as significant and confidence interval of 95%.

RESULTS

A total number of 91 patients were included in the study. Mean age at diagnosis was 48.00 ±12.49 years. Age of patients ranged between 18-84 years with most frequent age of presentation was 50 years (16 patients/17.6%). Sixty-six (72.5%) patients underwent tru-cut biopsy, 14 (15.4%) had modified radical mastectomy, 9 (9.9%) had wedge-biopsy, and 1 (1.1%) patient each had incisional and excisional biopsy. Only one (1.1%) patient had grade I disease, 43 (47.3%) had grade II tumor and 47 (51.6%) patients had grade III tumor, at the time of presentation. Forty (44%) patients had stage II disease, 32 (35.2%) had stage IV disease, and 19 (20.9%) had stage III disease (Table I). Fifty-five (60.4%) patients had ER positive tumors, 36 (39.6%) were ER negative. Fifty-three (58.2%) had PR positive status, 38 (41.8%) were negative. Same number was seen in HER2 neu staining. Out of 91 cases, 36 (39.6%) were p53 positive and 55 (60.4%) were negative (Table II). Out of 55 (60.4%) ER positive cases, 11 (12.1%) were positive for p53 and 44 (48.4%) were p53 negative ($p<0.001$). PR positive cases (n=53/58.2%) showed p53 positivity in 7 (7.7%) and negativity in 46 (50.5%) cases ($p< 0.001$). HER2 positive cases were positive for p53 stain in 8 (8.8%) and negative in 45 (49.5%) cases ($p<0.001$).

When triple negative breast carcinomas were statistically evaluated, 15/91 patients' (16.48%) biopsies were negative for all three hormone receptors. Twenty percent (3/15) each were at stage II and III. Sixty percent (9/15) were at stage IV. P53 was positive in all of these hormone receptor negative tumors (15/15, 100%), although 9 (60%) showed strong positivity and 6 (40%) exhibited weak staining intensity of p53. Minimum age of this group was 30 years, maximum was 50 years. Mean age was 48.60 with SD ±12.76. Most frequent age group was 40 and 55 (20% each). Ages ranged from 30 to 80 years. Histological grade II was seen in 9 (60%) and III was in 6 (40%) of tumors.

Table I: Demographic data of triple negative breast cancer subjects.

	All patients	TNBC only
Total number of subjects n (%)	91 (100%)	15 (16.48%)
Age		
Mean ±SD	48 years ±12.49	48 ±12.76 years
Range	18 years - 84 years	30 years - 80 years
Clinical stage		
II	40 (43.9%)	9 (60.0%)
III	19 (20.9%)	3 (20.0%)
IV	32 (35.2%)	3 (20.0%)
Histological grade		
Grade 1	1 (1.1%)	0
Grade 2	43 (47.3%)	9 (60.0%)
Grade 3	47 (51.6%)	6 (40.0%)
Receptor status		
ER +	55 (60.4%)	
PR+	53 (58.2%)	
HER2 Neu+	53 (58.2%)	
Source of sample tissue (at initial presentation)		
Wedge biopsy	9 (9.9%)	
Tru-cut Biopsy	66 (72.5%)	
Modified radical mastectomy	14 (15.4%)	

Table II: Receptor positivity in breast cancer (n=91).

Receptor status	Positive	Negative	p-value	Confidence interval
Estrogen receptor	60.4% (55/91)	39.6% (36/91)	<0.001	95%
Progesterone receptor	58.2% (53/91)	41.8% (38/91)	<0.001	95%
Her2neu receptor	58.2% (53/91)	41.8% (38/91)	<0.001	95%
P 53 Overexpression	39.6% (36/91)	60.4% (55/91)	<0.001	95%

DISCUSSION

The demographic features of the study group were similar to the data presented in previous literature. The mean age of breast cancer at presentation was 48 years ±12.49 in this group and other studies report mean age to be around 45-47 years.[12] A small proportion, 15.4% of these patients underwent mastectomy and rest had biopsies taken. It is in contrast to other studies where 91.35% underwent mastectomy or breast conserving procedures.[13] It was probably because most of these patients presented with inoperable large metastatic tumors that contraindicated mastectomy. Majority *i.e.* 79.9% of these patients, presented with advanced stage disease (stage II, III, IV) and only 20.9% are at stage I. This is contrary to international data where effective screening helps in diagnosing early stage cancer and proper management.[14]

It was observed that p53 was positive in 20% (11/55) of ER positive, 13.2% (7/53) of PR positive and 15.1% (8/53) of HER2 neu positive tumors. This percentage is lower when compared to international data where it is reported to be around 35-50%.[15] This discrepancy could be due to longer five-year survival studies conducted internationally. However, overall percentage of p53 in this study in 91 patients is 39.6%, which matches with other studies.[15] According to Esfehani *et al.*, p53 is more frequently present in triple-negative tumors (74.8%) and HER2 neu positive tumors (55.4%), which supports the discovery of isogenic gene signature located between triple negative and HER2 positive breast cancers.[16]

Triple negative breast cancer (TNBC) with p53 protein overexpression is more aggressive and resistant to conventional treatment as compared to other types of cancer.[17] This special type is receiving more attention because there is evidence of tumor regression in patients who received p53 MVA vaccine in combination with standard chemotherapy.[18] Frequency of p53 mutation in TNBC is variable in literature ranging from 30-80% in various studies.[19] In this study, all of TNBC (15/15, 100%) were immunopositive for p53 over-expression. Only one mastectomy specimen was received that turned out to be triple negative. Later on, when data was collected of wedge and incisional biopsies with triple negative status, respective patients were contacted to collect tumor blocks from mastectomy specimens. Out of them, 60% (9/15) showed diffuse and 40% (6/15) showed focal p53 staining. This is higher as compared to a study conducted in Korea (2017) which showed 61.9% (39/63) breast biopsies to be positive for p53 immunostain.[20] This could be due to smaller sample size in this study. On the contrary, since high levels of p53 protein expression are associated with somatic mutations, this could suggest that the studied patient population had higher frequency of p53 mutation in TNBC and thus deserved newer anti p53 targeted therapy.[21,22]

In this study, minimum age of presentation of TNBC with p53 overexpression was 30 years and maximum was 50 years with 40 years being the most frequent age group. Minimum and most frequent age group of presentation is similar to international studies but maximum age of presentation is more than 60 years in various studies.[23] Hence, receiving TNBC with p53 mutation at earlier age, which is resulting in young deaths. A study conducted in Argentina, showed p53 to be more frequent in advanced stage *i.e.* 47% positive in stage II patients. This is similar to our findings that shows 60% (9/15) positivity in stage IV, while 20% (3/15) each in stages II and III. This shows that as stage advances, p53 positivity also increases. Sadhigi and Gabriela *et al.* found no significant difference between expression of p53 and grade of tumor.[24,25] Similarly, the present study did not reveal any significant correlation between p53 expression and advancing grade of tumor as we observed 60% (9/15) positivity in grade II tumors and 40% (6/15) in grade III.

The challenge in treating TNBC breast cancers is that they are not responsive to antiestrogens or anti-Her2neu drugs, secondary to negative receptor status, and as a result have a poor prognosis. Therefore, the presence or absence of supplementary markers could help predict which therapies (platinum based) are best suited for TNBC patients.

It is proposed hereby that all patients over the age of 40 years, with advanced stage of breast cancer (indicated by mammography and ultrasound) should be tested for p53 mutations by non-invasive method, like serum testing. This can be useful in predicting prognosis, follow-up after treatment, and selecting candidates for p53 vaccines and targeted therapies.

CONCLUSION

P53 overexpression is less frequent in hormone receptor positive breast cancers. However, hormone receptor negative (triple negative) tumors overwhelmingly over-express p53 protein in tumor cells. P53 detected either immunohistochemically or serologically, can serve to predict poor prognosis and survival in breast cancer patients, unless newer anti-p53 treatments are introduced in Pakistan.

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