IMMUNOHISTOCHEMICAL EXPRESSION OF P53 IN INVASIVE DUCTAL CARCINOMA OF BREAST IN PAKISTANI WOMEN

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ABSTRACT

Objective: To determine the frequency of immunohistochemical expression of *p*53 in invasive ductal carcinoma of breast in Pakistani women.

Study Design: Descriptive, cross-sectional study.

Place and Duration of Study: Department of Histopathology, Combined Military Hospital, Lahore, from Jul 2015 to Jan 2016.

Methodology: This study involved 80 histopathological samples of invasive ductal carcinoma breast which were tested for p53 expression. Sample size with 80 cases was calculated using 95% confidence level and 10% margin of error while expected percentage of p53 expression will be 71.67%. Specimens were selected by non-probability, consecutive sampling. All specimens of newly diagnosed cases of invasive ducal carcinoma at CMH Lahore on histopathological examination of female patients aged between 15-80 years. IHC application of p53 was done as per technique mentioned in the manual given by the DAKO.

Results: Patient age was calculated which was between 25 years and 80 years while mean age was 50.76 ± 11.80 years. Majority of the patients were aged between 25-50 years (n=44, 55.0%). In 54 (67.5%) patients lymph nodes were positive for tumor metastasis. Majority 64 (80%) of the tumors were Grade-II followed by Grade-III in 15 (18.7%) patients. The size of the tumor ranged from 0.2 cm to 14 cm with a mean of 4.16 ± 2.59 cm. p53 expression was positive in 38 (47.5%) patients. The expression of *p*53 was higher in patients who have positive lymph nodes (59.3% vs. 23.1%; *p*=0.002). However, expression of *p*53 was not so significant for age (*p*=0.079), tumor size (*p*=0.414) and tumor grade (*p*=0.176) groups.

Conclusion: The frequency of p53 expression was found to be 47.5% in invasive mammary cancer in Pakistani population. The expression of *p*53 was higher in patients who have positive lymph nodes (59.3% vs. 23.1%; *p*=0.002). However, expression of *p*53 was not so significant for age (*p*=0.079), tumor size (*p*=0.414) and tumor grade (*p*=0.176) groups.

Keywords: Immunohistochemical expression, Invasive ductal carcinoma, *p*53.

INTRODUCTION

Breast cancer is the most common cancer in women in western world[1]. Incidence of breast cancer in Pakistani women is 24.4% which makes it the most common cancer among Pakistani women[2]. Carcinoma of breast is a heterogeneous disorder with multiple biological types, natural course, pathological and molecular characteristics with different prognostic factors[3]. Carcinoma of the breast is a complex disease. There is large intertumoral and intratumoral variations which causes variable clinical course and response to treatment[4].

TP53 causes progression of tumour by two mechanisms. Firstly it reduces tumour suppression. Secondly it is a pro-oncogene. Due to these mechanisms, p53 leads to tumorigenic effects[5]. TP53 gene can be measured by immuno-histochemistry due to longer half-life[1]. TP53 mutation is also related to response to various anticancer

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therapies. Breast carcinoma with TP53 mutation respond in a variable way to anticancer therapy due to different reasons[5]. After DNA damage induced by anticancer therapies, apoptosis is controlled by TP53 gene. After inducing DNA damage, radiotherapy and chemotherapy uses apoptotic ability of TP53. Breast cancer with TP53 mutation will be more resistant to radiation and chemotherapy. Hence *p*53 function loss is related to poor response to chemotherapy and endocrine therapy. *p*53 expression is also related with a poor prognosis in breast carcinoma and patient with *p*53 expression also presents with high grade tumour and lymph node invasion[1].

Different studies have shown different percentages of *p*53 mammary carcinoma[6]. A research conducted in India overexpression of *p*53 was noted in 22% cases of invasive ductal carcinoma[1]. In another study carried out in Romania, immunohistochemical expression of *p*53 was present in 44.44% of all invasive ductal carcinoma patients[6]. In one of the studies carried out in India, *p*53 was positive in 71.67% of invasive ductal carcinoma[7].

Rationale of the study was to determine the expression of *p*53 in invasive ductal carcinoma in Pakistani population as invasive ductal carcinoma is common malignancy in Pakistan and *p*53 would help to determine prognosis and response to therapy.

METHODOLOGY

This research was conducted at Pathology department of CMH Lahore between July 2015 to January 2016. Ethical committee approval was taken. A total of 80 females, with newly diagnosed invasive ductal carcinoma of breast,who aged between 25-80, were included in the study by non probability, consecutive sampling technique. Poorly fixed specimens and specimens with scanty tumor tissue, or patients on treatment were not included in the study.WHO calculator was used to calculate the sample. Confidence interval was 95%, while error margin was 10%. Expected percentage of *p*53 expression was taken as 71.67%.

IHC expression for p53 was done by DAKO kit as per the guidelines as follows: The FFPE tissue sections were cut at 3µm thickness and placed on clean glass slide with pre-attached adhesive on its surface. They were incubated at 58 degrees Celsius for 4 hours. The sections were deparaffinized with xylene 1 and 2, for 3 minutes each. They were rehydrated in decreasing concentrations of alcohol, 90%, 80% and 70% for 3 minutes each, followed by running tap water for 5 seconds. The slides were placed in coplin jar with 0.01 MTris-EDTA at 9.0pH. 750W domestic microwave was used to treat the slides for 20-30 minutes for heat mediated antigen retrieval. Slides were washed with distilled water for 20-40 minutes. After cooling down the sections, they were brought to phosphate buffered saline (PBS) at pH 7.3 for 5 minutes. PBS was washed and excess was wiped off the sections. Endogenous peroxidase activity blocked by incubating in 0.5% hydrogen peroxide in methanol for 5 minutes. 100uL of primary antibody of *p*53 was instilled on the sections and incubated for 60 minutes. The slides were again washed in three series of PBS for one hours. The slides were then incubated in avidin-biotin complex for 10 minutes. They were rinsed with distilled water. They were incubated in DAB (diaminobenzidine). Then the slides were put under water and counter stained with haematoxylin for 40 seconds. The slides were dehydrated by 70%, 80%, 90% and 100% alcohol for 3 minutes each. Clearing was done by placing slides in xylene for 3 minutes. The slides were mounted with Canada balsam. IHC results were verified by two histopathologiston high power, by calculating the proportion of tumor cells with nuclear stain. Any value above 10% of tumor cells was taken as positive[7].

Data was entered and statistically interpreted using SPSS version 22. Numerical variables which includes age are shown as mean ± SD. Categorical variables which include expression of *p*53 are shown as frequency and percentage. Stratification of data is done for age, lymph node positivity/negativity, size of tumor and grade to address effect modifiers. Post stratification Chi-square was taking *p*-value ≤0.05 as significant.

RESULTS

In the present study, the age of the patients ranged from 25 years to 80 years with a mean of 50.76 ± 11.80 years. A similar mean age among patients of invasive ductal carcinoma breast has been reported by Badar *et al*[8], in 2014 (48.6 ± 12.2 years), Khokher *et al*[9], in 2012 (47 ± 12 years) and Mamoon *et al*[10], in 2009 (48 ± 12 years). In 54 (67.5%) patients lymph nodes were positive for tumour metastasis. A similar proportion of positive lymph node status at presentation among patients of invasive ductal carcinoma breast has been reported by Badar *et al*, 8 (62.0%), Mamoon *et al*, 10 (74.6%) and Radha *et al*, 1 (52.0%).

Majority 64 (80%) of the tumours were Grade-II followed by Grade-III in 15 (18.7%) patients. Siddiqu *et al*[11], and Khokher *et al*[9], also reported Grade-II being the most frequent tumour grade observed in 65% and 55% of such patients at presentation respectively. Badar *et al*[8], observed relatively equal proportion of grade-II (41.4%) and Grade-III (49.6%) tumours among patients presenting with invasive ductal carcinoma at Shaukat Khanum Memorial Cancer Hospital and Research Center, Lahore. Kakarala *et al*[12], also reported a relatively similar distribution of tumour grade among Indian such patients; Grade-II (40.8), Grade-III (41.9%). Radha *et al*[1], observed much higher frequency of Grade-I tumours (48%) followed by Grade-II (40%) tumours with only a small proportion of Grade-III tumours (12.0%).

Table: Stratification of *p*53 expression according to age groups, lymph node status, tumour grade and tumour size.

Clinicopathological Variable	Cases (n)	Percentage (%)	*p*53 Expression		*p*-value
			Positive	Negative	
Age Groups (years) (n=80)					
25-50	44	55	17 (38.6%)	27 (61.4%)	0.079
51-80	36	45	21 (58.3%)	15 (41.7%)	
Lymph Node Status (n=80)					
Positive	54	67.5	32 (59.3%)	22 (40.7%)	0.002
Negative	26	32.5	6 (23.1%)	20 (76.9%)	
Tumour Grade (n=80)					
Grade-I	1	1.3	0 (0.0%)	1 (100%)	0.176
Grade-II	64	80	28 (43.8%)	36 (56.2%)	
Grade-III	15	18.7	10 (66.7%)	5 (33.3%)	
Tumour Size (n=80)					
<4 cm	52	65	22 (42.3%)	30 (57.7%)	0.414
4-9 cm	22	27.5	13 (59.1%)	9 (40.9%)	
9-14 cm	6	7.5	3 (50%)	3 (50%)	

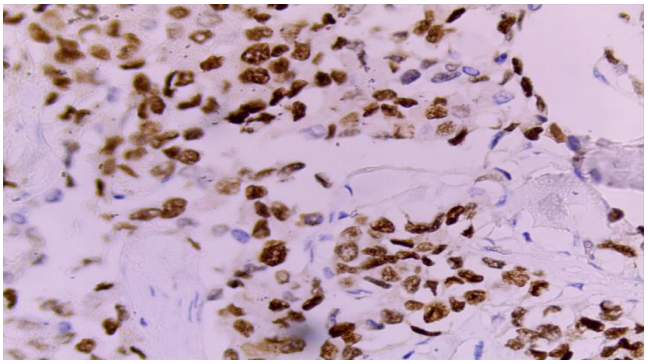

Figure-1: *p*53 positive IDC grade-III (magnification 40x).

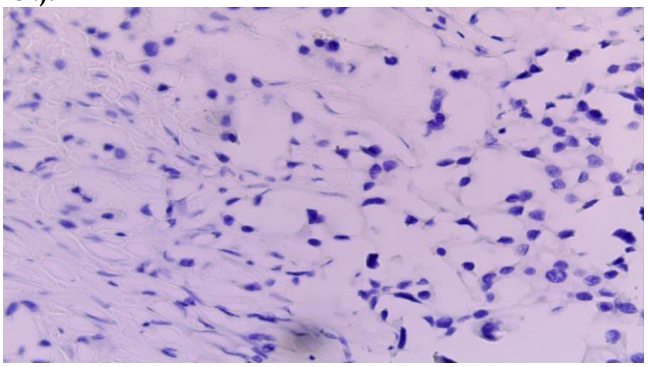

Figure-2: *p*53 negative IDC grade-II (magnification 20x).

The size of the tumour ranged from 0.2 cm to 14 cm with a mean of 4.16 ± 2.59 cm. Mamoon *et al*[10], mentioned same tumour of 4.6 ± 2 cm among such patient at Armed Forces Institute of Pathology, Rawalpindi, Pakistan.

*p*53 expression was positive in 38 (47.5%) patients (fig-1 & 2). The frequency of *p*53 was significantly higher in samples with positive lymph node status (59.3% vs. 23.1%; *p*=0.002). However, expression of p53 was not so significant for age (*p*=0.079), tumor size (*p*=0.414) and tumor grade (*p*= 0.176) groups.

DISCUSSION

In the present study *p*53 expression was positive in 38 (47.5%) patients. It was negative in 42 (52.5%) patients. Our results match with those of Plesan *et al*. In 2010 who reported similar frequency of p53 expression among Romanian such patients[6]. Previously Lipponen *et al*[13], (1995) also reported similar frequency of *p*53 expression in Finland patients. A much higher expression of *p*53 in invasive mammary cancer has been reported by Bertheau *et al*[14], in 2013 (69%) among French and Sekar *et al*[7], in 2014 (71.67%) among Indian such patients. While Radha *et al*[1], in 2014 (22%), Yamamoto *et al*[15], in 2014 (21.0%) and Yang *et al*[16], in 2013 (29.9%) reported relatively lower frequencies of p53 expression among Indian and Chinese such cases.

The frequency of *p*53 was significantly higher in samples with positive lymph node status (59.3% vs. 23.1%; *p*=0.002). However, expression of *p*53 was not so significant for age (*p*=0.079), tumor size (*p*=0.414) and tumor grade (*p*=0.176) groups.. Previously, Sirvent *et al*[17], in 1995 reported insignificant difference in the *p*53 expression with lymph node status (*p*>0.05). But they reported significant difference across tumour grade (*p*= 0.0048) where higher tumour grade was associated with increased frequency of *p*53 expression. Radha *et al*[1], in 2014 did notobserve any difference in *p*53 expression across age (*p*=0.806), tumour size (*p*=0.947), tumour grade (*p*=0.940) and lymph node status (*p*=0.848).

The present study is in local population and has found that 47.5% of breast cancer specimens were positive for *p*53. The conclsionof the present study matches to various populations with minor differences which can be due to population differences. As known from previous research *p*53 expression is associated with worst outcome[18]. Tumour with IHC detected *p*53 show more metastatic behavior[19]. Several studies have suggested that anti *p*53 drugs can restore the tumour suppression ability of *p*53 gene[20-23]. This higher proportion of *p*53 expression in the present study necessitates routine testing of *p*53 and anticipated measures in the management of patients with a positive *p*53 expression so that morbidity and mortality associated with breast cancer can be diminished. A very important finding in the present study was significantly higher proportion of *p*53 expression in specimens with a positive lymph node status which may imply an association between positive *p*53 expression and nodal involvement. However, further studies are required in this regard.

The strengths of the this research includes strict exclusion criteria to exclude the confounders and stratification of results for age, tumour size, grade and lymph node status to address effect modifiers which make the present study more reliable. However, there was a very strong limitation to this research that we did not compare the outcome of patients with and without *p*53 expression.

CONCLUSION

The frequency of p53 expression was 47.5% in invasive ductal carcinoma of breast among Pakistani women. It was significantly higher in samples with positive lymph node status (59.3% vs. 23.1%; *p*=0.002). However, significant difference in the frequency of *p*53 expression across age (*p*=0.079), tumor size (*p*=0.414) and tumor grade (*p*=0.176) groups was not noted.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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