ORIGINAL ARTICLE

Correlation of P53 Expression with Histological Grade and Histological Type of Invasive Breast Carcinoma

TAYABA JAVED[1], ROZINA JAFFER[2], MUNAZZA HASSAN[3], ALI AKRAM
[1]Assistant professor of Pathology, CMH Medical College, Lahore, [2]Professor of Pathology, SIMS, Lahore, [3]Associate Professor of Pathology, PGMI, Lahore.
Correspondence Author: Dr. Tayaba Javed, Assistant Professor of Pathology, CMH Medical College, Lahore.

ABSTRACT

Objective: To evaluate expression of P53 in different types of breast CA and correlate it with different grades of invasive ductal CA.
Study design: It is a descriptive study
Place and duration of study: Pathology Department, Post Graduate Medical Institute (PGMI) from 2nd January 2014 to 3rd January 2015.
Material & Methods: A total of 71 specimens diagnosed with invasive breast carcinoma in all surgical specimens (excisional biopsy, incisional biopsies mastectomies) were included in the study. P53 immunohistochemical staining was applied on all the cases. DAKO FLEX monoclonal mouse anti human P53 protein kit was used. P53 staining was done on 4mm sections and interpreted under the microscope. Typing and grading of carcinoma was done according to WHO classification and P53 expression was observed in different types and grades of breast carcinoma. Statistical package for social sciences 20 Data was used for analysis. Qualitative expression was presented in the form of frequency and percentage.
Results: From 71 patients, all 09 cases of grade I carcinoma were P53 negative, 22 cases of grade II carcinoma were P53 negative whereas 12 cases were positive. Among 22 cases of grade III breast carcinoma, 16 were P53 positive while 06 were negative. P53 showed different expression in different types of breast carcinoma. P53 positivity was seen in 28 of 65 cases while rest (37) was negative for it. Mucinous carcinoma showed positivity in 50% of the cases (one was positive and one was negative). Similar results were seen in invasive lobular carcinoma as seening mucinous carcinoma. P53 showed 100% positivity in poorly differentiated (1/1) and metaplastic carcinoma (1/1).
Conclusion: P53 positive expression is a poor prognostic marker. It is seen more frequently in carcinoma with poor prognosis and less frequently in carcinomas with better prognosis.

Key Words Invasive breast CA, p53, histological grade, Histological type

INTRODUCTION

Breast carcinoma is very diverse when we consider genetic and clinical aspects. In order to organize this diversity and, breast cancer classification systems have been developed. These classification criteria have been revised over decades to advance in management and prognosis. [1]

In 12% of breast carcinomas inheritance of susceptibility gene is an important cause – Genetic make-up is very important, almost 90% of the genetic change is observed in breast malignancy. [2]

The genes which are regularly mutated are BRCA I, BRCA 2 and p53 in women with positive family history of disease. [3] The probability to have breast carcinoma by age 70 is greater than 90% in whom p53 mutation is present. In breast carcinoma diagnosis and prognosis, P53 mutation is a vital immuno marker. The gene is located on short arm of chromosome 17 and encodes a nuclear phosphoprotein (375 aminoacids) [2]. P53 is a tumor suppressor protein; it plays an important role in regulating genomic stability by controlling the cell cycle. When cell damage is beyond repair, P53 induced apoptosis. P53 has a short life in normal cell. Within p53 gene, missense mutation converts it into a stable protein by posttranscriptional modification and accumulation within the cell nucleus. About 18%–25% of primary breast carcinomas have been established to have mutations in p53 tumor suppressor gene. The prognostic factors of breast carcinoma have been analyzed and discovered the absence of p53 mutations for sees prolonged disease-free and overall survival following primary therapy.

P53 is a critical tumor suppressor since it has been found to be lost in partly in all human malignancies. [4] It is a major role in malignancy prevention; it suppresses tumor development by blocking cell cycle and apoptosis. So the p53 quietus is the critical key in carcinogenesis. P53 is also a predictor of survival along with prognostic factors like stage, grade, tumor subtype and thus help in distinguishing a group with greater risk of mortality. Its positivity is considered to be a sign of poor prognosis. [5] It has been reported that each year over 1.5 million are diagnosed with breast carcinoma worldwide and 502,000 die from the disease.

The overall incidence of breast CA is about 22.9% of all Carcinomas. Studies done for cancer prvalance in Asians, Indians, Pakistanis and immigrants to various countries including Canada, US, Singapore, UK have documented a rise in breast CA in premenopausal women. Women of Indian and Pakistani origin are younger than 40 in comparisons to local Caucasian women. Hariset al 2007 reported that positivity of P53 had no impact on prognosis while study by Dookeran et al showed that itspresence correlated with higher grade of tumor, negativity of ER and PR and basal subtypes.

This study was designed to evaluate expression of P53 in different types of breast CA and correlate it with different grades of invasive ductal CA.

MATERIAL & METHODS

This study was conducted at Pathology Department, Post Graduate Medical Institute (PGMI) from 2nd January 2014 to 3rd January 2015.

All diagnosed cases of breast carcinoma were included in the study. The cases of recurrent breast carcinoma or receiving chemotherapy, radiotherapy or hormonal therapy were excluded. Suboptimally fixed tissues and inadequate materials were also excluded.

Hematoxyline and eosin stained slides were prepared and reviewed for confirmation of the diagnosis of breast carcinoma. The most representative section was used for immunohistochemical analysis. P53 (Dako Flex) monoclonal mouse anti Human P53 protein kit was used. It was in piqued form in buffer consisting steadying protein and 0.015mol/litsodiumazide. Centroblast in follicular lymphoma were taken as positive control while endothelial cells and fibroblasts in normal and reactive mesothelium were taken as negative control. Sections of approx. 4mm were cut on to poly L-lysine coated slides and were deparafinized and rehydrated. Microwave method was used for Antigen retrieval. Slides were permitted to cool for 20 minutes and then placed in UV block for 5 minutes. Tissues were covered with primary antibody at dilution 1:50 and were incubated for one hour at room temperature. Slides were then incubated first with amplifier and then with HRP polymer for 10 minutes. Chromogen was applied for 20 minutes and all slides were counter stained with hematoxyline, dehydrated and mounted. Between each step, the slides were washed with phosphate buffer solution. The staining was quantitatively assessed according to percentage of positive cells (nuclear staining).

Data was collected in a well custom designed Performa and analyzed using SPSS version 20. P- Value of <0.05 was considered statistically significant at 95% confidence interval.

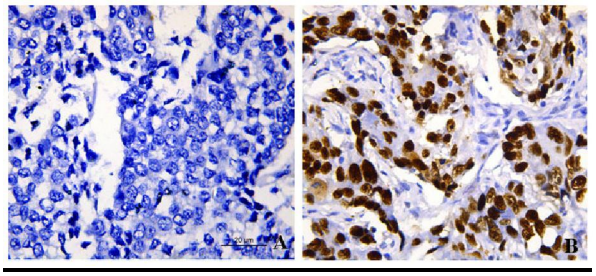

Figure A showed the negative expression. Figure B showed the Positive expression. Cells with the brown stain contain P53.

RESULTS

From 71 patients, all 09 cases of grade I carcinoma were P53 negative, 22 cases of grade II carcinoma were P53 negative whereas 12 cases were positive. Among 22 cases of grade III breast carcinoma, 16 were P53 positive while 06 were negative. P53 showed different expression in different types of breast carcinoma. P53 positivity was seen in 28 of 65 cases while rest (37) was negative for it. Mucinous carcinoma showed positivity in 50% of the cases (one was positive and one was negative). Similar results were seen in invasive lobular carcinoma as seen in mucinous carcinoma. P53 showed 100% positivity in poorly differentiated (1/1) and metaplastic carcinoma (1/1).

Table 1: Expression of p53 in different Grade of invasive ductal CA

Grade	Number of Case	P53 Positive	P53 Negative
I	09	00	09
II	34	12	22
III	22	16	06
Total	**65**	**28**	**37**

Table 2: Expression of p53 in different types of breast CA

Type	Number of Cases	P53 Negative	P53 Positive
Invasive ductal CA	65	37	28
Mucinous CA	02	01	01
Invasive Lobular CA	02	01	01
Poorly duff CA	01	00	01
Meta Plastic CA	01	00	01
Total	**71**	**40**	**32**
Percentage	**56%**	**56.11%**	**46.4%**

Table 3: p53 Scores in Different Grades of Breast Carcinoma

Grades	P 53 Score			
	1	2	3	4
Grade II	6	6	-	-
Grade III	-	10	6	-

Table 4

%age of Positive Cell	P53 Score
None	0
< 10%	1
10-25%	2
26-50%	3
> 50%	4

Table 5: Association between expressions of p53 with Grades of invasive ductal carcinoma

P53	Grade 111	Grade <111
P53 negative	06	25
P53 positive	16	12

DISCUSSION

Breast carcinoma is a disease with incredible heterogeneity in its clinical manners. Size of tumor, histological type and grade, lymph node involvement, vascular space invasion, tumor cell proliferation, extent of ductal carcinoma in situ are the pathological variables which are the predictors of prognosis and 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. [6]

It is a documented fact that most women are over the age of 60 years when diagnosed, therefore progression of age raises the risk of breast cancer. [7] Asian female are most probably to develop breast cancer at earlier ages than their Western world [8] Breast carcinomas arise in the upper outer quadrant of the breasts and it is supported the hypothesis that underarm cosmetics cause breast cancer. The greater amount of breast tissue is present in this quadrant. [9]Tumor size is one of the greatest predictors of tumor behavior in breast cancer. Larger tumor size has poor 5 year survival rate. 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.

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 describes the abnormal appearance of tumor under the microscope and highlights tumor growth 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 tumor diameter, the greater the number of axillary lymph nodes metastatic, also the worse the outcome. [9]. In our study, the entire grade I invasive ductal CA was p53 and majority in grade II were negative however, in Grade III majority were positive. Mucinous + Lobular CA poorly differentiated and Meta Plastic CA were p53 positive. 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. [10] 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. [11]

In our study, a significant relationship was obtained between tumor grade and p53 expression. 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. [12] 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. [13]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 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. [14]

CONCLUSION

The entire grade I invasive ductal CA and majority in grade II were p53 negative. Majority of Grade III were positive. Mucinous + Lobular CA poorly differentiated and Meta Plastic CA were p53 positive. 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.

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